Volumen 51. Nº 1. Marzo 2001
Suplemento 1

ALAN

ARCHIVOS LATINOAMERICANOS DE NUTRICION

Organo Oficial de la Sociedad Latinoamericana de Nutrición

Continuación de Archivos Venezolanos de Nutrición

SLAN

ALAN-VE ISSN 0004-0622
Depósito Legal: pp 199602DF83

Archivos Latinoamericanos de Nutrición (ALAN) es editado como órgano oficial de la Sociedad Latinoamericana de Nutrición (SLAN), para la divulgación de conocimientos en el campo de la alimentación y de la nutrición principalmente en el Hemisferio Americano. En sus páginas se acogen manuscritos en español, inglés, portugués y francés, tanto de miembros como de aquellos que no sean miembros de la Sociedad, y de cualquiera de las siguientes categorías: 1. Trabajos generales (revisiones científicas críticas); 2. Trabajos de investigación (originales); 3. Trabajos de nutrición aplicada (resultados analíticos de programas de intervención y discusión de recomendaciones de aplicación práctica), y 4. Cartas al Editor (comentarios cortos de interés general o relacionados con resultados o conceptos científicos publicados previamente en *Archivos*).

Archivos Latinoamericanos de Nutrición (ALAN) is the official publication of the Sociedad Latinoamericana de Nutrición (SLAN), for the dissemination of knowledge in the fields of food and nutrition, principally throughout the American Hemisphere. Articles in Spanish, English, Portuguese and French are accepted, both from the Society members and from nonmembers, in the following categories: 1. General articles (critical scientific reviews); 2. Research articles (originals); 3. Papers in applied nutrition (analytical results from intervention programs and discussion of recommendations of practical application), and 4. Letters to the Editor (short comments of general interest or about scientific facts and concepts previously published in *Archivos*).

Dirección: Archivos Latinoamericanos de Nutrición
Apartado 62.778. Chacao.
Caracas 1060. Venezuela, S.A.
Fax (58.212) 286.00.61

ENTIDADES PATROCINANTES

- **Fundación para la Alimentación y Nutrición "José María Bengoa"**
  Caracas, Venezuela
- **Instituto de Nutrición de Centro América y Panamá (INCAP)**
  Guatemala, Guatemala C.A.
- **INSTITUTO NACIONAL DE NUTRICION.** Venezuela
- **CONICIT.** Venezuela
- Kellogg's América Latina
- Roche **PRODUCTOS ROCHE,** América Latina
- **Fundación POLAR**
- **Protein Technologies International**
  Caracas, Venezuela
- **Centro de Atención Nutricional Infantil Antímano. CANIA**
- **Alimentos LE BISCUIT C.A.**
- **PARMALAT de Venezuela**
- **BASF Venezolana S.A.**
- **Cadipro Milk Products, C.A.**
- **ADM Protein Specialties**
- **Coca-Cola de Venezuela**

ARCHIVOS LATINOAMERICANOS DE NUTRICION se complace en destacar y agradecer el apoyo económico recibido del CONICIT para la edición sostenida de la revista.
Bajo la responsabilidad del Capítulo Venezolano de la Sociedad Latinoamericana de Nutrición.

Archivos Latinoamericanos de Nutrición

Organo Oficial de la Sociedad Latinoamericana de Nutrición

VOL 51 **MARZO 2001** **Suplemento N° 1**

Contenido

Archivos Latinoamericanos de Nutrición

Official Publication of the Latin American Society of Nutrition

VOL 51 MARCH 2001 Supplement Nº 1

Contents

PRESENTATION

The Latin American Center for Nutrition and Metabolic Studies, CELANEM, is proud to present this supplement of the Latin American Archives of Nutrition, that is completely devoted to the latest research on an iron amino acid chelate, commercially known as Ferrochel®, and chemically as iron bis-glycinate chelate, CAS No. 20150-34-9, now recognized as G.R.A.S. by the U.S. Food and Drug Administration (No. GRN 000019).

Since the later part of the 1980s, CELANEM has participated in research on the effectiveness of Ferrochel and other iron chelates such as the TF iron amino acid chelate (taste free iron chelate), compounds that are produced and registered by Albion Laboratories, Inc, of Clearfield, Utah, U.S.A.

During this time, a significant amount of investigation has been carried out, followed by publication in scientific journals that assisted in defining the advantages of the amino acid chelates for the control of iron deficiency and iron deficiency anemia all around the world.

In spite of all our efforts, some uncertainty persists about the effectiveness and safety of the amino acid chelates that in our opinion is the results of a poor understanding of what these chelates are, how they are absorbed, how they exert their effect in the human body, and how they are regulated in their absorption by the iron reserves of the body, and how toxic they may be. There has been a strong effort to show and publish the very low toxicity of these compounds as established by research in experimental animals that include acute, semichronic and chronic toxicity. It has been proven that the iron amino acid chelates show a very low toxicity, and has been successfully used in fortification of a number of food matrixes.

In the first part of the supplement we present an introduction, written by one of the leading experts in the field of iron deficiency control that focuses on the importance of iron deficiency and anemia in the world, followed by papers on the chemistry of chelation, the bioavailability, the absorption, the regulation, the toxicity and stability of Ferrochel in the presence of different compounds.

In the second part we present papers on the effectiveness of Ferrochel in clinical trials for control of iron deficiency and iron deficiency anemia in pregnant women, field trials in Tanzania, and several papers on the use, effectiveness and evaluation of the fortification of wheat flours, bread and sugar, as a mean of controlling iron deficiency anemia, and finally a discussion by the director of CELANEM on the implications of the research presented.

Oscar Pineda
Director of CELANEM

Note: All the iron chelates used in the investigations presented as well as other chelates discussed in some of the papers are produced, patented and registered by Albion Laboratories, Inc. of Clearfield, Utah, U.S.A., that kindly provided all the chelates used in the investigations and for which the authors and CELANEM are greatly indebted.

ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición Suplemento Vol. 51 N° 1, 2001

Iron deficiency and the developing world

A. Patrick MacPhail

Department of Medicine, University of Witwatersrand, Johannesburg, South Africa

SUMMARY. The dietary intake of iron in underdeveloped countries is based mainly on non-hem iron which is absorbed to a lesser degree that hem iron and is subjected to many interferences from inhibitors generally present in the diets, such as phenols, phytates, fibers, etc.
Food fortification with iron is considered to be the best and cheapest long-term approach for correcting the deficiency. The iron source selected for this purpose has to be soluble, and of high bioavailability, even in a diet rich in inhibitors. Ferrochel may prove to be this type of compound.
Key words: Iron deficiency, anemia, fortification, Ferrochel.

RESUMEN. Deficiencia de hierro y el mundo en desarrollo. La ingesta dietética de hierro en el mundo en desarrollo se basa esencialmente en hierro no hemínico el cual se absorbe en menor grado que el hierro del heme y está sujeto a interferencia por componentes normales de las dietas tales como fenoles, fitatos, fibra, etc.
La fortificación de alimentos con hierro se considera como el mejor enfoque a largo plazo para corregir la deficiencia. La fuente de hierro seleccionada para este objeto debe ser soluble y mostrar alta biodisponibilidad aún en dietas de alto contenido de inhibidores. Es posible que Ferrochel demuestre ser este tipo de compuesto.
Palabras clave: Deficiencia de hierro, anemia, fortificación, Ferrochel.

INTRODUCTION

Iron deficiency is the most commonly encountered nutritional deficiency in humans. More than half a billion people have iron deficiency anemia (1), and many more have depleted iron stores and are at risk for the development of anemia. Paradoxically, iron is the second most common metal in the earth's crust and is present in all foods. Furthermore, the prevalence of iron deficiency anemia varies widely (2). Over the years there has been a steady drop in the prevalence of iron deficiency in industrialized countries, with less than 3% of fertile women in the United States being affected (3). In contrast, iron deficiency is a major problem in the developing world and affects almost all segments of the population including men (4). It is particularly severe in infants and childbearing age women. For example, the prevalence of iron deficiency anemia in preschool children has been reported to be 70% in the Caribbean (5) and between 45 and 70% in Ecuador (6). In pregnant women in West Africa the prevalence ranges between 20 and 45% (2). To comprehend why iron deficiency is so common in the developing world in the face of a plentiful supply of iron and to appreciate the dilemmas that beset iron fortification, it is necessary to have an understanding of basic iron metabolism and particularly the factors that influence the absorption of iron from the diet.

Basic iron metabolism

Iron owes its importance in biology to its remarkable reactivity. The reversible one electron oxidation-reduction reaction between ferrous (Fe (II)) and ferric forms (Fe (III)) is exploited by most iron-dependent enzyme and transport systems and is managed by highly specialized proteins involved in the storage and transport of iron. However, it is this reactivity that produces unacceptable organoleptic changes in food, making the fortification of food with iron difficult. Normally iron can only enter the body through the diet. Four phases of iron absorption are recognized. In the *luminal* phase, food iron is solubilized, largely by acid secreted by the stomach, and is presented to the duodenum and upper jejunum where most iron absorption takes place. Factors which maintain the solubility of iron in the face of rising pH, such as valency (ferrous iron is better absorbed), mucin secreted by the mucosa and chelators (ascorbic acid), appear to be important in this phase. It is in this phase that the presence, or absence, in the diet of promoters and inhibitors of iron absorption is of great importance. In the second phase, *mucosal uptake*, iron is bound to the brush border and transported into the mucosal cell. In the third *intracellular phase*, iron is either stored in cellular ferritin or is transported directly to the opposite side of the mucosal cell and released. In the last phase iron is *released* from the mucosal cell into the portal circulation where it is bound to the transport protein, transferrin. Both iron uptake and release by the mucosal cell appear to be inversely related to the amount of iron stored in the body. Most of the absorbed iron is transported directly to the bone marrow where it is incorporated

into hemoglobin in the red cell. At the end of its life span the red cell is engulfed by cells of the reticuloendothelial system (RES), located mainly in the liver and spleen. The iron is separated from hem and either stored in ferritin or as hemosiderin, both in the RES and in hepatocytes, or released back into the circulation where it is again picked up by transferrin. It should be noted that this cycle is never reversed. There is normally only one way in and there is no way out except through blood loss or, in pregnancy, to the fetus. In reality, a small amount of iron is lost. In men this amounts to about 1 mg/day mainly through loss of blood and surface cells of the gut, urinary tract and skin. The loss is relatively easily balanced by iron absorption. In women, additional losses through menstruation (0.5 mg Fe/day) and the cost of pregnancy (2 mg Fe/day) and lactation (0.5 mg Fe/day) make it more difficult to balance the loss through iron absorption.

Iron balance

Iron requirements must be balanced by iron supply if iron deficiency is to be avoided. Several factors combine to influence iron balance (Table 1). Obligatory iron losses, the requirements of growth and pregnancy as well as pathological losses such as that due to hookworm infection (7) must be balanced against iron supply. Iron supply is heavily influenced by the amount and type of iron in food and the combination of various inhibitors and promoters of iron bioavailability. The major reason for the variation in the prevalence of iron deficiency is to be found in the bioavailability of iron in the staple diets consumed in different regions of the world. Subjects in many developing countrys subsist largely on cereals (8). The iron in which is of low bioavailability because of the presence of inhibitors such as phytates and polyphenols (8). On the other hand, mixed diets of high bioavailability are consumed in Western countries. They contain hem iron, which is well absorbed, together with ascorbic acid and meat, both of which are potent enhancers of non-hem iron absorption (9,10). In essence the problem is one of supply and demand. In regions dependent on cereals the supply of absorbable iron in the diet is limited and may not be sufficient to meet physiological requirements, especially when they are increased by growth, menstruation and pregnancy. While iron balance can also be disturbed at times of increased demand in subjects eating Western diets, the problem is usually a less serious one. Infants are, however, at risk in all populations, since the demand for iron is high and the bioavailability of iron in the infant diet is low (11). Figure 1 shows the changing median iron requirements of males and females with age (12). The upper dashed line represents the amount of iron supplied by a Westernized diet while the lower dashed line represents the iron supplied by a cereal diet typical of the developing world. Neither diet is able to meet the demands of infancy and pregnancy. In women, even the highly bioavailable Western diet is not able to meet the demands of more than 50% of women at menarche, while the cereal diet can not match the requirements of most adult women and some men.

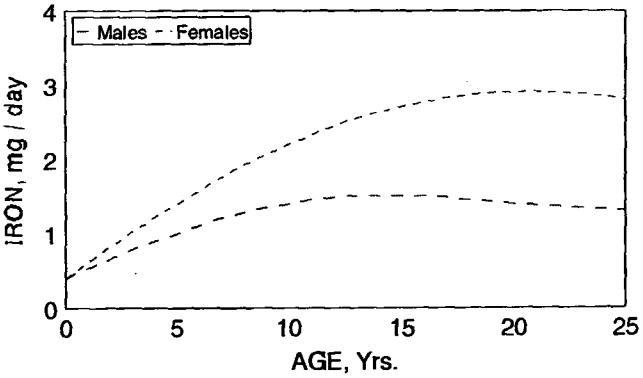
TABLE 1
Iron balance in the body

Iron requirements	Iron supply
Iron losses	**Food iron content**
Menstruation	**Type of iron**
Bleeding	
Hookworm	hem iron
	non-hem iron
	Iron requirements
Iron absorption	
Growtrh	
Pregnancy	Innbhibitors
Lactation	Promoters

Absorption of dietary iron

Fortification of basic foodstuffs with iron is considered to be the best and cheapest long term approach for correcting iron deficiency (13,14) particularly where low iron intake or bioavailability is the primary cause (15,16). However, the fortification of food with iron is not a simple matter and it is worthwhile reviewing the factors involved in the luminal phase of dietary iron absorption since they affect the methods that can be used for fortification.

FIGURE 1
Median iron requirements by age and sex



Common pools in iron absorption

In developing countries the single most important cause of iron deficiency is the low bioavailability of iron in major staple foodstuffs such as cereals and legumes (8). Unfortu-

nately any fortification iron added to such a diet enters the "common pool" of non-hem food iron and, in consequence, is absorbed as poorly as the native iron in the diet. This is due to the fact that the added iron is exposed to the same influences, both inhibitory and enhancing, that influence the absorption of non-hem iron in the diet. Hem iron, which is derived from hemoglobin and muscle, forms a separate pool in the lumen of the gut and is not subjected to inhibitory dietary factors, it is thus far more bioavailable (Reviewed by Layrisse and García-Casal (17)).

Enhancers and inhibitors of iron absorption

The two most important enhancers of dietary non-hem iron absorption are meat and ascorbic acid (9), Hem iron, derived from bovine hemoglobin, has been used successfully to fortify cereals (18,19). The two most important dietary inhibitors of iron absorption are phytates (20) and polyphenols (21). Phytates are widely present in foods and are found in particularly high concentrations in bran (22). Polyphenols are not only responsible for the marked inhibitory effect of tea on iron absorption (23) but are present in a number of vegetables, including sorghum and legumes. They are responsible, in a dose dependent manner, for the poor bioavailability of the non-hem iron present in these foodstuffs (21,24). Polyphenols are also responsible for the marked color changes that may develop in foods fortified with iron (21).

Food fortification strategy

The strategy that should be followed in establishing an iron fortification program has been carefully defined (13,14) (Table 2). It is perhaps significant that those fortification programs and trials that have had only limited success have usually disregarded one or more of the steps. Perhaps the most difficult step in iron fortification is to find a combination of iron compound and food vehicle in which the two are compatible (Step 2). The attributes of the ideal food vehicle are listed in Table 3 (14). The recommended criteria are particularly difficult to meet in developing countries, since the production of staple foods is rarely centralized. The choice of an iron compound presents additional problems. For example a simple iron salt such as ferrous sulfate, is both cheap and bioavailable but its high solubility may lead to the production of colored compounds in fortified food. On the other hand, compounds like ferric orthophosphate which are more stable and therefore do not adversely affect food, are poorly bioavailable (25,26). It follows that it is essential that the bioavailability of the iron compound be assessed in the setting in which it is to be used (Step 4). Both radioisotopic absorption studies prior to a trial and measurement of iron status before and after a controlled pilot trial are useful ways of assessing this. Ferrous iron bis-glycine chelate has potentially great advantages. Unlike other non physiological chelates, one atom of iron is chelated to two molecule of glycine forming coordinated covalent bonds and originating two heterocyclic rings in which the iron atom is central. Glycine is a natural constituent of most foods and is easily metabolized. This compound is already in widespread use as an iron fortificant (27) and is the subject of the articles that follow.

TABLE 2
Steps in developing an iron fortification strategy

1. Determine the iron status of the population
2. Choose an appropriate iron compound and food vehicle combination
3. Establish the acceptability and stability of the fortified vehicle
4. Assess the bioavailability of iron from the vehicle in the appropriate dietary setting
5. Carry out controlled field trials
6. Implement a regional or national fortification program

Effect of iron deficiency on human development

While no one will deny that severe anemia is deleterious to health, there continues to be a considerable debate around the question of weather iron deficiency alone or mild iron deficiency anemia are bad for you. This controversy has been highlighted in recent years by highly publicized claims, unsubstantiated in subsequent work (28,29), of the ill effects of minimally raised iron stores on the generation of atherosclerosis (30) and the prevalence of cancer (31). Apart from ill health endangered by severe anemia, recent reviews have highlighted three areas in which iron deficiency is thought to play an important role. These are pregnancy (27), infection (32) and psychomotor development (33).

TABLE 3
Considerations in the choice of a food vehicle

Consumption	Technical
High proportion of population	Centrally processed
Minimal regional variation	Few production facilities
Unrelated to socioeconomic status	Minimal segregation of fortificant
Minimal individual variation	Good masking qualities
Low potential for excess intake	Low cost
Contained in all meals	Limited stoirage
Linked to caloric intake	High bioavailability

Much of the evidence that iron deficiency is detrimental to the health of pregnant women or to the developing fetus is circumstantial, and well-designed, controlled studies to support the evidence are lacking (27). Equally, there is little evidence to support strongly held views, emanating from the developing world, that supplementation with iron dur-

ing pregnancy is unnecessary or harmful (34,35). The major problems associated with anemia in pregnancy include increased perinatal mortality and morbidity (36), low births weight (37,38), prematurity (39) and the development of iron deficiency during infancy (40). While neonates born to iron deficient mothers appear to have sufficient iron, there is evidence that their iron stores may be reduced (41,42) and that anemia is more likely to develop during infancy (43,40). This has particular relevance when the effects of iron deficiency anemia on psychomotor development are considered. The demands of pregnancy on iron supply are such that women entering a pregnancy with low iron stores will become anemic (44) and there is little doubt that supplementation in these women is necessary. In developing countries, where the background prevalence of iron deficiency is high, routine supplementation would appear to be essential. The impact of iron fortification programs on the outcome of pregnancy has yet to be studied. Indirect evidence that an improvement in general iron nutrition is likely to have a positive effect can be gleaned from the fact that physicians in the iron replete developed world think that iron supplementation in pregnancy is unnecessary (34).

Infants between the age of six months and two years, in both industrialized and the developing world, are at risk for the development of iron deficiency anemia. This is a period of rapid brain growth and the development of cognitive and motor skills. Two important case-control studies have show impairment in psychomotor skills in infants with iron deficiency anemia during this period (45,46). In addition, electrophysiological measurements, performed on iron deficient and non-iron deficient infants, suggest that neurological development is delayed in anemic infants (47,48). Studies showing scholastic performance in school-going children with iron deficiency anemia indicate that this problem continues beyond infancy (49). A worrisome finding in these studies is the lack of improvement in scores on reversal of the anemia although in another psychomotor study, where more prolonged iron supplementation was given, some improvement was demonstrated (50). Although beset by problems of methodology and interpretation, these studies indicate that preventive measures, in the form of adequate iron fortification programs targeted to infants and young children, could play a vital role in improving psychomotor development with long range benefits to the developing world.

In conclusion, iron deficiency appears to be an important factor in retarding the realization of the full potential of people. The key to the improvement of iron nutrition is the development of well- designed iron fortification programs targeted to high risk groups. The development of an iron compound that is bioavailable in the face of a diet rich in inhibitors of iron absorption and with minimal organoleptic problems, is central to achieving this goal. The possibility that ferrous bis-glycine chelate may fill this role deservers further intense investigation.

REFERENCES

1. ACC/SCN 1992. Second report on the World Nutrition Situation. Geneva: ACC/SCN.
2. MacPhail AP, Bothweell TH. The prevalence and causes of iron deficiency anaemia. In: Nutritional Anaemias. S Fomon and S. Zlotkin, eds. Nestle Nutrition workshop series, vol 30, Nestec Ltd. Raven Press, New York, 1992. pp 1-12.
3. Cook JD, Skikne BG, Lynch SR, Reusser MG. Estimates of iron sufficiency in the U. S. Population. Blood 1986; 68:726-31.
4. Hurrell RF. Preventing iron deficiency through food fortification. Nutr Rev 1997; 55:210-22.
5. Simmons WK, Gurney JM. Nutritional Anaemia in the English-speaking Carib bean and Suriname. Kingston: Caribbean Food and Nutrition Institute J-46-80. Pan-American Health Organization, 1980.
6. Freire WB. Strategies of the Pan American Health Organization/World Health Organization for the control of iron deficiency in Latin America. Nutr Rev 1997; 55:183-88.
7. Stoltzfus RJ, Dreyfus ML, Chwaya HM, Albonico M. Nutr Rev 1997; 55:223-32.
8. INACG. International Nutritional Anemia Consultative Group. The effects of cereals and legumes on iron bioavailability. The Nutrition Foundation, New York, 1982.
9. Sayers MH, Lynch SR, Jacobs P, Charlton RW, Bothwell TH, Walker RB, Mayet F. The effects of ascorbic acid supplementation on the absorption of iron in maize, wheat and soya. Br H Haematol 1973; 24:209-18.
10. Cook JD, Monsen ER. Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on nonheme iron absorption. Am J Clin Nutr 1976; 29:859-67.
11. Dallman PR. Changing iron needs from birth through adolescence. In: Nutritional Anemias. S Fomon and S. Zlotkin, eds. Nestle Nutrition workshop series, vol. 30. Nestle Ltd. and Raven Press, New York, 1992.
12. MacPhail AP. Iron. In: Essentials of human nutrition. Mann J and Truswell AS, eds. Oxford University Press, Oxford, 1998.
13. INACG. International Nutritional Anemia Consultative Group. Guidelines for the eradication of iron deficiency anemia. The Nutrition Foundation, New York, 1977.
14. Cook JD, Reusser ME. Iron fortification: an update. Am J Clin Nutr 1983;38:648-59.
15. Hallberg L. Bengtsson C, Garby L, Lennartsson J, Rossander L, Tibblin E. An analysis of factors leading to a reduction in iron deficiency in Swedish women. Bull WHO 1979; 57:947-54.
16. Viteri FE, Alvarez E, Bulux J, Pineda O, Batres R. Iron fortification in developing countries, In: Harper AE, Davis GK, eds. Nutrition in Health and Disease and International Development. Symposia from the XII INternational Congress of Nutrition. Alan R. Liss, New York, 1981, pp. 345-54.
17. Layrisse M, Gargía-Casal MN. Strategies for the prevention of iron deficiency through foods in the household. Nutr Rev 1997;55:223-39.

18. Calvo E, Hertrampf E, Pablo S, Amar M, Stekel A. Haemoglobin-fortified cereal: An alternative weaning food with high iron bioavailability. Eu J Clin Nutr 1989; 43:237-43.
19. Hertampf E, Olivares M, Pizarro F, Walter T, Cayazzo M, Heresi G, Llaguno S, Chadud P, Stekel A. Haemoglobin fortified cereal: A source of available iron to breast-fed infants. Eur J Clin Nutr 1990;44:793-8.
20. Hallberg L, Rossander L, Skanber AB. Phytates and the inhibitory effect of bran on iron absorption. Am J Clin Nutr 1987;43:988-96.
21. Gillooly M, Bothwell TH, Charlton RW, Torrance JD, Bezwoda WR, MacPhail AP, Derman DP, Novelli L, Morral O, Mayet F. Factors affecting the absorption of iron from cereals. Brit J Nutr 1983;51:37-46.
22. Bjorn-Rasmussen E. Iron absorption from wheat bread. Influence of various amounts of bran. Nutr Metabol 1974;16:102-10.
23. Disler PD, Lynch SR, Charlton RW, Torrance JD, Bothwell TH. The effect of tea on iron absorption. Gut 1975;16:193-200.
24. Gillooly M, Bothwell TH, Torrance JD, MacPhail AP, Derman DP, Bezwoda WR, Mills W, Charlton RW. The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. Br J Nutr 1983;49:331-41.
25. Cook JD, Miniich V, Moore CV, Rassmussen A, Bradley WB, Finch CA. Absorption of fortification iron in bread. Am J Clin Nutr 1973;26:861-72.
26. Foy H, Dondi A. Hookworm and iron deficiency anemias in Mauritius - the effects of dietary iron fortification. 1981; Soc Protozoologuists Special Puiblication No. 1.
27. Allen LH, Ahluwalia N. Improving iron status through diet. Monograph US Agency of International Development. John Snow Inc./OMNI Project 1997. Arlington, U.S.A.
28. Meyrs DG. The iron hypothesis. Does iron cause atherosclerosis? Clin Cardiol 1996; 19:925-29.
29. Corti MC, Gaziano M, Hennekens CH. Iron status and risk of cardiovascula disease. Ann Epidemiol 1997;7:62-8.
30. Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Sppänen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in Eastern Dinnish men. Circulation 1992;86:803-11.
31. Stevens RG, Jones DY, Micozzi MS, Taylor PR. Body iron stores and the risk of cancer. N Engl J Med 1988;319:1047-52.
32. Walter T, Olivares M, Pizarro F, Muñoz C. Iron anemnia and infection. Nutr Rev 1977;55:111-24.
33. De Andraca I, Castillo M, Walter T. Psychomotor development and behavior in iron-deficient anemic infants. Nutr Rev 1997;55:125-32.
34. Barret JFR, Whittaker PG, Williams JG, Lind T. Absorption of non-heme iron from food during normal pregnancy. 1994; Br Med J 1994;309:79-82.
35. US Preventive Services Task Force. Routine iron supplementation during pregnancy. USPSTF 1993. Washington, D.C.
36. Hughes A. Anemia in pregnancy: Maternal health and safe motherhood programme. World Health Organization 1991. Geneva.
37. Hemminki E, Starfield B. Routine administration of iron and vitamins during pregnancy: review of controlled clinical trials. Br J Obstet Gynecol 1978;85:404-10.
38. Howe D, Wheeler T, Osmond C. The influence of maternal haemoglobin and ferritin on mid-pregnancy fetal volume. Br J Obstet Gynecol 1995;102:213-19.
39. Scholl TO, Hediger ML, Fischer RL, Shear JW. Anemia vs iron deficiency: in creases risk of preterm delivery in a prospective study. Am J Clin Nutr 1992; 55: 985-88.
40. Colomer J, Colomer C, Gutierrez D, Jubert-Dardet C. Anaemia during pregnancy a risk factor for infant iron deficiency: report from Valencia Infant Anaemia Cohort (VIAC) study. Paediat Perinatal Epidemiol 1990;4:196-204.
41. MacPhail AP, Charlton EW, Bothwell TH, Torrance JD. The relationship between maternal and infant iron status. Scand J Haematol 1980;25:141-50.
42. Agrawal RMD, Tripathi AM, Agrawal KN. Cord blood haemoglobin, iron and ferritin status in maternal anaemia. Acta Obstet Gynecol Scand 1993;72:534-53.
43. De Benaze C, Galan P. Wainer R, Hercberg S. Prevention de l'anemie ferroprive au course de la grassesse par un supplementtation martiale precoce:un essaie controle. Rev Epidemiol Sant Publique 1989;27:109-19.
44. Guidozzi F, Pate; R, MacPhail AP. A prospective study of iron status in white and black pregnant women in an urban hospital. S Afr Med J 1995;85:170-73.
45. Lozoff B, Brittenham GM, Wolf AW, McClish DK, Kuhnert PM, Jimenez E, Jiminez R, Mora LA, Gómez I, Krasuskph D. Iron deficiency anemia and iron therapy effects on infant development and test performance. Pediatrics, 1987;79:981-95.
46. Walter T, de Andraca I, Chanud O, Perales CG, Iron deficiency anemia: adverse effects on infants psychomotor development. Pediatrics 1989;84:7-17.
47. Rodriguez E, Garrido M, Williamson AA. Ciclo sueño vigilia en lactantes de 6 meses de edad: efecto de la deficiencia de hierro. V Congreso de la Soc. Latinoamericana para el Estudio del Sueño. Viña del Mar Chile, 1994. Libro de resúmenes, 30.
48. Garrido M, Rodriguez E, Williamson AA. Actividad motora durente el sueño y la vigilia en lactantes deficientes en hierro. V Congreso de la Soc. Latinoamericana de Estudio del Sueño. Viña del Mar, Chile 1994. Libro de resúmenes, 9.
49. Pollit E. Iron deficiency and educational deficiency. Nutr Rev 1997;55:113-41.
50. Idjaradinata P, Pollit E. Reversal of development delays in iron-deficient anemic infants treated with iron. Lancet 1993;341:1-4.

ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición

Suplemento Vol. 51 N° 1, 2001

The chemistry of ferrous bis-glycinate chelate

Stephen D. Ashmead

Albion Laboratories, Inc., Clearfield, Utah U.S.A.

SUMMARY. In order to produce a ferrous chelate four criteria must be met: 1) the ligand must contain two functional groups which are capable of entering into covalent and coordinate covalent bonds; 2) a ring structure with the ferrous ion being the closing member of the ring must be created; 3) the chelate must be sterically possible; and 4) the chelation reaction must be energetically possible. In addition to the above, a totally nutritionally functional ferrous chelate must meet three further criteria: 1) it must have low molecular weight; 2) its stability constant must be nutritionally functional; and 3) the ligand must be metabolizable by the body. When ferrous iron is reacted with glycine and forms a bis-glycinate chelate, it meets all of the requirements of being both a chelate and being a totally nutritionally functional chelate.
Key words: Chelation, criteria for chelation, Ferrochel, amino acid chelates.

RESUMEN. La química del hierro ferroso bis-glicinato quelado. Para producir un hierro ferroso bis-glicinato quelado es necesario llenar los siguientes cuatro criterios: 1) el ligando debe contener dos grupos funcionales capaces de producir uniones covalentes y covalentes coordinadas; 2) debe poder crearse una estructura en la cual el ion ferroso sea el elemento que cierra una estructura en anillo; 3) el quelado debe ser estéricamente posible, y 4) la reacción de quelación debe ser energéticamente posible. Adicionalmente, un quelado ferroso para ser nutricionalmente funcional debe también llenar los siguientes criterios: 1) debe tener un bajo peso molecular; 2) su constante de estabilidad debe ser nutricionalmente funcional, y 3) el ligando debe poder ser metabolizado en el organismo. Cuando el hierro ferroso se reaciona con glicina para formar un bis-glicinato quelado, este llena todos los requerimientos tanto para ser un verdadero quelado como para ser nutricionalmente funcional.
Palabras clave: Quelación, criterios de quelación, Ferrochel, quelados con amino ácidos.

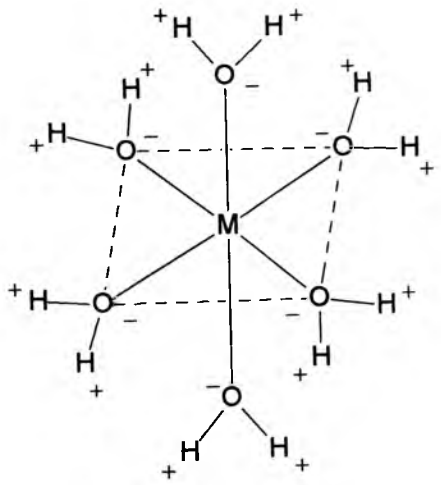
The chemistry of chelation is not novel. As a chemical phenomenon, various iterations of it have been studied for a little more than a century, beginning in the early part of the last decade of the nineteenth century. It was recognized as early as then that certain atoms could have more than one valence state. It was not, however, understood until the theory of chelation was proposed just how these particular atoms could form highly stable compounds.

In 1893, Alfred Werner postulated a new molecular structure to describe those stable molecules. He had noted that certain structural entities, which he called "complexes", remained intact through a series of chemical transformations. As a result of those observations, he wrote, "If we think of the metal ion as the center of the whole system, then we can most simply place the molecules bound to it at the corners of an octahedron" (1). His initial concept is illustrated in Figure 1, wherein the soluble metal ion (M) exists in a hydrato-complex bound to a number of water molecules in water with the negative oxygen of the water dipole oriented towards the positive metal. This results in a certain spatial configuration of the donor ligand around the central metal atom.

In the ensuing years, from 1894 through 1914, Werner refined his concept. Ultimately, he concluded that the cation was characterized by two valences. The first of these he termed the "principal valency". This corresponds to the oxidation state, or oxidation number of the metal. Werner's second valency was called the "auxiliary valency". This referred to the number of atoms from the ligand associated with the central metal ion and today is known as the coordination number of the metal (2-7).

A few years later, in 1920, Morgan and Drew applied the term "chelate" to the molecular structure postulated by Werner (8). In order to form a chelate, it was recognized that the ligand must have two points of attachment to the metal ion. It was this caliper-like mode of attachment that led to the use of the Greek word "chele", meaning lobster claw, to describe how the ligand was attached to the metal ion. When the claw, or ligand, held the cation, the metal was restricted in its ability to enter into other chemical reactions. Once chelated, the metal's chemical and physical characteristics changed (9). The metal chelates are coordination compounds in contrast to metal salts where the cation is bound by electrostatic attraction. In a chelate, the ligand donates electrons to the cation. More than one donor atom must come from the ligand so that a heterocyclic ring is formed with the metal being part of that ring. Comprehension of the structure and characteristics of metal chelates has had far reaching consequences in medicine, biology, chemistry, environmental chemistry, and, particularly, nutrition, due to the stability of the metallic molecule formed.

FIGURE 1
Complexation of a metal by water in an octahedral geometry as described by Werner



The American Association of Feed Control Officials (AAFCO), an organization composed from all 50 individual state (U.S.) chemists and the United States and Canadian Food and Drug Administrations, have officially defined a metal amino acid chelate as "the product resulting from the reaction of a metal ion from a soluble salt with amino acids with a mole ratio of one mole of metal to one to three (preferably two) moles of amino acids to form coordinate covalent bonds. The average weight of the hydrolyzed amino acids must be approximately 150 and the resulting molecular weight of the chelate must not exceed 800" (10). This definition states that the metal and amino acids must be reacted before a chelate can be formed.

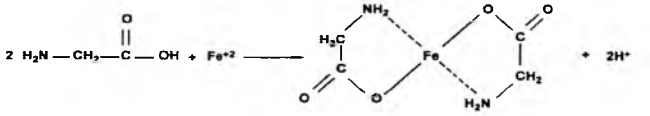
Since, by definition, the formation of a chelate requires chemical reactions to occur, four inviolate chemical requirements must be met. If any one of the four criteria is not fulfilled, the resulting molecule will not be a chelate, but simply a metal complex, a salt, or perhaps even an admixture.

The first of these criteria requires that the ligand must possess two functional groups, each capable of donating electrons to bond with the metal ion (11). The elements in the ligand that commonly function as donors are the more electronegative ones in the right hand side of the periodic table, primarily in Group V (12,13). The most important of these ligands contain N or O or both (14). The donor atoms may form a part of either an acidic or a basic functional group. Additionally, approximately 65% of the various types of amino acid side chains contain potential metal binding sites such as the sulfhydryls and hydroxyls binding groups. The common backbone of the naturally occurring amino acids contain the α-carboxyl and α-amino groups each of which can bind metal ions (11). In an aqueous environment the α-amino acid exists in the zwitterionic state with both the α-carboxyl and the α-amino groups ionized with opposite charges. Both reactive groups can thus participate in the chelation of the metal ion. The carboxyl group contains an electron that can be shared with the metal ion through a covalent bond. While the amino group has a pair of electrons that can be donated to the metal ion to form a coordinate covalent bond. The amino acid ligand is considered to be bidentate. The amino moiety meets the criteria of Lewis base when joined to the metal in accordance to current chemistry theory.

There are three classes of bidentate ligands: (1) two basic groups, (2) one acidic and one basic group, and (3) two acidic groups. Amino acids fall into the second category of bidentate ligands. Using glycine (NH_2CH_2COOH) as an example, in water at a pH of between 3 and 9, this ligand exists as the zwitterion, $H_3N^+CH_2COO^-$. The amino acid has lost a proton from the COOH group and is thus capable of chelating a metal ion and forming a five member ring through donation of one or more electrons to the metal (15).

A second prerequisite for chelation to occur requires that the functional groups of the ligand be located so that a ring structure can be formed with the metal atom being the closing member of the ring (12). Logically if the ligand has two donor atoms that must attach to a single metal ion, then a ring structure must be formed. Due to the nature of the elemental constituents of the ring members, this ring, by definition, must be heterocyclic. The formation of a ferrous bis-glycinate chelate is shown in Figure 2 and illustrates this concept.

FIGURE 2
The formation of ferrous bis-glycinate chelate from two ligands of glycine and a ferrous (+2) iron atom. The resulting chelate rings are five member, heterocyclic rings



Each heterocyclic ring in Figure 2 contains two bonds which extend between the ferrous ion and the glycine ligand in each ring. The first bond between the cation and the anionic, or polar portion, of the ligand is covalent in nature because they share one electron from the carboxyl group and one electron from the ferrous ion. This is the bond between the oxygen from the COO^- group, and the Fe^{++}. The second bond is a coordinate covalent bond. In this case, the iron behaves as Lewis acid, and the glycine as a Lewis base. The

donation of both electrons from the same atom in the amino group of the ligand to the metal ion establishes the coordinate covalent bond. This donation of electrons will go to the lowest energy orbital of the iron ion that is unfilled, which in this case is a p-orbital (15,16).

The third requirement for chelation to occur necessitates that the potential reaction between the metal ion and the ligand must be sterically possible. The ferrous iron has an atomic radius of between 74 and 77 nanometers (nm) making it one of the larger transition metal ions (17,18). Nevertheless, it is not a large cation when compared to many other ions, such as the alkaline earth metal ions. The size depends on either high spin or low spin configurations according to the magnitude of the surrounding ligand field. All non-heme iron ions are high spin (20). The larger the metal ion the greater the number of ligands that can surround it and still have contact with the metal ion (17).

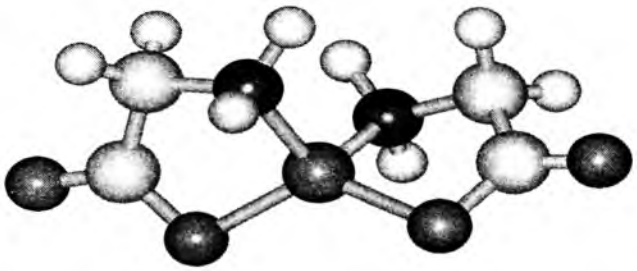
The size of the ligand will also affect the stereochemistry of the chelate. While one ligand may be able to attach itself to a metal ion without problem, the addition of a second or third may be prevented by a clash between the first ligand and parts of the second or third when the latter ligands attempt to position themselves properly for attachment (12). In the case of the ferrous bis-glycinate chelate formed from the reaction in Figure 2, only the amino acid backbone is involved in the bonding of the ligand to the ferrous ion. This results in the amino acid backbone assuming a configuration that sterically allows it to function as a ligand without straining the bonds within the amino acid.

Using x-ray diffraction spectrometry, it has been determined that when the two glycine ligands are chelated to the ferrous ion, the ligands orient themselves in the least sterically hindered conformation possible. The covalent and coordinate covalent bonds are tied to the metal at uniform tetrahedral angles (11,21,22). Figure 3 illustrates a ferrous bis-glycinate chelate molecule drawn from a computer generated model that employed thermodynamic algorithms to determine the most thermodynamically stable configuration. This figure is consistent with the models previously developed by Pettit and Hefford describing the steric orientation of amino acid ligand in this class of bidentate chelates and with x-ray diffraction studies of known chelates (22,23).

The fourth requirement for chelation to occur is that the potential reaction between the metal ion and ligands must be energetically possible. As noted by Werner, the charge on a metal ion influences its coordination number. If this charge is low, then only a few lone pairs of electrons from a small number of ligands could prevent the bonding of greater numbers of ligands. Where the bonding between the metal and the ligand is primarily covalent, as in the case of an amino acid chelate, the coordination number of the metal is determined by the number of bonding orbitals available on the metal for combination with the ligand orbitals (19). In the case of the ferrous ion, it is satisfied by a bonding of two glycine ligands as demonstrated in Figure 2. If a molar ratio (ligand to iron) of one glycine molecule or less is employed in the reaction, the ferrous ion has the capability of being bonded to some other ligand which may ultimately displace or interfere with chelation by the original glycine ligand. Thus in most situations a ferrous bis-glycinate chelate is relatively stable since the charges on both the ion and ligands are balanced and the molar ratio of ligand to metal is stoichiometrically correct. However in the presence of a strong oxidizer or reducer, the valency of the metal may change.

FIGURE 3

Ferrous bis-glycinate chelate drawn to correctly depict the tetrahedral relationship of the bonds of O, N, C and the metal and the resulting perpendicular orientation of the two ligand ring structures. The figure is based on x-ray diffraction spectrometry of pure metal - bis-glycinate chelate crystals



Not all chelates of iron have equal bioavailability. Chelation does not guarantee mineral absorption from the gut or its subsequent metabolism if absorbed (24). After comparing various iron chelates (EDTA, fructose, citric acid) to ferrous sulfate, Bates, et al. concluded, "Chelation does not, in itself, ensure efficient uptake [of iron]. . . " (25). Rubin et al. reported that an EDTA chelate of iron may bypass the normal absorptive mechanism in the intestines and be absorbed by diffusion, but due to its high stability constant most of that EDTA chelated iron later appears in the urine unmetabolized (26,27). Because some FeEDTA is absorbed intact through the intestine but subsequently deposited in the urine, Kratzer and Vohra report this form of iron cannot be effectively used in the treatment of anemia (28). In order for mineral absorption and metabolism to occur, the chelate must be nutritionally functional. There are several criteria that must be met for a chelate to be classified as a nutritionally functional chelate.

The first of these requirements is that the iron chelate must have a low molecular weight. In 1970, it was suggested

that in order for an iron amino acid chelate to cross cell membranes intact it must have a molecular weight of less than 1500 daltons (29). Subsequently, others have reported that small molecular weight chelates facilitate the transfer of iron from serum to tissues (30). Kratzer and Vohra reported that in order for a ligand from a chelate to promote metal absorption (which goes beyond simply protecting the metal ion in the gastrointestinal tract), it must have a molecular weight of under 1000 daltons. They have added that the higher molecular weight metalloproteins, such as ferritin or hemosiderin facilitate storage of the absorbed iron but suggested that these proteinaceous ligands are too large for intact transport of the iron molecule across cell membranes. For the transfer to occur the iron must be removed from the protein complex and bonded to another ligand that has a lower molecular weight (28).

It is relatively easy to calculate the molecular weight of a ferrous bis-glycinate chelate. The iron atom has an atomic weight of about 56 daltons. Each glycine ligand has a molecular weight of 75 daltons. Thus using the formula illustrated in Figure 2, the ferrous bis-glycinate chelate has molecular weight of about 204 daltons. This molecular weight has been confirmed in the laboratory (31). This in turn confirms that the molecular weight of the ferrous bis-glycinate chelate is well below the maximum molecular weight of 800 daltons established by AAFCO for a molecule to be classified as an amino acid chelate and well below the postulated absorptive limits (10,29,30).

The second requirement for a nutritionally functional chelate relates to its stability constant. If the chelate is to be classified as nutritionally functional, it must have a stability constant that is higher than the potential formation constants of the ligands in the intestinal chyme. This higher stability constant of the amino acid chelate prevents the molecule from being destroyed in the gut and allows the chelate to cross the intestinal cell membrane intact with the metal. Once absorbed into the mucosal cell, a nutritionally functional chelate must have a stability constant which is lower than those ligands in the storage systems of the mucosal cells and the transport systems that deliver the iron to target tissues (29). In this way, the chelate can be metabolized (degraded into the ligands and metal) after absorption across the mucosal cell membrane, and still participate in the regulatory pathways of the metal.

The stability constant can be affected by the size of the chelate ring. Ligands, which form saturated five and unsaturated six member rings are the most stable (32). The glycine ligand forms a saturated five member ring with the ferrous ion.

The number of chelate rings that can potentially be formed with one cation will also affect stability. As the number of rings increase, so does stability of the molecule (32). This concept, of course, is limited by sterochemistry and the valence state of the cation. If a ligand is too large to allow the bonding of a second ligand, then chelate stability may be sacrificed. So too, if the valence of the cation exceeds that of the available ligand(s), stability is reduced. The ferrous bis-glycinate has the ideal combination. The +2 charges of the ferrous iron are satisfied with an equal number of glycine ligands that each have a -1 change.

The Lewis basic strength of the ligand will also affect stability. The greater Lewis basicity of the ligand, the more stable the resulting chelate molecule (32). Glycine is an weak Lewis base, overall. This moderates the stability of the bis-glycinate chelate, and while it is more stable than the food ligands, it has a lower stability constant than the iron storage and transport ligands in the body. In a study in which $^{59}FeSO_4$ and ^{55}Fe bis-glycinate chelate were mixed in a cornmeal porridge and fed to 10 volunteers at breakfast, the absorption of the iron from the chelate was significantly ($p<0.001$) higher (5.3 times) than was iron absorption from the sulfate. There was no exchange of the radiolabelled irons from the ferrous bis-glycinate chelate and ferrous sulfate in the intestinal pool before absorption demonstrating that this bis-glycinate chelate was not affected by food ligands and was absorbed intact into the mucosal cells (33). Once absorbed into the mucosal tissue there is significant ($p<0.05$) hydrolyzation of the iron amino acid chelate into its individual components: iron and amino acids with the rates of transfer to the serosa from the mucosal tissue being different for each component (34).

The size and charge of the ligands will also affect the stability of the chelate. More stable chelates are formed by smaller ligands than are formed by larger ligands (32). If a large ligand is bonded to the reactive site of a metal ion, then the number of ligands able to chelate the metal ion are restricted due to steric hindrance. As noted above, this will also decrease chelate stability. Stability constants tend to become lower as the bulk of the ligand increases, suggesting a decrease in coordination sites of the ligand to the metal due to stearic hindrance (32). Glycine is the smallest of all of the amino acids. Its size favors the stability of the chelate.

Multidentate ligands, if not stearically hindered, form more stable chelates than do monodentate ligands (32). Glycine is a bidentate ligand, and as shown in Figure 3, when the ferrous ion is chelated with two glycine ligands there is no stearic hindrance to either ligand (35). The stability constant is also affected by the π-bonding strength of the central metal atom (32). It has been shown that, among the transition metal ions, copper has the highest stability. Iron is relatively low: $Zn^{2+} < Cu^{2+} > Ni^{2+} > Co^{2+} > Fe^{2+} > Mn^{2+}$ (36).

The nature of the ligand will also affect the overall stability of the chelate (32). Different ligands have different

potential formation constants at the same pH (37). EDTA, for example, will form a much stronger chelate than will glycine, but the ability of the body to extract the iron nutrient from the EDTA chelate is severely limited, whereas in the case of the iron bis-glycinate chelate, it is relatively easy (26-28,34).

It is estimated that a nutritionally functional chelate must have a stability constant of between 10^7 and 10^8, in order for that chelate to survive the environment of the stomach and intestine and still be hydrolyzed within the mucosal cells or other tissues (38). An absorbed iron chelate, if it is functional, must have a bonding constant which is lower than that of transferrin (39). The iron bis-glycinate chelate meets the criteria of having the ideal stability constant for a nutritionally functional chelate. It has a stability constant of approximately $10^{7.5}$ at pH 7 (37).

The final requirement for a totally nutritionally functional chelate is that the ligand must be easily metabolized by the body and also be utilized as a nutrient in addition to the metal contained in it (15). Amino acids are represented throughout mammalian biochemistry and physiology. Thus, a continuous dietary supply of amino acids is essential for the well being of the individual (40). It was previously noted that, after absorption into the mucosal cell, the majority of the iron amino acid chelate is hydrolyzed into its individual components before the iron is transferred to the serosa (34). The small amount of amino acid chelate that escapes this initial hydrolysis is broken apart later in the various other cells of the tissue which receive the absorbed chelate (38). The amino acid portion of the chelate is then free to be metabolized as it normally would if it had been absorbed from the lumen as a free amino acid.

Glycine is considered to be a conditionally essential amino acid. While it is absolutely essential for human nutrition, it is not generally required in the diet because under normal circumstances the body can synthesize it from more complex precursors. The body's ability to synthesize this amino acid from a precursor is, however, limited by the availability of the precursor, which in the case of glycine, is a nonessential amino acid. Furthermore, the synthesis can also be limited and potentially constrained by developmental or pathophysiological factors. For example low birth weight infants lack the ability to synthesize adequate quantities of glycine from more complex amino acids. When this lack of physiological development or pathological interference occurs, and when foods low in glycine, such as cow's milk, are mainly consumed, a glycine deficiency can result (40-43). Thus, when provided as part of the iron bis-glycinate chelate, the glycine in and of itself has nutritional value to the individual. Unlike EDTA, it is not excreted into the urine unmetabolized.

The ferrous bis-glycinate is a proven chelate. More importantly, however, it is a totally nutritionally functional chelate. By its atomic structure and chemistry it protects the ferrous ion from undesirable chemical reactions in the stomach and intestines that limit iron absorption. Once absorbed into the mucosal cells and to a limited extent, the tissues, it is easily hydrolyzed into its individual components. Each component is then employed by the body as normal metabolism would dictate.

REFERENCES

1. Werner A. Beitrag zur Konstitution Anaorganischer Verbindunge, Z, Anorg U Allgem Chem 1893;3:267.
2. Werner A & A Miolati. Z physik Chem Leipzig, 1894;14:506.
3. Werner A & Z Vilmos. Beitrag zur Konstitution Anaorganischer Verbindunge, A anorg U Allegem Chem 1899;21:153.
4. Werner A. Ueber Acetylacetonverbindungen des Platins, Ber Deut Chem Ges 1901;34:2584.
5. Werner A. Zer Kenntnis des Asymmetrischen Kobaltatoms V. Ber Deut Chem Ges, 1911;45:121.
6. Werner A. Über spiegelbild-isomerie bei chromverbindungen. III. Ber Deut Chem Ges, 1912;45:3065.
7. Werner A. Zur Kenntnis des Asymmetrischen Kobaltatoms XII. Uber Optische Aktivitat bei Kohlenstoffreien Verbindugen. Ber Deut Chem Ges, 1914;47:3087.
8. Morgan G. & H Drew. Research on residual affinity and co-ordination. II. Acetylacetones of selenium and tellurium. J Chem Soc, 1920;117:1456.
9. Bell CF. Principles and Application of Metal Chelation. *Oxford, Clarendon* Press, 1977, p. 3.
10. Bachman PM. (Ed.) Official Publication 2000. Oxford, IN, American Association of Feed Control Officials, 2000, p. 257.
11. Glusker JP. Structural Aspects of Metal Liganding to Functional Groups in Proteins. In Anfinsen, CB, JT Edsall, FM Richards & DS Eisenberg, (Eds.) Advances in Protein Chemistry. San Diego, CA, *Academic Press,1991*, V.42, p. 4.
12. Dwyer FP & DP Mellor. Chelating Agents and Metal Chelates. NY, Academic Press, 1964.
13. Bell CF. Principles and Application of Metal Chelation. Oxford, Clarendon Press, 1977, p. 9.
14. Van Uitert LG & WC Fernelius. Coordination compounds. IX. Solution stabilities of the chelate compounds of a number of organic ligands. J Am Chem Soc 1954;76:375.
15. Bell CF. Principles and Application of Metal Chelation. Oxford, Clarendon Press, 1977, p. 11-18.
16. Bailar JC, T Moeller, J Kleinberg, CO Guss, ME Castellion & C Metz. Chemistry. Orlando, FL, Academic Press, 1984, p. 246-253.Ahrens, LH. Ionization Potentials: Some Variations, Implications, and Applications. Oxford, UK, Pergamon Press, 1983.
17. Ahrens RD. Ionization potentials: Some Variations, Implications, and Applications. Oxford, U.K. Pergamon Press. 1983.
18. Shannon RD & CT. Prewitt. Effective ionic radil in oxides and flourides. Acta Crystallogr 1969;25:925-46.

19. Bell CF. Principles and Application of Metal Chelation. Oxford, Clarendon Press, 1977, p. 33-35.
20. Howard JB & DC Rees. Perspectives on Non-Heme Iron Protein Chemistry. In C.B. Anfinsen, J.T. Edsall, F.M. Richards & D.S. Eisenberg, (Eds.) Advances in Protein Chemistry. San Diego, CA, Academic Press, 1991, V.42, p.206.
21. Dalley NK. X-ray diffraction of iron amino acid chelate, Albion Laboratories, Inc., Unpublished Research Report, 1978.
22. Dalley NK. X-ray diffraction crystallography of Albion Zinc Amino Acid Chelate. Albion Laboratories, Inc., Unpublished Research Report, 1991.
23. Pettit LD & RJW Hefford. Steroselectivity in the metal complexes of amino acids and dipeptides. In: Metal Ions in Biological Systems. H. Sigel (Ed.) NY, Marcel Deker, 1979; V.9 p. 173-212.
24. Ashmead H, D Ashmead & N Jensen. Chelation does not guarantee mineral metabolism. J App Nutr 26:5-21, 1974.
25. Bates GW, J Boyer, JC Hegenauer & P Saltman. Facilitation of iron absorption by ferric fructose. Am J Clin Nutr, 1972;25:983.
26. Rubin M., J Houlihan & JV Princiotto. Chelation and Iron Metabolism I; Relative iron binding of chelating agents and siderophilin in serum. Proc Soc Exp Biol and Med 1960;103:663.
27. Rubin M & JV Princiotto. Synthetic amino acid chelating agents and iron metabolism. Ann NY Acad Sci 1960;88:450.
28. Kratzer FH & P Vohra. Chelates in Nutrition. Boca Raton, FL, CRC Press, 1986, p. 42-44.
29. Hofner W. Eisen und manganhaltige Verbindungen im Blutungassafit von Helianthus annuus, Physiol Plant, 1970;23:673-677.
30. Tiffin LO. Translocation of micronutrients in plants. In: Micronutrients in Agriculture. RC Dinauer (Ed.) Madison, WI, Soil Science Society of America, Inc., 1972, p. 207.
31. Johnson B. Molecular weights of several commercial chelates. Albion Laboratories, Inc. Unpublished research, 1974.
32. Kratzer FH & P Vohra. Chelates in Nutrition. Boca Raton, FL, CRC Press, 1986; p. 25-27.
33. Allen LH. Properties of iron amino acid chelate as iron fortificants for maize. In: Proceedings International Conference on Human Nutrition. Salt Lake City, UT, Albion Laboratories, Inc., 1998, p. 96-108.
34. Ashmead HD, DJ Graff & HH Ashmead. Intestinal Absorption of Metal Ions and Chelates. Springfield, IL, Charles C Thomas, 1985, p. 205-212.
35. Jeppsen RB. Organic minerals and their bioavailability on the basis of chemistry. In: Proceedings International Animal Conference. Salt Lake City, UT, Albion Laboratories, Inc., 1997, p. 8.
36. Irving H & RJP Williams. Order of stability of metal complexes. Nature 1948;162:746.
37. Kragten J. Atlas of Metal-ligand Equilibria in Aqueous Solution. Chichester, Ellis Horwood, 1978.
38. Ashmead SD. Metabolism of zinc amino acid chelate: A preliminary study. Masters Thesis, University of Utah, 1994.
39. Williams RJP. An introduction to the nature of iron transport and storage. In: Iron Transport and Storage. P Ponka, HM Schulman & RC Woodworth. (Eds.) Boca Raton, FL, CRC Press, 1990, p.1-15.
40. Reeds PJ & PR Beckett. Protein and amino acids. In: Present Knowledge in Nutrition. EF Ziegler & LJ Filer (Eds.) Washington, DC, ILSI 1996, p. 67-68.
41. Jaksie T, F Jahoor, PJ Reeds & W Heird. The determination of amino acid synthesis in human neonates with a glucose stable isotope. Surg Forum 1993;44:642-686.
42. Jackson AA, JCL Shaw, A Barber & M Golden. Nitrogen metabolism in pre-term infants fed human donor breast milk: The possible essentiality of glycine. Ped Res 1981;15:1454-1461.
43. Davis T, HV Nguyen, R Garcia-Bravo. The amino acid composition of human milk is not unique. J Nutr 1994;124:1128-1134.

ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición

Suplemento Vol. 51 Nº 1, 2001

The absorption and metabolism of iron amino acid chelate

H. DeWayne Ashmead

Albion Laboratories, Inc. Clearfield, Utah U.S.A.

SUMMARY. This paper summarizes several studies which describe significant increases in the intestinal absorption of iron from iron amino acid chelate compared to inorganic iron salts. While these increased uptakes of iron from the amino acid chelate into mucosal tissue are highly significant, this paper also demonstrates that there is a mechanism in the mucosal tissue which controls the quantity of iron from the amino acid chelate that is transferred to the plasma. For example, the higher the hemoglobin value, the less iron transferred. When considered together these studies demonstrate that iron amino acid chelate is both a safe and effective source of iron for treatment of iron deficiencies.
Key words: Ferrochel, absorption mechanism, iron deficiency anemia, iron deficiency, hemoglobin.

RESUMEN. Absorcion y metabolismo del hierro aminoquelado. Este artículo resume algunos artículos que describen incrementos significativos en la absorción del hierro aminoquelado comparado con la absorción de sales inorgánicas de hierro. También se demuestra la existencia de un mecanismo en la mucosa intestinal que controla la cantidad de hierro aminoquelado que es transferida al plasma. Por ejemplo, a mayor nivel de hemoglobina, menor cantidad de hierro es transferida al plasma. Considerados en la luz de los estudios discutidos, se demuestra que el hierro aminoquelado es una fuente segura y efectiva para el tratamiento de la deficiencia de hierro y la anemia ferropriva.
Palabras clave: Ferrochel, mecanismo de absorción, anemia ferropriva, deficiencia de hierro, hemoglobina.

Iron, as a therapeutic agent, was first documented about 2735, B.C., when it was declared by the Chinese Emperor, Shen Nung, as a cure for "anemia."(1). The ancient Greeks, Romans, Byzantines, and Arabs began consuming iron to reverse symptoms of anemia about 1500 B.C.(2). In the sixteenth century, Monarde proposed a relationship between iron and blood (3). Lemery and Geoffroy subsequently demonstrated the presence of iron in the erythrocytes (4). In the 1700's, Sydenham employed iron as a specific therapy for "chlorosis," a term he used to describe iron deficiencies. Symptoms of this malady included pallor, edema, muscular weakness, headache, rapid pulse, dyspnea, prolonged sleep, and cessation of the menses. Although iron became an accepted treatment for chlorosis, it was not until 1886, that Zinoffsky discovered that equine hemoglobin crystals actually contained 0.335% iron. Subsequently, in 1893, Stockman demonstrated that ingestion of iron increased hemoglobin in women (5). In parallel research, Menghini reported that administration of oral doses of iron augmented total red blood cells (4).

Iron is not only an essential component of erythrocyte hemoglobin for the transfer of oxygen and carbon dioxide, but it also has an important function or functions in every other cell of the body. For example, iron in either the heme or nonheme form plays important roles in cellular metabolism and growth due to its enzymatic involvement in energy production and DNA synthesis. Some other equally essential but frequently overlooked functions include catalyzing the conversion of carotene into vitamin A, the synthesis of purines into nucleic acid, the synthesis of carnitine to transport fatty acids, synthesis of collagen, its participation in antibodies, and the metal's involvement in detoxication of drugs in the liver (6). Ponka, *et al.,* have postulated that, since iron is a cofactor in numerous cellular enzymatic reactions, it is essential for the evolution of aerobic life on earth (7).

An adult male body contains about 50 mg Fe/kg of body weight, whereas a female adult body contains about 35 mg Fe/kg (8). Over two thirds of this iron is concentrated in the blood, primarily in hemoglobin. About 3% of the heme iron resides in the muscles as myoglobin. The remainder of the functional iron is found in specific enzymes of every living cell due to the essentiality of iron for cellular respiration (6).

Iron is found in abundance on our planet and, relative to the nutritive amount required by man, is theoretically plentiful. In spite of this natural abundance, iron deficiency anemia is still the largest single nutrient disease affecting the world today (9-12). It is estimated that more than 500 million people throughout the world suffer from severe iron deficiency anemia (13). In some geographical areas, as high as 58% of the young healthy women have been found to be iron deficient, with the percentages of deficiency being even higher during pregnancy (14).

This worldwide deficiency is, in part, a result of diet. The availability of iron from many foods is very low. Generally speaking, no more than 5% of vegetable iron is absorbed, and while the absorption of iron from meat, poultry, and fish may be somewhat higher, significant worldwide consumption of animal proteins is limited to the more affluent

(15). Consumption of certain foods, such as coffee or tea, will generally reduce iron absorption. The phenols in the tea, for example, bind dietary iron and render it insoluble. Phosphates, phytates, and bran will inhibit iron absorption, similarly (16,17). Conversely, organic acids, such as ascorbic acid, (16) amino acids, (6) or meat protein, (18) will generally enhance absorption of iron. Because of these and many other dietary factors, the variance in iron uptake may be as great as tenfold (19).

Besides diet, an individual's iron status is also related to his or her needs. Age and the sex will affect iron requirements and potential deficiency. Other individual differences include physical activity ranging from sufficient exercise to a sedentary lifestyle, lactations, etc. (16).

The general health of an individual, as well as the drugs he or she is consuming, is a third factor affecting iron status. The use of oral contraceptives, aspirin, antacids, anti-inflamatories, anticoagulants, and steroids may all increase the risk of iron deficiency. Diseases of the gastrointestinal tract, including cancer, hemorrhoids, or gynecological diseases, such as intrauterine fibrosis, will result in a greater risk of iron deficiency due to increased iron requirements and/or an increased inability to efficiently absorb iron (20). Illness associated with a fever may also reduce iron utilization, even if the iron is absorbed. This was shown in an experiment on the nutritional response to infection. Within hours after inoculation, iron levels began to decline in the plasma and accumulate as hemosiderin, primarily in the liver. Throughout the period of infection, this iron remained sequestered. Its incorporation into new hemoglobin was blocked throughout the infection and absorbed iron was sequestered in body storage depots (21).

Finally, the social-economic habits of a population including customs, religious practices, attitudes, etc., may affect the iron status of the members of the group. Included in this category is the living environment of the group (20).

Because of the global magnitude of iron deficiency, and because a dietary supplement is unavailable to the majority of the population, many governments have mandated iron fortification of basic foods (22,23). Frequently, however, in order to retain palatability and retard oxidation of certain food components, the iron salts selected for fortification have had low solubility. Low solubility generally equates to low bioavailability (24,25). For example, ferrous fumerate, ferrous glycine sulfate, ferrous sulfate, ferrous citrate, ferrous tartrate, ferrous pyrophosphate, ferric coline citrate, ferric sulfate, ferric citrate, and ferric ethylenediaminetetraacetate showed decreasing degrees of iron absorption, respectively, with the ferric compounds being absorbed at less than half of the first three ferrous sources (26). Increasing iron levels in a meal does not proportionately increase iron absorption. The mean absorption of nonheme iron has been reported to decrease from 18% to 6.4% as the nonheme iron content of the meals was increased from 1.52 mg to 5.72 mg.(27). Although 6.4% of 5.72 mg is higher than 18% of 1.52 mg (0.37 mg versus 0.27 mg), the amount of increased absorption is not proportional to the increased dose. It is, in fact, greatly suppressed. Increasing doses of unprotected iron is, thus, far more likely to elicit toxicity, than significantly increasing absorbed iron.

In order to enhance iron bioavailability and still avoid interactions with food ingredients, chelating iron with amino acids has been employed. Chelation was first described in 1893, when Werner wrote, "If we think of the metal ion as the center of the whole system, then we can most simply place the molecules bound to it at the corners of an octahedron"(28). In 1920, Morgan and Drew applied the name, "chelate" to the molecule described by Werner (29).

Not all chelates have the same biological consequences. Chelates can be produced that have little or no nutritional value. A nutritionally viable iron amino acid chelate must have a stability constant that is higher than the potential formation constants which would result if the iron were chelated or complexed to the food ligands found in the stomach and intestines (30). This is necessary for the original chelate to remain intact in the gastrointestinal tract prior to absorption. If the chelate dissociates in the gut, it has no more value than ionized iron from a soluble salt. The stability constant should also be high enough to allow the chelate to cross the intestinal cell membrane into the cytoplasm, and yet be low enough that the cytoplasmic ligands are capable of removing the iron from the absorbed amino acid chelate by complexing with the absorbed iron. In this fashion, the rate of delivery to the target tissue or enzyme from the mucosal cell is controlled (31).

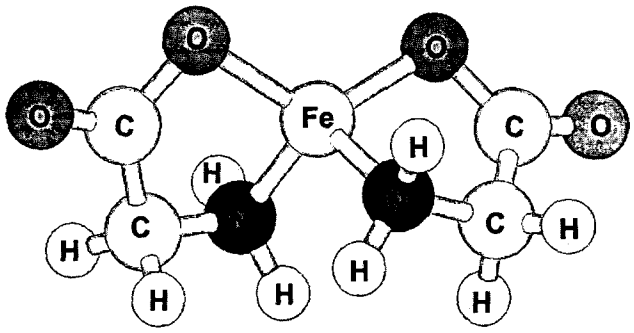
A stability constant indicates logarithmically the affinity of a metal to bond with a ligand. A ligand with a potentially higher stability or formation constant will displace a metal from a ligand with a lower stability constant. The strength of a stability constant is dictated by the number of members in the chelate rings (5- and 6- member rings being most stable), the number of rings, formed with a single cation, the basic bonding strength of the ligand(s), the size and charge of the ligand(s), the metal being chelated, the resonance effect of the chelate ring, and the steric orientation of the rings (32). *In vitro* and *in vivo* studies have demonstrated that the Albion® iron amino acid chelate developed via patented technology and described in this paper appears sufficiently strong to pass through the acid pH of the stomach into the duodenum and still provide reasonable protection of the iron from unwanted chemical reactions with dietary phosphates, phytates, fiber, etc. (33). Radioactive isotope studies have demonstrated that once the iron amino acid chelate has been absorbed into the mucosal tissue, its stability constant is low

enough to allow hydrolyzation of the chelate with subsequent transport of the iron to tissues (34).

To form such an iron amino acid chelate, each amino acid or ligand, must furnish at least two reactive moieties to combine with the iron atom. The carboxyl group (COOH) of the ligand forms an ionic bond with the cation, whereas the alpha-amino group (NH_2) donates an electron pair back into a vacant d-orbital of the metal ion, thus forming a coordinate covalent bond (35). Each amino acid ligand forms two bonds with the iron ion to produce a stable 5-member ring with the iron atom being the closing member of the ring (36). The two ligands tend to orient themselves in the least sterically hindered conformation possible and are tied into the metal at uniform tetrahedral angles (Figure 1) (37,38). This configuration provides a degree of protection to the iron by limiting its reactivity with dietary components. The same ligands that protect the cation from reacting with dietary substances also reduce the potential for gastric irritability of the iron on the mucosal lining because the cation is less likely prevented from coming in contact with the mucosal lining by the amino acid ligands.

FIGURE 1

Iron amino acid chelate composed of 2 glycine molecules to 1 iron atom drawn to depict the tetrahedral relationship of the bonds of carbon, nitrogen, and the iron and the resulting perpendicular orientation of the two ring structures. This figure is based on computer generated models resulting from X-ray diffraction analysis of metal amino acid chelate crystals



Because an iron amino acid chelate is a different form of iron molecule compared to a non-heme salt, the absorption characteristics of an iron amino acid chelate are also different, as demonstrated in a study involving 10 adult male volunteers. Each was given 1.5 μCi $^{59}FeSO_4$ and 3.0 μCi ^{55}Fe amino acid chelate simultaneously in 100g of dry cornmeal which was fed as a breakfast porridge. Neither isotope source of iron affected the absorption of the other. Absorption of iron from $FeSO_4$ was 1.34% compared to 8.68% for the chelates. The difference was highly significant ($p<0.0001$). The investigator concluded, "there was no exchange between the $^{59}FeSO_4$ and Ferrochel [^{55}iron amino acid chelate] in the intestinal pool or before entering the mucosal cell. If the $FeSO_4$ and the [iron amino acid] chelate exchanged iron between themselves, the same proportion of label would be absorbed from both compounds. However, [iron amino acid chelate] is consistently absorbed about 5.3 times more than $FeSO_4$, and this is not modified by the simultaneous mixing with $FeSO_4$. The [iron amino acid chelate] is probably entering the mucosal cell as a chelate. Although no subjects in this experiment were iron deficient, the results also suggest that iron absorption from the [chelate] and ferrous sulfate was regulated similarly by iron status" (24).

For an iron amino acid chelate to be absorbed into the mucosal tissue, it must be a low molecular weight chelate. Kratzer and Vohra cite the upper limit for this intact absorption of chelates as being 1,000 daltons (31). Tiffin reported that the amino acid chelate must have a molecular weight of less than 1,500 daltons if it is to be absorbed in humans. The iron amino acid chelate described in Figure 1 has a molecular weight of less then 800 daltons (40).

Generally, more iron can be absorbed from the gastrointestinal tract into the mucosal cell as a chelate than as a salt because, as demonstrated above, the rate of uptake for the two sources of iron is different (34). Once absorbed into the mucosal tissue, the body regulates how much is transferred to the plasma, thus preventing iron overload and toxicity. This regulation is seen in a double blind cross-over study in which seven (7) adult males and five (5) females ingested 18 mg Fe/day as ferrous sulfate for seven (7) days, had a washout period of seven (7) days, and then received 18 mg Fe/day as an amino acid chelate for seven (7) days. Mean absorption of the iron amino acid chelate was 59% greater than that of the ferrous sulfate source, as determined by fecal analysis (47). The percent of increased absorption of the iron amino acid chelate compared to $FeSO_4$ was highly significant ($p<0.001$) in males (7.24% ± 2.2% *vs.* 12.7% ± 3.6%) and significant ($p = 0.02$) in females (13.8% ± 1.8% *vs.* 18.2% ± 3.7%) (42). Regression analysis demonstrated that both iron compounds followed the same absorption pattern and were possibly subject to the same type of regulation ($R^2 = 0.9411$ with slope = 0.8445 and intercept = 7.6) (41).

This process of absorbing the iron amino acid chelate into the mucosal cell can be described as R_o+Fe-C→Fe C $(R)_o$→Fe-C $(R)_i$→Fe+C+R_i, where C is the ligand, and R is the receptor inside (i) or outside (o) of the cell membrane. If C is metabolized in the cytoplasm, or if the pH is changed while Fe is still attached to C, then the iron uptake into the cell is irreversible (24,30).

The hydrolysis of absorbed iron amino acid chelate within

mucosal tissue is clearly demonstrated in a double isotope *in vitro* study (34). In this study, replicated five times, jejunal tissue was removed from Sprague-Dawley rats, washed, and everted. The ends were tied off and the intestines were placed in mucosal solution baths. Serosal solution was injected into the everted intestines. ^{59}Iron chelated to ^{14}C-lysine was injected into the mucosal solution bathing each everted intestine. The ratio of iron to lysine in the mucosal solution was 1:2.7. Samples of the serosal solution were removed hourly for five hours, and each sample assayed for ^{59}Fe and ^{14}C. The mean ratio of iron to lysine in the serosal solution dropped from what it had been in the mucosal solution to 1:1.56 $\pm$ 0.65. At the end of the five hours, the everted intestines were also assayed for ^{59}Fe and ^{14}C, after being washed to remove external contamination. The mean ratio of iron to lysine in the mucosal tissue was higher than what it had been in the mucosal solution: 1:3.05 $\pm$ 0.049.

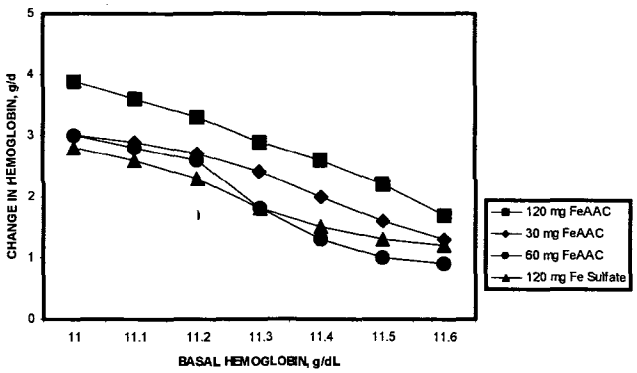
This study demonstrated that there was a significant ($p < 0.05$) hydrolysis of the iron amino acid chelate as it passed from the mucosal solution through the mucosal tissue before delivery of either the iron or amino acid to the serosal solution (42). More lysine was retained in the mucosal tissue than iron, indicating that some of the ^{59}Fe was separated from the lysine after absorption into the tissue, and that iron was transferred by other means to the serosal solution without being bonded to the lysine. Less lysine was found in the serosal solution than in the mucosal solution, also indicating hydrolysis of some of the amino acid chelate (34).

When hydrolysis of the amino acid chelate occurs within the mucosal cell, then the movement of the metal out of the mucosal cells and into body tissues is metabolically regulated similarly to the regulation of iron absorbed into mucosal cells from salts or from other food sources. If there were no regulatory mechanism in the mucosal cells to control the transfer of iron to the plasma, regardless of the source, there would immediately be iron over-loading and subsequent toxicity from iron ingestion, regardless of whether the iron originated from food or from an amino acid chelate.

This similarity in regulation of iron transferred to the plasma from iron amino acid chelate or $FeSO_4$ is seen in a dose-response study in which 100 anemic adolescents received either 120 mg of iron as $FeSO_4$ (n=27), (or 120 mg of iron (n=26), 60 mg of iron (n=21), or 30 mg of iron (n=26) as iron amino acid chelate, daily, for 28 days (43). Each source and quantity of iron was equally effective ($p<0.01$) in restoring the anemic adolescents' hemoglobin levels to normal (<11 to <13.5 g/dL), implying the greater bioavailability of the iron amino acid chelate compared to ferrous sulfate. When the changes in hemoglobin were plotted, however, the slopes showing these changes after treatment with the $FeSO_4$ or with different dose levels of the iron amino acid chelate were essentially the same, regardless of the quantity of iron administered (Figure 2). In each case, as hemoglobin levels increased, the uptake of iron, regardless of the source, decreased (44). These similar data suggest that all of the absorbed iron sources were regulated similarly by the body after absorption.

FIGURE 2

Line graphs showing that the absorption of iron from three dosage levels of amino acid chelate or from ferrous sulfate is directly related to the hemoglobin level



The transfer of absorbed iron from the mucosal cells to the plasma is controlled by the body's iron need. After cellular hydrolysis, the iron is first delivered to plasma transferrin in the mucosal cell, if the body has an immediate need for iron. Each transferrin molecule can bind to two atoms of iron. When approximately 33% of the transferrin is saturated with iron, it will not pick up any more iron. The remaining ferrous iron is oxidized to ferric iron. This iron remains complexed to ferritin within the mucosal cells until transferrin calls for more iron. The iron is then released from the ferritin through reduction of the ferric iron to ferrous iron and is subsequently bonded to transferrin in the blood (6).

The mucosal cells aggressively conserve iron that is not immediately required by the body, the iron being "trapped" in the mucosal cell. This could potentially result in toxicity within the mucosal tissue if the iron remained there indefinitely. Nature has, however, devised a plan to remove this non-transferred iron from the intestinal tissue. Mucosal cells migrate up the intestinal villus from the crypt, the site of their formation, to the tip of the villus, replacing older cells as they move towards the villus tip. After three to four days, these cells (and their iron, if it has not been transferred to the plasma) will reach the top of the villus where they will be sloughed off and excreted in the feces (6).

If all iron absorbed into mucosal tissue is generally handled and regulated similarly, what, if any, are the advantages of ingesting the iron in the form of an amino acid chelate?. There are two basic advantages. The first is that the

amino acid chelated iron is not as reactive with food ingredients, which leaves more of the iron potentially available for absorption. The second advantage is that the iron amino acid chelate is absorbed into mucosal cells in greater quantities, due, in part, to entering into fewer absorptive inhibiting reactions in the gut. The greater amount of absorbed iron is of great importance when iron deficiency or anemia exists. In these cases, more iron is made available for repletion of the iron need. These advantages are examined in greater detail below.

When dietary iron is ingested with phytic acid, bran, etc., the formation of an insoluble precipitate may leave the iron unavailable for absorption (45). In those cases, the iron is simply eliminated in the feces as part of a phytate, oxalate, phosphate, etc. There is less likelihood of insoluble compounds forming when that iron is ingested as an amino acid chelate because this form of iron is shielded by the amino acid ligands of the chelate and is made electrically neutral by the charge balancing effects of the chelating ligands. To illustrate, an experiment was devised in which cookies containing known iron absorption inhibitors (bran fiber, phytates, phosphates, phenols, and tannins) and 30 mg of iron as an amino acid chelate were fed to 10 anemic children. Blood samples from each child before and after treatment were obtained by venipuncture and assayed for hemoglobin. Each child then received one of the above described cookies per day, for 30 days. There was a significant ($p<0.01$) increase in the mean hemoglobin level of the group as the hemoglobin rose over the 30 day study from 8 g/dL to 10.1 g/dL, even though the iron amino acid chelate was combined with a multitude of dietary iron absorption inhibitors (41).

The same characteristics that reduce the reactivity of the iron amino acid chelate in food also reduce its oxidative effect on other food ingredients, such as vitamins (25,46). When the iron amino acid chelate was stored with vitamins A, D_3, tocopherol, K, ascorbic acid, niacinamide, biotin, folic acid, and pyroxidine at 20°C, the degradation of these vitamins was significantly less ($p<0.01$) at 180 days, than when stored with iron sulfate under the same conditions. At a temperature of 37°C, the differences between the two sources of iron on vitamin oxidation were even more significant ($p<0.001$). The iron sulfate form stimulated more rapid oxidation and inactivation of the vitamins compared to the iron amino acid chelate.

Besides being less affected by dietary absorption inhibitors, the absorption of the iron amino acid chelate is not inhibited by the presence of other minerals in the diet (47-49). For example, normally, ionic iron and ionic copper are mutually antagonistic in the small intestine where a high dietary level of either will reduce the absorption of the other (50-55). This antagonism can be manifested as a decrease in hemoglobin values (56).

The lack of copper and iron antagonism as amino acid chelates was seen in a double blind study involving 30 healthy nonanemic adult volunteers divided into three groups (5 males and 5 females/group). Blood samples were obtained by venipuncture at the commencement and conclusion of the study, and assayed for hemoglobin (57). One group each received 30 mg Fe/day. The second group each received 9.9 mg Cu/day. The third group each received 30 mg Fe and 9.9 mg Cu per day. Both the iron and copper were chelated with amino acids. Daily supplementation continued for 90 days. Changes in hemoglobin were analyzed statistically. As seen in Table 1, when the copper and iron were ingested as amino acid chelates, there were no significant changes in hemoglobin, leading to the conclusion that there had been no antagonistic competition between the iron and copper amino acid chelates during intestinal absorption, due to both metals being in the form of amino acid chelates.

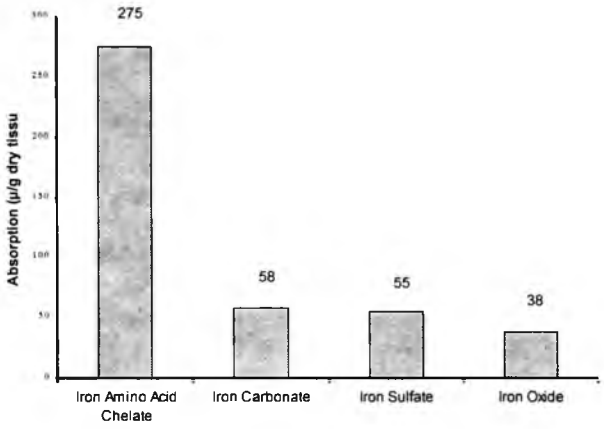
TABLE 1
Mean 90 day changes in hemoglobin (g/dL ± S.D.) following Fe and Cu amino acid chelate supplementation

	Group A (30 mg Fe/day)	Group B (9,9 mg Cu/Day)	Group C (30 mg Fe, 9,9 mg Cu/day)
Before supplementation	13,90 ± 0,85 [a]	16,03 ± 0,90 [a]	15,30 ± 1,61 [a]
After supplementation	14,50 ± 0,85 [a]	16,73 ± 0,94 [a]	15,29 ± 1,02 [a]

[a] Means not significant ($p>0,10$)

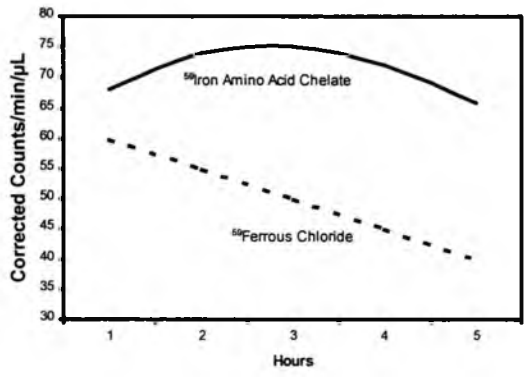
The second major advantage of iron amino acid chelates over iron salts is that the absorption of the chelates into mucosal tissue is greater, even under controlled conditions (58). In an *in vitro* study, replicated three (3) times, rat jejunal segments were randomized, washed, and then exposed for 120 seconds to identical gastric solutions containing iron from either a sulfate, an oxide, a carbonate, or from iron amino acid chelate. The control tissue was not exposed to any iron source. After exposure, each jejunal segment was washed thrice to remove external unabsorbed iron and then assayed by atomic absorption spectroscopy for the quantity of iron in the mucosal tissue. The mean net absorption of iron into the mucosal tissue (after subtracting the amount of iron in the control tissues) ranged from 4.7 to 7.2 times more for the amino acid chelate than from the inorganic salts (Figure 3).

FIGURE 3
Mucosal tissue uptake of iron after 120 seconds of exposure to iron from an amino acid chelate, carbonate, sulfate, or oxide mixed in gastric solution. The absorption represents the mean quantity of three (3) replications



Although excess iron in the mucosal tissue will be lost as the mucosal cells migrate to the tips of the intestinal villi, nevertheless, greater deposition of iron into the mucosal tissue potentially allows for a quicker recovery from iron deficiency. This is seen in a study in which twenty fasted 2-day-old anemic pigs (10 per group) were administered a single oral dose of 10 µg of ^{59}Fe as either $FeCl_2$ or as an amino acid chelate (59). The ^{59}Fe was in the form of $^{59}FeCl_2$. This $^{59}FeCl_2$ was divided into two parts, and one part used to make the iron amino acid chelate. Beginning one hour after dosing with either the $FeCl_2$ or iron amino acid chelate and continuing hourly for five hours, blood samples were obtained from each pig, centrifuged, and the red blood cells washed and then assayed for ^{59}Fe incorporation. Significantly more ($p<0.05$) iron from the amino acid chelate was found in the red blood cells compared to the chloride (Figure 4) (42).

FIGURE 4
Mean five hour incorporation of ^{59}Fe from iron amino acid chelate or ferrous chloride into washed red blood cells of 20 (10/group) two-day old pigs



At the end of 20 hours, all of the above pigs in both groups were sacrificed. Whole blood, livers, spleens, and bone marrows obtained at 20 hours were assayed for ^{59}Fe. The 20 hour blood samples were also assayed for hemoglobin. The data summarized in Table 2 demonstrate that ^{59}Fe tissue deposition from the amino acid chelate was significantly greater in the whole blood ($p < 0.01$) and red bone marrow ($p<0.01$). There was significantly less ($p<0.10$) ^{59}Fe from the amino acid chelate in the liver, suggesting less toxicity. These data also indicated that more iron was absorbed from the chelate in the 20 hour period and was immediately available for hemoglobin production, which was also significantly higher ($p<0.05$) (42).

A possible reason for the lower ^{59}Fe liver levels observed in Table 2 is suggested from a study conducted by Fairweather-Tait, *et al* (60). They fed two groups of 16 each weanling male Wistar rats a diet containing marginal levels of iron for four weeks and then added 30 mg Fe/kg as ferrous sulfate or iron amino acid chelate to the food for 4 weeks. At the end of the second four week period all of the animals were sacrificed and their livers removed for iron analysis by atomic absorption spectroscopy. Their blood was also analyzed for hemoglobin and packed cell volume. The mean hemoglobin concentration was significantly higher in the group fed the iron amino acid chelate ($p<0.001$), but there was no difference in the packed cell volume. These investigators concluded that most of the absorbed iron from the chelate was immediately incorporated into the 13% increase in hemoglobin which they observed, rather than being stored in the liver. They also noted, that the amount of iron absorbed from the chelate source must have been far greater than was reflected in the increased hemoglobin in order to meet the anabolic needs of the rapidly growing animals.

A similar response to the iron amino acid chelate can be seen in anemic human subjects. A group of 40 infants (Hb < 11 g/dL) were paired for age, sex, weight, and hemoglobin. After random assignment to 1 of 2 groups, each was given 5 mg Fe/kg body weight/day as $FeSO_4$ or as iron amino acid chelate for 28 days. Both treatments significantly increased ($p<0.001$) hemoglobin levels, but only the chelate significantly increased ($p<0.005$) serum ferritin. Calculated bioavailability of the iron amino acid chelate was 75.0% compared to 27.8% for $FeSO_4$. As suggested by Fairweather-Tait, *et al.* above, this study also demonstrated that the greater bioavailability of the iron amino acid chelate allows for the rapid incorporation of iron into hemoglobin first, followed by a quicker repletion of depleted iron pools (i.e. serum ferritin) than is possible with $FeSO_4$ (61).

Multiple regression analyses of the hemoglobin changes in the above 40 infants demonstrated that those changes were dependent upon the form of iron administered as well as the hemoglobin level. Hemoglobin increases were greatest when

consuming iron amino acid chelate, but the higher the hemoglobin value, the lower was the iron absorption, regardless of the iron source. This suggested that in people with normal iron levels, there is little potential danger of iron overloading or subsequent toxicity when consuming foods fortified with nutritionally appropriate amounts of either $FeSO_4$ or iron amino acid chelate.

After first satisfying hemoglobin requirements, the greater bioavailability of the iron amino acid chelate allows for a more rapid repletion of the tissue iron stores. This is seen in a study in which two groups (N= 15/group) of gestating albino rats were fasted for 24 hours and then orally administered 4.4 μC_i ^{59}Fe as a single dose as either ^{59}Fe amino acid chelate or $^{59}FeCl_2$ mixed in 25 µL of water (62). Approximately 72 hours after dosing, or one day before expected parturition, all animals were sacrificed and their tissues and fetuses removed, dried and assayed for ^{59}Fe. Table 3 summarizes the results and demonstrates significant increases in iron deposition in certain tissues

TABLE 2

Mean ± S.D. hemoglobin levels and corrected counts per minute of ^{59}Fe incorporated into pig tissues 20 hours after oral dosing

	Whole Blood (^{59}Fe ccpm/mL)	Liver (^{59}Fe ccpm/mg)	Spleen (^{59}Fe ccpm/mg)	Red Bone Marrow (^{59}Fe ccpm/mg)	Hemoglobin g/dL
Fe AAC	402,7 ± 90,7	3,3 ± ,54	8,5 ± 2,5	16,7 ± 5,6	11,7 ± 2,7
$FeCl_2$	292.3 ± 61,2	4,7 ± 2,1	7,7 ± 1,9	10,9 ± 6,1	10,5 ± 1,8
% Increase or decrease Chelate *vs.* Cl_2	+37,8 [b]	-29,8 [a]	+10,4	+53,3 [c]	+11,4

[a] $p < 0,10$ [b] $p < 0,05$ [c] $p < 0,01$

TABLE 3

Mean ^{59}Fe incorporation into tissues (ccpm/g dried tissue) from gestating rats and their fetuses

Tissue	$FeCl_2$	Fe Amino Acid Chelate	% Increase $\frac{AAC}{FeCl_2}$
Uterus	3,333	4,926	48
Liver	8,167	9,675	18
Kidney	567	950	68
Spleen	134	325	143[b]
Heart	333	1,425	328[b]
Lung	1,367	2,925	114[a]
Fetus	16	46	188[c]

[a] $p < 0,05$; [b] $p < 0,01$; [c] $p < 0,005$

In spite of the highly significant total increase in iron deposition into the fetuses from the chelate, no analysis of individual fetal tissue was conducted in the above study, due to the size of the fetuses. Nevertheless, by combining the data from the two non-isotope studies in pigs, a pattern of iron deposition from the maternal blood into the fetus can be demonstrated. In the first study, Brady, *et al.*, sacrificed one piglet at birth from each of 12 sows (6 control and 6 treated). Each mother had been fed 85 mg of supplemental Fe daily in the feed as iron amino acid chelate or $FeSO_4$ for four weeks before expected parturition. At birth, the piglets farrowed from sows consuming the iron amino acid chelate had 34.9% more iron in their livers, 8.5% more iron in their spleens, and 3.2% more iron in their skeletal muscles. Hemoglobin levels were also 11.3% higher (63).

In the second pig study, Yamamoto, *et al.*, also gave gestating sows 60 mg of supplemental Fe daily in the feed as either iron amino acid chelate, or ferrous fumarate, commencing four (4) weeks before expected parturition (64). At parturition, the newborn piglets from sows fed iron amino acid chelate had significantly greater ($p<0.01$) mean hemoglobin levels compared to piglets from sows receiving ferrous fumarate. Their mean hematocrit values were also significantly higher ($p<0.01$), as were the mean increases in serum ferritin concentration ($p<0.001$).

When the data from these two pig studies are combined, it can be seen that greater placental transfer of iron from the mother to the fetus occurs when the iron is ingested by the pregnant mother as an amino acid chelate, and that greater amounts of iron are delivered to the fetus via maternal blood which are subsequently stored in the iron pools of the fetus for use after parturition. After birth the serum ferritin levels dropped dramatically in both groups as the piglets began producing hemoglobin to support their rapid growth rates. The chelate group, however, had serum ferritin values that were significantly ($p<0.05$) above the level of the fumarate group. At four (4) weeks of age, the mean hemoglobin

concentration in the chelate group was 14.9% higher ($p<0.01$) than in the fumarate group. Neither group of piglets in these two (2) studies received supplemental iron after parturation. The first study showed that more iron was stored in the piglets farrowed from mothers ingesting iron amino acid chelate than equivalent amounts of iron from salts. This potentially allowed for greater hemoglobin production after birth without the need for supplemental dietary iron, which the second study demonstrated.

In summary, the absorption of the iron as an amino acid chelate has been shown to be greater than iron absorption from salts, presumably due to less chemical reactions that can potentially interfere with iron absorption. The iron amino acid chelate is well absorbed, whereas the iron from the salt source may or may not be. Once absorbed into the mucosal tissue, the chelate is hydrolyzed, and the release of the iron into the plasma and the rest of the body tissues and organs is regulated similarly to that of any other source of iron. The amount of iron transferred to the body from the mucosal tissue is directly related to the body's iron needs. Greater need results in greater transfer. The advantage of the iron amino acid chelate over other sources of supplemental iron is that its greater bioavailability into the mucosal tissue cells results in more iron being quickly and safely delivered to target tissues of the body in times of need. This potentially allows for smaller doses of supplemental iron being required to achieve physiological results, which can also result in fewer gastric complaints and reduce risks of iron toxicity and iron overload. As the above data have demonstrated, iron deficiencies are less likely to occur when ingesting even small amounts of iron amino acid chelate because of its greater bioavailability.

REFERENCES

1. Mehler CC. History of Medicine. NY, McGraw-Hill, 1947, p. 177.
2. Bryan CP. The Papyrun Ebers. NY, D. Appleton and Co., 1931, p. 156.
3. Monarde M. Joyful newes out of the newe founde worlde. 1500 (Trans.) NY, A. Knopf, 1925, p. 135.
4. Herchberg S. La Carence en Fer en Nutrition Humaine. Paris, Editions Medicals Internationales, 1988, p. 3.
5. Combs, GF. Iron in Poultry Nutrition. En: Iron in Animal and Poultry Nutrition. HR Conrad, DR Zimerman, & GF Combs. West Des Moines, IA, NFIA, 1980, p. 2.
6. Guthrie HA. Introductory Nutrition. St. Louis, MO, Times Mirror, 1989, p. 290-293.
7. Ponka P, HM Schulman & RC Woodworth. (Eds.) Iron Transport and Storage. Boca Raton, FL, CRC Press, 1990, Preface.
8. Beutler E. Iron. En:Modern Nutrition in Health and Disease. RS Goodhart & ME Shils. (Eds.) Philadelphia, PA, Lea & Febiger, 1980, p. 324.
9. Charlton RW and TH Bothwell. Definition, prevalence and prevention of iron deficiency. Clin Haematol 1982;11:309.
10. Demaeyer EM. Epidemiologic, traitement et prevention de la carence ferriprive. Rev Epiden et Sante Publ. 1980;28:235.
11. Herchberg S & C Rousud. La carence en fer. Cab Nutr Dict, 1981;16:219.
12. World Health Organization. Control of nutritional anemia with special reference to iron deficiency. Technical Report No. 580. Geneva, WHO, 1975.
13. Finch CA & H Huebers. Perspectives in iron metabolism. N Engl J Med 1982;306:1520-1528.
14. Beutler E. Iron. En: Modern Nutrition in Health and Disease. RS Goodhart & ME Shils. (Eds.) Philadelphia, PA, Lea & Febiger, 1980, p. 339.
15. Layrisse M & C Martinez-Torres. Absorption of iron from foods. Prog Hematol 1971;7:37.
16. Gillooly M, TH Bothwell, JD Torrance, AP MacPhail, DP Derman, WR Bezwoda, W Mills, RW Charlton & F Mayet. The effects of organic acids, phytates, and polyphenols on the absorption of iron from vegetables. Br J Nutr 1983;49:331-342.
17. Monsen ER, L Hallberg, M Layrisse, DM Hegsted, JD Cook, M Mertz & CA Finch. Estimation of available dietary iron. Am J Clin Nutr 1978;31:134-141.
18. Cook JD & ER Monsen. Food iron absorption in human subjects. III Comparison of the effect of animal proteins on nonheme iron absorption. Am J Clin Nutr 1976;29:859-867.
19. Hallberg L & L Rossander. Improvements of iron nutrition in developing countries: comparison of adding meat, soy protein, ascorbic acid, citric acid, and ferrous sulphate on iron absorption from a simple Latin American-type of meal. Am J Clin Nutr 1984;39:577.
20. Fairbanks FV. Iron in medicine and nutrition. En: Modern Nutrition in Health and Disease. ME Shils, JA Olson & M Shike. (Eds.) Philadelphia, PA, Lea and Febiger, V1, 1994, p. 186.
21. Beisel WR. Magnitude of the host nutritional responses to infection. Am J Clin Nutr 1977;30: 1236-1247.
22. Morton I. How mineral deficient are we? Paper presented at Booker Conference, London, England, 1976.
23. Ashmead HD, EJ Rapp & JJ Name. Uso de amino acidos quelatos na fortificaco de alimentos. Proc O Seminario Brasilero de Alimentos Enriquecidos, 1994, p. 32-37.
24. Allen LH. Properties of iron amino acid chelate as iron fortificants for maize. In:Proceedings International Conference on Human Nutrition. Salt Lake City, UT, Albion Laboratories, Inc., 1998, p. 96-108.
25. Marchetti M, HD Ashmead, N Tossani, S Marchetti, & SD Ashmead. Comparison of the rates of vitamin degradation when mixed with sulfates or amino acid chelates. J Food Comp Analy. In Press.
26. Brise H & L Hallberg. Iron absorption studies II. Acta Med Scandinav, 1962;171 (Supp 376):1-73.
27. Bezwoda WR, *et al.* The relative dietary importance of heme and nonheme iron. S Afr Med J 1983;64:552.
28. Wemer A. Beitrag Zur Konstitution Anaorganischer Verbindungen. Z, Anorg. U Aligem. Chem 1893;3:267.
29. Morgan G & H Drew. Research on residual affinity and coordination. II Acetylacetones of selenium and telluriumn. J Chem Soc 1920;117:1456.
30. Williams RJP. An introduction to the nature of iron transport and storage. En: Iron Transport and Storage. Ponka, P, HM

Schulman & RC Woodworth. (Eds.) Boca Raton, FL, CRC Press, 1990, p.1-15.
31. Kratzer FH & P Vohra. Chelates in Nutrition. Boca Raton, FL, CRC Press, 1986, p. 43.
32. Chaberek S & AE Martell. Organic Sequestering Agents. NY, John Wiley & Sons, 1959.
33. Jensen N. Biological Assimilation of Metals. US Patent 4,167,564, 11 September, 1979.
34. Ashmead HD, DJ Graff & HH Ashmead. Intestinal Absorption of Metal Ions and Chelates. Springfield, IL, Charles C Thomas, 1985, p. 171-232.
35. Bailar JC, T Moeller, J Kleinberg, CO Guss, ME Castellion & C Metz. Chemistry. Orlando, FL, Academic Press, 1984, p. 223.
36. Bell CF. Principles and Application of Metal Chelation. Oxford, Clarendon Press, 1977, p. 17-18.
37. Rogers K & J Lancaster. Reports on electron paramagnetic resonance spectra of Albion Metal Amino Acid Chelates. Albion Laboratories, Inc. Personal Communication.
38. Dalley NK. Report on X-Ray Diffraction Crystallography of Albion Zinc Amino Acid Chelate. Albion Laboratories, Inc., Unpublished Research Report.
39. Tiffin LO. Translocation of micronutrients in plants. En: Micronutrients in Agriculture. RC Dinauer (Ed.) Madison, WI, Soil Science Society of America, Inc., 1972, p.199-228.
40. Johnson B. Albion Laboratory Chelates, Physical Characteristics versus Product K. Unpublished Research Report.
41. Pineda O. Effectiveness of Ferrochel (Iron Amino Acid Chelate) in the treatment of iron deficiency and iron deficiency anemia. Proc Arab Republic of Egypt Ministry of Supply and Internal Trade Conference on Food Fortification for a Better Health. Cairo,16 September 1995.
42. Ashmead S. Statistical analysis of previously published data. Albion Laboratories, Inc., Unpublished Research Report.
43. Pineda O, HD Ashmead, JM Perez & CP Lemus. Effectiveness of iron amino acid chelate on the treatment of iron deficiency anemia in adolescents. J Appl Nutr 1994;26:2-13.
44. Pineda O. Regulation and Toxicity of Ferrochel. Unpublished Report, 1996.
45. Layrisse M. Iron bioavailability from a breakfast enriched with iron bis-glycinate chelate. Effect of phytate and polyphenols on iron absorption in humans. Submitted for publication.
46. Marchetti M, N Tossani, & S Marchetti. Degradazione delle vitamins A,E,B_1,C, e K_3 negli integratori zootecnici in funzione del tipo di oligoelementi presenti. Zool Nutr Anim 1995;21:67-73.
47. Mervyn L.The clinical significance of chelated minerals as nutritional supplements in medical practices. Proc of Malaysian Medical Assoc, 1978. Later published as: Human therapeutic applications of amino acid chelates. En: Proceedings International Conference on Human Nutrition, Salt Lake City, UT, Albion Laboratories, Inc., 1995.
48. Lowe JA, J Wiseman & DJA Cole. Zinc sources influence zinc retention in hair and hair growth in the dog. J Nutr 1994;124:2575S-2576S.
49. DiSilvestro RA, J Marten, & M Skehan. Effects of copper supplementation on ceruloplasmin and copper-zinc superoxide dismutase in free-living rheumatoid arthritis patients. J Am Coll Nutr 1992;11:177-180.
50. Cuser WO & A Resurrecion. En:Influence of copper, zinc, and protein on biological response to dietary iron. En: Nutritional Bioavailability of Iron. Washington, DC, American Chemical Society, 1982, p. 97.
51. Greger JL, ML Storey, JL Stahl, ME Cook, SE Gentry-Roberts & JC Lynds. Zinc, iron and copper interactions in humans, rats, and chicks. En: Trace Elements in Man and Animals. LS Hurley, CL Keen, B Lönnerdal & RB Rucker. (Eds.) NY, Plenum Press, 1988, p. 231-232.
52. Howell JM & JM Gaiothoma. Copper in Animals and Man. Boca Raton, FL, CRC Press, 1987, Vl, p. 90.
53. O'Dell, B. Copper and zinc in poultry nutrition. En: Copper and Zinc in Poultry, Swine and Ruminant Nutrition. B. O'Dell, ER Miller & WJ Miller. West Des Moines, IA, NFIA, 1979, p. 5.
54. Bezkorovainy A. Biochemistry of Nonheme Iron, 1980, p. 5.
55. El-Shobaki FA & W Rummel. Binding of copper to mucosal transferrin and inhibition of intestinal iron absorption in rats. Res Exp Med 1979;174:187-195.
56. Grassman E & M Kirchgessner. On the metabolic availability of absorbed copper and iron. En:Trace Element Metabolism in Man and Animals. Canberra City, Australia Academy of Science, 1981, p. 593.
57. Ashmead HD. A lack of competition in the absorption of Albion iron and copper amino acid chelates. Clearfield, UT, Albion Laboratories, Inc., Prepared for publication.
58. Graff D, HD Ashmead & C Hartley. Absorption of minerals compared with chelates made from various protein sources into rat jejunal slices, *in vitro*. Paper presented at Utah Academy Arts, Letter, Sciences, April 1970. Later published in:Intestinal Absorption of Metal Ions and Chelates. HD Ashmead, DJ Graff & HH Ashmead. Springfield, IL, Charles C Thomas, 1985, p. 118-125.
59. Ashmead HD & RB Jeppsen. Enhanced tissue metabolism of minerals chelated to amino acids. Proc. Bioavailability 93, Ettlingen, Germany V2, 1993, p. 63.
60. Fairweather-Tait SJ, TE Fox, SG Wharf & NA Ghani. A preliminary study of the bioavailability of iron- and zinc-glycine chelates. Food Add Contam 1992;9:97-101.
61. Pineda O & HD Ashmead. Treatment of iron deficiency anemia in infants and young children with bis-glycine iron chelate or ferrous sulfate. Submitted for publication.
62. Graff D & HH Ashmead. Transplacental movement of iron59 chelated with amino acids versus iron59 in inorganic form in synchronously impregnated rats. Paper presented at Nutrition Conference, Cancun Mexico, November, 1977.
63. Brady PS, ER Miller, PK Ku, FF Green & DE Ullrey. Evaluation of an amino acid iron chelate hematinic. Report of Swine Research, 1975, p. 4-6.
64. Yamamoto M, Y Yoshino & K Migawaki. A comparative study of iron proteinate and ferrous fumerate on pig performance. En: Chelated Mineral Nutrition in Plants, Animals and Man. Ashmead, D, (Ed.) Springfield, MO, Charles C Thomas, 1982, p. 210-222.

ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición

Suplemento Vol. 51 N° 1, 2001

Bioavailability of iron bis-glycinate chelate in water

Manuel Olivares G., and Fernando Pizarro A.

Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile

SUMMARY . Iron amino acid chelate is being increasingly considered in programs for iron fortification of foods. The bioavailability of iron bis-glycinate chelate given in water was studied using a double-isotopic method in a group of 14 women. Iron absorption from aqueous solutions of 15 mg/L of elemental iron as either iron bis-glycine chelate or ferrous ascorbate was not significantly different (34.6% and 29.9% respectively). Standardized iron absorption of the iron bis-glycinate was 46.3% (standardized to 40% absorption of the reference dose). There was a significant correlations between (ln) iron absorption of iron bis-glycinate chelate with (ln) serum ferritin ($r = -0.60$, $p < 0.03$) and with (ln) iron absorption from ferrous ascorbate ($r = 0.71$, $p < 0.006$), suggesting that iron bis-glycinate chelate absorption is indeed regulated by the iron stores of the body.
Key words: Ferrochel, absorption, bioavailability, regulation.

RESUMEN. Biodisponibilidad del hierro bis-glicinato quelado dado en agua. El hierro aminoácido quelado está siendo considerado en forma creciente para programas de fortificación de alimentos. La absorción del hierro del bisglicinato dado en agua fue estudiada en un grupo de 14 mujeres utilizando la técnica doble isotópica. Las absorciones de soluciones acuosas de 15 mg/L de hierro elemental como bis-glicinato o como ascorbato ferroso no fueron significativamente distintas (34,6% y 29,9% respectivamente). La absorción estandardizada del bis-glicinato de hierro fue 46,3% (estandarizado a 40% absorción de la dosis de referencia de ascorbato ferroso). Existió una correlación significativa entre el logaritmo de la absorción del bis-glicinato y el logaritmo de la ferritina sérica ($r = -0,60$, $p < 0,03$) y el logaritmo de la absorción del ascorbato ferroso ($r = 0,71$, $p < 0,006$), sugiriendo que la absorción del bis-glicinato de hierro es regulada por los depósitos de hierro del organismo.
Palabras clave: Ferrochel, absorción, biodisponibilidad, regulación.

INTRODUCTION

Iron deficiency continues to be one of the most prevalent nutritional deficiencies in the world. For physiological reasons the most commonly affected groups are infants, children, adolescents and women of childbearing age (1).

Fortification of foods with iron is considered the best sustainable way of preventing iron deficiency (2). The sequential steps that should be followed in establishing an iron fortification program have been well defined. Perhaps the most difficult technical hurdle is finding the adequate combination of iron compound and food vehicle. Consequently, the use of an iron compound that is less influenced by inhibiting dietary ligands is an appealing strategy.

Iron amino acid chelate is a compound formed by two glycine molecules bound to an iron atom, resulting in a double heterocyclic ring compound. It has been proposed that this configuration protects the iron from dietary inhibitors and intestinal interactions (3). Recent studies have shown that iron bis-glycinate chelate is well absorbed when it is added to foods with a predominance of inhibitors (4,5).

The aim of the study was to etablish the bioavailability of iron bis-glycinate chelate when given in water.

SUBJECTS AND METHODS

Subjects

Iron absorption studies were performed in a group of 14 women between the ages of 27 and 51 years. None were pregnant, all used contraceptive intrauterine devices and were in apparent good health. Written informed consent was obtained from each volunteer before participation in the study. The protocol was reviewed and was found in accordance with the standards set by the Institute of Nutrition and Food Technology's Ethics Committee on Human Research. Radioactive doses were approved by the Chilean Nuclear Energy Commission.

Isotope studies

Iron isotopes (^{55}Fe and ^{59}Fe) of high specific activity were used as tracers. Both isotopes are iron (III) chlorides as purchased (Du Pont de Nemours & Co. Inc., Wilmington, DE). Isotopes were mixed with water immediately before administration. Iron bis-glycinate chelate (Ferrochel ®, Albion Laboratories, Ine., Clearfield, Utah) was intrinsically labeled during the synthesis of iron amino acid chelates. This process was performed by the manufacturer. The specific activity of the labeled iron bis-glycinate chelate was 37 kBq

of ^{59}Fe/mg elemental iron. The preparations were consumed after an overnight fast, and no food or beverages other than water were permitted for 4 hours following consumption. The amounts of aqueous solutions ingested were calculated by differential weight of the glasses. For the calculation of total radioactivity ingested, aliquots of the aqueous solutions were counted in sextuplicate as standards. Measurement of blood radioactivity was performed in duplicate venous samples according to the method of Eakins and Brown (6), and were counted for sufficient time to ensure less than 3% counting error. A liquid-scintillation counter (Beckman LS 5000 TD, Beckman Instruments, Inc., Fullerton, CA) was used for the double isotope measurements. Percent absorption was calculated based on the blood volume estimated from height and weight, assuming an 80% red cell utilization of radio iron (7).

On day 1 the subjects received 200 mL of an aqueous solution of 15 mg/L of elemental iron as iron bis-glycinate chelate labeled with 111 kBq of ^{55}Fe and on day two 200 mL of a reference dose of 15 mg/L of elemental iron as ferrous ascorbate (molar ratio 1:2 iron to ascorbic acid) labeled with 37 kBq of ^{59}FeCl3. A venous blood sample was obtained two weeks later (day 16) to measure the circulating radioactivity and to determine the iron status of the subjects.

Hemoglobin, mean cell volume, free erythrocyte protoporphyrin, serum iron, total iron binding capacity and serum ferritin were determined in the venous blood obtained on day 16 (8).

For comparative studies of iron bioavailability, the absorption of 3 mg of elemental iron as ferrous ascorbate is used to offset the effect of differences in iron status among individuals (9). For purposes of comparison all studies currently refer to 40% absorption of the reference dose of ferrous ascorbate. This absorption percentage is used because it corresponds to that which is obtained in borderline iron deficient populations.

Because the percentages of iron absorption and serum ferritin concentrations have a skewed distribution, these values were converted to natural logarithms before performing mean and SD calculations, and the results were transformed back using antilogarithms to recover the original units, and expressed as geometric means ±1 SD (7). Statistical analyses included paired Student t test and Pearson correlation. Statistical analyses was performed on logarithmically transformed data using the program SPSS for Windows, release 6.0, SPSS Inc., Chicago, IL, 1993.

RESULTS

The iron bioavailability of the aqueous solutions of iron bis-glycinate chelate and ferrous ascorbate were not significantly different (34.6% and 29.9% respectively, t = 0.80, p NS). When iron absorption was standardized to 40% absorption of the reference dose using the data shown in Table 1, the corresponding percentage of iron absorption for the iron bis-glycinate chelate given in water was 46.3%.

There was a significant inverse correlation between (ln) serum ferritin and (ln) iron absorption from iron bis-glycinate chelate given with water ($r = -0.60$, $p < 0.03$). The correlation between the absorption of (ln) ferrous ascorbate and (ln) iron bis-glycine chelate given with water was ($r = 0.71$, $p < 0.006$).

DISCUSSION

The efficacy of an iron compound for supplementation or food fortification interventions can be predicted from iron bioavailability studies of the supplement or the fortified food. Bioavailability of iron is influenced by the characteristics of the iron compound, total amount of iron in the diet, iron status of the individual, rate of erythropoiesis, and the presence of inhibitors or enhancers of iron absorption present in the intestinal lumen or in the diet (9,10). If soluble inorganic iron salts are given with water solutions without food, absorption of iron is high, but, absorption of iron salts decreases markedly when given with foods (11).

Our results showed that the iron bioavailability of iron bis-glycinate chelate given in water is high, compared to the absorption of the reference dose of ferrous ascorbate. The iron absorption of this compound was calculated to be 46.3% in a population with low iron stores (a population absorbing 40% of the reference dose of iron ascorbate).

It has been proposed that the iron amino acid chelate is absorbed in the jejunum rather than as inorganic iron in the duodenum (3). Therefore, there has been some concern about the role of iron stores on the regulation of iron absorption from the chelate. The results of this study, suggest that the absorption of the iron bis-glycinate chelate is likely to be controlled by the iron stores of the subjects. There was an inverse relationship between the iron stores of the body, as reflected by serum ferritin, and the absorption of the iron bis-glycinate chelate. This holds true as well when we compare the absorption of the iron bis-glycinate chelate with that of ferrous ascorbate which showed an excellent correlation (r=0.71). However, this correlation may not necessarily prove causality. Another study performed in adult women also showed an inverse correlation between serum ferritin and the absorption of iron bis-glycinate chelate (12). The demonstration that regulation of iron absorption by iron stores occurs with the iron bis-glycinate chelate should dispel the risk of iron overload if this compound was to be used in food fortification programs for the population at large. Further research is needed to elucidate the mechanism of absorption of iron bis-glycine chelate.

We can conclude that in water the absorption of iron bis-glycine chelate is high. Studies to compare the bioavailability of the iron amino acid chelate when added to different foods are needed.

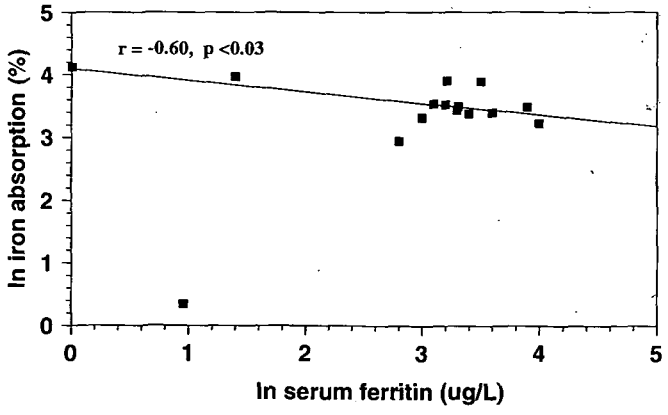
TABLE 1
Iron absorption from iron bis-glycinate chelate [1]

Subject	Age y	Hb g/L	FEP ug/dL Rbc	Sat %	SF ug/L	Iron Absorption % Iron bis-glycinate ^{55}Fe (A)	Iron ascorbate ^{59}Fe (B)	Ratio I A/B
GR	48	133	57.0	26.7	24	33.8	6.0	5.63
EM	43	150	42.8	15.4	29	29.3	8.5	3.45
AC	51	140	60.0	23.1	53	25.1	14.4	1.74
BM	38	132	48.6	20.4	36	29.8	17.6	1.69
LM	49	142	37.1	18.1	21	27.6	28.4	0.97
GR	33	138	54.3	34.3	23	34.3	29.1	1.18
MA	43	148	42.9	17.1	48	32.6	30.3	1.08
YR	45	144	57.2	27.1	16	19.0	32.7	0.58
XM	46	147	88.6	23.9	26	31.0	35.0	0.89
MLG	40	131	45.6	15.2	25	49.5	36.5	1.36
GM	42	143	65.7	25.2	27	32.8	46.2	0.71
PG	43	144	62.9	27.8	32	49.0	72.6	0.67
PA	45	143	49.4	26.1	4	52.9	79.4	0.67
LA	43	118	191.4	9.9	1	61.8	124.0	0.50
Mean	43.5	139.5	64.5	22.2	19 [2]	34.6 [2]	29.9 [2]	1.16 [2]
SD	4.4	8.2	37.3	6.2	7-53	25.4-47.2	13.4-66.6	0.60-2.25

[1] Abbreviations used Hb, hemoglobin; FEP, free erythrocyte protoporphyrin; Sat, transferrin saturation; SF, serum ferritin.
[2] Geometric mean and range 1 SD.

FIGURE 1

Relationship between natural logarithm (ln) iron absorption of iron amino acid chelates given in water and ln serum ferritin



ACKNOWLEDGMENTS

The authors sincerely appreciate the very useful editorial suggestions provided by Ms. Virginia Gidi.

REFERENCES

1. DeMayer E, Adiels-Tegman M. The prevalence of anemia in the world. World Health Statist Q 1985;38:302-316.
2. International Nutritional Anemia Consultative Group (INACG). Guidelines for the eradication of iron deficiency anemia: a report of the International Nutritional Anemia Consultative Group. The Nutrition Foundation, Inc. Washington, DC, 1977:1-29.
3. Ashmead HD, Graff DJ, Ashmead HH. *Intestinal absorption of metal ions and chelates*. Charles C. Thomas Publisher, Springfield, Il, 1985:1-251.
4. Olivares M, Pizarro F, Pineda O, Name JJ, Hertrampf E, Walter T. Milk inhibits and ascorbic acid favors ferrous bis-glycine chelate bioavailability in humans. J Nutr 1997;127:1407-1411.
5. Bovell-Benjamin AC, Allen LH, Viteri FE. Iron is well absorbed from ferrous bisglycinate (Ferrochel) added to a high phytate whole-maize meal. FASEB J 1997;11:A606 (abstract)
6. Eakins JD, Brown DA. An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. Int J Appl Radiact Isotopes 1966;17:191-197.
7. Cook JD, Layrisse M, Finch CA. The measurement of iron absorption. Blood 1969;33: 421-429.
8. International Anemia Consultative Group (INACG). Measurement of iron status: a report of the International

Anemia Consultative Group. The Nutrition Foundation, Washington, DC, 1985:1-49.
9. Cook JD, Bothwell TH. Availability of iron from infant foods. In: Iron nutrition in infancy and childhood. Stekel A, ed., pp. 119-145. Nestlé/Raven Press, Vevey/New York. 1984:119-145.
10. Bothwell TH. Iron absorption. Ann Rev Med 1983;34: 55-68.
11. Hurrell RF. Bioavailability of different iron compounds used to fortify formulas and cereals: technological problems. In: Stekel A, ed. Iron nutrition in infancy and childhood. Nestlé/ Raven Press, Vevey/New York, 1984:147-178.
12. Allen LH. Properties of iron amino acid chelates as iron fortificants for maize. International Conference on Human Nutrition. Salt Lake City, TU, January 24-25, 1998, pp 96-108.

ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición

Suplemento Vol. 51 Nº 1, 2001

Toxicology and safety of Ferrochel and other iron amino acid chelates

Robert B. Jeppsen

Albion Laboratories Inc. Clearfield, Utah U.S.A.

SUMMARY. Iron is estimated to be deficient in the diets of one fifth of the world's population. Iron is commonly provided as a supplemental nutrient in industrialized countries for uses of choice. In other countries of the world, it may be required as an overt addition to the diet to prevent iron deficiency. This may be accomplished through fortification of a common food. As a micronutrient, iron has a relatively narrow range of safety – whether given as a supplement or fortificant, it must be in a high enough dose to be appreciably absorbed, but low enough to avoid toxicity. This concern can be ameliorated by careful choice of the form of iron administered. A source of iron which has proven to be highly bioavailable, yet regulated by dietary need, is iron chelated with amino acids. The structural integrity and longevity of these compounds have been proven by valid chemical and instrumental tests. Proofs of safety of iron amino acid chelate in the dietary administration of iron to swine in both multigenerational and longevity studies are reported. Formal tests of toxicity utilizing ferrous bisglycinate chelate (Ferrochel) carried out in accordance to US-FDA guidelines are also summarized. Ferrochel has been demonstrated to have a No Observable Adverse Effect Level (NOAEL) of at least 500 mg per kg rat body weight, the highest dose tested. This and other results of the detailed toxicity test, as well as other tests of safety and efficacy, have resulted in the US-FDA acknowledging that this product is Generally Recognized As Safe (GRAS) under its approved conditions of use as a source of iron for food enrichment and fortification purposes.
Key words: Iron, Ferrochel, iron amino acid chelate, NOAEL, GRAS.

RESUMEN. Toxicidad e innocuidad de Ferrochel y de otros aminoquelados de hierro. Se estima que el hierro es deficiente en las dietas de una quinta parte de la población del mundo. En paises industrializados el hierro es comúnmente usado como un suplemento dietético; en otros paises la adición de hierro a la dieta se hace necesaria para prevenir la deficiencia de hierro y la anemia ferropriva, lo que se puede lograr fortificando alimentos seleccionados. El hierro presenta límites estrechos de seguridad y cuando se administra ya sea como un suplemento dietético, o como un fortificante, debe darse en una cantidad suficiente para que la cantidad absorbida sea adecuada, y suficientemente baja para prevenir toxicidad. Una fuente de hierro que ha mostrado tener una alta biodisponibilidad y ser regulada por las reservas de hierro del organismo es el hierro quelado con amino ácidos. La innocuidad de este hierro ha sido demostra-da por su administración a generaciones sucesivas de cerdos en los cuales no se ha encontrado ningún efecto nocivo. Pruebas formales de toxicidad del hierro bis-glicinato quelado se han llevado a cabo en ratas siguiendo como guía protocolos del FDA de los E.E.U.U. En estas pruebas se ha encontrado que un nivel de ingesta diaria de 500 mg por kg de peso corporal no producen ningún efecto adverso observable (NOAEL). Con base en estos y otros resultados, el FDA ha reconocido al compuesto como GRAS (Generalmente Reconocido como Innocuo) y ha aprobado su uso para suplementación y fortificación de alimento.
Palabras clave: Hierro, Ferrochel, hierro aminoquelado, NOAEL, GRAS.

INTRODUCTION

Four aspects of iron have long orchestrated the significance of its use in nutrition. The first is that it is absolutely essential to life. It is a required part of hemoglobin, myoglobin, ferredoxins, cytochromes, and several enzymes active in porphyrin synthesis, oxygen regulation and immunity. If iron is absent from its niche within these molecules, they are nonfunctional. The ubiquitous metabolic needs for these molecules make the lives of all aerobic organisms impossible without them. Second, the deliberate intervention of additional bioavailable iron into metabolic systems which are deficient in iron will improve both the functional ability of the metabolites as well as the systems they support, thus reducing iron deficiency and improving the general health of the individual. Third, as a micronutrient, the range of iron sufficiency is relatively narrow. Absorption of iron into the body, transport to its sites of need and the placement of elemental iron into the actual molecules requiring it are subject to narrow ranges of concentration tolerances. Additions of iron which are larger than these tolerances can lead to toxicity expressed as discomfort, pathology or even death.

The fourth aspect of iron nutrition promotes a way to ameliorate the negative effects of the third aspect (toxicity) with the positive consequences of the second aspect (the

possibility of iron remediation). It is important that extradietary iron be supplied to populations who need it, either as short term intervention (supplementation) or long term support (food fortification). The prospect of intervention and the possibility of toxicity need to be favorably balanced, the iron needs to be sufficiently bioavailable to be efficacious and it must be sufficiently free from the toxicities associated with iron to be safe to consume. The fourth aspect, therefore, is the form, or source, of the nutritional iron which dictates its relative bioavailability and safety.

Iron nutrition versus iron toxicity

Although the beneficial effects of iron in the body have been recognized from antiquity and its physiological effects have been further elaborated upon since the Renaissance (1,2), the recommended dosage has been widely disparate. Blaud recommended an $FeCO_3$ concoction delivering 193 to 771 mg iron/day as suitable remediation for 'green sickness' or 'chlorosis' (1). Bunge thought all iron supplementation was ineffective and worthless, while Quincke and von Noorden were convinced that no more that 1,5 grains (0,1 mg) of daily iron were sufficient (3). It was only as recent as the 1930's that the quantitative incorporation of iron into hemoglobin was conclusively proven (4,5).

Typical pharmacological recommendations for therapeutic sources of iron are ferrous sulfate heptahydrate, coated anhydrous ferrous sulfate, ferrous gluconate, polysaccharide-iron complex, and finely ground elemental iron. The average dose for treatment of iron deficiency anemia in adults is around 200 mg iron/day, given in three equal doses of around 65 mg iron. Children weighing 15 to 30 kg are presumed to tolerate half of the adult dose, while small children and infants are cited as being able to handle a larger relative proportion (5 mg/kg/day). The main consideration of these recommended doses is that they represent "a practical compromise" between the therapeutic action desired and the toxic effects of the iron (2). Therefore, maximal doses are recommended for the highest absorption of iron ions while the toxic effects are parlayed to their highest tolerance levels. The above recommendations are also made on the presumption that the iron doses are taken on an empty stomach. This is because the absorption rates of iron from its salts are typically limited to 2-10% of the dose in individuals with sufficient iron stores (6), and 10-20% in people suffering iron deficiency anemia (2). These absorbed amounts of iron shrink by an additional 50-67% when taken with meals (7). However, it is acknowledged that when adverse gastrointestinal effects of iron salts are encountered, either the dose should be reduced or the high-dosed iron should be consumed with food (2,8). These adverse effects include nausea, heartburn, abdominal cramping, vomiting, diarrhea and constipation (8). In the event of acute toxicity, the initial feature is still gastrointestinal irritation which confounds the perception that a toxic dose has been taken – the initial signs of toxicity are the same as common side effects of iron supplementation.

Children are more inclined to lethal iron toxicities due to ingesting relatively high doses compared to their weights. Acute toxicities from oral iron preparations include the above side effects of gastrointestinal irritation. Vomiting may be the first sign. These may be followed by gastrointestinal bleeding, lethargy or restlessness, and gray cyanosis. Following these signs, there may be a seeming recovery period of several hours to one to two days. The third phase of toxicity then commences and may include pneumonitis, jaundice, additional signs of liver toxicity and/or convulsions. Additional symptoms are gastrointestinal bleeding and neurological manifestations, including coma. Most deaths occur during this phase. Where individuals survive from three to four days after the acute ingestion of iron, recovery is generally rapid, although long-term leukocytosis may occur. Pyloric constriction and gastric fibrosis may occasionally result. Acute iron toxicity is treated with a combination of induced emesis, gastric intubation with $NaHCO_3$ lavage, and parenteral chelation of the iron with deferoxamine (8,9).

From a therapeutic standpoint, some side effects (toxic symptoms) are expected from the ingestion of ionic sources of iron due to the close proximity of therapeutic and toxic doses of iron salts. Adults taking doses of 200 mg iron/day divided into three equal doses could expect these symptoms to occur in approximately 25% of the cases. If the dose were doubled to 400 mg iron/day, then the incidence of toxic symptoms would increase to approximately 40% (2), although individual tests may indicate greater toxicities. For instance, adolescents and younger children could expect even higher incidences of toxicity at these dose levels or even at lower doses. In a double-blind nutritional study with adolescents having iron deficiency anemia there was a 33,3% incidence of side effects in just 120 mg iron/day from $FeSO_4$ (given in two daily doses of 60 mg iron, each contained in two enteric coated tablets containing 30 mg of iron, each) (10).

Chelation of the iron source for improved bioavailability and safety

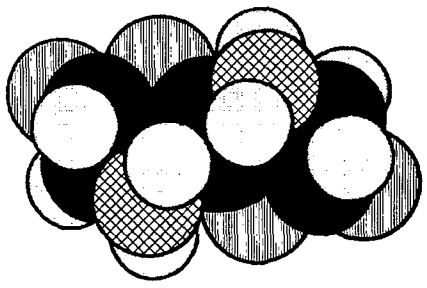
Oral iron sources which are inorganic salts with at least a degree of solubility are typically absorbed from the duodenal segment of the small intestine where the luminal pH is low and the iron is ionized. If the iron is held in a larger molecule, such as in a polysaccharide complex, then the iron needs to be severed from its position in the larger molecule before it can be absorbed by the small intestine. If the iron could be covalently bound to organic ligands which would reduce the charge of the cation and provide some spacial protection on the side of the attachment, then less toxicity due to

gastrointestinal irritation would be expected. The degree of protection inherent in the molecule would vary according to the strength of the ligand bond protecting at least one spacial side of the metal ion and the potential of the remaining charges of the iron cation to irritate the surfaces of the gastrointestinal mucosa or engage in free reactions with metabolites which could contribute to toxic effects in the body.

Chelates are formed when the same ligand molecule bonds to the same metal atom through more than one reactive site on the ligand. The ligand thus bends around the metal atom forming a molecular ring structure of atoms. The molecular ring sterically protects the metal atom where the ligand moieties are attached, as well as by the physical barrier of the ligand, itself, between the two moieties. When an additional ligand chelates the same metal atom, the atom is sterically more protected and sequestered within the molecule. Thus, a chelate of elemental iron, preferably having a metal to ligand ratio of 1:2 or greater would provide more sequestration and protection than either a covalently bound organic complex or an organic or inorganic salt. This is illustrated in the space filling structure of an iron bisglycinate chelate shown in Figure 1.

FIGURE 1

Space filling structure of an iron bisglycinate chelate molecule. The iron atom appears in the center with nitrogen, carbon, oxygen and hydrogen atoms of the glycine ligands surrounding the iron atom and bound to it via each nitrogen atom and each carboxyl oxygen atom. The protective capacities of the glycine amino acids with a metal to ligand ratio of 1:2 on each chelated iron atom are illustrated



Provided that the chelating bonds are sufficiently strong to resist cleavage by digestion or through reactive natural foodstuffs (such as, phytic acid, phosphates, tannic acid, and other luminal components which can potentially bind nutritive minerals), the chelates can protect the mineral atoms sufficiently long to be absorbed and utilized nutritionally. It is readily apparent that a 1:1 metal to ligand ratio with glycine or any other amino acid would leave one side of the chelated metal unprotected and vulnerable –both 1) to attack by phytic acid, phosphates, etc., with subsequent loss of nutrient capability and 2) to a greater potential for toxicity due to retaining a reactive site (and, therefore, irritative site) on the metal. The 1:2 metal to ligand ratio restricts unwanted reactions with dietary components, neutralizes the valence of the ferrous iron and protects the gastrointestinal surfaces from being irritated by close contact with the iron atom. Supplemental or fortifying levels of orally administered iron given as a bioavailable chelate would thus provide more protection from iron damage to the body that requires the iron.

There is precedence demonstrating the protection gained from chelates (as well as for improved bioavailability) in the natural iron source from animals known as heme. In this molecule, iron is chelated by a cyclic porphyrin ligand which is a breakdown product of blood hemoglobin and muscle myoglobin. Heme iron is far more bioavailable than inorganic or ionic sources of iron. It has been shown that in a diet containing only 6% of the total iron as heme, 30% of the iron absorbed was acquired from the heme only, at the exclusion of other dietary sources (2). The absorption of iron as heme has been shown to be independent of the presence or composition of concurrently eaten foodstuffs as well as being independent of iron absorption from supplemental organic or inorganic iron salts. These traits are also shared with mineral (metal) amino acid chelates which demonstrate different uptake pathways than either salts or complexes of the same minerals and which do not compete with the uptake of inorganic mineral sources (11-13).

When any mineral atom chelates with reactive ligands, a new molecule is formed which does not have the same properties as either the mineral ion or the free ligands. The new compound better stabilizes both the mineral and the ligands over that of their free forms. The overall properties of the chelated molecule mainly resemble those of the ligand(s), however, since these contribute the most bulk to the new molecule. This may explain the predominant uptake of metal amino acid chelates in the jejunum where amino acids and other products of protein hydrolyzation are typically absorbed (11).

Structural integrity of iron amino acid chelates

The molecular structures of the iron amino acid chelates have been proven through a variety of tests which elucidate chemical structures. Electron paramagnetic resonance (EPR) has been used to determine electron orientations between iron and the chelating amino acids in an animal feed supplement. In addition to proving the presence of chelation, the experiment was designed to determine the structural integrity of the product in premix, mixed loose feed and pelleted (extruded) feed. Both upper and lower g-values demonstrated that the bond orientations of the iron atoms in each case were tetrahedral, which vouched for the integrity of the 1:2, iron:amino acids, molar ratio of the chelated

animal nutrient. Collaborative evidence for the chelation of metal amino acid chelates has been given through a combination of x-ray crystallography and Fourier-transformed infrared spectrometry (FT-IR).

X-ray diffraction was first applied to prove the chelated structure of zinc bisglycinate chelate. This compound was used to verify published infrared absorption peaks for metal amino acid chelates. The appropriate absorption peaks were then applied to the other metal amino acid chelates to determine the structural integrity of these compounds. The molecular structures of these chelates (including iron amino acid chelates manufactured for both animal and human uses) have been proven by these combined instrumental techniques.

The safety of iron amino acid chelates including Ferrochel

The safety of iron amino acid chelates has been demonstrated through a variety of animal tests and human clinical studies. Three kinds of chelated iron products have been used in these tests. For clarity, these are herein identified as animal grade iron amino acid chelate for the animal feed grade product made from feed grade ingredients and human grade iron amino acid chelate which utilizes USP ingredients. The third form is Ferrochel, which is also made from USP ingredients and predominately maintains the chelated iron in the ferrous state. Ferrochel may also be referred to as ferrous bisglycinate chelate. These products have been validated as amino acid chelates by FT-IR and other appropriate instrumental methods.

Studies utilizing animal grade iron amino acid chelate. Long-term multigenerational feeding of iron amino acid chelate in sows

Two breeding sow operations from Ontario Province in Canada were chosen as treatment and control farms to conduct a toxicological assessment of animal grade iron amino acid chelate (also referred to as Iron Metalosate) as well as other metal amino acid chelates (14,15). Pigs on the treatment farm had received this dietary iron amino acid chelate for several years, resulting in five generations of pigs which had received dietary minerals in the form of metal amino acid chelates (Metalosates). The pigs on both farms were raised under similar confinement housing systems and the farms were within 20 km of each other. All animals received inoculations against erysipelas, leptospirosis, *Parvo* virus and *Escherichia coli.* The treatment animals additionally received Ivomec® for deworming and lice control. The breeds of the sows on both farms were similar, being mixtures of both York and York-Landrace stock. The nutritional constituents of the feeds were similar for both treatment and control sows with the exception that the treated sows received iron amino acid chelate as a supplemental source of iron, as well as other metal amino acid chelates.

Four feed rations were formulated for the pigs at the treatment farm. The first two, Weaner and Grower, were fed sequentially to all production offspring. Production pigs were marketed upon achieving 105 kg weights. Once the sows which became production sows were grown, they mostly received Dry Sow ration. Lactation ration was given to sows starting three weeks prior to parturition and continued for 25 days after birth, at which time the litters were weaned and began receiving the Weaner ration. While all four of the rations contained iron amino acid chelate, the greatest amount was formulated into the Lactation ration with the equivalent amount of 183,86 mg iron per kg of finished feed. Additionally, nursing sows were allowed to consume 7,257 kg of the Lactation ration per day (which supplied 1334,27 mg iron per day) to nourish their litters, while sows on the Dry Sow ration were allowed just 2,722 kg of feed per day (contributing 272,2 mg iron per day). Due to a change in the Lactation ration, prior to assessing the contributions of iron amino acid chelate to the health of the sows, all of the sows in the test group, except one, received 756,54 mg iron per day from the Lactation ration during their last farrowing. All of the sows also received the iron which was inherent in their food. In comparing the Dry Sow ration with the Lactation rations, it can be seen that the total consumption of iron as iron amino acid chelate was largely dependent on the number of farrowings (litter births) which the sows achieved in their lifetimes.

Six breeding sows were chosen for assessment from each of the farm herds. The two sets of six sows each were delivered to a local abattoir (slaughter house) on the same morning. Three of the six treatment animals represented the fourth filial generation of breeding sows to receive iron amino acid chelate. One of the treatment sows was of the third filial generation and the other two were of the second filial generation. The total amounts of iron from iron amino acid chelate consumed by the sows on the basis of their age and number of farrowings (parity) were less in the fourth generation animals since these tended to be the youngest sows. One of these animals had lived long enough to have six farrowings, however, so her total consumption was greater that the other two. Sows from the fourth filial generation thus consumed 131, 193 and 556 g of iron. The single individual of the third filial generation consumed 734 g, while the two individuals of the second filial generation consumed 580 and 687 g.

A local veterinarian, who had been certified with the Canadian Veterinary Medical Association, was enlisted to conduct both premortem and postmortem examinations of the sows. The veterinarian was kept blind as to which pigs had received iron amino acid chelate (treatment sows) and which had not (control sows). He was not appraised of the sow identifications until he had submitted his written report to Albion Laboratories Inc.

Following euthanasia of the sows by cerebral electrocution

and exsanguination, internal organs and skeletal tissues were excised for histopathological examination. The tissues excised included: brain, duodenum, jejunum, ileum, large intestine, muscle, heart, liver, spleen, bone marrow, mesenteric lymph node, kidney and ovary. The certified veterinarian excised all of the tissues from all of the animals and made all of the collections from the same parts of the organs utilized in order to standardize the assessments of both the treatment and control animals. He also made internal examinations as to the health of the animals. The tissues were trimmed into small blocks (approximately,1 cm X 1 cm X 2,5 cm) for ease of infiltration of the tissue fixative and for subsequent microtoming for examination slides. Following excision, the tissue blocks were submerged in 10% formalin solution in small capped bottles and shipped the next day by overnight courier to Albion Laboratories. Following receipt at Albion, the fluid in the bottles was changed out for 10% buffered formalin and shipped to a certified veterinary histopathologist in the United States. The veterinary report on premortem and postmortem examinations was also reviewed by the histopathologist although he was kept blind as to the identifications of treatment and control sow groups in both the on-site veterinarian's report and his own tissue samples until he had issued his draft report. At that time, he was made aware of the sow identifications so he could finalize his report as to group and sow identifications.

This study was conducted in order to assess two conditions: 1) the long term effects of continuous feeding of iron amino acid chelate on single individuals, and 2) possible cumulative effects of this iron amino acid chelate on multiple generations of production sows. The certified veterinary histopathologist's conclusion was that, "No histopathologic tissue alterations were observed that could be attributed to dietary administration of Iron Metalosate® to sows." He also noted that, "When the histopathologic findings in pigs of Group I [Control] were compared to those of Group II [Treatment], there were no biologically significant differences between the groups.. It was concluded that no histopathologic tissue alterations were observed that could be attributed to Iron Metalosate® given as a mineral premix to sows " (16).

Long-term feeding of iron amino acid chelate in sows achieving six or greater parity

This investigation was a companion study to the long-term multigenerational study described above. As noted in the previous study, three of the test animals were of the fourth filial generation of production sows which had received iron amino acid chelate throughout their lives. Two of these animals had only been alive for a third to a half of the time of other test animals in the study. It was considered important to organize an additional investigation to verify that production sows which were uniformly older, at least by production standards, and which had received this iron amino acid chelate throughout their production lives, would be likewise free from pathology (17). Parity is an additional manner of expressing the number of farrowings which a sow has achieved over her productive lifetime. Six parities denotes a sow that has lived a longer than average production life.

Two farms in Ontario Province of Canada were chosen for this study. The control farm was the same one used for the multigenerational study, but the treatment farm was different. Both farms were still within 20 km of one another, however. Pigs at both farms had been inoculated for erysipelas, leptospirosis, *Parvo* virus and *Escherichia coli*. The control animals additionally received Ivomec® for internal and external parasites. Of the four sows selected for assessment from the control farm, three were of the Landrace breed and one was a York-Landrace cross. Eight sows were taken for assessment from the treatment farm. All of these were of the York-Landrace cross. The nutritional constituents of the feeds were similar for both treatment and control sows with the exception that the treated sows received iron amino acid chelate as a supplemental source of iron, as well as other minerals in the metal amino acid chelate form.

Four feed rations were formulated for the pigs at the treatment farm. These included Pig Starter, Grower, Gestation (dry) and Lactation rations. Iron amino acid chelate was supplied in all but the Grower ration. Production pigs were fed Pig Starter ration from weaning at 12-14 kg until they were 18-20 kg. This ration contained 110 mg iron per kg of finished feed from the iron amino acid chelate source. Pigs in this weight range consumed an average of 0,888 kg of finished feed per day. Grower ration was given to the production pigs from 18-20 kg until marketed at 100 kg, or above. Sows which were kept on the farm for litter production were then placed on either Gestation (dry) ration or the Lactation ration depending on the stage of their pregnancies. Two weeks prior to parturition, pregnant sows received an additional product called Litter Booster which also contained iron amino acid chelate. This was supplemented at the rate of 64,5 mg iron per kg finished feed. The Litter Booster addition was continued in each the sow's feed until her litter was weaned at a mean of 26 days after birth. Lactation ration was given to sows starting one week prior to parturition and continuing until the litters were weaned and began receiving Pig Starter ration. The greatest amount of iron from iron amino acid chelate was formulated into Gestation (dry) ration at the inclusion rate of 225 mg iron per kg of finished feed. The same iron chelate was formulated into Lactation ration at 189,5 mg iron per kg of finished feed. Sows on Gestation (dry) Sow ration were allowed just 2,04 kg of feed per day, while nursing sows were allowed to consume 7,71 kg of Lactation ration per day to nourish their litters. With the

consumption of more Lactation ration being allowed on a per day basis, more of the chelated iron was consumed via Lactation diet during the days of its administration. Since Litter Booster commenced two weeks prior to parturition and since 2,04 kg of the Gestation (Dry) ration was being given to the gravid sows, they received 591 mg iron per day from two weeks to one week prior to parturition. At the commençement of one week prior to parturition, the gravid sows were put on Lactation diet with Litter Booster and were allowed to consume 7,71 kg feed per day, until a mean of 26 days postparturition. During this time, the adult sows received 1958 mg iron per day from the amino acid chelated source. All pigs also received iron which was intrinsic in their feed. At times other than two weeks prior to parturition and during lactation, the sows received Gestation (Dry) ration and were limited to 2,04 kg of feed per day. During this time the sows received 459 mg iron per day.

The achievement of six parities for the animals used in this study was the minimum requirement. Of the four control sows used in the study, three had achieved six parities while the fourth one had reached ten parities. Among the eight treatment sows, three had achieved six parities, two had passed seven parities, one had achieved eight parities and two were at nine parities. Lifetime ingestion of iron as iron amino acid chelate reflected the age of the sows as apparent from the respective parity numbers (farrowings) which they had achieved. The three treatment sows with six parities ingested 768, 776 and 785 g iron as iron amino acid chelate throughout their lives. The two sows with seven parities received 893 and 907 g of iron in this form while the sow with eight parities reached 1073 g iron. Lastly, the two treatment sows which achieved nine parities ingested 1074 and 1103 g iron from the iron amino acid chelate source over the courses of their lives. All of these lifetime ingested amounts of iron were more than the highest amount of iron ingested by any of the six treatment sows assessed in the long-term multigenerational study reported above.

A local certified veterinarian was enlisted to conduct both premortem and postmortem examinations of the sows. The veterinarian was kept blind as to which pigs had received iron amino acid chelate (treatment sows) and which had not (control sows). He was not appraised of the sow identifications until he had finalized his written report to Albion Laboratories Inc.

Following euthanasia of the sows, their internal organs and skeletal tissues were excised by the certified veterinarian for histopathological examination. The veterinarian made all of the collections from the same parts of the organs utilized in order to standardize the assessments of both the treatment and control animals. He also made internal examinations as to the health of the animals. The tissues were trimmed into small blocks (approximately, 0,5 cm X 1 cm X 2,5 cm) for ease of infiltration of the tissue fixative and microtoming for examination slides. The tissues excised included brain, duodenum, jejunum, ileum, large intestine, muscle, heart, liver, spleen, bone marrow, mesenteric lymph node, kidney and ovary. Following excision, the tissue blocks were submerged in 10% buffered formalin solution in small capped bottles and these were shipped the next day by courier to Albion Laboratories. Following receipt at Albion, the fixed tissues were shipped to the same certified veterinary histopathologist in the United States who had assessed the slides made from tissues of pigs used in the long-term multigenerational study. In order to keep the veterinary histopathologist blind as to the identifications of the sow tissues, they were sent to him as three sets of four animals each (comprising one set for control animals and two for the treatment animals). The veterinary report on premortem and postmortem examinations was also reviewed by the histopathologist although he was kept blind as to the identifications of the three groups of sow tissues which he had received. After he had issued his draft report, he was made aware of the sow identifications so he could finalize his report as to group and sow identifications.

This study was conducted to assure that production sows receiving supplemental iron as iron amino acid chelate did not show any cumulative pathological effects due to the iron source. The certified veterinary histopathologist's conclusion was that, "No histopathologic tissue alterations were observed that could be attributed to dietary administration of the test article [Iron Amino Acid Chelate]."

Studies utilizing Ferrochel

LD_{50} determination of Ferrochel in rats

The LD_{50} for Ferrochel (human grade ferrous bisglycinate chelate) was determined using good laboratory practices (GLP) as specified for non-clinical studies by the US-FDA. Five male and five female rats were used for each of four dosing levels. The dosing levels of Ferrochel were calculated to yield 150, 300, 600 and 1200 mg iron/kg body weight. These doses were administered to the rats via oral gavage (gastric lavage) on the first day of the study. All of the animals were observed for toxic effects at 1, 2,5 and 4 hours on the day of dosing and twice a day for the next 14 days. All surviving animals were sacrificed at the end of the 14 day observation period, necropsied, and given a complete examination for gross pathology (18).

The oral LD_{50} for iron in Ferrochel was calculated to be the same for both male and female rats. It was 560 mg iron (as Ferrochel) per kg body weight of rat.

90-Day subchronic toxicity study of Ferrochel in rats administered via diet according to US-FDA guidelines

An acute dose LD_{50} toxicity test supplies relative

information as to susceptibility of animal physiological systems to relatively large single doses of the test article. A further assessment of the cumulative effects of receiving potentially toxic or subtoxic doses can be gained by supplying daily (chronic) doses of the test article over a period of time. The standard 90-day (3-month) subchronic toxicity test in rats has been designed to provide this information. The term, subchronic, implies that the assessment is being made over a finite period of time in an effort to answer specific physiological questions about the toxicity of the test article rather than supplying the article to the rats chronically for one to two years. When young rats are used for the test, 90 days can bracket the period of their highest growth rate. In addition to a control group of rats which do not receive the article being tested, the actual daily doses of the test article are set by best efforts to bracket a benign dose, a median dose and a compromised or toxic dose.

A 90-day subchronic toxicity test was carried out for Ferrochel (19). The rat breed used was Sprague Dawley CD® strain. In a preliminary study on three rats of each sex at 0, 300 and 500 mg Ferrochel/kg body weight, one female was found with signs of internal irritation at the 500 mg/kg dose. No deaths or other signs of toxicity were apparent. It was determined that the highest dose should be set at 500 mg Ferrochel/kg rat body weight in anticipation that some toxicity would be generated at this level over the course of the 90-day study allowing an assessment of the toxicity of Ferrochel. For the 90-day subchronic study, 20 male and 20 female rats per dosing level received either 0, 100, 250 or 500 mg Ferrochel/kg rat body weight mixed in their diets, making a total of 160 rats involved in the study. Iron levels from Ferrochel were confirmed by atomic absorption spectrometry on alternate weeks throughout the study. Structural integrity of the Ferrochel in the feed was confirmed periodically by Fourier-transformed infrared spectrometry (FT-IR).

No deaths occurred in any group. Both growth rates and feed consumption for the treatment animals matched the control animals for all dosage groups. Premortem and postmortem examinations failed to reveal any pathology that could be attributed to the iron dosages. Forty-eight tissues from each of ten males and ten females randomly selected from the 500 mg Ferrochel/kg dose level and the controls were excised, fixed, mounted on microscope slides and examined. None of the tissues revealed signs of iron pathology. Additionally, blood chemistries and clinical chemistries were also free from changes due to the increased iron in the rat diets. The No Observable Adverse Effect Level (NOAEL) was determined to be at least 500 mg Ferrochel/kg body weight for both male and female rats. Further details of this 90-day subchronic toxicity study and its conclusions have been published elsewhere (20).

Physiological control of absorption of Ferrochel

When an iron source shows high bioavailability, a commonly expressed concern is for toxic overload of iron. At issue is the question as to whether the highly bioavailable iron source is regulated in a similar fashion to heme iron or inorganic salt sources of iron. Subjectively, this has not been a concern for the iron amino acid chelates. Early LD_{50} estimates for several of the metal amino acid chelates routinely indicated that these sources were safer than inorganic mineral sources (21). Similar inferences have been gained in additional toxicological tests, such as, the multigeneration pig study (14-16) and the six-parity pig study (17), summarized above. A very strong implication of physiological regulation of these iron amino acid chelates is the 90-day subchronic toxicity study (19,20) assessing the impact of continual high doses of dietary Ferrochel. The finding of no toxicological evidence, no hematological or biochemical aberrations and virtually no impact other than normalcy speaks very strongly for the regulation of tissue uptake of iron taken as Ferrochel.

More direct measures of the regulation of iron uptake when in the amino acid chelated form were made by Pineda(22) on data that had been reported by Mervyn (23). The data were from a crossover study of 6 male and 6 female non-anemic individuals in the Mount Sinai School of Medicine in New York who received 18 mg iron daily from $FeSO_4$ for a week, followed by a washout period, then a replicate week-long dose of 18 mg iron/day from human grade iron amino acid chelate. Fecal iron was measured as the reciprocal of absorbed iron. In all of the individuals, more iron was absorbed from the amino acid chelated source. The mean improvement in absorption was 59%. Since the data were obtained from the same individuals, Pineda arranged the pairs of absorbed amounts of iron from the two sources from the least to the greatest. Then he calculated linear regressions for both sets. Presuming different inherent needs for iron among the subjects of the test, the data could be expected to plot the iron needs of a small sampling of a population. This resulted in two regression lines which differed in position (due to the higher absorption from the iron amino acid chelate source), but which were very similar in slope. Pineda additionally plotted the correlation of iron absorbed from the iron amino acid chelate against the respective data for iron absorption from $FeSO_4$, with the paired data arranged from least to highest. The correlation plot had an r^2 of 0,9411. These paired data indicate a linear correlation between the amounts of iron absorbed from the two iron sources and also imply that both iron sources are similarly regulated.

Additional data supporting the regulation of iron absorption from the amino acid chelated source have been provided by Olivares *et al* (24). These researchers noted that

unmodified cow's milk would inhibit the absorption of any non-heme source of iron, due to the high concentration of iron absorption inhibitors, including casein, calcium, whey protein and phosphates. In comparing the iron absorbed from Ferrochel (termed: ferrous bis-glycine chelate in this paper) versus $FeSO_4$, they found that, while absorption of iron as Ferrochel was decreased by the cow's milk, iron from this source was still absorbed from 2 to 2,5 times higher than iron from $FeSO_4$. This suggests that the Ferrochel iron source was less influenced by the inhibitors than was ferrous sulfate. They additionally found an inverse relationship between serum ferritin content and iron absorption as Ferrochel. This was correlated to the absorption of iron from ferrous ascorbate, thus further suggesting the regulation of Ferrochel iron absorption.

Data from a study by Iost *et al.* describing the successful repletion of hemoglobin levels in anemic young children with low fortificant doses of Ferrochel also indicate regulation of iron uptake against iron stores (25). One hundred and eighty-five young children (134 being 1-year old at the commencement of the study) were given 3 mg iron as Ferrochel in 1 L of cow's milk per day. Hemoglobin amounts were measured initially and at 133 ± 13 and 222 ± 2 days into the study. Mean and standard deviations for each sampling were 9,3 ± 1,5, 10,5 ± 1,6 and 11,2 ± 1,5 g hemoglobin/dL, respectively, demonstrating the repletion of iron deficiency anemia in the children over the course of the study. The data were additionally divided by degree of anemia, ≤ 9,4 g hemoglobin/dL whole blood being the most severe with 9,5 - 11,0 g hemoglobin/dL being less severe. Children having hemoglobin levels ≥ 11,1 g/dL were considered normal. Over the course of the study, the greatest changes were noted in the most severely anemic group. However, among children with normal hemoglobin values, there were no significant differences in hemoglobin amounts at any of the measurement times ($P > 0,10$). While ferritin assays were not a part of this study, the hemoglobin concentrations among the normal children implied that less amounts of iron were absorbed where there were less needs for iron.

The absorption of iron as Ferrochel has additionally been assessed in whole maize meal porridge by Bovell-Benjamin, Viteri and Allen (13). Whole maize is high in phytates which normally bind iron fortificants and may greatly limit their absorption. In an experiment involving 10 non-anemic men who consumed porridge containing $^{59}FeSO_4$ on the first day of the study and porridge containing ^{55}Fe-ferrous bisglycinate chelate on the second day, blood analyses done on the sixteenth day revealed that iron from the bisglycinate chelated source was absorbed greater than was iron from the $FeSO_4$ source. The two sources of iron were additionally consumed together in whole maize porridge by the same 10 men following the above blood sampling and final blood samples were taken 14 days later. The second blood sampling demonstrated that there was no exchange of the differently labeled iron isotopes between sources in the whole maize porridge when consumed at the same time. The lack of label exchange in the second experiment demonstrated that there was no breakdown of the ferrous bisglycinate in the intestinal pool before entering the mucosal cell. The $FeSO_4$ source was expected to breakdown in the intestine, resulting in free cationic iron being presented to the intestinal mucosal cells for absorption. The absence of label exchanging also implies that the Ferrochel iron was entering the mucosal cell intact as the bisglycinate chelate. The mean of the differences in iron absorption between the separate and mixed blood samplings was 4,7 times greater from the ferrous bisglycinate chelate than from ferrous sulfate. Additionally, scatter plots of the natural logarithm (ln) of percent iron absorption versus the ln of serum ferritin concentration for both the ferrous bisglycinate and $FeSO_4$ sources of iron yielded regression lines with similar correlation coefficients and slopes, although the ferrous bisglycinate data potted higher in absorption. This demonstrated that the absorptions of both iron as Ferrochel and iron as $FeSO_4$ were effectively regulated by the iron reserves of the body.

Safety of ferrous bisglycinate chelate determined

In 1997, after its review of many scientific papers on the efficacy and safety of Ferrochel (ferrous bisglycinate chelate), a panel of scientific experts nationally recognized in the USA and representing physiological, toxicological and medicinal disciplines concluded that Ferrochel was affirmed as "Generally Recognized As Safe" (GRAS) as both a food fortificant and dietary supplement. In 1999, following the self-affirmation of Ferrochel as GRAS and the subsequent review of the findings of the expert panel, the Office of Premarket Approval of the Center for Food Safety and Applied Nutrition of the US-FDA acknowledged the GRAS self-affirmation of Ferrochel (ferrous bisglycinate chelate) and advised that it had no questions or concerns regarding the GRAS status of this product under its proposed conditions of use for food enrichment and fortification purposes.

CONCLUSIONS

The importance of iron to the well being and healthy functionality of the body cannot be underestimated. Among the pathologies which may result from the deficiency of any physiologically required mineral, of paramount importance and of paramount occurrence are the morbidities associated with iron deficiency anemia. A fifth of the world's entire population is estimated to present this anemia. This is the major mineral deficiency to address and solve.

Inherent in iron are the seeds of its own noncompliance to efforts to reduce the effects of compromised absorption. In the inorganic form, which has been the form of choice for supplementation and fortification attempts in diets where it has been low or lacking, iron consistently demonstrates restricted bioavailability with ever greater toxic symptoms if the supplementary sources are increased in an attempt to increase its absorption. Modifications on the inorganic forms of iron, such as, the addition of ascorbic acid to keep the iron in the ferrous state prior to absorption, gain some improvements in absorption, but apply additional costs to the intervention.

Ferrochel may be the best form for supplementation and fortification of iron into human diets. It presents iron in the ferrous state, resists cleavage once ingested, is absorbed intact in its protective state, repletes iron levels at relatively small doses, has been proven remarkably nontoxic, even at relatively high daily doses, demonstrates the characteristics of being physiologically regulated, and increases iron absorption over that obtainable from ferrous sulfate, the current standard for iron intervention.

REFERENCES

1. Herbert V. Drugs effective in iron-deficiency and other hypochromic anemias. In: The Pharmacological Basis of Therapeutics, 4th edition. LS Goodman & A Gilman (Eds.) NY, MacMillan, 1970, p. 1397-1413.
2. Hillman RS. Hematopoietic agents; growth factors, minerals, and vitamins. En: Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th edition. JG Hardman, LE Limbird, PB Molinoff, RW Ruddon & AG Gilman (Eds.) NY, McGraw-Hill, 1996, p.1311-1340.
3. Haden RI. Historical aspects of iron therapy in anemia. JAMA 1939;111:1059-1061.
4. Heath CW, MB Strauss & WB Castle. Quantitative aspects of iron deficiency in hypochromic anemia. J Clin Invest 1932;11:1293-1312.
5. Reimann F, F Fritsch & K Schick. Eisenbilanzversuche bei Gesunden und bei Anämischen. II. Untersuchungen über das Wesen der eisenempfindlichen Anämien ("Asiderosen") und der therapeutischen Wirkung des Eisens bei diesen Anämien. Z Klin Med 1936;131:1-50.
6. Guthrie HA. Introductory Nutrition. St. Louis, MO, Times Mirror/Mosby College Publishing, 1989, p. 294.
7. Grebe G, C Martinez-Torres & M Layrisse. Effect of meals and ascorbic acid on the absorption of a therapeutic dose of iron as ferrous and ferric salts. Curr Ther Res 1975;17:382-397.
8. Beliles RP. Metals. En: Toxicology; The Basic Science of Poisons. LJ Casarett & J Doull (Eds.) NY, Macmillan, 1975, p. 454-502.
9. Grossman BJ & TM Brody. Drugs to treat anemia. En: Human Pharmacology; Molecular to Clinical, 2nd edition. TM Brody, J Larner, KP Minneman & HC Neu (Eds.) NY, Mosby, 1994, p. 861-870.
10. Pineda O, HD Ashmead, JM Perez & CP Lemus. Effectiveness of iron amino acid chelate on the treatment of iron deficiency anemia in adolescents. J Appl Nutr 1994;46:2-13.
11. Ashmead HD, DJ Graff & HH Ashmead. Intestinal Absorption of Metal Ions and Chelates. Springfield, IL, Charles C Thomas, 1985.
12. Ashmead, HD, HS Hsu & N Jensen. A lack of competition in the absorption of Albion® iron and copper amino acid chelates. Albion Laboratories Inc., Unpublished Research Report.
13. Bovell-Benjamin, AC, FE Viteri & LH Allen. Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status. Amer J Clin Nutr 2000;71:1563-1569.
14. Jeppsen RB. Assessment of Long-Term Feeding of Iron Metalosate® in Sows. Clearfield, UT, Albion Laboratories Inc., Unpublished Research Report, 1987.
15. Jeppsen RB. An assessment of long term feeding of amino acid chelates. En: The Roles of Amino Acid Chelates in Animal Nutrition. HD Ashmead (Ed.) Park Ridge, NJ, Noyes Publications, 1993, p. 106-113.
16. Jeppsen RB. Assessment of Long-Term Feeding of Iron Metalosate® in Sows. Appendix 5, Histopathology Report, p. 4, 6. Clearfield, UT: Albion Laboratories Inc. Unpublished Research Report, 1987.
17. Jeppsen RB. Examination of Long-Term Feeding of Iron Amino Acid Chelate (Iron Metalosate®) in Sows Achieving Six or Greater Parity. Clearfield, UT, Albion Laboratories Inc., Unpublished Research Report, 1990.
18. Kukulinski M. Acute Oral Toxicity Study in the Sprague Dawley Rat (LD 50), Test Article: Ferrochel. Muskegon, MI, Biologic Safety Research, (BSR), Unpublished Research Report, 1993.
19. Mandella RM. A Subchronic (3-Month) Toxicity Study of Ferrochel in the Rat Administered via Diet. East Millstone, NJ, Huntingdon Life Sciences Inc., Unpublished Research Report,1997.
20. Jeppsen RB & JF Borzelleca. Safety evaluation of ferrous bisglycinate chelate. Food Chem Tox 1999;37:723-731.
21. Larson AE. L.D.50 studies with chelated minerals. En: Chelated Mineral Nutrition in Plants, Animals and Man. D Ashmead (Ed.) Springfield, IL, Charles C Thomas, 1982, p.163-169.
22. Pineda, O. Effectiveness of Ferrochel (iron amino acid chelate) in the treatment of iron deficiency and iron deficiency anemia. En: Toxicity and regulation, Conference sponsored by the Arab Republic of Egypt, Ministry of Supply and Internal Trade, September 16, 1995.
23. Mervyn L. Human therapeutic applications of amino acid chelates. En: International Conference on Human Nutrition, Proceedings, January 21, 22, 1995, Salt Lake City, UT. Clearfield, UT, Albion Laboratories Inc., 1995.
24. Olivares, M, F Pizzaro, O Pineda, JJ Name, E Hertrampf & T Walter. Milk inhibits and ascorbic acid favors ferrous bisglycine chelate bioavailability in humans. J Nutr 1997;127:1407-1411.
25. Iost C, JJ Name, RB Jeppsen & HD Ashmead. Repleting hemoglobin in iron deficiency anemia in young children through liquid milk fortification with bioavailable iron amino acid chelate. J Amer Col Nutr 1998;17:187-194.

ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición

Suplemento Vol. 51 N° 1, 2001

The effect of change in pH on the solubility of iron bis-glycinate chelate and other iron compounds

María Nieves García-Casal and Miguel Layrisse

Instituto Venezolano de Investigaciones Científicas (IVIC). Centro de Medicina Experimental.
Laboratorio de Fisiopatología- Caracas, Venezuela

SUMMARY. The effect of a pH change from 2 to 6 was tested on the solubility of ferrous sulfate, ferrous fumarate, iron bis-glycine chelate (Ferrochel) and sodium-iron ethylenediaminetetraacetic acid (NaFeEDTA). It was found that at pH 2 ferrous sulfate, Ferrochel and NaFeEDTA were completely soluble and only 75% of iron from ferrous fumarate was soluble. When pH was raised to 6, iron from amino acid chelate and NaFeEDTA remained completely soluble while solubility from ferrous sulfate and ferrous fumarate decreased 64 and 74%, respectively compared to the amount of iron initially soluble at pH 2.These results suggest that iron solubility from iron bis-glycine chelate and NaFeEDTA is not affected by pH changes within the ranges tested, probably because iron remained associated to the respective compounds.
Key words: Iron solubility, pH changes, iron bis-glycine chelate, NaFeEDTA, ferrous fumarate, ferrous sulfate.

RESUMEN. Efecto de cambios de pH sobre la solubilidad del hierro bis-glicinato quelado y de otros compuestos de hierro. Se estudió el efecto del cambio de pH de 2 a 6 sobre la solubilidad del hierro de sulfato ferroso, fumarato ferroso, hierro bis-glicinato quelado (Ferrochel) y ácido etilendiaminotetraacético sódico-férrico (NaFeEDTA). Se encontró que a pH 2 el hierro proveniente de sulfato, Ferrochel y NaFeEDTA estaba completamente soluble, mientras que para el fumarato ferroso solo el 75% del hierro se hallaba en solución. Cuando se incrementó el pH a 6, el hierro del glicinato quelado y del NaFeEDTA permaneció completamente soluble mientras que la solubilidad a partir de sulfato y de fumarato disminuyó 64 y 74%, respectivamente comparado con la cantidad de hierro soluble a pH 2. Estos resultados sugieren que la solubilidad del hierro proveniente del glicinato quelado y del NaFeEDTA no fue afectada por los cambios de pH estudiados, probablemente debido a que el hierro permanece asociado a los respectivos compuestos.
Palabras clave: Solubilidad de hierro, cambios de pH, hierro bisglicinato quelado, NaFeEDTA, fumarato ferroso, sulfato ferroso.

INTRODUCTION

According to their solubility iron compounds have been classified as water soluble (ferrous sulfate, ferrous gluconate, ferrous lactate, ferric ammonium citrate), poorly water soluble but soluble in diluted acids (ferrous fumarate, ferrous succinate, ferric sacharate), poorly soluble in water acid solutions (ferric pyrophosphate, ferric orthophosfate, elemental iron) and protected compounds (hemoglobin, ethylenediaminetetraacetic acid (NaFeEDTA), iron bis-glycinate chelate). To establish relative iron bioavailability of these compounds, absorption values are compared to a standard compound which is ferrous sulfate, with a relative bioavailability of 100 (1).

During the last two years the Nutritional Anemia Laboratory of the Instituto Venezolano de Investigaciones Científicas (IVIC), has been studying the properties of various iron compounds, including iron bis-glycine chelate (Ferrochel), for food fortification under various conditions. This iron compound has the advantage of being soluble in water and does not change the organoleptic properties of the food vehicle(2). The purpose of the present study was to evaluate the solubility of iron compounds (ferrous sulfate, ferrous fumarate, Ferrochel and NaFeEDTA) currently used in food fortification programs, when the pH is changed from 2 to 6.

MATERIAL AND METHODS

Iron solubility when the pH is changed from 2 to 6 was evaluated under equal conditions for ferrous sulfate, ferrous fumarate, iron bis-glycine chelate (Ferrochel) and ethylenediaminetetraacetic acid (NaFeEDTA). Iron solutions of each of the compounds mentioned containing 5 mg iron, were prepared in 0.1 mol/L HCl. Duplicate 1-mL aliquots were taken after 30 min at room temperature to measure soluble iron at pH2, and to the remaining solution, the pH was adjusted to 6 with careful addition of NaOH (3). After standing 10 min at room temperature, duplicate 1-mL aliquots from the top of the solution were taken to measure iron concentration by a published digestion method (4).

RESULTS AND DISCUSSION

The selection of the iron compound for fortification is important in order to avoid interactions with the food vehicle or the total meal, because a minor change in organoleptic characteristics of the food, will result in consumers rejection. When the iron compound is added, it is necessary to evaluate possible changes in food color, taste or appearance with time and storage on adverse temperature and humidity conditions. Solubility, chemical reactivity, bioavailability and cost are other important issues when selecting an iron compound. For instance, ferrous sulfate is a highly bioavailable and relatively inexpensive compound, but because of its reactivity produces undesirable changes in some fortified foods. On the other hand, elemental iron (reduced, electrolytic or carbonyl) is also inexpensive but it has been reported to have a low bioavailability depending on particle size and the food vehicle to be fortified. Protected iron compounds have been reported to have a high bioavailability, but they are usually expensive (5-7).

Variations in iron solubility when pH was changed from 2 to 6 are presented in Table 1. At pH 2 iron from ferrous sulfate, Ferrochel and NaFeEDTA was completely soluble while the amount of soluble iron from ferrous fumarate was 76.6% of the expected amount of 5 mg, after 30 min incubation. Solubility of this compound in acidic solutions increases with longer incubation time.

TABLE 1
Iron solubility from ferrous sulfate, ferrous fumarate, NaFeEDTA and Ferrochel at pH 2 and 6

pH	Ferrous sulfate		Ferrous fumarate		Ferrochel		NaFeEDTA	
	mg	%	mg	%	mg	%	mg	%
2	6.22±0.2 [a]	100.0	3.83±0.1 [a]	100.0	5.71±0.3 [a]	100.0	5.40±0.1 [a]	100.0
6	2.24±0.2 [b]	36.0	0.99±0.2 [b]	25.8	5.33±0.2 [a]	93.3	5.12±0.1 [a]	94.8

[1] Values are means ± SEM. n = 10. Values in any given column with different letters are significantly different. $p<0.05$. Initial iron content: 5 mg.

Increasing pH to 6 produced a decrease in solubility for ferrous sulfate and ferrous fumarate. After 10 min incubation at pH 6, 64% of the iron from ferrous sulfate became insoluble while 74% of the iron from ferrous fumarate, was precipitated. This percentage for ferrous fumarate is based on the amount soluble at pH 2, that was already reduced in approximately 23%. For Ferrochel and NaFeEDTA solutions, the change in pH from 2 to 6 did not modify the amount of soluble iron, showing in both cases that more than 90% remained in solution.

These results indicate that iron from Ferrochel and NaFeEDTA remains soluble even when the pH is changed over a wide range as happens when food moves from the stomach to the small intestine. This finding may indicate that the observed high iron absorption from these two compounds is related to their resistance to pH changes and that within the studied range, the molecules are not altered, maintaining their capability to keep iron in a protected, soluble form.

REFERENCES

1. Hurrell, R. Preventing iron deficiency though food fortification. Nutr. Rev. 1997;55(6): 210-222.
2. Pineda O. Studies using iron bis-glycine chelate. Absorption, regulation and toxicity. Proc. International Conference on Human Nutrition. January 24-25. Salt Lake City, Utah, 1998.
3. Garcia-Casal M, Layrisse M, Solano L, Barón M, Arguello F, Llovera D, Ramírez J, Leets I, Tropper E. Vitamin A and β-carotene can improve nonheme iron absorption from rice, wheat and corn in humans. J Nutr 1998; 128: 646-650.
4. Bothwell T, Carlton R, Cook J, Finch C. Iron metabolism in man. Blackwell Scientific Publication, Oxford, UK. 1979.
5. Cook J, Reusser M. Iron fortification: an update. Am J Clin Nutr. 1983;38 :648-659.
6. Hallberg L. Iron nutrition and food fortification. Sem Hematol. 1982;19 :31-41.
7. Hallberg L, Brune M, Rossander L. Low bioavailability of carbonyl iron in man: studies on iron fortification of wheat flour. Am J Clin Nutr. 1986;43:59-67.

ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición

Suplemento Vol. 51 N° 1, 2001

Micronutrient dietary supplements - A new fourth approach

Michael C. Latham, Deborah Ash, Godwin Ndossi, Haile Mehansho and Simon Tatala

Cornell University, Ithaca, NY, USA- Tanzania Food an Nutrition Center, Tanzania
The Procter and Gamble Company, Ohio, USA

SUMMARY. Currently the three main widely used strategies to control micronutrient deficiencies are food diversification, fortification, and consumption of medicinal supplements. In Tanzania a fourth strategy has been evaluated in school children, and is to be studied in pregnant and lactating women. The dietary supplement comes in the form of a powder used to prepare a fruit flavored drink. Children consumed for six months 25 grams per school day attended, the powder being added to 200 ml of water. The dietary supplement provides between 40 and 100 percent of the RDA of 10 micronutrients, which includes iron, vitamin A and iodine. Unlike medicinal supplements it provides the multiple vitamins and minerals in physiologic, not megadoses. In a well conducted randomized double blind placebo controlled trial, a dietary supplement in the form of a fortified powder fruit drink produced statistically significant differences not only in vitamin A and iron status, but also in the growth of young school age children.
Key words: Multiple supplementation, Ferrochel, nutritional deficiencies, fortified beverage.

RESUMEN. Suplementación dietética de micronutrientes – Un cuarto nuevo enfoque. Hasta este momento, las tres estrategias mas frecuentemente utilizadas para el control de las deficiencias de micronutrientes son: diversificación de alimentos, fortificación y consumo de suplementos medicinales. En Tanzania, una cuarta estrategia fue evaluada en niños escolares y se estudiará en mujeres embarazadas y en mujeres lactantes. El suplemento dietético se prepara como un polvo usado para preparar una bebida con sabor a naranja. Los niños estudiados consumieron durante seis meses, 25 gramos del polvo disueltos en 200 mL de agua por día de asistencia a la escuela. El suplemento provee entre 40 y 100% del RDA de 10 micronutrientes entre los cuales se encuentra hierro, vitamina A y yodo. Contrario a los suplementos medicinales, la bebida provee vitaminas y minerales en cantidades fisiológicas y no en dosis mayores. En una prueba de campo bien efectuada, randomizada, doble ciega con control de placebo, la bebida fortificada produjo diferencias estadísticamente significativas, no solo en la condición nutricional con relación a hierro, vitamin, a A y yodo, sino también en el crecimiento de los niños estudiados.
Palabras clave: Suplementación múltiple, Ferrochel, deficiencias nutricionales, bebida fortificadas.

INTRODUCTION

Malnutrition is increasingly recognized as a very prevalent and important health problem in many countries, a problem that has serious long-term consequences for the child and may adversely influence development itself. That more than 2,000 million people live at risk of diseases related to micronutrient deficiencies-most commonly vitamin A, iron, and iodine-and that most of these people are women of childbearing age and young children in developing countries, are facts that are often stated and much studied (1). Malnutrition in its severe and not-so-severe forms still remains a significant cause and determinant of mortality, short- and long-term morbidity, and permanently lost productivity and intellectual capacity in societies which can ill afford such losses and costs.

Despite these devastating consequences, the range of methods by which micronutrient deficiencies are addressed have remained much the same and almost consistently fall into one of the following categories (2).

Food diversification - in some cases this will simply come about by increasing overall food intake while in other cases it comes about through behavioral change which increases the consumption of nutrient rich foods. This may be affected through diversifying household and/or regional food production strategies to ensure that foods rich in the nutrients identified as deficient become available.

Food fortification. The deliberate addition of a specific nutrient to a food vehicle which is identified as being widely consumed in adequate quantities by populations at risk of the deficiency. The classic examples being the iodization of salt; and the fortification of milk with vitamin A and vitamin D.

Medicinal supplements. (a) periodic administration of large doses of a specific micronutrient, such as vitamin A or iodine, which either provide some protection from a prolonged period of deficient intake or treats a deficiency which has already resulted in clinical symptoms, or (b) medicinal amounts far above the RDA for the nutrient, for example iron and folate supplements during pregnancy.

These methods are, for the most part, technically easy and cost-effective given the economic gains which can be

achieved. Significant achievements in reducing micronutrient malnutrition have been made using them.

Each of these three standard approaches has its disadvantages and limitations. Most people agree that food diversification offers the best long term approach that is likely to be sustainable. But often it requires major changes either in agricultural production, including home gardens, or in higher incomes for the poor, allied with nutrition education. So in many non-industrialized countries progress is slow, and in some African countries with a deteriorating economic situation, food diversification is unlikely to substantially reduce micronutrient deficiencies in the near future.

Conditions for fortification vary depending on the nutrient and the foods eaten in a country. In some countries several commonly eaten foods do pass through commercial processing where fortification is feasible. Salt iodination has greatly reduced iodine deficiency disorders in many countries. But in many non-industrialized countries it is difficult to find a suitable food vehicle to fortify with iron or vitamin A. A suitable food has to be consumed regularly by those at risk of the deficiency who are often children and women in poor families. Especially in rural areas those suffering from micronutrient deficiencies may purchase few manufactured or processed foods.

Medicinal supplements are of two kinds. First there are those taken in pharmacological doses daily or at frequent intervals, and second those prescribed to be consumed in large doses at intervals of 4 to as long as 24 months. Ferrous sulfate and folate are examples of the former, and vitamin A and lipiodol (containing iodine) are examples of the latter. Medicinal supplementation is dependent on a delivery system which is often relatively costly if the supplement is to reach those at risk. Other problems include poor compliance which is common with iron prescribed during pregnancy, and low participation rates for example when massive dose vitamin A supplements are offered over time.

A World Bank review of micronutrient programs (3), found that three common problems arising from the implementation of any or all of these strategies were: (i) the lack of appropriate consumer demand; (ii) the lack of appropriate delivery infrastructure with adequate access for poor women and isolated populations; and (iii) the lack of honest, efficient and technically competent enforcement systems for food fortification. Furthermore, a key feature of successful programs was consistently found to be "supply": that is supply of the food itself or seeds and other necessary inputs to allow dietary diversification; supply through secure access to fortified foods via appropriate food vehicles in adequate quantities; and/or supply through cost-effective, professionally prescribed and readily available medicinal supplements.

Dietary supplements - it's been done before

Micronutrient dietary supplements offer a fourth approach, and one which can control deficiencies using a single intervention.This approach is unique since it delivers micronutrients that fill the nutritional gap via a vehicle that is, or becomes well accepted by the target group. The supplementation of diets with a specific food substance high in one or more micronutrients recognized as potentially deficient in the regular diet is not a new concept. Not so long ago children in industrialized or industrializing countries received a regular dose of cod-liver oil to stave off the effects of vitamin A and D deficiencies. At the turn of the century, rickets, the consequence of prolonged deficient vitamin D intake or lack of sunshine was very common amongst children in the poor communities of industrialized cities where the diets comprised a small range of foods and there was limited access to outdoor areas and thus direct sunlight. In some countries, on the shelves of remote, small rural and urban shops, one can still find bottles of Haliborange - a concoction of halibut oil high in vitamins A and D or of Ribena, to provide vitamin C. In many countries north and south similar products, some labeled as "tonics," provide micronutrient supplements. However these tonics often do not include those minerals and vitamins most lacking in local diets. Often, this is a very costly way of providing micronutrients to target groups that have limited income.

In Europe and North America, the promotion of cod-liver oil and other healthful dietary supplements empowered mothers with affordable options to prevent rickets in their children and where such solutions were not affordable those dietary supplements were available free of charge through public health clinics. The development of better health care systems, affordable and diversified food supplies and a growing appreciation of the health benefits of outdoor play presumably also underlay the decline of rickets in industrialized countries (4). Unfortunately, the concept of regular dietary supplement consumption has not been translated from industrialized countries to the populations of non-industrialized countries which continue to be at risk, or suffer from micronutrient deficiencies.

Trial of a micronutrient supplement in Tanzania

In Tanzania a randomized double blind placebo controlled trial of a multiple-micronutrient fortified dietary supplement in school children has been completed (5). A similar trial has been planned to assess the feasibility and efficacy of a similar micronutrient dietary supplement in pregnant and lactating women.

Micronutrient deficiencies including iron deficiency anemia, vitamin A deficiency and iodine deficiency disorders are recognized as important public health problems in

Tanzania (6). The project was a collaborative study involving the Tanzania Food and Nutrition Centre, Cornell University, UNICEF, the Micronutrient Initiative and the Procter and Gamble Company.

The dietary supplement used was a fortified powdered fruit drink. It was developed and produced especially for this project by scientists at the Procter and Gamble Company in Cincinnati, Ohio, USA. The product tested consisted of 25 grams of a fine white powder in individual serving sachets. One sachet contained 5.4 mg of iron from bis-glycinate chelate, 1750 IU of vitamin A, 45 μg of iodine, 5.25 mg of zinc, 72 mg of ascorbic acid, 0.6 mg of riboflavin, 0.14 mg of folic acid, 3 μg of vitamin B_{12}, 0.7 mg of vitamin B_6 and 10.5 mg of vitamin E. Nutrient and product stability evaluations demonstrated the product is stable up to one year.

In the Mpwapwa District of Dodoma Region 830 children attending 6 primary schools participated in the study. A baseline examination included the collection of clinical, biochemical and anthropometric data. Eligible children were then randomly assigned to one of two groups either to receive one sachet of the micronutrient dietary supplement each school day attended, or to consume a non-fortified product, identical in appearance and taste. The research team, schoolteachers, and participants were blinded. Six months later a final examination was conducted on 775 children.

The results, in terms of measures of iron and vitamin A status, plus anthropometric findings are being published elsewhere (5). In summary there were no significant differences at the baseline in serum retinol levels, nor in terms of measures of iron status (including hemoglobin, hematocrit, zinc protoporphyrin, and serum ferritin).

Six months later there were highly significant differences between the two groups always in favor of the micronutrient supplemented children. In the group with mild and moderate anemia (less than 11g/dL hemoglobin), there was a significant increase in hemoglobin only in the group that received the fortified dietary supplement (by 0.92 g/dL) as compared to that of the placebo group (by 0.02 g/dL). This was confirmed by a significant increase in ferritin in the treatment group (by 16μg/L) versus the placebo group (by 2 μg/L). Also, low serum vitamin A levels were significantly lowered in the fortified group but not in the non-fortified group. There was a significant decrease (by 50%) in vitamin A deficiency as expressed by serum retinol of <20 μg/dL in the group that received the micronutrient fortified beverage.

Somewhat surprisingly, although weight, height and BMI did not differ between groups at the baseline, the fortified group, at follow-up, had gained significantly more in all three parameters as compared with the non-fortified group. The highly significant gains attributable to micronutrient supplementation were weight gain of 0.55 kg, height gain of 0.57 cm, and BMI (Body Mass Index) of 0.88 (wt/ht2).

This trial suggests that the micronutrient supplement was effective in improving iron and vitamin A status, as well as growth of children. The supplement was extremely popular. The fact that primary school children would not, compared to pre-school children or pregnant and lactating women, usually be considered the prime beneficiaries of such a mechanism, is recognized. However, the benefits of the school setting in providing a secure delivery mechanism (schoolteachers prepared the drink and supervised consumption) and thus high compliance were considered critical for this initial trial. A trial with pregnant women is planned.

Some principles and considerations relating to this approach

The objective of the trial in Tanzania was to deliver adequate levels of bioavailable iron, stable vitamin A and iodine via a beverage that children find highly palatable, and that is of low cost. The multiple nutrient fortified powder fruit drink delivers nutrients, taste, convenience and affordability all together. The dietary supplement strategy recognizes some of the key principles incorporated in one or all of the three commonly used intervention methods discussed above: behavioral change via social marketing; diversification of food intake; supply of specific vitamins in specific foods; and regular doses of vitamins and minerals specific to regionally or locally recognized deficiencies. These are the underlying mechanisms by which micronutrient deficiencies are addressed. Just as food diversification seeks to create a supply, demand and taste for a new food item so should the promotion of a dietary supplement; and, just as medicinal supplements aim to provide a significant (albeit pharmacological) dose of specific nutrients a well developed supplement could do the same.

Important factors in the development and promotion of a dietary supplement include:

Nutrient composition

A single serving of the product should deliver adequate levels of three critically needed (iron, vitamin A and iodine) micronutrients.

Product acceptance

The addition of the micronutrients should not affect the accepted taste and color of the vehicle. The preparation as well as the consumption of the product have to be culturally accepted. It should not replace, but rather complement other foods and beverages.

Product shelf life and nutrient stability

The stability of the nutrients added and the product shelf life should be evaluated in a condition that mimics the envi-

ronment under which it will be stored and prepared for consumption.

Product efficacy

For a dietary supplement to have an impact on the micronutrient status of the population who consume the product, the nutrients have to be bioavailable. Thus, the ability of the fortified product to improve micronutrient status should be demonstrated by carrying out a well designed clinical study.

Product package and convenience

The product should be of a kind that can be transported easily to poorly accessible remote areas and from stores to homes, and should not be bulky. It should be easy to prepare and serve in the home. The package should be strong and air tight.

Quality assurance and surveillance

The product should be made by following an established quality assurance program. The quality of the starting materials and the process of making the product determines the quality of the finished product. To make sure that the product is delivering the micronutrients claimed in the package at the time of consumption, monitoring should be done by a third party such as a regulatory agency.

Affordability

This is key to success of the dietary supplement strategy. It should be within the economic reach of the target groups. The cost of the product should reflect the value of the product. This has to be recognizable by the targeted consumer.

Raising awareness

The reality is that many of the populations suffering from micronutrient deficiencies are not aware of the problem. For the consumers to make an informed decision in choosing between a fortified and similar but unfortified products, they have to be educated about the prevalence, consequences and prevention of micronutrient malnutrition. Through schools, social marketing and media, the benefits of the product and the consequence of micronutrient malnutrition have to be communicated to the consumers and the professionals.

Partnerships

To accomplish all these elements, a partnership has to be established among the key players, which includes government agencies, industry, international agencies, non-government organizations and the scientific community. Each organization has expertise and these often complement each other. The goal is to succeed in accomplishing a common goal, which is combating micronutrient deficiency by leveraging each stake holder's strength.

Reviewing some of the problems identified with the other approaches to micronutrient deficiencies, the dietary supplement may potentially overcome some of these. Where sufficient appreciation and desire for the food item is generated - developed through commercial marketing strategies - supply should be ensured through market demand. Political will and external, public resources are not necessary inputs although they could be of benefit in generating a rapid development of demand and supply or for ensuring access through subsidies and/or free supply where income is not sufficient. The argument for a contribution from commercial manufacturers to the cost of subsidies and for social-marketing type promotions should not be overlooked given the potential for longer term sales development.

As with the dietary supplement practices of old, this approach can empower mothers and families with a healthful, care-giving practice which they control and can, ideally, access with security and at reasonable cost. Importantly, it is technically possible to include several micronutrients within a single food item, thus the process of addressing a situation where there are several deficiencies is simplified - a clear advantage over current fortification and medicinal supplementation strategies.

CONCLUSION

Tanzania is currently taking steps to address the serious problems of iron, vitamin A and iodine deficiencies. Wisely a variety of strategies are being used. Among the interventions being used are iron and folate supplements routinely administered to pregnant women; programs are in place to deworm children in part to reduce anemia; fermentation and germination or grains is being advocated to improve iron utilization and to reduce the action of phytates; vitamin A supplements administered to high risk children in many health units; efforts to increase the production and consumption of carotene rich foods; legislation in place to ensure iodination of salt from the major manufacturers; and other actions to address diseases such as malaria which influence nutritional status.

This study is considered an important first step in testing and further developing the mechanism of dietary supplementation for addressing micronutrient deficiencies. A distinction is being made here between medicinal supplements such as ferrous sulphate tablets and dietary supplements. Differences include the fact that medicinal supplements are taken under medical supervision and control, whereas dietary supplements for children are controlled by the mother or family, and for the pregnant woman by the mother herself. Another important difference is that this dietary supplement provided physiological "doses" of each nutrient, where medicinal supplements provide doses of micronutrients much above the RDA.

It is not expected or intended that this approach will replace current programs and strategies but instead that it will provide policy-makers, health-planners, and more importantly mothers and families, with an additional option. The decision on which strategy should be pursued or promoted, and how public dollars should be directed for nutrition interventions must be assessed on a case by case basis. Moreover, consistent with current understandings and experiences which show that no single approach will be effective in all settings and at all times, the development of a 'fourth option' can provide an effective means to fill the gaps left by other approaches. It should be noted that the Procter and Gamble Company developed and supplied the supplement as a prototype for this project. The product is not available in the marketplace.

ACKNOWLEDGMENTS

The authors are grateful to the Micronutrient Initiative, Ottawa, Canada and UNICEF, New York for financial support. UNICEF and their staff in Dar es Salaam provided logistical and other support. SmithKline Beecham donated albendazole to deworm children. Many colleagues at all of our three institutions provided advice and support. Finally we are appreciative of the help of staff in the Ministries of Health and Education in Mpwapwa, the school teachers, and the children who participated.

REFERENCES

1. ACC/SCN (United Nations Administrative Committee on Coordination/ Sub-Committee on Nutrition). Second report on the world nutrition situation – Volume 1: global and regional results. Geneva: World Health Organization, October 1993.
2. Latham MC. Human Nutrition in the Developing World. Rome: FAO, 1997.
3. World Bank. Enriching lives: overcoming vitamin and mineral deficiencies in developing countries. Washington, DC: World Bank, 1993.
4. Latham MC. Nutrition, national development and planning. In Nutrition, national development and planning. Edited by Scrimshaw, NS and Call, D. MIT Press 313-328, 1973.
5. Ash, D, Tatala, SR, Frongillo, Jr., EA, Ndossi, GD, and Latham, MC. Random-ized trial of a micronutrient dietary supplement in primary school children in Tanzania. Lancet (submitted), 1998.
6. Kavishe FP. The control of micronutrient: the experience of Tanzania. In: Proceedings of Conference on "Ending Hidden Hunger. Montreal 1991. Ottawa: The Micronutrient Initiative, 1991; 89-130.

ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición
Suplemento Vol. 51 N° 1, 2001

Relative effectiveness of iron bis-glycinate chelate (Ferrochel) and ferrous sulfate in the control of iron deficiency in pregnant women

Sophia Cornbluth Szarfarc, Luz Marina Núñez de Cassana, Elizabeth Fujimori, Elvira Maria Guerra-Shinohara, Ida Maria Vianna de Oliveira

Sao Paulo University. Sao Paulo - Brazil, National University of Cajamarca , Perú

SUMMARY. The relative effectiveness of daily supplementation of iron deficiency during pregnancy using 15 mg/day of iron from iron-bis-glycinate chelate (71 pregnant women), or 40 mg iron from ferrous sulfate (74 pregnant women) was evaluated by measuring hemoglobin, transferrin saturation and serum ferritin, at the beginning of the study (<20 weeks of pregnancy) and at 20-30 weeks and 30-40 weeks thereafter. Ingestion for 13 weeks or more was considered adequate. Seventy three percent of the Ferrochel consuming group and 35% of the ferrous sulfate consuming group were considered to have taken the treatment adequately. The decrease in levels of all the measured parameters was significantly less pronounced in the group that consumed Ferrochel in spite of the lower treatment dose. Iron depletion was found in 30.8% of the women treated with Ferrochel and in 54.5% of the women than consumed ferrous sulfate. Of the factors responsible for non compliance taste was reported in 29.8% of the ferrous sulfate consumers and none in the groups that consumed Ferrochel. It is concluded that daily supplementation with Ferrochel was significantly more effective, in spite of the lower dose, than supplementation with ferrous sulfate.

Key words:Ferrochel, iron supplementation, iron deficiency anemia, iron deficiency, pregnancy, Ferrochel's tolerance, Ferrochel effectiveness.

RESUMEN. Efectividad relativa del hierro bis-glicinato quelado (Ferrochel) y del sulfato ferroso en el control de la deficiencia de hierro en mujeres embarazadas. La efectividad relativa de la suplementación diaria con sulfato ferroso (FeSO4) o con hierro aminoquelado (Ferrochel, FeAAC) se estudió en 145 mujeres de menos de 20 semanas de embarazo distribuidas en dos grupos. Un grupo (71 mujeres) fue suplementado con 15 mg de hierro por día provenientes de Ferrochel, y el otro (74 mujeres) con 40 mg de hierro por día provenientes de sulfato ferroso. Se efectuaron mediciones de hemoglobina, ferritina y saturación de trasferrina al ingreso al programa (<20 semanas de embarazo), entre 20-30 semanas y entre 30-40 semanas. La ingesta ininterrumpida por 13 semanas o más se consideró adecuada. La disminución en los valores de todos los parámetros medidos fue menor en el grupo tratado con Ferrochel, a pesar de que la dosis fue mas pequeña. Depauperación de hierro se encontró en 30.8% de las mujeres tratadas con Ferrochel y en 54.5% de aquellas tratadas con sulfato ferroso. Entre los factores informados como responsables del abandono del tratamiento se encontró que el sabor fue el factor mas importante para las consumidoras de sulfato. Sabor indeseable no fue informado por las mujeres que consumieron Ferrochel. Se concluye que la suplementación diaria con Ferrochel fue sifnificativamente mas efectiva a pesar que la dosis usada fue menor que la dosis de sulfato ferroso.

Palabras clave: Ferrochel, suplementación con hierro, anemia ferropriva, deficiencia de hierro, embarazo, tolerancia a Ferrochel, efectividad del Ferrochel.

INTRODUCTION

Pregnant women constitute the most vulnerable group for iron deficiency. According to WHO estimates (1), in developing countries 56% of pregnant women suffer from iron deficiency anemia, which indicates that a much higher proportion may be iron deficient.

Iron deficiency anemia affects the fetus and the pregnant mother leading to impairment in oxygen supply to the fetus favoring the development of fetal hypoxia which has been associated with prematurity, low birth weight and neonatal and perinatal death (2-5).

The increased need of iron during pregnancy, specially after the second trimester, makes iron supplementation mandatory (6). In many countries, including Brazil, mandatory supplementation programs have been established to control the development of iron deficiency during pregnancy (7). Following WHO suggestions (8), the iron compound more frequently used is ferrous sulfate due to its low cost and reasonable availability (9,10).

In spite of its efficiency from a hematological point of view, ferrous sulfate supplementation shows low efficacy for the control of iron deficiency anemia (11,12), due to poor compliance with the treatment because of its disagreable

flavor and collateral effects such as nausea, vomit, constipation, diarrhea, abdominal pain.

Besides the above problems, usually there are also administrative problems such as deficient coverage in the health services, lack of compliance and logistiç problems in the supplement distribution (6). It has to be stressed, that iron deficiency anemia does not present specific signs or symptoms. This associated with the lack of knowledge of the general population on the importance of the disease for the health of the mother and the development of the fetus, and the presence of the normal discomfort associated with pregnancy results in a high rate of abandonment of the treatment.

Ideally, a supplementation with a well accepted iron compound accompanied with educational information could improve the compliance of the supplementation resulting in a significant reduction in the prevalence of iron deficiency and iron deficiency anemia.

Brazil has endorsed the compromise to reduce by 30% the prevalence of iron deficiency during pregnancy before the year 2000 (6), and has made a great effort for the implementation of alternative, efficient measures that can be rapidly incorporated to the prenatal care programs, aiming at reaching the established goal of iron deficiency reduction in pregnancy.

Among the possible alternatives to control iron deficiency, iron bis-glycine chelate (Ferrochel) has been used and successfully evaluated in Brazil in iron fortified foods (13,14). As a supplement has been tested in other countries and has shown a great efficacy in reducing iron deficiency and iron deficiency anemia in very short-time treatments with significantly low doses (15).

Structurally, Ferrochel is a non-ionizable, non reducing compound of good stability that does not change the organoleptic characteristics of the foods in which it has been used as a fortificant. Formal testing using radiolabeled Ferrochel has shown the compound to have a high bioavailability, 4-7 times greater than that of ferrous sulfate, and that its absorption is regulated by the iron stores in the body (16).

The present study was designed to compare the efficacy of iron supplementation using ferrous sulfate or Ferrochel in the control of iron deficiency during pregnancy.

MATERIAL AND METHODS

The study was conducted in the community of Santo André in the State of Sao Paulo, Brazil. It was designed as a prospective longitudinal study in a cohort of 145 pregnant women that assisted to the prenatal control program in 6 of the 13 Basic Health Units that had the prenatal programs implemented.

The study was approved by the Ethics Committee of the Infant Assistance Foundation of Santo André (FAISA), that is the institution responsible for the public health services of the locality. The study was carried out in a random sample of pregnant women that volunteered to participate and that filled the following criteria: a). Less than 20 weeks of pregnancy as recorded in their clinical record, b). Low obstetric risk evaluated by absence of hypertension, diabetes and a number of set clinical criteria, and c). Non use of any iron supplement prior to the enrollment in the Pregnancy Attention Program (PAG). In 3 of the health posts, ferrous sulfate in a dose of 200 mg/day (40 mg iron), as recommended by the Ministry of Health of Brazil was given (7). In the other 3 health posts a supplement of 75 mg of Ferrochel/day (15 mg iron), was given considering that on the bases of bioavailability this lower dose was equivalent to the 40 mg of iron from ferrous sulfate. The final number of pregnant women per group of treatment is shown in Table 1.

TABLE 1
Composition of sample, time and source of supplement

Sample size	Supplement source	Elemental iron
74 pregnant women	Ferrous sulfate	40 mg/day
71 pregnant women	Ferrochel	15 mg/day

To prevent administrative factors, the supplements were guaranteed to all women starting from the 20th. week of pregnancy.

At the time of enrollment, each enrolled woman received a printed message on the importance of anemia and a form to register the daily intake of supplement, and in the case the supplement was not taken an explanation of the reason for non compliance. A copy of the supplement intake form was attached to the clinical record of each pregnant women, and was actualized after each visit.

At each visit, the attending physician reinforced the information to each women on the importance of the supplement for her health and that of the fetus. He/she personally supervised that the proper blood sample was taken for biochemical analysis when required.

Hematological measurements were carried out in three occasions: at the time of enrollment in the program (less than 20 weeks of pregnancy), between 20 and 29 weeks of pregnancy, and at 30 or more weeks of pregnancy. Blood was extracted from a cubital vein and dispensed into two tubes. One containing EDTA, was used for the photometric determination of hemoglobin by the cyanmethemoglobin procedure (17), and the other with no anticoagulant for the separation of serum for the determination of iron, total iron binding capacity, and ferritin by standard methods (18,19). From these data, transferrin saturation was calculated.

As suggested by WHO (20), the criteria used to establish the presence of anemia was a hemoglobin level lower than 11 g/dL. All pregnant women with ferritin levels below 12 μg/L were consider to be iron depleted (18), and all those with transferrin saturation levels below 16% were consider to be iron deficient (21).

Following the norm adopted by the Prenatal Attention Program, a supplement intake for least 13 weeks was considered satisfactory (7).

RESULTS AND DISCUSSION

About 15% of all pregnant women in the community of Santo André receive prenatal attention in the health centers. This means that about 1200 new pregnant women participate in the Prenatal Attention Program per year. Osis et al, commented that the majority of women participating of the public health centers in Brazil come from the population of lower familiar income and that this is the only service available to them (22), We confirmed that about 40% of the women studied referred an income of less than U.S. $ 100 per month. Sixty three percent of these women did not complete elementary school (8 years). Most of the sampled women that worked (20%) were employed in general services or did domestic chores. Over 20% of the sample studied was conformed by adolescent women (less than 20 years), and 42% were primiparas.

The mean number of prenatal visits to the clinics was 6 and about 30% did not attend the clinics with the minimum frequency considered adequate for the control of their pregnancy. This, of course, affected the total number of blood samples analyzed.

As stated before, good compliance in the supplement intake is affected negatively by a number of factors related to gastric effects, bad taste or administrative failure. The lack of supplement in the Basic Health Units, and the impossibility of the pregnant women to buy it locally are cited as the principal causes of non compliance in supplementation programs. Gillespie, et al, analyzing the supplementation programs in India, observed that over 80% of the treated women abandoned the program due to lack of supplement (23). Nuñez de Cassana (24) also describes similar reasons for non compliance.

To prevent this type of problem, in the present study the distributed supplements were always available and non compliance was then due to other factors. The adequacy of supplement intake was related to the type of supplement. Seventy three point two percent of the women treated with Ferrochel consumed the supplements for at least 13 weeks, while only 35.1% of those treated with ferrous sulfate reached the 13 week limit. This is shown in Table 2.

Table 3 presents the frequency of factors responsible for noncompliance as informed by the subjects.

TABLE 2
Adequacy of supplement intake as related to iron source

Type of Treatment	n	Adequacy of intake 13 weeks or more	Inadequate intake less than 13 weeks
Ferrous sulfate	74	26 (35.1%)	48 (64.9%)
Ferrochel	71	52 (73.2%)	19 (26.8%)

TABLE 3
Factors that affected compliance of the supplement as related to iron source

Factor	$FeSO_4$	FeAAC*
Nausea, womiting,diarrhea	5 (7.5%)	2 (2.9%)
Supplement's taste	20 (29.8%)	0 (0.0%)
Abandon of prenatal care	18 (26.9%)	14 (20.9%)
Abortion, change of address forgetfulness	5 (7.5%)	3 (4.5%)
Total	48 (71.6%)	19 (28.4%)

* FeAAC = Ferrochel

Faulty flavor was the principal factor of noncompliance in the group of women that consumed the ferrous sulfate supplement. In contrast in the Ferrochel treated group, none of the women reported faulty flavor. Twice as many women reported nausea, vomit or diarrhea as a cause of noncompliance when the supplement was ferrous sulfate than when it was Ferrochel.

One important factor to consider is that Ferrochel can be consumed with food without altering its bioavailability. This fact facilitates its use and may be a contributing factor for adequate intake of the supplement. Independent of the type of treatment, desertion of prenatal attention was the principal cause for noncompliance.

The principal cause of information loses was unexplained desertion from the prenatal program. A mean of 22% of the registered women stopped their periodic visits to the Program of Assistance to Pregnant women. Five point five percent reported change of address, fetal losses or plain forgetfulness for interrupting their assistance to the program.

Table 4 presents the mean values and standard deviations for hemoglobin and the iron deficiency indicators. From the second half of gestation, fetal and placental growth are responsible for the increased need of iron, and is at this stage that iron reserves may be depleted, and limitation in adequacy of diet intake become more apparent. Hemoglobin concentration is diminished up to the end of pregnancy. The table shows that hemoglobin response was similar in both treatments, but the response in terms of serum ferritin and transferrin saturation was much better in the groups that received Ferrochel treatment, in spite of the lower dose of iron from the chelate.

TABLE 4
Changes in concentration of hemoglobin and serum ferritin and on percent saturation of transferrin during pregnancy, as related to type of iron supplement

Gestational age	Hemoglobin, g/dL		Serum ferritin µg/L		Transferrin saturation %	
	$FeSO_4$	FeAAC*	$FeSO_4$	FeAAC*	$FeSO_4$	FeAAc*
<20 weeks						
mean ± s.d	12.3 ± 1.1	12.7 ± 1.0	38.0 ± 30.4	38.4 ± 35.1	29.2 ± 13.2	27.6 ± 13.0
(N)	(65)	(67)	(58)	(49)	(64)	(62)
20-29 weeks						
mean ± s.d	11.4 ± 0.7	11.9 ± 0.7	14.7 ± 10.5	24.8 ± 37.6	23.4 ± 13.7	27.9 ± 16.4
(N)	(40)	(33)	(33)	(24)	(37)	(29)
30 weeks or more						
mean ± s.d.	11.6 ± 1.3	11.9 ± 0.9	10.8 ± 8.1	14.3 ± 10.7	17.7 ± 11.1	22.8 ± 13.9
(N)	(30)	(25)	(20)	(20)	(28)	(28)

*FeAAC = Ferrochel. The sample size decreased as pregnancy progressed due to not attendance of some women for blood sampling and to losses of samples in the laboratories, as explained in the text.

The diagnosis of iron deficiency during pregnancy presents some difficulties due to the large intra-individual variation associated with the normal blood volume expansion during gestation. This may explain the large variability of the results obtained (25).

According to Milne, et al.(26), serum ferritin concentration is the most sensitive test for the identification of body reserves of iron. Furthermore, the decrease in ferritin confirms the etiology of the anemia.

Contrary to what has been described by Puolakka, et al. (27), a decrease in the levels of serum ferritin was observed which was more evident in the groups supplemented with ferrous sulfate. This drop in serum ferritin reflects an increase in blood volume and a rapid utilization of the body iron reserves which may become depleted during gestation, even in the absence of anemia.

Blood iron transport as measured by transferrin saturation also decreased being more marked in the ferrous sulfate supplemented group.

The importance of sample loss in spite of the ample information supplied to the women enrolled in the program has to be stressed. There was some resistance to blood drawing which became evident even in the first laboratory examination (< 20 weeks of pregnancy), this became more serious as gestation progressed. As can be appreciated in Table 3, only about one third of the enrolled women gave a blood sample at the third control (30 weeks of gestation or more).

All these arguments become more evident when one analyses only those women that in each group gave blood samples at the three different testing times. The sample was further reduced by sample losses in the laboratory. The results of this analysis are presented in Table 5.

This type of problem was also reported by Papagallo and Bull (28) who observed lowering compliance even when the only test they carried out was hemoglobin.

When one analyses only those women that have results for all the analysis from the basal sample to the last, Table 5, there is a clear difference in response to the treatments. At 30 or more weeks of pregnancy the better effectiveness of the smaller dose of Ferrochel is clear. None of the biochemical parameters measured was significantly reduced, while in the group using ferrous sulfate at higher dose this was not the case.Table 6 shows the distribution of the pregnant women sample as related to the presence of iron deficiency anemia or iron depletion according to the type of supplement ingested. Here again, the greater efficacy of the treatment with Ferrochel is evident, even when the dose was only about one third of the dose of ferrous sulfate.

Among women that did not consume adequately the supplement about 50% had anemia and practically all had iron depletion.

CONCLUSIONS

Desertion from the supplementation program is very common in the Brazilian public health services. In most cases medical consultation is related to pathological episodes and not by compliance with the established program to follow the normal development of pregnancy. This is specially true when blood collection data is analyzed since there is always a great resistance to blood sampling. This results in a significant reduction of the initial sample as was shown in Tables 3, 4 and 5.

The finding of low prevalence of iron deficiency in the first trimester of pregnancy is not frequent. Lack of compliance in attending the health services, in taking the assigned supplement, and in permitting blood sampling prevented the present study from reaching the starting goals. Nonetheless, the results obtained strongly point to a higher efficacy of the chelated iron (Ferrochel) for which acceptance was not a problem. We imaging that the greater compliance with the chelate may be due to the fact that the treatment can be taken with the meals causing less negative gastric effects.

TABLE 5
Changes in concentration of hemoglobin and serum ferritin and on percent saturation of transferrin during pregnancy, as related to type of iron supplement

Gestational age	Hemoglobin, g/dL		Serum ferritin µg/L		Transferrin saturation %	
	$FeSO_4$	FeAAC*	$FeSO_4$	FeAAC*	$FeSO_4$	FeAAc*
<20 weeks						
mean ± s.d	12.3 ± 1.09 ad	12.6 ± 1.00 b	41.7 ± 34.20 ce	43.2 ± 41.80	29.9 ± 12.0 f	27.6 ± 12.0
(N)	(23)	(14)	(21)	(7)	(22)	(13)
20-29 weeks						
mean ± s.d	11.4 ± 0.73 ad	11.9 ± 0.70 b	17.7 ± 10.50 c	16.5 ± 11.01	23.3 ± 13.0	27.3 ± 14.2
(N)	(40)	(14)	(22)	(14)	(22)	(14)
30 or more						
mean ± s.d.	11.6 ± 1.27	12.2 ± 1.02	10.7 ± 8.14 e	15.1 ± 10.10	17.7 ± 11.10f	22.0 ± 16.3
(N)	(23)	(14)	(17)	(11)	(21)	(13)

These data refers to all those pregnant women in the sample that completed the three blood samplings of the study. The differences in sample size are due to laboratory loses of sample.
The bars indicate which differences are statistically significant. For ferrous sulfate, Hb1 -Hb2 = 0.001; Hb1-Hb3 = 0.05. For Ferritin, For FeAAC, Hb1-Hb2 = 0.004. FeAAC = Ferrochel treated group.

TABLE 6
Presence of iron deficiency anemia or iron deficiency in pregnant women with 30 or more weeks of gestation, according to treatment and adequacy of supplement intake

Adequacy of intake	Treatment	Iron deficiency anemia	Iron depletion
Adequate	$FeSO_4$	2 (11.1%)	6 (54.5%)
	FeAAC*	0	4 (30.8%)
Inadequate	$FeSO_4$	6 (50%)	8 (88.9%)
	FeAAC*	3 (50%)	4 (100.0%)

* FeAAC = Ferrochel

ACKNOWLEDGMENTS

To the Ministry of Health of Brazil and to Albion Laboratories, Brazil, that provided the treatments with ferrous sulfate and Ferrochel, to Laboratorios Buenos Aires that prepared the supplements and to the Infancy Assistance Foundation of Santo André in whose centers the study was carried out.

REFERENCES

1. World Health Organization. The prevalence of anemia in women: a tabulation of available information. WHO. Geneva, 1992.
2. Klein L. Premature birth and maternal prenatal anemia. Am J Obstet Gynecol 1962; 83:588-90.
3. Klebanoff MA, Shiono PH, Selby JV, Trachtenberg AI, Graubard BI. Anemia and spontaneous preterm birth. Am J Obstet Gynecol 1991;164:59-63.
4. Scholl TO, Hediger ML, Fisher RL. Shearer JW. Anemia vs iron deficiency: increased risk of preterm delivery. A prospective study. Am J Clin Nutr 1992; 57:135-9.
5. Waller MF, Haynes EM. Effects of heat and exercise on sweat iron loss. Sci Sports Exerc 1996; 28:197-203.
6. Organización Panamericana de la Salud. Plan the acción para el control de la anemia por carencia de hierro en las Americas. OPS/OMS, Washington. D.C.,1996.
7. Secretaria de Estado da Saúde. Resolução SS131 de 16 de maio de 1990; establece diretrizes e estratégias para combater a anemia ferropriva. Diario Oficial do Estado de São Paulo 1990, 15 de maio; Secção 1, p 12.
8. Organización Mundial de la Salud. Lucha contra la anemia nutricional, específicamente contra la carencia de hierro. OMS, Informe Técnico 580. Ginebra, 1975.

9. Sood S, Ramachandran K, Marthur M, Gupta K, Ramalingaswamy V, Swarnabai C. et al. Word Health Organization sponsored collaborative studies on nutritional anaemia in India. Q J Med 1975; 44:241-58.
10. International Nutritional Anemia Consultative Group. Iron deficiency in women. Washington, DC: The Nutrition Foundation, 1981.
11. Charloenlarp P, Dhanamitta S, Kaewvichit R, Silprosert A, Suwanaradd C, Na-Nakorn S, et al. A WHO collaborative study on iron supplementation in Burna and Tailand. Am J Clin Nutr 1988; 47:280-97.
12. Schultink W, Van der Ree M, Matulessi P, Gross R. Low compliance with an iron supplementation program: a study among pregnant women in Jakarta, Indonesia. Am J Clin Nutr 1993; 57:135-9.
13. Fisberg M, Braga JAP, Klianca PE, Ferreira AMA, Berezowski M. Utilização do queijo petit suisse na prevenção da anemia carencial em pré-escolares. Clin Pediatr 1995; 6(19):27-31.
14. Torres MAA, Lobo NF, Sato K, Queiroz SS. Fortificação do leite fluido na prevenção e tratamento da anemia carencial ferropriva em crianças menores de 4 anos. Rev Saúde Públ 1996; 30:350-7.
15. Pineda O, Ashmead HD, Perez JM, Ponce-Lemus C. Effectiveness of iron amino acid chelate on the treatment of iron deficiency anemia in adolescents. J Appl Nutr 1994; 46:2-11.
16. Christensen JM. Effects of divalent amino acids on iron absorption. J Pharm Sci; 1984, 73(9): 1245-7.
17. Dacie JV & Lewis SM Practical hematology. Churchill Livingstone, 1984.
18. Fenton V, Cavill I, Fisher J. Iron stores in pregnancy. Brit J Haematol (1977); 37:145-9.
19. Behringwerke AG N Latex-ferritina. Edición Junio 1994.
20. Organización Mundial de la Salud. Anemias nutricionales. OMS.Ginebra. Informe Técnico 405, 1968.
21. International Nutrition Anemia Consultative Group. Measurements of iron status. Washington DC: The Nutrition Foundation, 1985.
22. Osis DMJ, Hardy E, Faunde A, Alves G. Fatores associados à assistência pré-natal entre mujeres de baixa renda no Estado de São Paulo, Brasil. Rev Saúde Públ 1993; 27(1): 49-53.
23. Gillespie S, Kevany J, Mason J. Controlling iron deficiency. Geneva: United Nations Administrative Committee on Coordinations. Subcommittee on Nutrition, 1991.
24. Nuñez de Cassana LM. Intervenção nutricional no controle da deficiência de ferro em gestantes da Rede Básica de Saúde [tesis]. São Paulo: Universidade de São Paulo - Faculdade de Saúde Pública, 1998.
25. Roodenburg AJC. Iron supplementation during pregnancy. Eur J Obstetr Reprod 1995; 61: 65-71.
26. Milne DB, Gallagher SK, Nielsen FH. Response of various indices of iron status to acute iron depletion produced in menstruating women by low iron status, intake, and phlebotomy. Clin Chem 1990; 36:487-91.
27. Puolakka J, Janne O, Pakarinen A, Jarvinen PA, Vihko R. Serum ferritin as a measurements of iron stores during and after normal pregnancy with and without iron supplements. Acta Obstet Gynecol Scand 1980; 95 (Suppl): 43- 51.
28. Pappagallo S and Bull DL. Operational problems of an iron supplementation programme for pregnant women. Bull World Health Organ 1996; 74:25-33.

ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición
Suplemento Vol. 51 N° 1, 2001

The use of sweet rolls fortified with iron bis-glycinate chelate in the prevention of iron deficiency anemia in preschool children

Giorgini E., Fisberg M., De Paula RAC., Ferreira AMA., Valle J., Braga JAP.

Health and Nutrition Research Center, San Marcos University, Brazil.- Federal University Sao Paulo and State University of Rio de Janeiro, Brazil

SUMMARY. The effectiveness of bread fortified with iron bis-glycinate chelate for the control of iron deficiency and iron deficiency anemia was evaluated in 89 preschool children from families of low socioeconomic level attending 2 day nurseries of the Sao Paulo City Council. During the intervention's time of 6 months the children received besides their usual center's diet, a daily supplement of two sweet rolls fortified with 2 mg each of iron from the chelate for a total daily iron intake of 4 mg. After six months of intervention there was a significant decrease in the prevalence of iron deficiency anemia from 62 to 22%. There was a mean hemoglobin improvement of 1.1 g/dL, and in children with initial hemoglobin levels < 11 g/dL the mean increase in hemoglobin was 1.42 g/dL. The total mean plasma ferritin values increased from 11.34 to 20.2 µg/L, and in those children originally iron depleted the ferritin values normalized. A significant increase in the "z" score for weight/height was also observed. We concluded that the use of sweet rolls fortified with 2 mg of iron from the bis-glycinate chelate is highly effective for the control of iron deficiency and iron deficiency anemia in young children.

Key words: Ferrochel, bread fortification, flour fortification, Ferrochel effectiveness in bread, evaluation of bread fortification.

RESUMEN. Uso de bollos fortificados con hierro bis-glicinato quelado en la prevención de anemia por deficiencia de hierro en preescolares. La efectividad del pan fortificado con hierro bis-glicinato quelado para el control de deficiencia de hierro y la anemia ferropriva en infantes fue evaluada en 89 niños preescolares procedentes de familias de bajo nivel socio-económico, que asistían a hogares de día de la municipalidad del Estado de Sao Paulo, Brazil. La intervención duró 6 meses durante los cuales los niños del estudio recibieron dos bollos fortificados con 2 mg de hierro del quelado cada uno. Después de 6 meses de intervención, se observó una disminución significativa en la prevalencia de anemia ferropriva de 62 a 22%. Hubo una mejoría en los niveles de hemoglobina de 1.1 g/dL en el grupo total, y en aquellos niños que al principio mostraban valores <11 g/dL el aumento medio en hemoglobina fue de 1.42 g/dL. Los valores medios de ferritina en plasma aumentaron de 11.34 a 20.2 µg/L, y todos los niños con depauperación de hierro normalizaron sus niveles de ferritina plasmática. Al final de la intervención se demostró un aumento significativo en el valor de "z" para peso/talla. Se concluye que el consumo de los bollos fortificados con 2 mg de hierro del bis-glicinato quelado por unidad es altamente efectivo en el control de la deficiencia de hierro y la anemia ferropriva.

Palabras clave: Ferrochel, fortificación de pan, fortificación de harina, efectividad de Ferrochel en pan, evaluación de fortificación de pan.

INTRODUCTION

Mineral and vitamin nutritional deficiencies resulting from inadequate diets affect nearly two thirds of the world's population. Iron deficiency is the most prevalent deficiency and the main cause of nutritional anemias affecting almost 2,5 billion people in the world (1,2).

Studies have shown that iron deficiency is responsible for 80% of all nutritional anemias affecting children, and that 59,1% of the world's population is affected by iron deficiency anemia.

Although iron deficiency anemia is more prevalent in developing countries, it also affects industrialized countries where 8% of the population suffers from the disease. It is estimated that in developing countries, nearly 26% of men and 50% of women and children suffer from anemia. In industrialized countries 10% of children less than one year of age suffer from anemia, whereas in developing countries this figure can reach 30 to 80% (3,4).

In Brazil, iron deficiency anemia is considered a public health problem. Data from the National Epidemiology Division show that 1,3% of the total deaths in children result from anemia. In children under 5 years of age, and in women of childbear- bearing age, 50% of the total number of deaths is associated with iron deficiency anemia (5).

Studies carried out during the two last decades have shown a prevalence of more than 50% in the areas investigated (6-9).

An inadequate diet is the main cause of iron deficiency,

either because of its quality, or because of the insufficient amount of this micronutrient available in the population diet.

In 1988, Tudisco (10) examined the diets of Latin American countries and found, that in most places, including Brazil, the iron consumed came predominantly from non heme sources, and that the total amount ingested was below the recommended dietary intake (RDI). This fact confirmed dietary deficiency as the main cause of iron deficiency anemia in the populations studied.

Iron deficiency effects go beyond impairments in hemoglobin synthesis affecting besides other organic functions, causing behavioral alterations, reduced physical and intellectual performance, immunity and growth (11,12).

In the last decades, controlling iron deficiency anemia has been an important goal to achieved by any one concerned in solving the nutrition problems that affects large portion of the world population. In 1992, the International Conference on Nutrition held in Rome, attended by representatives of 160 countries, including Brazil, reached what was called a goal-compromise to reduce iron deficiency anemia in pregnant women to 1/3 of the prevalent at that time (3).

The World Health Organization (WHO) suggested that the disease be controlled by nutritional education programs combined with strategic actions to increase iron consumption of the populations at risk. This included iron supplementation and food fortification with iron (13).

Some studies on food fortification have shown promising results, leading many investigators in the world to believe that this is the most effective measure to be taken to prevent and control iron deficiency anemia (14-21). Fortification is defined as the addition of nutrients to food. It is a public health measure of rapid application, but it requires the selection of foods that are commonly consumed by the target population in relatively constant amounts, and to which de micronutrient in deficit can added without altering its organoleptic characteristics (22), and without significantly increasing its cost to be consider as a viable public health measure. The main criteria in selecting the nutrients are: proven need, safety and effectiveness. The effectiveness of the measure is, of course, directly connected to the selection of the food to be fortified. The cost of fortification, the nutrient bioavailability in the fortified food, the shelf life of the fortified food and its storage conditions are goals to be reached. It is also important to select the most adequate level of fortification, and to promote the development of adequate country legislation to ensure continuity of the fortification programs (23-25).

Several iron salts have been tested such as ferrous sulfate, fumarate, gluconate, and lactate. In Guatemala and in Egypt, NaFeEDTA was tested in the fortification of sugar and wheat flour (26,27). Bovine hemoglobin concentrate, isolated from blood obtained in slaughter houses has been used for the fortification of biscuits, but it is uncertain if the product is completely free from microbiological and viral contaminations Hem iron is known to be good source of iron (28,29), but its isolation from animal blood may pose large problems to comply with the need of control of biological contamination of the fortified product. Most of the studies with hem have been carried out in Chile where hem iron bioavailability in milk, biscuits and extruded cereals has been investigated (29-31).

In Brazil, fortified cookies containing 3% bovine hemoglobin were given to children 2- 4 years of age attending day care nurseries in the community of Piaui. The children were fed 5 cookies (8,3 mg of iron) per day, which resulted in a hemoglobin increase of about 2 grams, from 11 to 13,2 g/dL. The results of the trial show the effectiveness of hem in the prevention of iron deficiency anemia (32-34).

The development and availability in the market of an iron bis-glycinate chelate in which, an atom of iron is bonded to two molecules of glycine by means of stable coordinated covalent bonds forming two heterocyclic rings, offered greater fortification possibilities due to the high bioavailability of the chelate, with the added advantage that this compound does not provoke organoleptic changes in the fortified food (19,20).

In a study designed to evaluate the bioavailability of the iron amino acid chelated in whole milk, the authors observed that the iron absorption from 3 mg Fe/L was 2 to 2,5 times higher than the observed absorption from a similar milk fortified with ferrous sulfate (35,36). The results indicate that even in the presence of the common inhibitors inorganic of iron absorption present in milk (casein, calcium, serum proteins and phosphates) the absorption of the amino chelate was not hindered (36).

According to Olivares (35), in milk, the bioavailability of the iron bis-glycinate chelate alone is similar to that obtained with ferrous sulfate plus ascorbic acid (33,35). The organoleptic characteristics of milk fortified with the iron amino acid chelated remained unchanged (36).

Some additional studies of milk fortified with iron bis-glycinate chelate have been carried out in Brazil.The São Paulo's Department of Health conducted a six-month study with 2 year old children who were given a milk fortified with 9 mg of iron from the amino acid chelate and 65 mg of ascorbic acid per 100 g of powdered milk (3 mg of iron and 13.6 mg of ascorbic acid per 250 ml of milk). The results demonstrate the effectiveness of milk fortified with the amino acid chelate in the prevention of iron deficiency anemia (21).

In 1995, in a study using *Petit-Suisse* cheese fortified with iron bis-glycinate chelate at a level of 2 mg of iron in 90 g of cheese per day, it was found that after 3 months of fortified cheese consumption, there was a significant increase in plasma ferritin levels from 15.69 to 24,68 µg/L (20).

In the district of Angatuba, Sao Paulo, in a population trial in which milk fortified with 3 mg iron per liter from

iron bis-glycinate chelate was administered to children for a period of one year, a marked decline in the prevalence of iron deficiency anemia, from 62.3% to 41.8% was observed in six months. At the end of the year of consumption of iron-fortified milk, the anemia prevalence had dropped to 26.4 (36).

In 1995, Fisberg, *et al*, used iron bis-glycinate chelate to fortify wheat flour used to bake bread and cookies to a final iron level of 2 mg Fe/50 g of bread or 2 mg Fe/5 g biscuit. Nearly 900 children from 4 to 6 years of age received the fortified bread for three months at which time they showed an average increase in hemoglobin of 0.745 g/dL. Other 400 children from 6 to 36 months of age, received 2 fortified cookies per day for three months, and at the end of the intervention they presented a hemoglobin increase of 0,720 g/dL. The authors concluded that the use in the school meals, of bread or biscuits produced with flour fortified with iron bis-glycinate chelate was a safe and effective way of controlling iron deficiency and iron deficiency anemia, and that the fortification did not alter the organoleptic characteristics of biscuits or bread (21).

The aim of the present study was to evaluate the effect of consumption of sweet rolls fortified with iron bis-glycinate chelate, on the nutritional iron condition of preschool children.

MATERIAL AND METHODS

The study was carried out with preschool children of low socioeconomic level attending two day nurseries of the São Paulo's City Council. Children were kept in these day nurseries from 7 AM to 5 PM from Monday to Friday and received 5 meals per day.

When the study was started, 92 children were attending the nurseries, but three found severely anemic were treated and discarded from the study. These three children had an initial mean hemoglobin level below 9 g /dL. Of the remaining 89 children, 12 to 72 months of age, 52% were females and 48% were males.

Informed written consent and authorization for the study was secured, and signed by either the parents or the legal guardians of all children. The study's protocol was approved by the Medical Ethics Committee of the Federal University of São Paulo.

The study was divided into two phases: preintervention and the intervention proper that lasted 6 months. During the preintervention phase, all children were subjected to clinical examinations carried out by a pediatrician in order to evaluate the clinical status of each child and establish the presence of any preexisting illnesses that could interfere with their normal growth process.

At the beginning of the intervention, complete hematology tests were obtained from each child. All anthropometric measurements were carried out by well-trained personnel. The same measurements were repeated at the end of the six-month period of intervention.

During the nutritional intervention period children received their regular diet plus a sweet roll of 25 g fortified with 2 mg of iron from the amino acid chelate twice daily for five days a week. The total daily iron intake of 4 mg corresponded to 40% of the RDA) (37).

Body weight, recorded in kilograms, was obtained using a portable electronic digital scale with a total capacity of 150 kg and a sensitivity of 100 g. Height measurements were taken using a vertical stadiometer graduated in centimeters. The weight and height data was analyzed using EPI-INFO / EPI-NUT 6,02 (C.D.C. Atlanta, GA, U.S.A.). The weight/height, height/age and weight/age ratios data was evaluated according to the "z" score curve using the World Health Organization (WHO) reference standards (38,39).

For the analysis of hemoglobin and ferritin, 6 ml of blood were collected by venous puncture. Three mL of whole blood was kept in test tubes containing EDTA. This sample was used for hemoglobin determinations. From the rest of the 6 mL sample serum was obtained for the determination of ferritin. Hemoglobin concentration was measured electronically (Cell-Dyn 3000) (40), and the ferritin concentration by standard radioimmune assay procedures.

Fortified flour for the baking of the sweet rolls was obtained from Betamix (J. Macedo Alimentos Ltda. Sao Paulo). Each sweet roll weighed 25 g and contained 2 mg of iron from bis-glycinate chelate (Albion Laboratories, Inc. Clearfield, Utah, U.S.A.). Sweet rolls were selected because all subjects were used to their consumption.

RESULTS AND DISCUSSION

Nutritional condition

After the six months of intervention, mean values for weight/age and height/age of the children had increased significantly. At the beginning of the intervention, weight/age showed an average "z" score of - 0,23 and at the end of the intervention had risen to - 0,07. At the beginning of the intervention, the mean height/age "z" score was - 0,40 and at the end - 0,17 (Table1).

Iron condition

At the beginning of the study 28% of the children had hemoglobin levels < 11.0 g/dL, after 6 months of intervention the prevalence had decreased to 9%. The mean hemoglobin level at the beginning of the study was 11.5 g/dL and at the end 12.6 g/dL. (Table 2, Figure 1).

TABLE 1
Effect of 6 months of consumption of sweet rolls fortified with iron from Ferrochel on anthropometric indexes

	Weight/Height		Height/Age		Weight/Age	
Z score	Basal	Post-TX	Basal	Post-TX	Basal	Post-TX
≤ -2	2(2%)	1(1%)	7(8%)	2(2%)	3(3%)	2(2%)
>-2≤-1	8(9%)	8(9%)	20(23%)	20(23%)	18(20%)	13(15%)
>-1≤1	68(77%)	68(77%)	54(60%)	56(63%)	58(65%)	62(70%)
>1	11(12%)	12(13%)	8(9%)	11(12%)	10(11%)	12(13%)
Total	89	89	89	89	89	89
Mean ± S.D.	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)
Calculated z	-1,15		-5,5*		-4,8*	

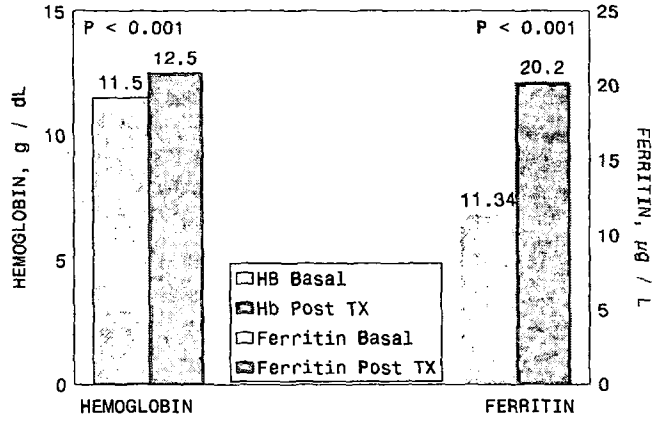
Wilcoxon critical z= 1,96; *P<0,001

TABLE 2
Effect of 6 months of consumption of sweet rolls fortified with iron from Ferrochel on the prevalence of anemia and levels of hemoglobin

	Hemoglobin		
Hemoglobin group	Basal N°. (%) g/dL ± s.d.	Post TX N°. (%) g/dL ± s.d.	Mean change in Hb level g/dL
<11.00 g/dL	25(28%)	8(9%)	
	10,00±0,70	11,42±1,23	1,42
≥11.00 g/dL	64(72%)	81(91%)	
	12,07±16,70	13,03±1,03	0,96
Total	89(100%)	89(100%)	
Mean±S.D.	11,50±1,11	12,60±1,30	1,10

Wilcoxon test (B) x (TX), calculated z= 6,80. Critical z= 1.96, P <0,001. All Post-Tx changes are significant.

FIGURE 1



At the beginning of the study the mean hemoglobin level was 11,50 g/dL, the average gain after intervention was 1,10 g/dL. (Table 2, Figure 1). The prevalence of moderate anemia cases (Hb = 9 -10 g/dL) dropped from 12% at the beginning to 1% at the end, and the prevalence of children with hemoglobin levels 10-11 g/dL, decreased from 16 to 7%. In those children with basal hemoglobin levels below 11 g/dL the change after intervention was 1,42 g/dL, and in the group with basal levels greater than 11 g/dL the change after intervention was 0,91 g/dL (Table 2).

At the beginning of the intervention, of the 89 children studied, 55 (62%) had ferritin values of less than 10 µg/L and 34 (38%) had values greater or equal to 10 µg/L. The mean value for all children (89) was 11,34 µg/L. After six months of intervention this mean value had significantly increased to 20,2 µg/L.

The children that at the beginning of the study had depleted iron stores, with ferritin levels below 10 µg//L (55 children), after the six months intervention had a mean increase in ferritin level of 13,03 µg/L (2,36 to15,35 µg/L), see Table 3.

TABLE 3
Ferritin prevalence of low levels and change after 6 months of consumption of fortified sweet rolls

	Ferritin		
Ferritin level group	Basal Prevalence (%) µg/L	Post TX Prevalence (%) µg/L	% change
<10.00 µg/L	255(62%)	22(25%)	
	2,32±2,29	15,35±11,57	661,6
≥10.00 µg/L	34(38%)	67(75%9)	
	25,93±16,70	28,04±10,30	8,1
Total	89(100%)	89(100%)	
	11,34±15,51	20,20±12,66	178,1

Wilcoxon test (B) x (TX), calculated z= -6,05. Critical = 1.96, P <0,001

It has previously been shown that iron absorption from the bis-glycinate chelate is regulated by the iron stores of the body (35). In line with this observation, the children with lower ferritin values that consumed the iron fortified sweet rolls, had the greater response in terms of increased serum ferritin levels.

CONCLUSION

After 6 months of consumption of sweet rolls fortified with 2 mg of iron from iron bis-glycinate chelate, we observed that:

1. The prevalence of anemia decreased significantly from 28 to 9%, and the prevalence of low iron stores decreased from 62 to 25%.
2. The mean hemoglobin levels of the children studied increased significantly (mean increase 1,10 g/dL). In anemic children, the increase was higher (1,42 g/dL).
3. The mean ferritin increase in the total group of children was 8,9 µg/L, and in children with depleted iron stores, the mean increase was 13,03 µg/L.
4 Anthropometrically, there was a significant increase in the "z" score for Weight/Age and Height/Age.
5. Sweet rolls fortified with 2 mg of iron from iron bisglycinate chelate are effective in the prevention and control of iron deficiency anemia at low cost and are, therefore, a good option for usage in programs of control of iron deficiency anemia.

REFERENCES

1. World Health Organization (WHO). National strategies for overcoming micronutrient malnutrition. 89th session of the executive board. Geneva,1992
2. Burton B. Human Nutrition. MacGraw Hill, São Paulo, 1979. p. 434-445
3. DeMayer EM. Preventing and controlling iron deficiency anaemia through primary health care. Geneva, World Health Organization, 1989. p. 58
4. Baker SJ & DeMaeyer EM. Nutritional anemia: its understanding and control with special reference to the work of the World Health Organization. Am. J Clin Nutr 1979;32:368-417.
5. Becker NA & Lechtig A. Evolução da mortalidade infantil no período de 1977–1984 (Infant Mortality evolution from 1977-1984. Brasília, DF. Min. da Saúde. 1986.
6. Monteiro CA, Szarfarc SC. Estudo das Condições de saúde das crianças do Município de São Paulo. S.P. (Brasil), 1984-1985. (Health Condition of São Paulo Municipality children). Rev. Saúde Publica 1987;21:255-260.
7. Cardoso MA, Ferreira MV, Camargo LM & Szarfarc SC. Anemia em população endêmica de malária, Rondônia . Brasil (Anemia in malaria endemic population). Revista de Saúde Pública 1992;26:161-66. .
8. Torres MA, Sato K, Sousa SQ. Terapêutica com doses profiláticas de sulfato ferroso, como medida de intervenção no combate à carência de ferro em crianças atendidas em Unidades Básicas de Saúde (UBS). (Prophylactic doses of Iron sulphate as means for combating iron anemia in children attended at Primary Health care Centers. Revista de Saúde Publica 1994;28:410-15.
9. Fisberg M, Braga JAP, Taddei JAC, Faria MP, Ferreira AMA, Blecher S, Nizutani L. Prevalência de anemia e desnutrição em pré – escolares de São Paulo (1994)-Anemia and Malnutrition Prevalence in preschool age children in São Paulo . In: XXIX Comgresso Brasileiro de Pediatria. 29 Congresso Goiano de Pediatria.Goiana, Temas Livres. Goías, Sociedade Brasileira de Pediatria, 1995. p.106 (Resumo, 094).
10. Tudisco ES. O papel da dieta na profilaxia da anemia ferropriva -The importance of the diet in the anemia's prophylaxis. Bol. Soc. Bras. Hemat. Hemoter 1988;149:129-33.
11. Walter T, De Andraca I, Cadud PMT. Iron Anemia: Adverse Effects on Infant Psychomotor Development. Pediatrics 1989;84:07-17. .
12. Walter T. Impact of iron deficiency on cognition in infancy and childhood. European Journal of Clinical Nutrition 1993;47:307-16.
13. World Health Organization. National strategies for overcoming micronutrient malnutrition. 89th session of the executive board. Geneva,1992
14. Andelman MB, Seed BR. Utilization of dietary iron by term infants. A study of 1048 infants from a low socioeconomic population. Am J Dis Child 1966;11:45-55.
15. Saariden UM. Need for iron supplementation in infants on prolonged breast feeding. J.Pediatr 1087;93:177-80.
16. Stekel A, Monckeberg F, Beyda V. Combating Iron Deficiency in Chile; A case Study. International Anemia Consultive Group Washington, D. C; 1986.
17. Hertrampf E, Olivares M, Walter T, Pizaro F, Heresi G, Llaguna S, Veja V, Cayazo M, Chadud P. Anemia ferropriva en el lactente: erradicación con leche fortificada con hierro (Iron deficiency anemia in children: erradication with iron enriched milk). Rev Med Chile 1990;118:1330-37.
18. Olivares M, Pizarro F, Walter T, Cayazzo M, Llaguno S, Chadud P, Cartagerna N, Hertrampf E, Veja V, Stekel A. Hemoglobin fortified biscuits: bioavailability and its effects on iron nutriture in school children. Arch Latinoam Nutr. 1990;40:208-220.
19. Queiroz SSQ, & Torres MMA. Anemia carencial ferropriva: aspectos fisiopatológicos e experiência com a utilização do leite fortificado com ferro (Iron deficiency anemia: physiopathology factors and experience with iron fortified milk). Pediatr Mod 1995;31:441-55.
20. Fisberg M, Braga JAP, Kliamca PE, Ferreira AMA, Berezowski. Utilização de queijo "petit suisse" na prevenção de anemia carencial ferropriva em pré-escolares (Petit Suisse cheese in the prevention of iron anemia in preschool children). Clin Pediátrica 1995;6:19-24.
21. Fisberg M, Pellegrini JAP, Cardoso R, Giorgini E. Uso do pão fortificado com ferro amino quelato em pré–escolares de 4 a 6 anos em Barueri SP., (The use of bread fortified with iron amino acid chelate in 4-6 year old children in Barueri, São Paulo) Anais- XII Congresso Latino Americano de Gastroenterologia Pediátrica e Nutrição., Barasil, São Paulo 1996. Abstracts 73 &104.
22. Guiland JC, Leynew B. As vitaminas (The vitamins). 1995. p. 357.
23. Organizaçao das Naçöes Unidas para Agricultura e Alimentaçao. FAO. Inserção de componentes de alimentação à nutrição nas políticas governamentais e na estatística nacional de desenvolvimento. Relatório final, Brasília(The Inclusion of Food and Nutritional components in governmental policies and in the national development statistics (Fev/ 1996).
24. Maberly GF. Programs against micronutrient malnutrition.

Ann ver 1994;15: 277-301.
25. Trowbridge FL, Haress SS, Cook J, Dunn JT, Florentino RF, Kodyat BA, Mannar MGV, Reddy V, Tontisirinni K, Underwood BA, Yip R. Coordinate strategies for controlling micronutrient malnutrition: a technical workshop. J.Nutr 1993;123:77587. -87,1993.
26. VIteri FE, Garcia-Ibañez R, Town B. Sodium iron NaFe EDTA as an iron fortification compound in Central America. Absorption studies. Am J Clin Nutr 1978;31:961-71.
27. Piske D. Aproveitamento de sangue de abate para alimentação humana: Uma revisão Bol. do Instituto de Tecnologia de Alimentos,19:253-308
28. Domene SMA. Utilização de sangue bovino para alimentação humana. (Cow's blood for human nutrition). Revista de Nutrição da Pontifícia Universidade Católica de Campinas(PUCAMP) 1988:1:163-79.
29. Asenjo JA, Amar M, Cartagena N, King J, Hiche E, Stekel A. Hiche E,Stekel A. Use of Bovine Heme Iron concentrate in the fortification of biscuits. J Food Sci 1985; 50:795-99.
30. Calvo E, Hertrampf E, Pablo S, Amar M, Stekel A. Haemoglobin-fortified cereal: an alternative weaning food with high iron availability. Eur J Clin Nutr 1989;43:237-43.
31. Hertramps E, Pizarro F, Pereira A, Veja V. Nueva formula lactea: biodisponibilidad del hierro y efecto en la prevención de su deficiencia en lactantes. Rev .Chil.Pediatr 1991;62:94-8.
32. Nogueira NN, Colli C, Cozzolino SM. Controle da anemia ferropriva em pré – escolares por meio da fortificação de alimento com concentrado de hemoglobina bovina (estudo preliminar). (Iron anemia control in preschool children by means of bovine hemoglobin concentrate). Cad. Saúde Públ 1992;8:459-66.
33. Stekel A, Olivares M, Pizarro F, Chadud A, Lopez I, Amar M. Iron absorption in infant formula. Am. J. Clin. Nutr 1986;43:917-22.
34. Hallberg L. Bioavailability of dietary iron in man. Ann Rev Nutr 1981;1:123-47.
35. Olivares M, Pizarro F, Pineda O, Name JJ, Hertrampf E, Walter T. Milk inhibits and ascorbic acid favors ferrous bis-glycinate chelate bioavailability in human. American Society for Nutritional Science Review 1997;13:1407-11.
36. Torres MA, Sato K, Lobo NI, Queiroz SS. Fortificação de leite fluído na prevenção e tratamento de anemia carencial ferropriva em crianças menores de 4 anos (Fortification of fluid milk in the prevention and treatment of infant iron deficiency anemia). Revista de Saúde Pública 1996;30:350-7.
37. National Research Council. Recommended Dietary Allowances. 10th ed. Food and Nutrition Board, Commission of Life Sciences. Washington- DC. National Academy Press, 1989
38. Dean AG, Dean JA, Burton AH, Dieker RC. Epi – Info, Version 5.0: word processing, database, and statistics program for epidemiology on microcomputers. Central for Disease Control. Atlanta, GA 1990. p 190-367.
39. World Health Organization. Measuring change in nutritional status: Guidelines for assessing the nutritional impact of supplementary feeding programs for vulnerable groups. WHO, Geneva, 1983. p.101..
40. Abbot Cell Dyn. Operator manual for Cell-Dyn 3000. Hematology Analyzer. Sequoia-Turner Corporation. California, 1989.

ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición

Suplemento Vol. 51 N° 1, 2001

The use of sugar fortified with iron tris-glycinate chelate in the prevention of iron deficiency anemia in preschool children

Regiane A. Cardoso de Paula, Mauro Fisberg

São Marcos University- São Paulo, Brazil

SUMMARY. In the present work, the effectiveness of consumption for 6 months of iron fortified sugar in the prevention or control of iron deficiency anemia was evaluated in 93 children (10-48 months old) attending a day care center in São Paulo, Brazil. Each child consumed 20 g of fortified sugar per day for 5 days a week in orange juice during breakfast. Two levels of fortification were tested using iron tris-glycinate chelate as the source of iron. Level one sugar contained 10 mg of iron /kg of sugar, and level 2, 100 mg of iron/kg. The children were assigned to either of the two groups. The first group (n=42) received level 1 sugar, and those of group two (n=52) received level 2 sugar. The daily iron intake corresponded to 2 and 20% of the RDA. At the end of the 6 months trial period, significant increases in weight/height ratio was observed in both groups. In the group consuming level 1 fortified sugar the mean change in hemoglobin concentration was 0,4 g/dL (from 11,3 grams to 11,7 g/dL), and in the group consuming level two fortified sugar the mean hemoglobin increase was also 0,4 g/dL (from 11,6 to 12,0 g/dL). Both changes were highly significant ($p<0.001$). When only the anemic children were considered (32/93), the increment of hemoglobin was 1,4g/dL. In anemic children there was a significant increase in the levels of serum ferritin. The increase was more notorious in group 2 children. We verified that the acceptability of the iron-fortified sugar was excellent. There were no detectable changes in the organoleptic characteristics of the fortified sugar as compared with unfortified sugar. No differences in response were observed between the two groups indicating that probably the lower level of iron was absorbed more efficiently that the higher level. The iron tris-glycinate chelate was very well tolerated with no side effects registered. It was concluded that even with low iron levels, the consumption of iron fortified sugar is an effective, low cost intervention for the control and prevention of iron deficiency anemia in preschool children.

Key words: Ferrochel, sugar fortification, effectiveness of iron fortified sugar, iron deficiency anemia control.

RESUMEN. El uso de azúcar fortificado con hierro tris-glicinato quelado en la prevención de anemia ferropriva en preescolares. En el presente estudio se evaluó la efectividad del consumo por 6 meses de azúcar fortificado con hierro tris-glicinato quelado en la prevención o control de anemia ferropriva en niños preescolares. Un total de 93 niños atendiendo una centro de cuidado diario fueron estudiados. Se utilizaron dos niveles de fortificación. En el nivel 1 el azúcar fue fortificado con 10 mg de hierro /kg de azúcar, y en el nivel 2 con 100 mg de hierro /kg. Los niños se distribuyeron en dos grupos. Grupo 1 (42 niños) consumió azúcar con el nivel 1 de fortificación. El grupo dos (52 niños) consumió azúcar con el nivel 2 de fortificación. En ambos grupos el azúcar fortificado fue usado por 5 días a la semana en jugo de naranja administrado durante el desayuno. El jugo de naranja contenía 20 de azúcar. Al final de los 6 meses del estudio ambos grupos mostraron incrementos significativos en la razón peso/talla y un incremento medio de hemoglobina de 0,4 g/ dL. Esta diferencia fue altamente significativa ($p>0.001$). Cuando el incremento en hemoglobina de los niños con anemia (32/93) fue analizado, este incremento fue de 1,4 g/dL. Dado que ambos grupos tuvieron un incremento igual en hemoglobina se postula que la absorción del hierro en dosis bajas es mas alta. En niñs anemicos hubo un increamenmte significative en la concentración de ferritina en suero. Se pudo verificar que no hubo ningún cambio en las características organolépticas del azúcar fortificado, y que este fue excelentemente tolerado por todos los niños. Se concluye que el azúcar fortificado con hierro tris-glicinato quelado puede ser un medio efectivo de control o prevención de anemia ferropriva en niños preescolares.

Palabras clave: Ferrochel, fortificación de azúcar, efectividad de azúcar fortificado con hierro, control de anemia ferropriva.

INTRODUCTION

Iron deficiency (ID) is a major public health concern due to its high prevalence and to the side effects it brings to the health of affected population groups. Many factors contribute to the high prevalence of ID in underdeveloped countries, among then, poor diets, diarrhea, intestinal infections and intestinal parasites (1-4). All these factors significantly contribute to children morbidity (3).

There are many non-hematological effects of ID, such as decrease capacity for physical activity, and alterations in the learning process and in motor and mental development. ID also has a depressing effect on cellular immunity, resulting in increased susceptibility to infection (5-7).

The high prevalence of ID makes it mandatory to seek new interventional strategies to control de deficiency (3).

Studies carried out by the World Health Organization (WHO), have shown that ID is a major cause of health related disorders, and decreased work capacity leading to significant economic loss in many populations. In the United States, 15-20% of the population under 18 years of age, suffers from ID, while in developing countries up to 51% of the population is affected (5,8).

In Brazil, important changes in the nutritional status of the population have taken place in the last few decades. There has been a gradual reduction in child mortality rates, a decrease in the total prevalence of protein-energy malnutrition, especially of the chronic forms, but at the same time, the prevalence of iron deficiency has increased (8,9).

Data from regional studies has shown that the high prevalence of iron deficiency anemia is a national concern, affecting all social groups, but predominantly in the most deprived population.

During the 80s, in the northeastern part of the country, anemia prevalence was 20-75% in preschool children, depending on the studied region (9,10). In the Amazon region, approximately 50% of the preschool children population suffered from this disease (11,12). In the Southern regions, where there is a high consumption of beef-based food, Turconi and Turconi (13), found that in the Municipality of Bento Gonçalves, 37.4 % of children aged 0-12 months suffered from anemia, and that in the Federal District of Brasilia, in a study of 279 children below 36 months of age attending public day-care centers the prevalence was 28.7% (14).

In 1978, in the city of Sao Paulo, Sigulem, *et al*, found an anemia prevalence of 22% in children up to 5 years of age (15), and ten years later, Montero and Szarfac, studying a similar population, found that anemia prevalence had increased to 35,7% (16). In 1992, the São Paulo State Health Agency, in a study that covered several areas of the State, found a prevalence of 59,1% in infants that attended the Basic Health Units (13,15,16). In another study, part of the National Survey of Malnutrition and Anemia, carried by the Nutritional and Health Research Center of São Marcos University in preschool children, it was found that in the city of São Paulo, with the best economic indexes in the country, the prevalence of anemia was up to 75% in children below 5 years of age (14). All these reports made it mandatory to start iron supplementation and food fortification programs considered to be the most appropriate measures to control the deficiencies.

The major technical difficulty in planning a fortification program is the selection of the iron compound to be used. When choosing the iron compound, the organoleptic alterations of the food vehicle, its bioavailability and its market price have to be evaluated (8, 24,37). The ideal food vehicle is regularly consumed by the target population in predictable amounts.

The fortification of foods with iron is the most effective measure to control the deficiency in a population. With this strategy, it is possible to reach all socioeconomic groups (8).

Ferrous sulfate is the most frequently used iron salt, but other salts such as ferrous fumarate and lactate can also be used, although at a higher cost. Sodium iron EDTA (NaFeEDTA) has been used in sugar fortification in Guatemala and in wheat flour in Egypt with limited success (18,8).

The development of more physiological iron chelates, such as iron bis- and tris-glycinate chelates opens new possibilities in food fortification, because of its higher bioavailability and its lack of alteration of the organoleptic characteristics of the selected foods (17,18). With these compounds, several types of foods have been tested in the last decade such as milk, sugar, bread, cookies, grains and salt (18-23,38).

In Brazil, several studies in food fortification with iron have been carried out in the last decade in an effort to find a viable way of controlling iron deficiency in the population. In 1994, Fisberg, *et al*, were able to show that three months consumption of "petit Suisse" cheese fortified with iron bis-glycinate chelate resulted in a significant decrease in anemia prevalence in preschool children (24).

In 1995, Torres, *et al*, evaluated the impact of the consumption of powdered cow's milk fortified with iron bis-glycinate chelate on anemia prevalence of preschool children. The study carried out under the auspices of the State of Sao Paulo Health Agency established that consumption for one year of fortified milk, significantly reduced the prevalence of IDA in the studied population (17).

In the city of Barueri, 1296 children ranging in age from 6 months to 6 years were studied. Eight hundred and ninety six of these children aged 4-6 years received bread fortified with 2 mg iron from the bis-glycinate chelate, and 400, aged 6 months to 3 years received cookies fortified with the same amount of chelated iron. At the end of the study it was found that the children that consumed fortified bread had a mean increase in hemoglobin of 0.74 g/dL, and those consuming the fortified cookies 0.72 g/dL (25).

In the present study we tested the effectiveness of the consumption of sugar fortified with either 10 or 100 mg of iron per kg from iron tris-glycinate chelate (a taste free iron chelate), on the iron nutritional status of preschool children. At the time of the study, sugar fortified with 10 mg iron per kg was available in the Brazilian market. We also studied the effect of a tenfold increase in the iron fortification level.

MATERIAL AND METHODS

A day care center from a Public Institution was chosen for the study. The sample was conformed by 93 children aged

10 to 48 months distributed in two groups. A pediatrician carried out physical examinations of all the children. The following factors were analyzed: clinical characteristics and signs related to previous and current diseases, anthropometry, and hematological condition. The pediatrician was in charge of supervision and training of the personnel involved in the intervention program. In two of the children in the sample no anthropometric data was collected due to their frequent absence at the time of gathering the data.

At the beginning of the intervention and six months after, all the children were subjected to clinical examination, anthropometric measurements and laboratory tests. All children with hemoglobin levels < 9,0 g/dL were excluded from the study, treated with ferrous sulfate and controlled by the pediatrician of the project. During the observation period, no changes were made in the diet of the day-care center, except for the use of sugar fortified with iron tris-glycine chelate as a sweetener in the orange juice ingested by the children. Children that failed to attend the day-care center for more than 10 consecutive days or 18 nonconsecutive days were excluded from the statistical analysis.

Blood samples were collected by venipuncture on a peripheral vein using discardable materials. Hemoglobin and ferritin determinations were carried out at the beginning and end of the study (6 months). Hemoglobin was measured by automated procedures (26), and serum ferritin by radioimmunoassay (27). Children were considered anemic when their hemoglobin levels were < 11 g/dL (28), and iron deficient when their serum ferritin levels were less than10 µg/L (8,27,29).

Data on weight and height was collected at the beginning and end of the trial. A digital, portable scale with capacity for 150 kg with 50 g divisions was used. Children were weighed naked or wearing the minimum amount of clothes possible, when weather was a concern. Estimates of the anthropometric ratios weight for age (W/A), height for age (H/A) and weight for height (W/H) were calculated using the program EPI – INFO 6,04 (CDC, Atlanta, GA, U.S.A.). The calculated z-score distribution was compared with WHO reference standards (30).

Each child received 20 grams of sugar fortified with either 10 mg of iron /kg of sugar (group 1), or 100 mg of iron /kg of sugar, both in orange juice. The iron used in the fortification was iron tris-glycinate chelate (Albion Laboratories, Inc. Clearfield, Utah, U.S.A.). This is a taste-free compound. The ingested fortified sugar provided 2% RDA of iron for group 1 and 20% RDA for group 2.

The comparison of the results in the two groups was carried out using parametric and nonparametric statistics. Student "t" test and two-way analysis of variance for a fixed significant level of 5% (31-33).

RESULTS AND DISCUSSION

The use of a staple food as a vehicle for iron fortification has always been the goal of researchers all over the world. The fortified food should be consumed by every risk group in a continuous and controlled way, and should have low price. Thus, milk, flours, salt and others are being used for this purpose.

Sugar is, in fact, a natural option, for it fills the described characteristics. The use of ferrous salts in sugar deeply alters its organoleptic characteristics preventing its use. This problem, was overcome by the use of iron tris-glycinate chelate since it produces only very slight variations in the physical characteristics of sugar and no organoleptic change.

This intervention using iron-fortified sugar is only the second world's trial and the first to overcome the organoleptic problems. Sugar fortified with 10 mg of iron from the amino acid chelated per kg was already available in the Brazilian market. Sugar fortified with 100 mg iron / kg of sugar was specifically prepared for this study.

Table I shows the anthropometric characteristics of the population studied.

TABLE 1
Anthropometric characteristics of the children enrolled in the study

Group	Age, months mean ± S.D. (Range)	Weight, kg mean ± S.D. (Range)	Height, cm mean ± S.D. (Range)
1	29,2 ± 9,5 (11,7-40,8)	12,9 ± 2,2 (9,2-17,5)	89,2 ± 8,1 (73,0-102,5)
2	31,4 ± 10,0 (10,4-44,8)	13,7 ± 2,4 (9,1-18,7)	91,7 ± 9,0 (71,5-103;5)
P*	n.s.	n.s.	n.s.

Student's "t" test for independent samples

It is noteworthy that in the present study the acceptability of the fortified sugar was excellent regardless of the iron dose used. There was no detectable change in taste in the orange juice, or side effect due to the use of the fortified sugar.

At the beginning of the study, the selected population presented a prevalence of anemia of 33.3%. This value was lower than that previously obtained by Torres, *et al*, (17), but very similar to the values previously obtained in the cities of Barueri (34,35) and Brasilia (14).

In the present study, despite the low level of iron added to the sugar (2 and 20% RDA), a mean increase in hemoglobin levels, of 0.40 g/dL in the six-month consumption period was observed in both groups. This increase is very close to that obtained in a similar time with milk fortified with this same iron chelate. Studies conducted

by us and several other groups have shown that even with higher fortification levels in milk, the increase in hemoglobin in a six-month period is about 0.5 g/ dL. (17,19,).

After the intervention with iron-fortified sugar, there was a mean decrease in the prevalence of anemia of 43.8% for group 1, which received the lower dose of iron, and of 66.7% for group 2. The total mean prevalence of anemia, came down from 33.3 to 18.3%. (Table 2).

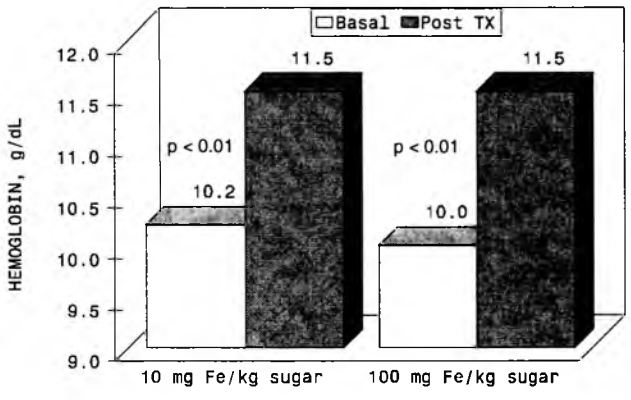
TABLE 2
Effect of intervention on the prevalence levels of low hemoglobin levels

Group		Hb basal N (%)	Hb post Tx N (%)	Prevalence change (%)
1	< 11	16 (38,1)	7 (16,7)	-43,8
	≥11	26 (61,9)	35 (83,3)	+34,6
2	< 11	15 (29,4)	10 (19,6)	-66,7
	≥11	36 (70,6)	41 (80,4)	+13,9
Total	<11	31 (33,3)	17 (18,3)	-54,8

ANOVA shows that the differences before and after treatment are significant, p = 0.01

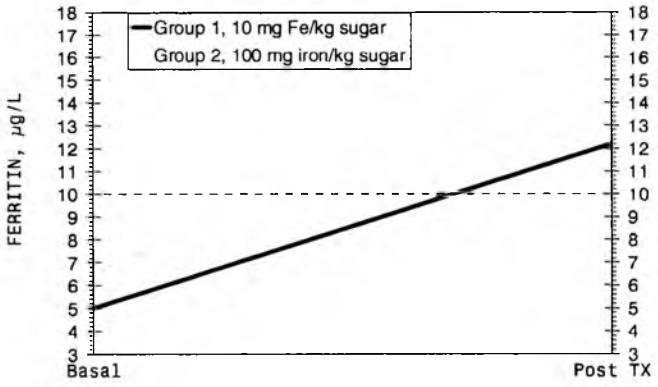
If only anemic children are analyzed, the increase in hemoglobin values obtained in the six months period of sugar consumption is 1.3 g/dL for group 1 and 1.5 g/dL for group 2. These results are highly significant, with the advantage that they can be achieved with a much lower cost and with no side effects (35). Moreover, the mean hemoglobin level reached normal values in both groups, with no significant difference between the groups (Figure 1).

FIGURE 1
Effect of 6 months consumption of 20 g of fortified sugar per day on hemoglobin levels



In the group, as a whole, there was no significant change in body iron reserves as measured by serum ferritin levels. Most probably, the period of intervention with levels of iron that are a small fraction of the RDA was not long enough to alter iron reserves. However, when children with iron deficiency at the beginning of intervention were analyzed, there was a significant increase in ferritin from 5,0 to 12,2 µg/L for group 1 and from 6,5 to 15,9 µg/L for group 2 (Figure 2). This shows a trend toward normalization of ferritin values in children with iron deficiency (36).

FIGURE 2
Effect of 6 months consumption of 20 g/day of iron fortified sugar on serum ferritin levels of iron deficient children



The trend towards increasing hemoglobin and ferritin levels of the children studied, do not seem to be related to the dose of iron used. This may be a reflection of the small doses used, since even with a fortification level of 100 mg iron per kg of sugar, the iron contribution in the dose taken represents only 20% of the iron RDA, and it is a know phenomenon that at low levels, iron is absorbed more.

When assessing the anthropometric results of only those children considered at nutritional risk at the beginning of the trial, only group 1 presented a significant progress for the weight/height ratio. The biologically meaning of these results may not have any important significance because of the short time of treatment. There was no relationship between anemia prevalence and nutritional status, probably due to the general adequate nutritional level of the population at the beginning of the trial.

As it was already mentioned, sugar is considered a staple food, with low cost, that can be easily distributed for populations at risk. Several studies have shown that its adequate use is not related to increase in prevalence of obesity or tooth decay (18,36).

The search for an effective iron compound with good bioavailability and of universal application has been the objective of worldwide research (5). The results obtained in this study show that the use of iron tris-glycinate chelate to fortify sugar is an extremely useful alternative for the control of iron deficiency anemia.

The fortification of sugar with iron tris-glycinate chelate is very satisfactory in preventing iron deficiency anemia. There is no detectable alteration in the flavor or in the organoleptic characteristics of sugar. There is a very slight change in color that does not prevent its acceptance in our day-care center. However, after months of storage of the fortified sugar, there was a tendency for the formation of dark or yellowish dots in the inner part of the packaging. This may affect the commercial aspect of the product, but does not modify the good results obtained in the present trial. We have to point out that the sugar used in Brazil is very white and powdered. For general crystallized sugar fortification, the necessary technology has been developed considering the specific characteristics of local sugar to prevent these changes from happening.

The trial using sugar fortified with iron tris-glycinate chelate resulted in a significant increase in hemoglobin levels and in a reduction in anemia prevalence. There was no observable dose related effect, maybe because of the very low relative doses of iron used. As expected from an absorption and regulation point of view, the effects were evident only in children who presented anemia at the beginning of the trial.

No side effects were encountered during the duration of the trial with either level of sugar fortification. On the contrary, there was an excellent tolerability and acceptance. We conclude, therefore, that sugar fortified with iron tris-glycinate chelate in low doses, is an effective means in preventing iron deficiency anemia in populations at risk.

REFERENCES

1. Burton B. Nutrição Humana. Ed. Mac Graw Hill, São Paulo, 1979;434-445.
2. Committee on Nutrition . Iron Supplementation. Pediatrics 1979; *58*:765-8.
3. Lozoff B, Jimenez B, Wolf AW. Long Term Developmental Outcome of Infants with Iron Deficiency. N Engl J Med 1991;94: 325:687.
4. Pollit E. Effects of a Diet Deficient in Iron on the Growth and Development of Preschool-age Children. Food and Nutrition Bulletin 1991;13:110-17. The United Nations University.
5. World Health Organization. Nutritional Anemias. Technical Report Series No. 405. Geneva,1968. p 37.
6. Walter T, Andraca I, Chadud P. Iron Anemia: Adverse Effects on Infant Psychomotor Development. Pediatrics 1989;84:07-17.
7. Lozoff B. Has Iron Defficiency been show to Cause Altered Behavior in Infants? In: Brain behavior and iron in the infant diet. Ed. J. Dobbing ,107-131.,-Spring Verlag, London ,1990.
8. DeMaeyer EM. Preventing and Controlling Iron De ficiency Anaemia Tthrough Primary Health Care. WHO, Geneva, 1989.
9. Instituto Nacional de Alimentação Nutrição. Ministerio de Saúde. Pesquisa Nacional sobre Saúde e Nutrição. Perfil de Crescimiento da População Brasileira de 0 a 25 anos. Brasília, 1990, p 60.
10. Braga, JAP. Avaliação do Estado Nutricional de Pré - Escolares submetidos a Intervenção com um Suplemento Lácteo Fortificado com Ferro. Doctorate thesis presented to Universidade Federal de São Paulo, 1996.
11. Salzano AC, Torres MAA, Batista FO, Romani SA. Anemia em Crianças em Dois Serviços de Saúde de Recife, PE (Brasil). Rev. Saúde Pública 1985;19: 499-507.
12. Silva NB, Maeinho HA, Castro JS, Rocha,YR, Ferraroni MJR Prevalência da Anemia Ferropriva em Crianças Pré - escolares da Cidade de Manaus, AM. Anais: I Congresso Nacional da SBAN, São Paulo, 1988. p.63.
13. Turconi SJ, Turconi VL. Anemia Ferropriva: Incidência em uma População Infantil. Pediatr Mod 1992;28:107-12.
14. Schimits BAS, Aquino KKCN, Picanço MR, Bastos J, Giorgini E, Cardoso R, Braga JAP, Fisberg M. Prevalência da Desnutrição e Anemia em Pré- escolares de Brasília, Brasil. Pediatr Mod 1998;34:155-64.
15. Sigulem DM, Tudisco ES, Goldenberg P, Athaide MMM, Vaisman E. Anemia Ferropriva emCrianças do Município de São Paulo. Rev. Saúde Púbica 1978;12:168-78.
16. Monteiro CA, Szarfarc SC. Estudo das Condições de Saúde das Crianças do Municipio de São Paulo. S.P. (Brasil), 1984-1985. Rev. Saúde Publica 1987;21:255-
17. Torres MA, Sato K, Lobo NI, Queiroz SS. Efeito do Uso de Leite Fortificado com Ferro e Vitamina C sobre Níveis de Hemoglobina e Condição Nutricional; de Crianças menores de 2 anos. Revista de Saúde Pública 1995;29:301-7.
18. Viteri FE, Alvarez E, Batres R, Torún B, Pineda O, Mejia LA, Sylvi J. Fortification of Sugar with Iron Sodium Ethylendiaminotetraacetate (FeNaEDTA) Improves Iron Status in Semirural Guatemalan Populations. Am J Clin Nutr 1995;61:1153-6.
19. Queiroz SSQ, Torres MAA. Anemia Carencial Ferropriva: Aspectos Fisiopatológicos e Experiencia com a Utilização do Leite Fortificado com Ferro. Pediatr Mod 1995;31:441-5.
20. Layrisse M, Marinez-Torres C, Renzi M, Velez F, Gonzales M. Sugar as Vehicle for Iron Fortification. Am J Clin Nutr 1976;29:8-18.
21. Calvo E, Hertrampf E, Pablo S, Amar M, Stekel A. Haemoglobin-fortified cereal: an Alternative Weaning Food with High Iron Availability. Eur J Clin Nutr 1989;43:237-243.
22. Tuntawiroon M, Sritongkul N, Rossander-Hulten L, Pleehachinda R, Suwanik R, Brune M, Hallberg L. Rice and Iron Absorption in Man. Eur J Clin Nutr 1990;44:489-97.
23. Peña GG, Pizarro F, Hertrampf DE. Aporte del hierro del pan a la dieta Chilena. Rev Med Chile 1991;119: 753-57.

24. Fisberg M, Braga JAP, Kliamca PE, Ferreira AM, Berezowski. Utilização de Queijo "petit suisse" na Prevenção de Anemia Carencial Ferropriva em Pré - escolares. JAMA,-Peediatria 1995;2:14-24.
25. Fisberg M, Velloso EP, Ribeiro RMS, Zullo M, Braga JAP, Soraggi C, Kliamca PE, Chedid EA, Schuch M, Valle J, Cardoso R, Krumfli M, Graziani E. Projeto Barueri Pediatria Atual 1998:11:19-26.
26. Abbott Cell Dyn. Operator Manual. Abbott Diagnostics Operation Reference Manual for Cell – Dyn 3000 – Hematology Analyser 1989, Sequoia -Turner Corporation, California, USA.
27. Franco CD.- Ferritin. In: Kaplan LA and Pesce, A J., Eds. Methods In Clinical Chemistry, 1980, p 124-42.
28. Dallman PR, Reeves JD. Laboratory Diagnosis of Iron Deficiency. In: Stekel A. Iron Nutrition in Infancy and Childhood. (Nestlé Nutrition Workshop Series, No. 4). Raven Press, New York, 1984.
29. Oski FA, Honig A. The Effects of Therapy on the Developmental Scores of Iron Deficient infants. J Pediatr 1978;21:92.
30. Dean AG, Dean JA, Burton AH, Dieker RC. Epi – Info, Version 5.0: A Word Processing, Database, and Statistics Program for Epidemiology on Micro – Computers. Center for Disease Control. Atlanta, GA 367 p., 1990.
31. Sokal RR, Rohlf FJ. Biometry: the Principles and Practice Statistics in Biological Research. Chapter 7: Estimation and Hypothesis Testing. 124-174. Chapter 11: Two-Way analysis of Variance. W. H. Freeman and Co., 1969.
32. Siegel S. Estatística não-paramétrica (para as ciências do comportamento). Cap. 6: Amostras independentes. McGraw - Hill, Brasil, 1975.
33. Snedecor GW, Cochran WG. Statistical Methods. 7th edition, The Iowa State University Press, 1982.
34. Fisberg M, Velloso EP. Barueri Project.-Programa de Intervenção Nutricional com Farinhas Enriquecidas com Ferro para Pré-escolares. Anais do Encontro Estadual sobre Alimentação do Escolar, 1997. p 45-50.
35. Fisberg M, Velloso EP, Ribeiro RMS, Zullo, M, Braga JAP, Soraggi C, Kliamca PE, Chedid EA, Schuch M, Valle, Cardoso R, Krumfli M, Graziani E. Projeto Barueri. Pediatria Atual 1998;11:19-26.
36. Vega-Franco L. Deficiência de Hierro en la Infancia: Manisfestaciones Clinicas, Tratamento y Prevencion. Parte II. Bol Med Hosp Inf Mex 1989;46:690-95.
37. Cook JD, Reusser ME. Iron Fortification: An Update. Am J Clin Nutr 1983 38: 648-59.
38. Fisberg M, Pellegrini JAP, Cardoso R, Giorgini E. Uso do pão fortificado com ferro aminoácido quelato em Pré-escolares de 4-6 anos em Barueri SP. Anais XII Congresso Latino Americano de Gastroenterologia Pediátrica e Nutrição, São Paulo, Brasil, 15-19 de Julho, 1996. Abstract 73,104.

FINAL COMMENT

Oscar Pineda

CELANEM, Director

As mentioned in practically all the papers presented in this supplement, Iron Deficiency and Iron Deficiency Anemia persist as the most important single nutrient deficiency in the world, in spite of several decades of efforts to achieve its control.

Food fortification with iron has been mandatory in a number of countries but no overall improvement has been obtained. We know now that the iron compounds used in countrywide fortification programs show low bioavailability, which is further reduced by compounds normally present in the diets.

Through the years, CELANEM has devoted a significant amount of time and effort to stimulate research on uses of Ferrochel (iron amino acid chelate, iron bis-glycinate chelate, which include formal bioavailability studies in water and in different food matrixes. These studies have consistently shown that Ferrochel is well absorbed, even in the presence of inhibitors, does not produce gastric discomfort, has low toxicity, is well tolerated, and its absorption is regulated by the iron stores of the body.

In this supplement work is presented on the chemistry of chelation, on the absorption, metabolism, regulation and toxicity of Ferrochel, and on important applications when the chelate is given as a supplement or in fortified foods, presenting further evidence of the effectiveness of the iron amino acid in the control of iron deficiency anemia.

When pharmaceutical preparations were marketed in the early 1990s (in tablets, syrups and pediatric drops) they rapidly gained recognition, confidence and support from the medical profession due to its great effectiveness in the control of iron deficiency anemia using smaller doses than with any other iron compound. The mean treatment time was reduced to 4-6 weeks. At the present time, in Central and South America and in South Africa, these preparations have gained medical recognition and have become the treatment of choice for iron deficiency anemia.

The application of iron bis- and tris-chelates in food fortification has been extensively studied and its effectiveness evaluated after short times of consumption of the fortified foods. The pioneering studies of milk fortification with low levels of iron proved to be so effective that in the State of Sao Paulo, Brazil, fortification became mandatory for state supported assistance programs for small children. At the present time, milk and dairies are fortified with Ferrochel in Argentine, Chile, Paraguay, Ecuador, Brazil, Colombia, Venezuela, Central America, Mexico, Europe, Saudi Arabia, South Africa, and Thailand and the use of Ferrochel is rapidly gaining recognition in a number of other countries.

The studies by Cornbluth, *et al* on pregnant women supplementation with Ferrochel's iron, presented in this supplement, show that 15 mg of iron from the chelate are more effective than 40 mg of iron from ferrous sulfate.

In food fortification two further examples are presented, fortification of bread and fortification of sugar. The consumption for six months of sweet rolls fortified with iron from Ferrochel resulted in a highly significant improvement in the iron status of the tested population. Studies carried out by Bovell-Benjamin, *et al,* have confirmed that even in the presence of high concentrations of phytates, the iron of Ferrochel is absorbed 4-7 times better than that of ferrous sulfate (1).

Regarding sugar fortification, the only other trial we are aware off is that of Viteri, *et al*, (2) carried out in the early 1970s and in which after 4 years of consumption of sugar fortified with NaFeEDTA, there was only a marginal improvement in the iron status of the tested populations. Furthermore, the organoleptic characteristics of the sugar were significantly altered.

In contrast, the studies by Cardoso de Paula and Fisberg presented here show a great effectiveness in improving the iron condition of the population tested, when 20 g per day of sugar fortified with low levels of iron tris-glycinate chelate (10 and 100 mg of iron per kg of sugar) were consumed. With the tris-chelate there was no detectable change in the organoleptic characteristics of the fortified sugar. Its effectiveness evaluated after only six months of sugar consumption showed highly significant improvements in the iron condition of the population tested.

It has been shown, that Ferrochel added to multivitamin preparations does not affect the stability of the vitamins. This is especially important in preparations containing vitamin A that deteriorates very rapidly in the presence of inorganic

iron compounds (3,4). As shown by Garcia-Casal and Layrisse, in this supplement, Ferrochel remains soluble at different pHs, and its bioavailability is not altered.

The characteristics and effectiveness of Ferrochel in clinical studies and field trials either presented here or previously published indicate that Ferrochel is, at the present time, the best available iron compound to use in the control of iron deficiency and iron deficiency anemia.

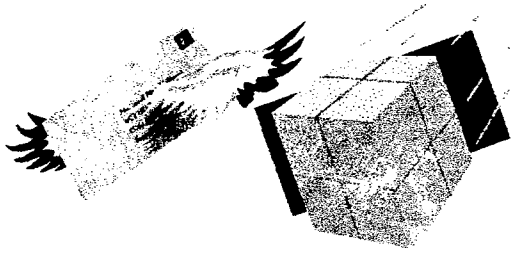
We consider that the information presented here will assist the public health community in gaining a better understanding of the chemical characteristics of the iron chelates and on its physiology, and stimulate its use in programs geared to a better control of iron deficiency and iron deficiency anemia in a short time.

REFERENCES

1. Bovell-Benjamin AC, Viteri FE, Allen LH. Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status. Am J Clin Nutr 2000;71:15163-69.
2. Viteri FE, Alvarez E, Pineda O, Torún B. Prevention of Iron Deficiency by Means of Iron Fortification of Sugar. In: Nutrition Intervention Strategies in National Development. B.A. Underwood, ed. Academic Press, New York, 1983. pp.287-314.
3. Bourdonnais A. Stabilité d'une solution de Vitamine A au cours du temps en presence de Métalosate de fer. 1994, Zootech Tech Bull.
4. Marchetti M, Ashmead HD, Tossani N, Marchetti S, Ashmead S. Comparison of the rates of vitamin degradation when Mixed with Metal Sulfates or Metal Amino Acid Chelates. J Food Composition and Analysis 2000;13:875-84.

En Venezuela y hacia y desde todo el mundo, en IPOSTEL le ofrecemos

SERVICIOS TRADICIONALES de Cartas, Impresos y Encomiendas.



... y además contamos con novedosos

SERVICIOS EXPRESOS

A nivel nacional ofrecemos: EEE (Entrega Especial Expresa) es un servicio que le permite enviar documentos y encomiendas a todo el Territorio del país de una forma expresa, económica y segura.

EMS, (Express Mail Service), es un servicio tipo courier que le pemite enviar documentos y mercaderías a nivel internacional, respondiendo a su necesidad de entrega en tiempo breve y a los precios más económicos del mercado.

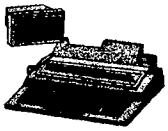
SERVICIOS ELECTRONICOS

CORREO ELECTRONICO Y KIOSCO INTERNET

Ipostel habilitó para aquellos usuarios que les gusta navegar en el mundo de la red, kioskos de internet, ubicados en Altamira, C.C.C.T y los Ruices, los cuales disponen de computadoras de fácil acceso a todas aquellas personas interesadas en explorar dentro del campo de la comunicación.

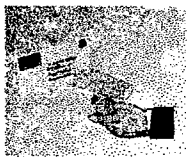
AUTOMATIZACION DE TAQUILLA

La automatización de taquillas cuenta con modernos accesorios como balanza electrónica e impresora de recibos; esta permite mejorar la atención en las taquillas postales y simultáneamente establecer un control eléctrico de los ingresos por venta de servicios.



SEGURIDAD Y RASTREO POR CODIGO DE BARRA

El Código de barra permite registrar los datos más importantes de los envíos EMS entrantes y salientes, este sistema actualmente es utilizado a nivel internacional, para envíos EMS.



Un Mundo de Servicios para Usted...

Artes Finales: Amerik Solutions C.A., Caracas, Venezuela
Teléfono (02) 993.81.43

Portada: Chávez & López, Diseño Gráfico, Caracas, Venezuela
Teléfono (02) 285.55.29

Impresión: Editorial Texto C.A., Caracas, Venezuela
Teléfonos (02) 62.24.85 - 62.87.30

SOCIEDAD LATINOAMERICANA DE NUTRICION (SLAN)

La Sociedad Latinoamericana de Nutrición (SLAN) fue creada el 10 de Noviembre de 1965 en ocasión de celebrarse el Primer Congreso de Nutrición del Hemisferio Occidental. El actual Consejo Directivo de la SLAN (2001-2003) está constituido por los siguientes miembros:

Presidente	Adolfo Chávez
Vicepresidente (Presidente Electo)	Helio Vannucchi
Secretario General	Abelardo Avila Curiel
Tesorero	Fernando Pérez-Gil Romo
Presidente Saliente	Alejandro O'Donnell
Vocal de Salud Pública	Juan Rivera Dommarco
Vocal de Alimentación y Dietética	Consuelo Velásquez
Vocal de Nutrición Infantil	Homero Martínez
Vocal de Ciencia de los Alimentos	Jóse Angel Ledesma Solano
Vocal de Tecnología de Alimentos	Eduardo Mendoza Martínez
Vocal en Nutrición Básica	Salvador Villalpando
Vocal en Nutrición Animal	Silvia Carrillo
Vocal en Estatutos y Organización	Natalia Berún de Tamez
Vocales de Informática	Teresa Shamah Levy
	José Félix Chávez Pérez (ALAN)
	Héctor Araya (AEN)
Vocales de Congreso y Reuniones	Miriam Muñoz Rivera
	Mara Rosa Ochoa Camposeco
	Claudia Hunot
	María Concepción Sánchez Rovelo

DIRECTORIO DE ARCHIVOS LATINOAMERICANOS DE NUTRICION

Editor General	José Félix Chávez Pérez
Editor Asociado	Maritza L. de Jiménez

MIEMBROS DE CUERPO EDITORIAL PERIODO 2001-2003

Juan de Dios Alvarado
Héctor Araya
María Laura Arias E.
Jaime Ariza M.
Guillermo Arroyave
José María Bengoa
Adriana Blanco M.
Héctor Bourges R.
Ricardo Bressani
Jesús Bulux
Benjamín Caballero
Germán Camejo
Sara J. Closa
Omar Dary M.
Elizabet Dini G.
J.E. Dutra de Oliveira
Juan Ignacio Egaña
Patricia R. de Ferrer
María N. García Casal
Marisa Guerra M.
Werner G. Jaffé
Gladys Henriquez P.
Eva Hertrampf
Patricio Hevia O.
Miguel Layrisse
Luis López Valladares
Julio Sergio Marchini
María L.P. Martín de Portela
Lilia Masson Salaué
Reynaldo Martorell
Rafael Monge R.
Josefina Morales de León
Santiago Muzzo B.
Rosa María Ortega A.
Saturnino de Pablo
Nelly Pak
Emma W. de Penna
Ernesto Pollitt
Myriam Puig A.
María Ester Río
María Elena Sambucetti
Nilson E. de Sousa
Nora Slobodianik
Liseti Solano R.
Noel W. Solomons
Luiz C. Trugo
Ricardo Uauy D.
Mirtha E. Valencia
Mauro Valencia J.
Yolanda H. de Valera
Mario Villarroel T.
Tomás Walter
Carolyn Jane Wyatt
Enrique Yáñez S.

Archivos Latinoamericanos de Nutrición

Suplemento -Volumen 51. N° 1, Marzo 2001

Contenido