AN ABSTRACT OF THE THESIS OF

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Title: CHELATING AGENTS IN THE DRUG DOSAGE DESIGN OF IRON PREPARATIONS

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The role of iron in the body is well known for it is a component of hemoglobin, myoglobin, cytochrome, enzyme catalase and peroxidase. Iron deficiency anemia is widespread among vulnerable groups in industrialized societies and in the general population of tropical developing areas. In the United States there are ten million women with no iron reserves to meet the physiological needs of menstruation and pregnancy.

The normal diet has five times the total amount of iron needed to maintain iron balance but, unfortunately, less than ten percent is absorbed. The absorption of iron has been increased by combining it with reducing agents such as ascorbic acid. Also it was found that protein uptake is necessary for adequate iron absorption.

Increase in absorption was noted when iron was combined with chelating agents. It was suggested that these chelating agents bind iron strongly, forming a stable ring

complex which seems to be necessary to maintain it in a soluble and permeable form.

The purpose of this thesis research was to determine the effect of amino acids on iron absorption. The amino acids were glycine, l. proline, d.l. tryptophan, l. asparagine, d.l. alanine, d.l. phenylalanine, d.l. leucine, serine, d.l. methionine, and l. isoleucine. Ferrous sulphate was combined with each of the amino acids in a molar ratio of 1:2. Iron-59 was used as a tracer. At various time periods after oral administration to rats, blood samples were collected from the rats' toes. At the end of four hours, the animals were sacrificed and liver, kidney, muscle, heart and brain samples were taken from the animals. All blood and organ samples were counted using a solid scintillation counter.

All amino acids increased iron absorption, but the increase was most notable in asparagine, and then glycine. No correlation was found between stability constants of iron with amino acids and initial rate of absorption or area under the curve. In the organs, iron was found mostly in the liver. Lesser amounts were found in the heart and kidney, and very small amounts in the muscle and brain.

Glycine - iron and control tablets were prepared using the direct compression method. Glycine was chosen rather than asparagine because it is much cheaper. These tablets were activated in the OSU TRIGA Reactor for four hours with

a neutron flux of $3x10^{12}$ neutron/cm^2/sec.

These tablets were administered to groups of four rabbits. Blood samples were collected from their ears using the same sampling times as for rats. At the end of four hours the animals were sacrificed and organ samples were taken from the animals. All these samples were counted as before. Results were analyzed using PROPHET[1] and show that iron is absorbed much better when administered with glycine than when administered alone.

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[1]The organization and analysis of the data base associated with this investigation were carried out in part using the PROPHET system, a unique national resource sponsored by NIH.

Chelating Agents in the Drug Dosage Design of Iron Preparations

by

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CHELATING AGENTS IN THE DRUG DOSAGE DESIGN OF IRON PREPARATIONS

I. INTRODUCTION

Statement of the Problem

Iron-deficiency anemia is widespread among vulnerable groups in industrialized societies (1) and in the general population of tropical developing areas. In the United States there are five million women with iron deficiency anemia while ten million have no iron reserves to meet the physiological needs of menstruation and pregnancy (2, 3). In Central America, 15% of the total non-pregnant population and 24% of pregnant women at term are anemic based on low serum iron and percent saturation of total iron binding capacity which are associated with anemia (4, 5).

The capacity of the body to excrete iron is very limited and therefore, the iron content of the body must be regulated by changes in iron absorption. For adult males, the normal daily loss of iron is 0.6 mg from the gastrointestinal tract, 0.2 mg from the skin and 0.1 mg via the urinary tract, totaling 0.9 mg daily. The menstruating female loses an additional 0.4 mg per day averaged over a month, to give a daily total loss of 1.3 mg (6). Absorption of these amounts is therefore sufficient to maintain iron equilibrium. However, the

recommended dietary allowance of 18 mg iron daily for women during the reproductive years is not always achieved and with a typical intake of 10-11 mg (1, 6) in the United States a woman must absorb 12-13% of the daily intake to maintain iron equilibrium, whereas the male obtains sufficient bodily iron content by absorbing 6% of the customary intake of 16 mg iron daily. This leaves the woman with less reserve capacity for increasing absorption still further to the maximum of 25% in order to meet added demands, such as pregnancy, and to maintain adequate iron stores. (6).

The standard dosage form available on the market contains ferrous sulphate (7). There are side effects associated with all dosage forms containing iron. These include nausea and gastrointestinal irriations (8).

It has been proposed that since amino acids have a chelating effect upon iron, then there is the possibility that iron absorption might increase if iron is administered with amino acids (9). Kroe et al. (9, 10) used phosphate buffer in experiments to study the effect of amino acids on iron absorption. The results may not be quantitatively correct since phosphate decreases iron absorption both in vivo and in vitro (11), which confounds the results (9, 10).

The only iron chelated tablets available are those produced by Fibertone and Hudson Companies (12). Iron in

these tablets is chelated to protein molecules. Unfortunately, there is no data available about these products. Also, the absorption of iron has been increased by combining it with reducing agents such as ascorbic acid (13-15) and iron with ascorbic acid tablets are also available (12).

Purpose of the Study

This study was initiated in order to formulate an oral iron preparation that has high absorption. The effects of ten amino acids on iron absorption were tested. The amino acids are glycine, l. proline, d.l. tryptophan, l. asparagine, d.l. alanine, d.l. phenylalanine, serine, d.l. methionine, d.l. leucine and l. isoleucine. Iron-59 was used as a tracer. Solid scintillation was used for counting.

The study entailed:

1. The determination of stability constants for the above mentioned amino acids and iron.
2. The effect of these amino acids on the absorption of iron, tested on groups of six rats. Blood and different organ samples were counted. The results were analyzed for correlation with the stability constants of the amino acids and iron.
3. Tablets containing iron and an amino acid were prepared. The amino acid of choice was the one which

increased iron absorption most. Control tablets were also prepared.

4. The above tablets were activated at the OSU TRIGA reactor.

5. These activated tablets were given to groups of four rabbits. Blood and organ samples were counted. Results were compared.

II. THEORY

Iron

Distribution and Types of Iron in Nature

Iron is widely distributed in the animal body. Iron is easily oxidized or reduced, and thus is found as a minute but vital part of certain enzymes concerned with electron transfer (the cytochromes, cytochrome oxidase, succinic dehydrogenase, xanthine oxidase). Normally, about 70% of human body iron is functional or "essential" iron (i.e., in hemoglobin, myoglobin, and intracellular iron containing enzymes) and 30% is storage or "nonessential" iron (i.e., in hemosiderin and ferritin). In women, the storage reserve tends to be less than half that in men (8).

Foods high in iron content include organ meats (liver, heart), wheat germ, egg yolks, meat, fish, milk and milk products and most nongreen vegetables (7, 8).

Quantitative Significance of Iron in the Body

In the adult man there is 4-5 g of iron. This makes iron one of the most important metals in mammals (see Table 1). Other biologically significant heavy metals are found in smaller amounts (11). The demand for iron varies during

Table 1. Content of iron in the body compared with other metals.

Metals*	Content per 70-Kg man (mg)	Intake with diet per day (mg)
Fe	3.9 -5.0	12-15
Cu	0.12-0.15	2- 5
Zn	1.9 -2.0	10-15

*Mn, molybdenum, cobalt and chromium occur in the human body in an amount of 2 mg or less.

the lifetime of man (Tables 2 and 3). This was calculated assuming that 10% of iron ingested with food is absorbed (16, 17). Therefore, the amount of iron ingested must be tenfold the daily requirement. There is 6 mg of iron per 1000 Kcal in the average American diet. Therefore, iron intake from dietary sources is borderline for teenage girls and women, and may be inadequate for infants and pregnant women (18).

Iron Metabolism

Absorption. Absorption of iron occurs mainly in the small intestine. Absorption can occur along the entire length of the alimentary tract, but it is greatest in the duodenum and becomes progressively less distally (8). Two mechanisms exist for iron absorption across the mucosa of the upper intestine. These occur simultaneously; an active transport process with enzymatic or carrier characteristics

Table 2. Daily demand for iron in man at various ages.

	Absorbed iron requirement, mg/day
Infants	0.5-1.5
Children	0.4-1
Adolescents	1 -2
Normal men and nonmenstruating women	0.5-1
Menstruating women	0.7-2
Pregnant women	2 -4.8

Table 3. Iron requirements for pregnancy.

	Average mg	Range mg
External iron loss	170	150- 200
Expansion of red blood cell mass	450	200- 600
Fetal iron	270	200- 370
Iron in placenta and cord	90	30- 170
Blood loss at delivery	150	90- 130
Total requirement*	980	580-1340
Cost of pregnancy+	680	440-1050

*Blood loss at delivery not included.

+Expansion of red-cell mass not included.

and a first-order passive transport process, perhaps diffusion, that is operative primarily at doses of iron exceeding those in a normal diet (8, 11).

Iron is absorbed more easily in its ferrous form; ferrous iron passes into and through the mucosal cell directly into the blood stream, where it is immediately bound to transferrin. Small excesses of iron within the villous epithilial cells are oxidized to the ferric state, where they combine with the protein apoferritin to yield ferritin, which is eventually excreted in the feces incorporated in villous absorptive columnal epithelial cells. This "mucosal block" mechanism for handling excess iron can cope with only small excesses, and it is overwhelmed by large amounts of iron (13, 14).

To have a composite picture of iron absorption, Figure 1 shows ionic iron in the human intestine, there it adsorbs to specific receptors in the brush border of the mucosal cell. From these receptors, the iron is transferred to the cytoplasm of the mucosal cell by an active process involving energy. The absorbed iron appears to be present in the cytosol in small molecular weight form, possibly chelated to amino acids, and in equilibrium with iron-poor ferritin present there. On reaching the serosal surface of the cell, it becomes attached to transferrin for transport in the plasma. The serosal transfer mechanism probably also involves receptors in the cell membrane and may be

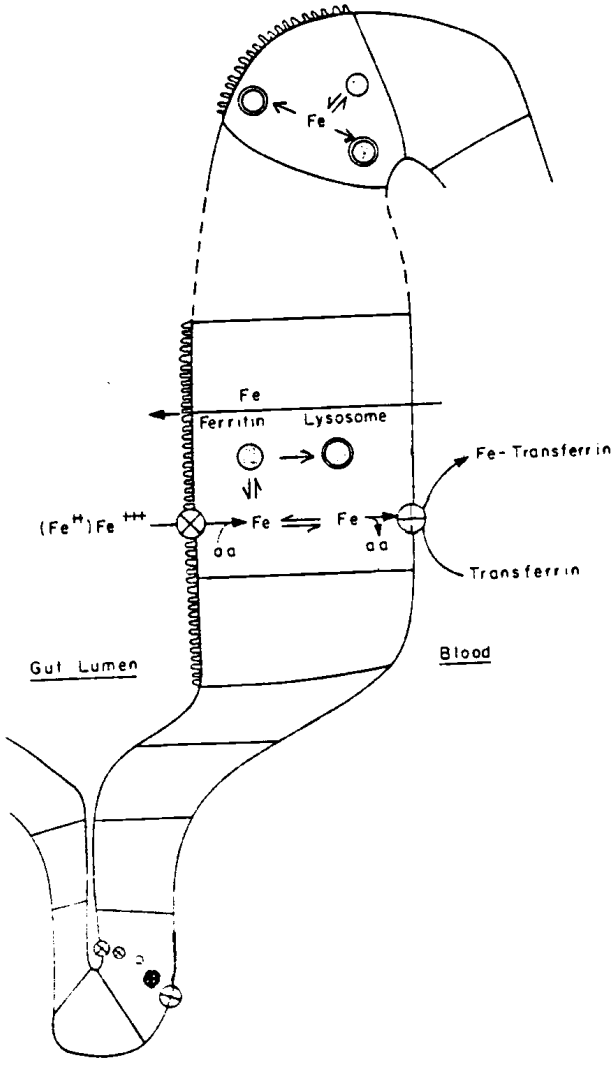


Figure 1. Composite picture of the mechanism and regulation of iron absorption in the intestinal mucosa (3).

independent of cell energy. Iron in the mucosal cell not transported to the plasma is retained in the cell, probably mostly as ferritin until it is sloughed off at the tip of the villus and thus returns to the lumen of the gut. The iron status and erythropoietic needs of the individual, as well as some other factors, regulate iron absorption, particularly at the level of serosal transfer. Finally, iron can enter the mucosal cell from the plasma,especially their mitochondria,and can pass into the lumen by active extrusion, notably in the lower part of the small intestine (3).

Transport. A glycoprotein bi-globulin called transferrin transports ferric iron after it has been absorbed to the bone marrow, where the iron is incorporated into hemoglobin (8).

Fate. The distribution of about 3.5 g of total body iron in the 70 kg male is shown in Figure 2. It exists exclusively complexed to protein (transferrin, ferritin) or in heme (hemoglobin, myoglobin, heme enzymes). Two-thirds is in hemoglobin, one-fourth is in the body's reserve stores as ferritin and hemosidein, 3% is in myoglobin, 0.5% is in heme enzymes, and a minute fraction is in transferrin (0.1% of body iron) (8).

Excretion. The body excretes only minimal amounts of iron. Normally this amounts to 0.5 to 1 mg daily. This excretion is in nails, hair, feces, and urine, mainly as

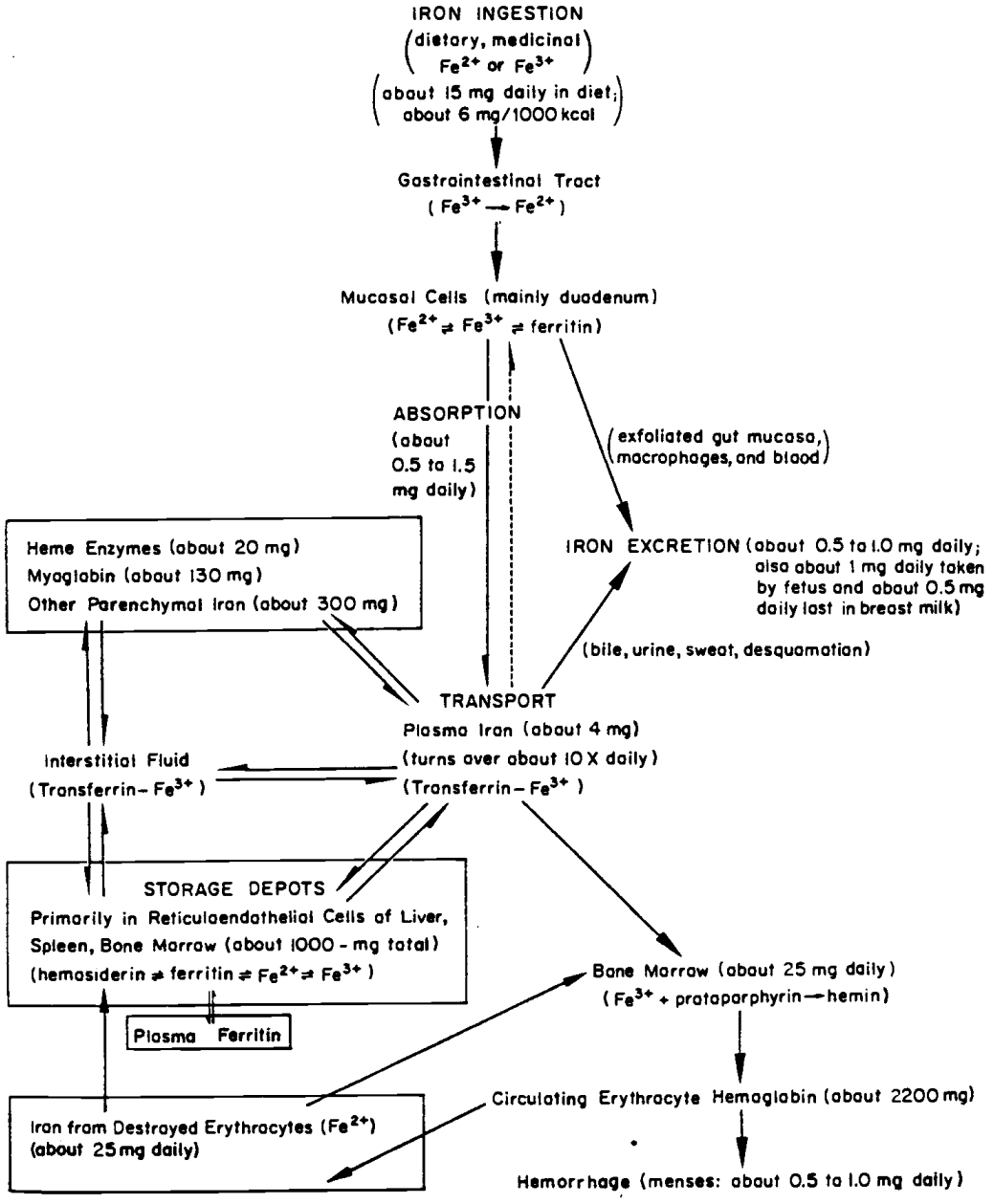


Figure 2. Normal absorption, metabolic pathways, and excretion of iron (8).

enzyme iron of exfoliated cells, but there are also trace doses in bile and cell-free sweat. The main excretory pathway is by way of epithilial cells sloughed from the skin and gastrointestinal tract, which carry out unneeded iron as ferritin (19, 20).

Adverse Reactions

Side Effects of Iron Therapy. Constipation occurs in about 10% of patients, diarrhea in about 5%, and nausea and epigastric pain in about 7% to 90% (8). This can be reduced by prescribing iron just after meals instead of between meals. When iron balance is restored to normal, therapy with iron should be stopped to avoid iron-overload hemosiderosis (19, 20).

Overdosage Toxicity. Iron poisoning is very rare in adults; it would be unlikely in adults with ingestion of doses of ferrous sulphate below 50 g. Acute poisoning in children can result from ingestion of doses in excess of 1 g (8). Signs and symptoms are those of gastrointestinal irritation and necrosis, often associated with nausea, vomiting and shock; they include pallor or cyanosis, lastitude, drowsiness, hematemesis, diarrhea of green and subsequently of tarry stools, and cardiovascular collapse. If death does not occur within 6 hours, there may be a transient period of apparent recovery followed by death in 12-48 hours. The corrosive injury to the stomach may result

in subsequent pyloric stenosis or severe gastric scarring (19). Hemochromatosis, i.e. the deposition of hemosiderin in the paranchyma of liver and other organs, has occurred in a few patients given iron for many years. It occurs idiopathetically and following multiple transfusion (7).

Treatment of Chronic Toxicity. Gastric lavage can be employed with a lavage solution that contains phosphate ion to combine with dissolved iron (8). Following lavage or induced emesis, a volume of phosphate solution appropriate to the size of the victim should be left in the stomach to decrease the solubility of iron remaining in the lumen. Specific treatment depends upon the use of chelating agents, which complex with metal ions, removing them from solution and incorporating them into less toxic chelate (7). Deferoxamine forms a nontoxic complex with iron with such avidity that it is able to extract iron from transferrin and ferritin. Figure 3 shows the chemical structure of ferroxamine (7).

Preparations and Dosages

Oral Preparations. Ferrous sulphate is usually the standard drug. Several preparations are available on the market (7). The most popular are shown in Table 4.

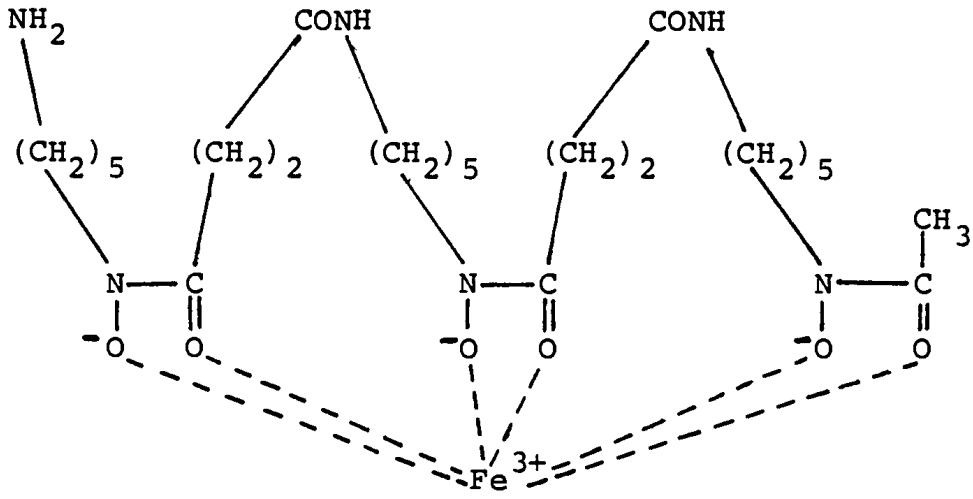


Figure 3. Chemical structure of ferroxamine. (Feroxamine without the chelated iron is deferoxamine-desferal.)(7)

Table 4. Iron for oral administration: dosages and preparations available (7).

	Iron content	Usual adult	Iron/ dose	Preparations available
Ferrous sulphate	20%	300 mg	60 mg	•Tablets (plain, coated or enteric-coated), 200, 300, and 325 mg •Controlled release tablets, 525 mg •Syrup or elixir, 125 mg/1 ml, 150mg/5 ml, and 220 mg/5 ml •Drops, 75 mg/0.6 ml
Ferrous sulphate, exsiccated (eg. Feosol)	30%	200 mg	60 mg	•Tablets and capsules, 200 mg; sustained release capsules, 150 mg
Ferrous gluconate (e.g., Fergen)	12%	300 mg	36 mg	•Tablets (plain, coated or enteric-coated), 325 mg •Elixir, 300 mg/5 ml
Ferrous fumarate (e.g., Toleron, Ircon)*	33%	200 mg	66 mg	•Tablets (plain, coated, or chewable), 200, 225, 250, and 325 mg •Suspension, 100 mg/5 ml
Ferroglycine sulfate (e.g. Ferronord)	16%	250 mg	40 mg	•Tablets (coated), 250 mg
Ferrocholinate (iron choline citrate; e.g., Ferrolip, Chel-Iron)	12%	330 mg	40 mg	•Tablets (coated), 330 and 360 mg •Syrup, 280 mg/1 ml and 417 mg/5 ml
Polysaccharide-iron complex (e.g., Niferex)			50 mg	•Tablets (coated), 50 mg elemental iron •Elixir, 100 mg elemental iron per 5 ml

Injectable Iron Preparations:

1. Iron-dextran injection (Imferon). The iron dextran complex contains 5% iron, i.e., 50 mg of iron per ml of solution.
2. Iron sorbit (Jectofer) is an iron-sorbiton complex comparable to iron-dextran complex. It also contains 50 mg of iron per ml.

Ways to Affect Iron Absorption

Extensive research has been done to study factors that affect iron absorption. It was found that iron is affected by the following:

Metal Precipitating Agents. Examples are: soluble carbonates, oxalates and phosphates which combine with metal ions to form insoluble complexes which are poorly absorbed. Also, the bicarbonates are effective in reducing iron uptake due to the formation of iron in a macromolecular form which cannot be absorbed (21).

Reducing Agents such as cysteine and hydroquinone increase the absorption of iron (11). The most famous example is ascorbic acid. The increase in iron absorption from a semisynthetic meal was directly proportional to the amount of ascorbic acid added over a range of 25 to 1000 mg (14). The ratio of iron absorption with/without ascorbic acid at these two extremes was 1.65 and 9.57 respectively. The reason behind this lies in the fact that

reducing agents keep iron in the reduced form (22-26).

Iron-Chelating Sugars such as fructose increase iron absorption (22). This was attributed to the fructose ability to form a stable complex with iron, thereby facilitating the transport of iron across the intestinal mucosa.

Composition of Diet influences the amount of iron absorbed. For example, iron fortification is necessary in Southeast Asian diets which contain a lot of rice (27-29). Alcohol and tea decrease iron absorption (30-32). Also, it was found that protein uptake is necessary for adequate iron absorption (33-35).

Deferoxamine. As mentioned before, it is used for iron toxicity (36). It binds strongly with iron forming a nontoxic complex which is eliminated from the body (Figure 3).

Ferrous iron is much more absorbed than ferric iron (37-39). In a medium with a pH value slightly above 3, solubility of ferric iron decreases because the sparingly soluble ferric hydroxides are formed (11). Ferric iron forms aggregates at pH 7.0 (40) and is much less soluble than ferrous iron at this pH (solubility products are approximately 10^{-36} for $Fe(OH)_3$ and 1.64×10^{-14} for $Fe(OH)_2$).

Amino Acids effect on the absorption of iron has been investigated by Kroe et al. (9, 10). The effect of

glutamine, aspargine, glutamic acid, serine, phenylalanine, proline, methionine, histidine and ethionine on iron absorption was tested. The activity of Fe-59 in the blood was found to be greater when the Fe-59 was administered orally with an amino acid than when it was administered alone (Figure 4). But, the results cannot be conclusive since phosphate was used as a buffer and it decreases iron absorption both *in vivo* and *in vitro* (11, 41). Peters *et al.* (41) studied the action of phosphate salts and phosphate-containing foods on iron absorption *in vivo* and compared the results to the interactions observed between iron and phosphate under conditions similar to those in the gastrointestinal tract. Phosphoproteins, calcium phosphates and phosphate salts were found to bind iron effectively and decrease dialysis across a membrane. Also, it was shown that phosphates act to accelerate the oxidation of ferrous iron to the ferric state and sequestering it as ferric phosphate which is precipitated (41).

Other Effects. Many other factors were studied that might affect iron absorption. The most famous are the decrease in absorption of iron due to calcium and phosphate salts, EDTA and antacids (42-44). Polychlorinated hydrocarbons stimulate iron absorption (45), while absorption of iron as supplements in infant cereal and infant formulas is low (46).

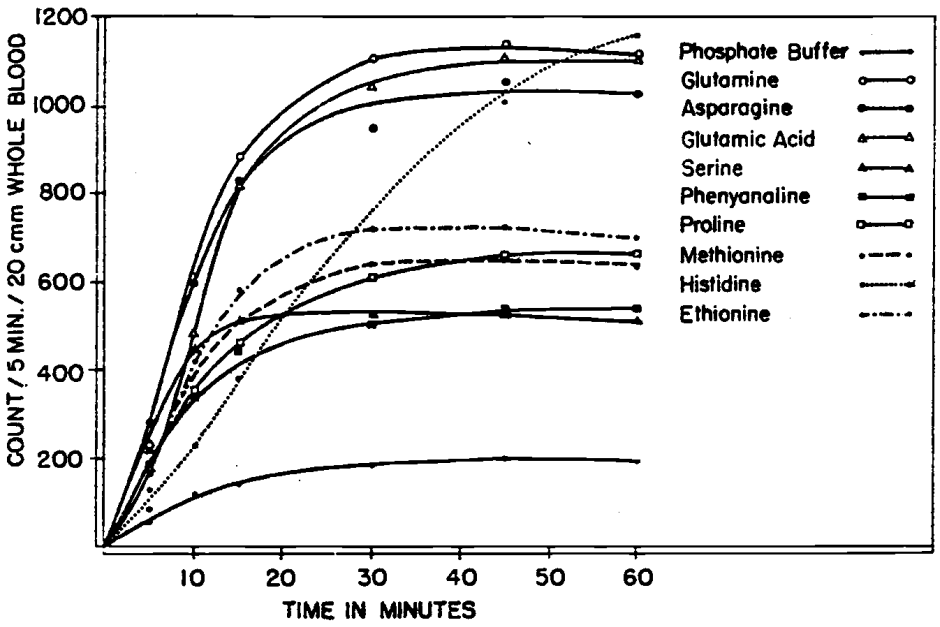


Figure 4. Fe^{59} activity curves in blood when various amino acids and phosphate buffers were administered (Figure 1 in reference (9)).

Stability Constants of Metal Complexes

During the past thirty years, much interest has been shown in obtaining dissociation constants for cations other than the hydrogen ion. The mass action equations are formally similar, but the results are customarily expressed as stability constants (47). It is convenient to write stability constants (which are the reciprocals of instability constants) as log K (and hence not as pK).

The Nature of Chelation

Any substance which can bind a proton can bind a metallic cation instead. At high proton concentrations, the proton will be greatly preferred, at low proton concentrations, the metallic cation may be preferred if it is present in great excess, or better, if a new ring can be formed by two groups in the molecule which simultaneously bind the metal. This simultaneous binding is termed chelation, and confers stability on the complex. If the pH is allowed to rise too high, the free metal cations may be precipitated as the hydroxide or as self-complexes (48).

In general, chelation through oxygen or nitrogen atoms takes place only when 5- or 6-membered rings can be formed, and of these, the 5-membered rings are more stable. In the presence of excess ligand, 2:1 complexes can be formed (47, 49). It is now agreed that the equilibrium

between a complex forming agent and an ion is usually thermodynamically reversible: it occurs instantly and without appreciable energy of activation. Hence these equilibria are correctly represented by mass action equations (47).

Designing a Chelate Drug

The medical importance of chelating agents hinges on the fact that metals play many critical roles in the life of living organisms. In the human body metabolism depends not only on sodium, potassium, magnesium and calcium but also to a considerable degree on trace amounts of iron cobalt, copper, zinc, manganese and molybdenum. On the other hand, certain other metals, even in minuscule amounts, are highly toxic to the body. It is apparent, therefore, that chelate drugs with appropriate properties could play several different therapeutic roles. Various chelating agents might be designed (1) to seek out toxic metals and bind them in compounds that will be excreted, (2) to deliver essential trace metals to tissues or substances that require them, and (3) to inactivate bacteria and viruses by depriving them of metals they need for their metabolism or by delivering metals to them that are harmful. All three of these hopes have been realized (17).

Obviously, in the design of chelate drugs for specific purposes, everything depends on the relative affinity of the chelating drug for the given metal. To be effective in removing a metal, the drug must seize and hold it more strongly than any biological substance that might be available to form a compound with the metal. To deliver a metal to the cells, on the other hand, the grip of the carrier drug must be comparatively loose; that is, the drug's affinity for the metal must be less than that of the substance to which the metal is to be surrendered. Hence an important part of the research on chelating agents has been the development of ways to measure the affinities between various substances and metal ions (17).

Stability Constants

Stability constants are determined in order to assess the affinity between ligands and metals, a property which varies between wide limits. For the 1:1 complex of glycine and ferrous ion, K_1 (the first stability constant), is given by equation (48)

$$K_1 = \frac{[H_2N \cdot CH_2 \cdot CO_2 \cdot Fe^+]}{[Fe^{+2}]\,[H_2N \cdot CH_2 \cdot CO_2^-]}$$

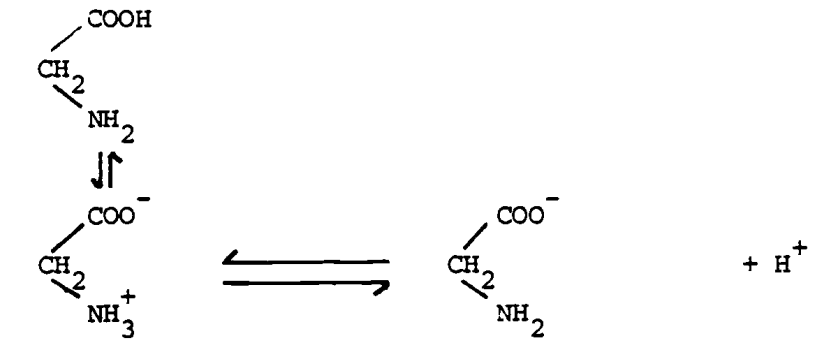
and for the 2:1-complex (48)

$$K_2 = \frac{[H_2N \cdot CH_2 \cdot CO_2 \cdot Fe \cdot O_2C \cdot CH_2 \cdot NH_2]}{[H_2N \cdot CH_2 \cdot CO_2 \cdot Fe^+]\,[H_2N \cdot CH_2 \cdot CO_2^-]}$$

In each case the formed complex is in the numerator, and the substances which form it are in the denominator. For our purposes, the product of the constants, i.e., the overall stability constant "K_s" is required. $K_s = K_1K_2$ (47-50).

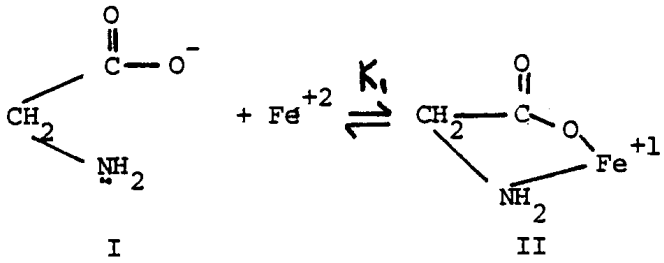
Amino Acid Chelation with Iron

To explain chelation of amino acid with iron, glycine will be used as an example. When amino acid is in solution a zwitterion and a free complex forming species (I) are formed. The free complex forming agent is the one which is capable of chelation. Two free complex forming agents bind and chelate with one iron forming chelate (III) (47-51).



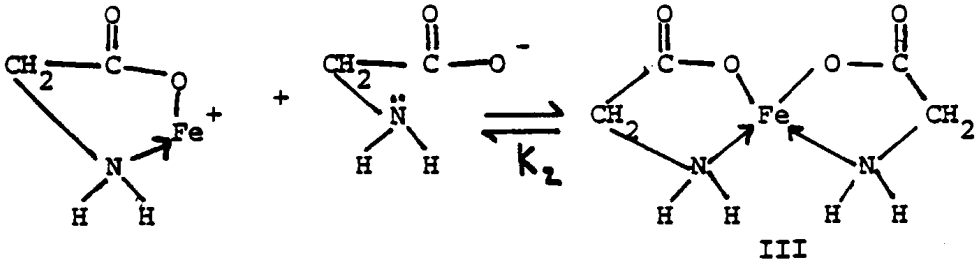
Zwitter iron

I Free complex forming species



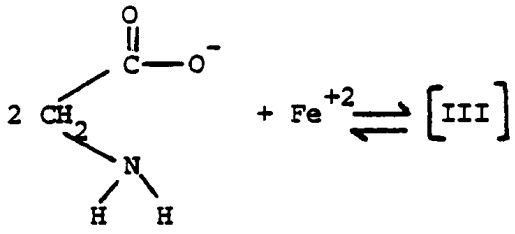
I

II



III

$$K_1 = \frac{[II]}{[I][Fe^{+2}]} \quad , \quad K_2 = \frac{[III]}{[I][II]}$$



$$2[I] + Fe^{+2} \rightleftharpoons [III] \qquad \text{TOTAL REACTION}$$

$$\therefore K_s = \frac{[III]}{[Fe^{+2}][I]^2} = \frac{K_2[II][I]}{[Fe^{+2}][I]^2} = K_1K_2$$

$$\therefore K_s = K_1K_2$$

Neutron Activation Analysis

Introduction

By bombardment with neutrons (charged particles and high-energy photons) stable atoms can be transformed into radioactive isotopes. By measuring the radiation emitted from a bombarded sample, the presence of one or more elements of particular interest in the sample often can be determined quantitatively. This approach to detection of trace

elements and analysis of the chemical composition of a sample is referred to as activation analysis. Activation analysis is now being used widely in a variety of analytical problems where high sensitivity is required. The greater part of this work is being performed with thermal neutrons. The reason for this is the general availability of nuclear reactors and the large cross section of many nuclides for thermal neutron capture. Sensitivity of 10^{-9} grams is not unusual (52-54).

Radiation Sources for Activation Analysis

Activation analysis of a sample may be performed with any type of radiation which transforms stable atoms into radioactive isotopes. For example, slow and fast neutrons, protons, deuterons[2], triton,[3], α particles, and high energy x- and γ-rays have been employed for activation analysis. Most often samples are activated with neutrons, especially slow (thermal) neutrons (53).

Most elements with Z[4] above 10 exhibit reasonable cross sections for activation by thermal neutrons. Usually, the activation is achieved by an (n,γ) reaction, and the product nucleus is a radioactive isotope of the target

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[2]Deuteron = nucleus of deuterium.

[3]Triton - nucleus of tritium.

[4]Z = atomic number

element. The four major compounds (H, C, N and O) of biologic tissue have atomic numbers less than 10 and are activated only rarely by thermal neutrons. Consequently, thermal neutrons are useful for analysis of trace elements in biologic tissue, because the higher Z trace elements can be activated and detected without interference from activated products of the four low-Z elements which constitute the major portion (96%) of biologic tissue (52, 55, 56).

Nuclear Reactors

In a nuclear reactor, a fissionable nuclear fuel such as ^{235}U is placed in a defined geometry within the reactor core. Fast-moving neutrons released as the ^{235}U nuclei fission are slowed to thermal energy ($\sim$0.025 eV) by a low-Z moderator such as water, heavy water or beryllium. The slowly moving neutrons are absorbed by additional ^{235}U and a chain reaction is maintained. The rate of absorption of slow neutrons by fissionable nuclei determines the fission rate for the reactor, and is controlled by placing inert neutron absorbers in the reactor core. Typical neutron absorbers, usually termed control rods, include boron and cadmium. Heat generated in the reactor core is removed by a coolant such as water or one of a variety of gases or liquid metals (53, 54).

Activation of Prepared Tablets

After preparing the iron tablets, there was the problem of testing them *in vivo* in rabbits. A new method which involves neutron activation analysis was designed. Tablets of two types were activated. The first type was iron tablets without glycine (control), and the second type was iron with glycine. The advantages of this method are numerous. To include some: it is safe, economical, fast, practical in elemental analysis, and last but not least, it is not destructive to the sample (57).

After having a computer search on the literature since 1960, no reports of activation were found of any type of tablets in order to test them *in vivo*.

Equation for Neutron Activation

Activation analysis equation:

$$A = N\phi\sigma(1 - e^{-\lambda T})e^{-\lambda t}$$

where A = activity at end of irradiation (dis/sec)

N = number of atoms

ϕ = flux (neutrons/cm^2-sec)

σ = cross section (cm^2/atom)

λ = decay constant (sec^{-1})

T = length of irradiation (sec)

t = time elapsed between activation and measurement

The induced activity depends upon the neutron flux density, the cross sections for activation of the target atoms, the irradiation time, and the number of target atoms in the sample. The activity at the time of measurement of the radiation depends also upon the half-lives of the isotopes of interest and the time elapsed between activation and measurements (53, 54). Activation analysis is subject to a variety of errors. These include errors due to flux gradients, self shielding, and interfering nuclear reactions (53).

III. EXPERIMENTAL PROCEDURE

Determination of Stability Constant of Ten Amino Acid Complexes with Iron

Ten amino acids: glycine, l.-proline, d.l.-tryptophan, l.-asparagine, d.l.-alanine, d.l.-phenylalanine, d.-serine, d.l.-methionine, d.l.-leucine and l.-isoleucine were obtained from J.T. Baker.

The method of determining the stability constant is a standard method and was used for determining all types of chelations between amino acids and metals (47-50).

The above amino acids were all dried for one hour before weighing. Then each amino acid (50 ml of 0.01 M - aqueous solution) was titrated with 0.1 N KOH, first in the absence of metals and then in the presence of one equivalent of the ferrous salt (0.005 M). Thus the molar ratio of the complex forming agent to metal was 2:1 for all bivalent ions. The amount of alkali used in each titration was 5 ml (i.e. the equivalent of the amount of complex-forming agent) and this was added in ten equal portions of 0.5 ml.

The experiment was done in a water bath that was at 37°C and the amino acid solution was stirred continuously by N_2 to maintain an inert atmosphere above the solutions. Boiled water was used since air must be removed from the

system to avoid change in base concentration. The pH was recorded after each addition (47-50).

Preparation of the Buffer

A potassium hydrogen phthalate, sodium hydroxide buffer solution was prepared with a final pH of 6.0. Such a buffer contains 50 ml of 0.1 M KHP (potassium hydrogen phthalate) and 43.7 ml of 0.1 M NaOH diluted to 100 ml (58).

Procedure Involving Rats

Solutions of iron complexed with each of the ten amino acids, as well as the control solution alone, were administered orally to groups of six rats. The amino acids were glycine, l.-proline, d.l. trypotphan, l.-asparagine, d.l.-alanine, d.l.-phenylalanine, serine, d.l.-methionine, d.l.-leucine, l. isoleucine. All these amino acids, except asparagine, have one amine and one carboxyl group. Thus, two molecules of each amino acid will complex with one atom of iron.

Male albino rats, weighing 250-270 gm each, were used. Rats were fasted 24 hours before the start of the experiment to prevent complexing agents present as food constituents in the intestinal lumen which may affect iron absorption (9). Iron in the form of ferrous sulphate was used in all experiments. Test solutions were prepared by

adding an amino acid and $Fe^{59}SO_4$ to aliquots of KHP-NaOH buffer so that the resulting Fe-59 activity was 10 μc/ml. The amino acid concentration was 0.4 M, ascorbic acid was 0.5 M and ferrous sulphate 0.2 M. A control solution was prepared using the same procedure, but not containing any amino acid.

An intubation tube[5] was put in the rat's mouth and 1 ml of test or control solution was delivered into his stomach. The tube was then removed and the rat was kept under anesthesia (ether) during the whole experiment.

At time periods of 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes, after administration of the solution, 20 lambda of blood samples were collected from the toes of the rat using capillary tubes. The capillary tubes were placed into counting vials. Each sample was counted for 10 minutes in a solid scintillation counter chamber.[6]

At the end of the four hours, the animals were sacrificed by ether and liver, kidney, heart, brain and muscle samples were taken from the animals, weighed and then counted for ten minutes.

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[5]Manufactured by Popper and Sons, Inc., New York.

[6]Tracer Northern TN-1705 MCA 40 cc NaI(Tl) detector.

Production of Tablets

Direct compression was used to produce tablets.[7] Two types of tablets were prepared. The first type has glycine and ferrous chloride. The second type, the control one, does not have glycine.

Type 1 (average weight of each tablet was 0.4152 gm)

Formula for 1 tablet of Type 1 -

Dipac[8]	296.6189 mg	71.44%
Mg Stearate	29.6452 mg	7.14%
Glycine	39.5270 mg	9.52%
Ferrous chloride	49.4088 mg	11.90%

Type 2 (average weight of each tablet was 0.4053 gm)

Formula for 1 tablet of Type 2 -

Dipac[8]	328.1309 mg	80.96%
Mg Stearate	28.9384 mg	7.14%
Ferrous chloride	48.2307 mg	11.90%

Disintegration tests were done for both types using the Vanderkamp USP tablet and disintegration tester (59).

Activation of Tablets

Tablets were put in small scintillation bags.[9] Each

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[7]Single-Punch Tablet Machine, Model TPK-12, manufactured by Chemical and Pharmaceutical Co., Inc.

[8]Manufactured by Armerstone, prepared by co-crystallization of 97% sucrose and 3% dextrios.

[9]Manufactured by New England Nuclear

bag was sealed by heat and then put in a polyvial.[10] Then the polyvials were sealed by heat. All these samples were put in the OSU TRIGA reactor in the Lazy Susan and activated with a neutron flux of $3x10^{12}$ neutrons/cm^2/sec. The irradiation time was four hours.

Procedure Involving Rabbits

The iron-glycine activated tablets, as well as the control activated tablets, were each tested on groups of four rabbits. Female rabbits were used, weighing 2.0-3.0 kg each. Each rabbit had his ears shaved using an electric shaver and Nair ^{R} ointment. Then, the rabbit was put in a rabbit restrainer leaving his head out, and was given four tablets that had a total activity of 1.0824 µcurie.

At time periods of 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240 minutes after administration, 100 lambda blood samples were collected from the rabbit's ears using capillary tubes. Bleeding was initiated by pricking the vein of the rabbits' ear with a 20 gauge needle. Procaine was used as a local anaesthetic. These samples were placed into counting vials. Each sample was counted for 4000 seconds in a solid scintillation counter chamber.

At the end of four hours, the animals were sacrificed by ether and liver, kidney, muscle and heart samples were

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[10]Manufactured by Olympia Plastic Co., Los Angeles, Calif.

taken from the animals, weighed and then counted in the same manner as before.

IV. RESULTS AND DISCUSSION

Figures 5 to 11 summarize the results of this project. Tables 5 to 26 show iron concentration in blood and different tissues foolowing the oral administration of each amino acid-iron complex. The iron concentration presented was calculated from the iron activity and does not include iron present before the administration. All data were analyzed using the National Institute of Health Computer Prophet System. Results show that there were greater amounts of iron absorbed when iron was administered with an amino acid than when it was administered with buffer alone.

For all these amino acid-iron complexes, the amount of iron in the blood versus time was found to fit a polynomial of degree 3 (r^2= 0.9857- 0.991). The empirical equation for all iron concentration curves is therfore:

$$Y= B_o + B_1T + B_2T^2 + B_3T^3$$

The initial rate of absoption is the tangent to the iron concentration curve at time zero and is equal to B_1 after differentiating dy/dt.

The effects of different amino acids on iron absorption from time zero to four hours were compared with the control by comparing the area under the curve for each amino acid-iron complex from time zero to four hours. When integrating the above equation over the experimental period of four hours,

the area under the curve (AUC 0-240 min) for each amino acid and amino acid-iron complex can be obtained as:

$$\int_0^{240} y\,dt = \int_0^{240} (B_0 + B_1T + B_2T^2 + B_3T^3)\,dt$$

$$= 240\ B_0 + 28800\ B_1 + 4608 \times 10^3\ B_2$$

$$+ 82944 \times 10^4\ B_3$$

It is evident from these experiments (Table 27 and Figure 5), that iron appears more quickly and in greater amounts in blood if it is administered with an amino acid than alone. The amount of circulating iron is a reflection of four factors: (1) the amount and rate of absorption, (2) the amount and rate of deposition in the tissues, (3) the release of iron from the tissues into the circulation, and (4) loss of iron from the body. Also, it can be seen from the curves (Figure 5) that the quantity of iron appearing in the blood was not identical for the different amino acids.

The amino acids could be grouped into three categories on the basis of increase of iron absorption over the control. Category I consists of asparagine and glycine which increase the area under the iron concentration curve by 265.22% and 224.52% respectively (Figure 5); Category II consists of serine where the increase was 114.29% (Figure 5); Category III consists of alanine, proline, isoleucine,

leucine, methionine, tryptophan and phenyl alanine where the increase was 64.46%, 44.89%, 34.82%, 29.79%, 24.66%, 20.89% and 24.28% respectively (Figure 5). These curves are enlarged in Appendix B.

The initial rate of absorption was in the following order starting with the amino acid that increased the initial rate of absorption most: glycine, serine, leucine, methionine, isoleucine, tryptophan, alanine, asparagine, phenyl alanine and proline (Table 27).

At the end of four hours, the rats were sacrificed and tissue samples collected for counting. The uptake of iron by the selected tissues is shown in Figures 6 to 11. The concentration of iron in the tissues of animals receiving amino acid-iron complexes was consistent with iron levels in the blood. Iron concentration was highest in the tissues of animals receiving asparagine-iron and glycine-iron complexes, while it was low in the tissues of animals receiving the other amino acid-iron complexes. The fact that the tissue iron content at the end of the experiment was significantly higher in the animals receiving amino acid-iron complexes indicates that the elevated blood-iron activity in these animals is a reflection of increased absorption from the gastrointestinal tract.

In the tissues, iron was found mostly in the liver (Figure 6). Lesser amounts were found in the heart and

kidney (Figures 7 and 8), and very small amounts in the muscle and brain (Figures 9 and 10). This was consistent for the control experiments as well as all ten amino acid-iron complexes. The stores of iron are mainly in reticule endothelial cells, and thus are found in organs rich in such cells, especially liver. The amount of storage iron varies with the sources and also with demands made upon it for hemoglobin formation (8).

The amount of iron in the gut was determined after administration of phenylalanine-iron and methionine-iron complexes where the increase in iron absorption was lowest and asparagine-iron complex where it was highest. The amount of iron in the gut was much higher in the case of phenylalanine-iron and methionine-iron complex (Figure 11). This too, is an indication that the elevated blood-iron activity in the animals is a reflection of increased absorption from the gastrointestinal tract.

Hober, in 1903, suggested that protein breakdown products participated in the absorption of iron (59). Also, the effect of proteins on iron absorption has been described as either positive or negative. Egg protein retards the absorption of iron (60, 61); it is not known whether the effect is related to its content of conalbumin. Conalbumin forms a stable complex with iron that in vitro drastically reduces the transfer of iron in jejenul segments of normal and anemic rats (11). Egg yolk also

contains phosphoproteins that form stable complexes with iron (41, 62).

An absorption-enhancing action of proteins has been reported repeatedly (9-11). This effect may account for the high fraction of iron in meat that is absorbed and for the enhancement of iron absorption from vegetable food by meat ingestion (63, 64). The differential effect of proteins on the absorption of iron probably can be attributed to the degree of proteolysis in different experiments (11).

It has been shown that amino acids in general are effective chelating agents (9-11) and that certain of these will affect the absorption of iron. It was suggested that amino acids facilitate absorption by chelating iron in the lumen of the gut, acting as vehicles for its absorption (9, 65). In 1977, Linder and Munro (3) suggested that iron-amino acid chelate goes into the muscosal cells. On reaching the serosal surface of the cell the amino acid leaves the iron and iron gets attached to transferrin for transport in plasma (Figure 1).

Theoretically, there are two types of iron complexes or chelates with different effects on the availability for iron absorption: 1) iron complexes or chelates of high effective stability that essentially do not release their metal for absorption; 2) iron complexes or chelates of lesser effective stability which do release iron for absorption. Various factors determine the degree of

metabolic utilization of iron, e.g. distribution in the organism, excretion, and, especially, ability of the organism to split the complexes and liberate the iron. As was mentioned before, EDTA and Deferroxamine (Figure 3) have high affinity for iron and thereby a very stable complex is formed. The organism cannot split such complexes and liberate the iron (7, 45). There are acceptor sites on the surface or within the mucosal cells that compete for iron with the ligands in the gut lumen. This is an important factor for understanding the role that iron competes with different stability complexes play by making iron available for absorption (17).

In the present study, no correlation was found between the stability constants for the amino acid-iron complexes tested and area under the iron concentration curve or with the initial rate of absorption (Figures 12 and 13). Thus, the stability constants are not predictive in the present study, because when the chelating drug is introduced into a living organism many interfering factors come into play. Substances in the organism will compete with the drug for the metal in which we are interested; conversely, various ions in the organism will compete with this ion for combination with the chelator. A famous example of this situation is the affinity between EDTA and mercury in the test tube is strong, in the body it is so weakened by interfering factors that EDTA is ineffective against

mercury poisoning. On the other hand, EDTA is quite effective in eliminating zinc from the body, because of the relative weakness of competing or interfering factors even though EDTA's absolute affinity for zinc is about a million times less than its affinity for mercury (12).

Effect of reducing power of food on iron absorption has been demonstrated repeatedly (19, 20, 21). Reduction of ferric to ferrous iron is favored by the reducing agents. The importance of reducing agents lies in the fact that iron is absorbed mainly in the divalent form (37-39). Also, it is now known that labilization of stable ferric complexes occur by reducing agents. This helps liberation of iron from ferritin (66,67). The most important reducing agent used is ascorbic acid. Ascorbic acid is added to many iron preparations in order to increase iron absorption. Tables 28, 29 and Figures 14, 15 show iron concentration in blood and different tissues following the oral administration of iron and ascorbic acid. It was found that the area under the iron concentration curves (time zero to four hours) increased by 31.59% (Figure 14). This occurred although ascorbic acid was given in much higher concentrations than ferrous sulphate (5:2), while the amino acid concentration compared to ferrous sulphate was (2:1).

Table 30 shows iron concentration in blood following oral administration of iron-glycine in the absence of

buffer. Figure 16 compares blood iron concentration of iron-glycine in the absence and presence of buffer. The area under the iron concentration curve of glycine-iron complex (time 0-4 hours) in the presence of buffer is 11.4% more than AUC (time 0-4 hours) of glycine-iron complex in the presence of buffer. The reason behind this lies in the fact that in the presence of buffer, the metallic cation binds better (48).

It was found that asparagine was much more expensive than glycine. Glycine costs $22.02/kg while asparagine was $114.84/kg[11]. So, glycine was used in the preparation of tablets. Also ferrous chloride was used rather than ferrous sulphate when preparing the tablets. The reason is because the tablets were to be activated, and the less radioactive the tablets are, the more it is convenient for handling them. S-35, with a half life of 88 days, is produced when activating ferrous sulphate. Cl-38, with a half-life of 37.3 minutes, is produced when ferrous chloride is activated (68). So, Cl-38 decays very quickly after the end of bombardment and is very easy to handle.

After the tablets were prepared using direct compression, disintegration tests were conducted. The Vanderkamp tablet disintegration apparatus was used. Eighteen tablets of each type were tested. Seventeen glycine-iron

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[11]J. T. Baker prices.

tablets disintegrated within 18 minutes, while the 18th tablet disintegrated in 23 minutes. On the other hand, 16 control tablets disintegrated within 15 minutes, while the other two were within 19 minutes. The reason for this slow disintegration is that the tablets were harder than usual (15 kg/in^2).

A new technique using neutron activation analysis was introduced to test the prepared tablets *in vivo*. Glycine-iron and control tablets that didn't have amino acid, were activated for four hours in the Lazy Susan in the OSU TRIGA reactor. The activated tablets were administered to groups of four rabbits. Blood and tissue samples were collected in the same manner as that for rats. After having a computer search on the literature since 1960, no reports of activation were found of any type of tablets in order to test them *in vivo*. Neutron activation analysis is very useful in analytical problems where high sensitivity is required. Sensitivity can reach 10^{-9} gm. It is safe, economical, fast, practical in elemental analysis and non-destructive (57). Also, as was discussed in Chapter I, thermal neutrons do not activate isotopes with atomic numbers less than 10. Therefore, carbon, hydrogen, oxygen and nitrogen, which have atomic numbers less than 10 are not activated.

Tables 31-34 and Figures 17 and 18 show iron concentration in blood and different tissues following the oral

administration of glycine-iron and control activated tablets to rabbits. It is evident from these experiments that involved rabbits, that iron appears more quickly and in greater amount in blood when the tablets had glycine. The area under the iron concentration curve for the time during which data was collected increased by 67% when the tablets had glycine (Figure 17).

The amount of iron in the different tissues was also consistent with iron levels in the blood. Iron concentration was highest in the tissues of the rabbits that received the glycine-iron tablets (Figure 18).

Table 5. Iron concentration in blood following oral administration of 0.2 M ferrous sulphate (55.6 mg/ml).

Sample time (min)	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 25 µl of blood	Average amount of iron (mg/ml of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	119	129	144	88	97	139	119	0.0095	0.0016	16.8421
10	273	198	212	250	311	189	238	0.0191	0.0034	17.8010
15	332	351	371	266	289	294	317	0.0253	0.0029	11.4624
20	356	420	310	321	333	322	343	0.0279	0.0035	12.5448
30	486	388	423	499	467	462	454	0.0363	0.0030	8.2644
45	581	620	567	633	542	553	582	0.0414	0.0058	14.0096
60	771	802	688	743	759	863	771	0.0616	0.0042	6.8181
90	1080	930	1120	833	1199	1233	1065	0.0852	0.0113	13.2629
120	1163	1065	1233	963	1244	1369	1172	0.0938	0.0104	11.0874
150	1204	1132	1219	1168	1268	1246	1206	0.0964	0.0036	3.7344
180	1209	1119	1334	1189	1234	1198	1213	0.0970	0.0051	5.2577
210	1233	1144	1343	1142	1259	1011	1188	0.0950	0.0083	8.7368
240	1188	1123	1166	1263	1283	1152	1195	0.0956	0.0046	4.8117

[1]Each R represents a different rat.

Table 6. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml).

Organ	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 1 gm organ	Average amount of iron (mg/1 gm organ)	SD for iron (mg/gm)	CV% for iron (mg/gm)
Liver	1811	1862	1808	1420	1624	1769	1716	0.1372	0.0132	9.6209
Heart	2388	2864	2360	1852	1531	2503	2250	0.1799	0.0383	21.2896
Kidney	403	417	499	416	265	436	406	0.0324	0.0061	18.8271
Muscle	423	430	433	373	363	424	408	0.0325	0.0024	7.3846
Brain	160	139	125	122	131	180	143	0.0114	0.0018	15.7894

[1]Each R represents a different rat.

Table 7. Iron concentration in blood following oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M glycine (30.0 mg/ml).

Sample time (min)	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 25 µl of blood	Average amount of iron (mg/ml of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	216	167	207	199	150	185	188	0.0150	0.0018	12.0000
10	384	194	246	230	221	269	257	0.0206	0.0049	23.7864
15	--	259	342	283	239	466	318	0.0254	0.0066	25.9842
20	765	584	506	655	433	687	605	0.0484	0.0089	18.3884
30	1476	778	636	944	1233	1391	1076	0.0861	0.0250	29.0360
45	2310	1430	824	1723	1936	1863	1681	0.1345	0.0371	27.5836
60	2838	2362	2529	2521	2624	2481	2559	0.2047	0.0117	5.7156
90	3078	2762	2736	2850	3244	2936	3101	0.2347	0.0143	6.0928
120	3972	3139	3099	3340	3632	3341	3420	0.2736	0.0241	8.8084
150	3894	4397	3281	3562	3944	3672	3792	0.3033	0.0279	9.1988
180	3924	4085	3437	3455	4233	3869	3834	0.3067	0.0238	7.7600
210	3904	4008	3422	3489	3844	3732	3733	0.2986	0.0171	5.7967
240	3828	4053	--	3480	4259	3763	3877	0.3100	0.0212	6.8387

[1]Each R represents a different rat.

Table 8. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M glycine (30.0 mg/ml).

Organ	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 1 gm organ	Average amount of iron (mg/1 gm organ)	SD for iron (mg/gm)	CV% for iron (mg/gm)
Liver	--	8239	--	8723	6734	9169	8216	0.6573	0.0847	12.8860
Heart	--	6814	--	8320	5439	7435	7002	0.5601	0.0969	17.3000
Kidney	--	1750	--	1222	2824	1358	1788	0.1430	0.0580	40.5591
Muscle	--	958	--	1259	1079	733	1009	0.0807	0.0176	21.8091
Brain	--	337	--	368	267	381	338	0.0270	0.0040	14.814

[1]Each R represents a different rat.

Table 9. Iron concentration in blood following oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M leucine (52.4 mg/ml).

Sample time (min)	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 25 µl of blood	Average amount of iron (mg/ml) of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	78	104	99	22	118	82	117	0.0093	0.0039	41.9354
10	220	301	289	433	304	270	303	0.0242	0.0051	23.1818
15	334	394	379	499	411	459	413	0.0311	0.0041	13.1832
20	429	479	475	602	509	569	511	0.0408	0.0047	11.5196
30	644	684	811	811	717	762	719	0.0575	0.0043	5.4782
45	854	891	879	1033	927	987	929	0.0742	0.0050	6.7385
60	956	1034	987	1146	1072	1288	1081	0.0864	0.0088	10.1851
90	1193	1259	1254	1359	1286	1405	1293	0.1034	0.0056	5.4158
120	1345	1387	1392	1519	1414	1445	1417	0.1145	0.0049	4.2794
150	1378	1418	1441	1530	1452	1523	1457	0.1158	0.0043	3.7132
180	1356	1433	1443	1545	1460	1556	1466	0.1172	0.0054	4.6075
210	1367	1459	1455	1561	1449	1522	1472	0.1175	0.0048	4.0851
240	1382	1480	1478	1563	1470	1519	1482	0.1190	0.0052	4.3697

[1]Each R represents a different rat.

Table 10. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M leucine (52.4 mg/ml).

Organ	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 1 gm organ	Average amount of iron (mg/1 gm organ)	SD for iron (mg/gm)	CV% for iron (mg/gm)
Liver	1822	1911	1867	1795	1825	1858	1846	0.1475	0.0032	2.1694
Heart	2633	2604	2584	2658	2489	2258	2588	0.2069	0.0047	2.2716
Kidney	341	246	279	353	329	214	289	0.0234	0.0044	18.8030
Muscle	449	489	536	523	467	428	482	0.0385	0.0033	8.5716
Brain	154	134	186	219	144	176	169	0.0483	0.0669	13.8509

[1]Each R represents a different rat.

Table 11. Iron concentration in blood following oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M tryptophan (81.2 mg/ml).

Sample time (min)	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 25 µl of blood	Average amount of iron (mg/ml of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	98	139	124	177	79	131	125	0.0099	0.0025	25.2525
10	211	267	243	294	187	261	244	0.0195	0.0028	14.3589
15	294	356	339	389	288	358	337	0.0268	0.0030	11.1940
20	389	478	433	511	373	408	432	0.0345	0.0039	11.3043
30	544	633	591	661	429	687	591	0.0472	0.0068	14.4067
45	804	894	847	904	767	874	848	0.0678	0.0039	5.7522
60	1001	1123	1061	1144	955	1087	1062	0.0764	0.0097	12.6963
90	1222	1313	1284	1333	1122	1464	1290	0.1031	0.0083	8.0504
120	1355	1405	1372	1411	1244	1480	1361	0.1102	0.0057	5.1724
150	1341	1385	1351	1395	1211	1461	1352	0.1085	0.0060	5.5299
180	1289	1313	1281	1356	1154	1415	1301	0.1041	0.0064	6.1479
210	1267	1288	1245	1311	1133	1267	1252	0.1001	0.0045	4.4955
240	1160	1222	1179	1288	1089	1199	1189	0.0951	0.0048	5.0473

[1]Each R represents a different rat.

Table 12. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M tryptophan (81.2 mg/ml).

Organ	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 1 gm organ	Average amount of iron (mg/1 gm organ)	SD for iron (mg/gm)	CV% for iron (mg/gm)
Liver	2123	2781	2945	1633	1521	2397	2233	0.1786	0.0468	26.203
Heart	2565	2324	3139	2956	2279	2601	2644	0.2075	0.0248	11.9518
Kidney	432	956	844	566	762	691	709	0.0565	0.0151	26.725
Muscle	543	411	449	585	622	437	508	0.0405	0.0069	17.037
Brain	204	147	163	179	156	153	167	0.0133	0.0017	12.78

[1]Each R represents a different rat.

Table 13. Iron concentration in blood following oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M asparagine (52.8 ml).

Sample time (min)	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 25 µl of blood	Average amount of iron (mg/ml of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	322	378	286	389	454	442	379	0.0302	0.0047	15.5629
10	754	796	844	772	762	708	772	0.0618	0.0033	5.3398
15	922	982	966	940	844	1156	978	0.0774	0.0075	9.6899
20	1164	1244	1238	1224	1196	1256	1220	0.0976	0.0025	2.5614
30	1688	1786	1812	1756	1728	1766	1756	0.1404	0.0032	2.2792
45	2312	2552	2422	2362	2168	2354	2362	0.1889	0.0092	4.8703
60	2866	3122	2936	2898	2638	2930	2898	0.2318	0.0013	4.8748
90	3478	3720	3622	3510	3240	3570	3523	0.2818	0.0119	4.2228
120	3984	4216	4216	4018	3866	3924	4037	0.3229	0.0108	3.3446
150	3976	4222	4224	4002	3876	2888	4031	0.3225	0.0114	3.5348
180	3924	4226	4318	4998	--	3886	4070	0.3256	0.0136	4.1769
210	4112	4224	4356	4036	--	3676	4083	0.3266	0.0183	5.6031
240	4008	4232	4352	4016	--	3726	4087	0.3253	0.0172	5.2874

[1]Each R represents a different rat.

Table 14. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M asparagine (59.8 mg/ml).

Organ	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 1 gm organ	Average amount of iron (mg/1 gm organ)	SD for iron (mg/gm)	CV% for iron (mg/gm)
Liver	10230	11414	9631	10743	--	12293	10862	0.8689	0.0827	9.5177
Heart	11654	11121	12034	11077	--	11841	11545	0.9235	0.0343	3.7141
Kidney	5327	6394	7517	6644	--	7101	6597	0.5276	0.0664	12.5852
Muscle	1206	1353	1146	1412	--	1227	1269	0.1014	0.0088	8.6785
Brain	789	701	593	620	--	698	680	0.0543	0.0061	11.2338
Gut	3624	--	5431	4632	--	6731	5104	0.4083	0.1049	25.6918

[1]Each R represents a different rat.

Table 15. Iron concentration in blood following oral administrative of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M serine (43.6 mg/ml).

Sample time (min)	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 25 µl of blood	Average amount of iron (mg/ml of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	931	808	735	780	845	804	817	0.0653	0.0048	7.3506
10	1432	1323	1265	1311	1389	1204	1320	0.1027	0.0057	5.5501
15	1565	1497	1345	1377	1603	1588	1496	0.1196	0.0080	6.6889
20	1622	1596	1472	1507	1624	1640	1577	0.1236	0.0044	3.5598
30	1802	1678	1567	1632	1731	1662	1679	0.1343	0.0059	4.3931
45	1943	1862	1794	1813	1942	1819	1862	0.1489	0.0048	3.2236
60	2085	1973	1886	1970	2003	1860	1963	0.1570	0.0059	3.7579
90	2155	1924	1944	2060	2023	1888	1999	0.1599	0.0072	4.5028
120	2201	2080	1921	2133	2202	1902	2073	0.1658	0.0097	5.8504
150	2224	2062	1919	2137	222	1945	2085	0.1668	0.0097	5.8153
180	2249	2011	1904	2148	2206	1948	2078	0.1662	0.0104	6.2575
210	2277	2111	1887	2170	2244	1935	2104	0.1683	0.0117	6.9518
240	2280	2094	1958	2199	2262	1922	2119	0.1695	0.0112	6.6076

[1]Each R represents a different rat.

Table 16. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M serine (43.6 mg/ml).

Organ	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 1 gm organ	Average amount of iron (mg/1 gm organ)	SD for iron (mg/gm)	CV% for iron (mg/gm)
Liver	2553	2581	2329	2486	2591	2196	2456	0.1964	0.0127	6.4663
Heart	3443	3594	3522	3481	3511	3335	3481	0.2784	0.0069	2.4789
Kidney	793	845	733	624	667	674	723	0.0577	0.0067	11.6117
Muscle	581	753	634	694	605	498	628	0.0501	0.0071	14.171
Brain	187	243	230	255	170	273	226	0.0180	0.0032	17.777

[1]Each R represents a different rat.

Table 17. Iron concentration in blood following oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M proline (45.6 mg/ml).

Sample time (min)	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 25 µl of blood	Average amount of iron (mg/ml of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	312	450	420	415	435	319	391	0.0313	0.0044	14.0575
10	533	546	555	574	551	564	553	0.0443	0.0010	2.2573
15	603	621	626	681	598	629	626	0.0500	0.0021	4.2000
20	623	740	681	624	678	694	673	0.0538	0.0032	5.9479
30	732	812	769	746	774	784	770	0.0615	0.0020	3.2520
45	1098	1174	1118	1002	1134	1166	1115	0.0892	0.0045	5.0448
60	1222	1344	1288	1252	1296	1222	1270	0.1016	0.0034	3.3464
90	1286	1322	1302	1264	1320	1280	1296	0.1036	0.0017	1.6409
120	1512	1588	1558	1498	1572	1538	1544	0.1235	0.0025	2.0242
150	1564	1622	1588	--	1590	1584	1590	0.1261	0.0025	1.9825
180	1592	1630	1602	--	1612	1582	1603	0.1283	0.0013	1.0132
210	1580	1634	1606	--	1620	1578	1604	0.1282	0.0017	1.3260
240	1578	1640	1624	--	1622	1592	1611	0.1289	0.0018	1.3964

[1]Each R represents a different rat.

Table 18. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M proline (45.6 mg/ml).

Organ	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 1 gm organ	Average amount of iron (mg/1 gm organ)	SD for iron (mg/gm)	CV% for iron (mg/gm)
Liver	1820	1736	1955	2134	2034	1852	1922	0.1537	0.0118	7.677
Heart	2654	2565	2791	2904	2855	2887	2776	0.2220	0.0109	4.909
Kidney	394	377	477	620	545	408	470	0.0375	0.0077	20.53
Muscle	419	386	484	599	563	503	492	0.0393	0.0065	16.53
Brain	133	119	153	197	123	187	152	0.0121	0.0026	21.48

[1]Each R represents a different rat.

Table 19. Iron concentration in blood following oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M methionine (59.6 mg/ml).

Sample time (min)	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 25 µl of blood	Average amount of iron (mg/ml of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	212	182	216	284	242	202	191	0.0178	0.0025	14.0449
10	260	188	284	344	279	213	261	0.0208	0.0040	19.2307
15	266	212	296	412	314	367	311	0.0255	0.0062	24.3137
20	280	244	312	486	368	522	368	0.0295	0.0082	27.7966
30	540	412	605	629	672	704	594	0.0484	0.0074	15.2892
45	812	912	798	1134	1089	985	955	0.0764	0.0102	13.3507
60	1068	960	1124	1233	1132	1096	1102	0.0881	0.0065	7.3779
90	1220	1008	1256	1323	1296	1244	1225	0.0979	0.0082	8.3758
120	1284	1123	1324	1412	1224	1304	1278	0.1022	0.0071	6.9471
150	1322	1194	1388	1483	1237	1354	1329	0.1063	0.0076	7.1495
180	1340	1202	1312	1512	1292	1374	1338	0.1071	0.0075	7.0028
210	1484	1216	1424	1533	1383	1398	1406	0.1124	0.0079	7.0284
240	1454	1236	1437	1539	1419	1424	1418	0.1134	0.0072	6.3492

[1]Each R represents a different rat.

Table 20. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M methionine (59.6 mg/ml).

Organ	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min in 1 gm organ	Average amount of iron (mg/1 gm organ)	SD for iron (mg/gm)	CV% for iron (mg/gm)
Liver	1737	1969	1832	1923	1759	1664	1814	0.1450	0.0093	0.4137
Heart	2511	2432	2545	2521	2655	2602	2544	0.2035	0.0062	3.0466
Kidney	522	443	471	387	514	381	453	0.0361	0.0048	13.296
Muscle	351	452	431	476	459	343	419	0.0334	0.0045	13.473
Brain	184	131	172	145	170	151	159	0.0126	0.0015	11.904
Gut	17431	18221	19433	20564	18651	--	18860	1.5087	0.0957	6.343

[1]Each R represents a different rat.

Table 21. Iron concentration in blood following oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M alanine (35.6 mg/ml).

Sample time (min)	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 25 µl of blood	Average amount of iron (mg/ml of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	142	202	164	122	111	192	155	0.0125	0.0027	21.6000
10	364	382	422	372	312	412	377	0.0302	0.0028	9.2715
15	520	534	458	462	474	502	492	0.0393	0.0023	5.8524
20	536	512	468	482	506	514	503	0.0402	0.0017	4.2288
30	664	678	690	720	646	760	693	0.0554	0.0030	5.4151
45	984	978	960	1032	984	1012	992	0.0795	0.0018	2.2641
60	1134	1065	1096	1128	1174	1134	1122	0.0897	0.0027	3.0100
90	1458	1512	1498	1420	1476	1432	1466	0.1172	0.0026	2.2184
120	1690	1682	1688	1704	1703	1684	1696	0.1357	0.0013	0.9579
150	1964	1644	1744	1864	1832	1688	1789	0.1431	0.0087	6.0796
180	1982	1732	1801	1799	1850	1733	1816	0.1453	0.0067	4.6111
210	1984	1855	1960	1787	1811	1754	1858	0.1486	0.0068	4.5760
240	2011	1821	1847	1829	1862	1766	1856	0.1485	0.0060	4.0404

[1]Each R represents a different rat.

Table 22. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M alanine (35.6 mg/ml).

Organ	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min in 1 gm organ	Average amount of iron (mg/1 gm organ)	SD for iron (mg/gm)	CV% for iron (mg/gm)
Liver	1970	1992	2054	1662	2088	2052	1970	0.1575	0.0125	7.9365
Heart	2927	3035	3136	3932	3064	2938	3005	0.2537	0.0304	11.9826
Kidney	473	507	359	499	466	494	466	0.0373	0.0043	11.5281
Muscle	542	539	351	473	567	561	506	0.0404	0.0066	16.3366
Brain	213	135	144	135	176	183	164	0.0131	0.0025	19.0839

[1]Each R represents a different rat.

Table 23. Iron concentration in blood following oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M isoleucine (52.4 mg/ml).

Sample Time (min)	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 25 µl of blood	Average amount of iron (mg/ml of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	122	173	165	144	178	255	173	0.0138	0.0033	23.9130
10	322	361	346	335	372	423	360	0.0288	0.0025	8.6805
15	411	478	562	423	485	523	480	0.0375	0.0045	12.0000
20	511	598	704	531	610	653	601	0.0480	0.0052	10.8333
30	680	746	794	705	755	804	747	0.0597	0.0035	5.8696
45	906	975	1071	920	982	1011	978	0.0782	0.0044	5.6265
60	1055	1112	1131	1076	1122	1223	1120	0.0896	0.0042	4.6875
90	1267	1342	1373	1299	1351	1437	1345	0.1076	0.0043	3.9962
120	1355	1434	1493	1385	1447	1522	1439	0.1145	0.0043	3.7554
150	1411	1511	1622	1461	1519	1589	1518	0.1215	0.0057	4.6913
180	1420	1476	1631	1466	1485	1604	1514	0.1211	0.0061	5.0371
210	1431	1522	1645	1479	1532	1611	1537	0.1228	0.0059	4.8045
240	1418	1514	1596	1495	1504	1620	1525	0.1219	0.0053	4.3478

[1]Each R represents a different rat.

Table 24. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M isoleucine (52.4 mg/ml).

Organ	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 1 gm organ	Average amount of iron (mg/1 gm organ)	SD for iron (mg/gm)	CV% for iron (mg/gm)
Liver	1945	1853	1886	1871	1944	1858	1893	0.1513	0.0033	2.1810
Heart	2743	2634	2659	2769	2678	705	2698	0.2091	0.0138	6.5997
Kidney	423	367	445	355	345	401	389	0.0311	0.0031	9.9678
Muscle	459	534	509	422	498	413	473	0.0377	0.0039	10.3448
Brain	233	211	149	167	213	145	186	0.0148	0.0029	19.5946

[1]Each R represents a different rat.

Table 25. Iron concentration in blood following oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M phenylalanine (66.0 mg/ml).

Sample time (min)	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 25 µl of blood	Average amount of iron (mg/ml of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	119	92	88	109	105	111	104	0.0083	0.0008	9.6385
10	273	145	123	225	175	182	177	0.0141	0.0028	19.8581
15	332	212	205	214	262	358	260	0.0208	0.0045	21.6346
20	356	279	254	279	321	377	313	0.0250	0.0037	14.8000
30	486	523	503	562	563	605	662	0.0449	0.0032	7.1269
45	581	698	685	685	731	820	728	0.0583	0.0038	7.0631
60	771	841	811	854	878	938	879	0.0703	0.0040	5.6899
90	1080	1088	1162	983	1142	1222	1133	0.0906	0.0063	6.9536
120	1163	1196	1233	1198	1252	1312	1251	0.1000	0.0038	3.8000
150	1342	1223	1277	1233	1298	1441	1302	0.1041	0.0059	5.6676
180	1361	1230	1284	1233	1309	1447	1310	0.1043	0.0057	5.4650
210	1354	1243	1288	1241	1289	1430	1307	0.1045	0.0053	5.0717
240	1351	1246	1291	1239	1311	1428	1311	0.1048	0.0051	4.8664

[1]Each R represents a different rat.

Table 26. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M phenylalanine (66.0 mg/ml).

Organ	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 1 gm organ	Average amount of iron (mg/1 gm organ)	SD for iron (mg/gm)	CV% for iron (mg/gm)
Liver	1674	1765	1723	1805	1947	1842	1793	0.1433	0.0076	5.3035
Heart	2569	2389	2323	2459	2532	2478	2458	0.1966	0.0072	3.6622
Kidney	331	449	379	438	513	439	425	0.0339	0.0050	14.7493
Muscle	521	477	321	392	445	375	422	0.0337	0.0058	17.2107
Brain	168	158	161	169	173	145	162	0.0129	0.0007	5.4263
Gut	19732	22432	23584	26531	--	24560	23367	1.8701	0.2026	10.8336

[1]Each R represents a different rat.

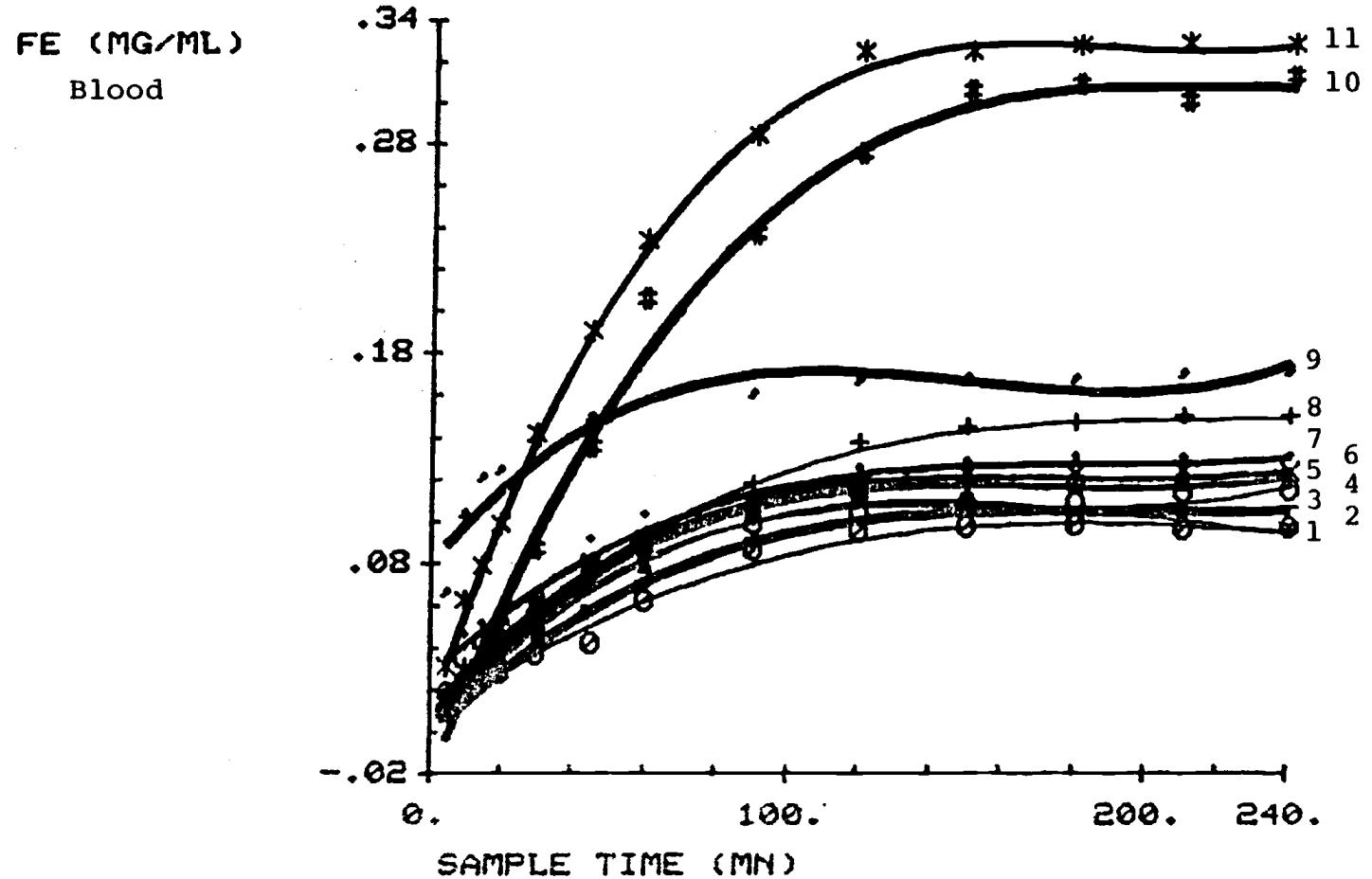

Figure 5. Comparison of blood iron concentration when 0.2 M ferrous sulphate was given as (1) control and with 0.4 M of (2) phenylalanine, (3) tryptophan, (4) methionine, (5) leucine, (6) isoleucine, (7) proline, (8) alanine, (9) serine, (10) glycine, and (11) asparagine.

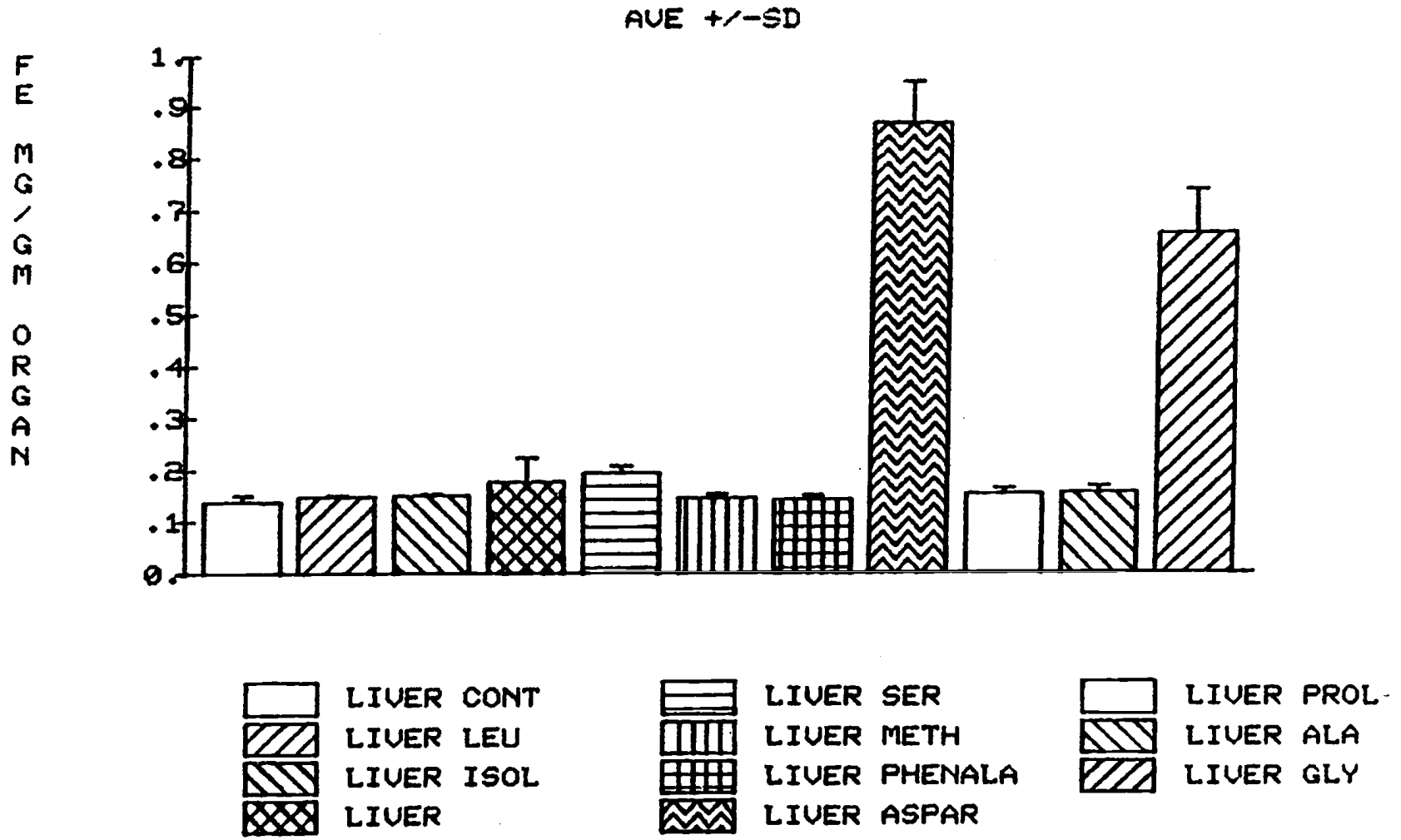

Figure 6. Comparison of iron concentration per 1 gm of liver, four hours after the oral administration of 0.2 M ferrous sulphate when it was given as control and when it was administered with 0.4 M of various amino acids.

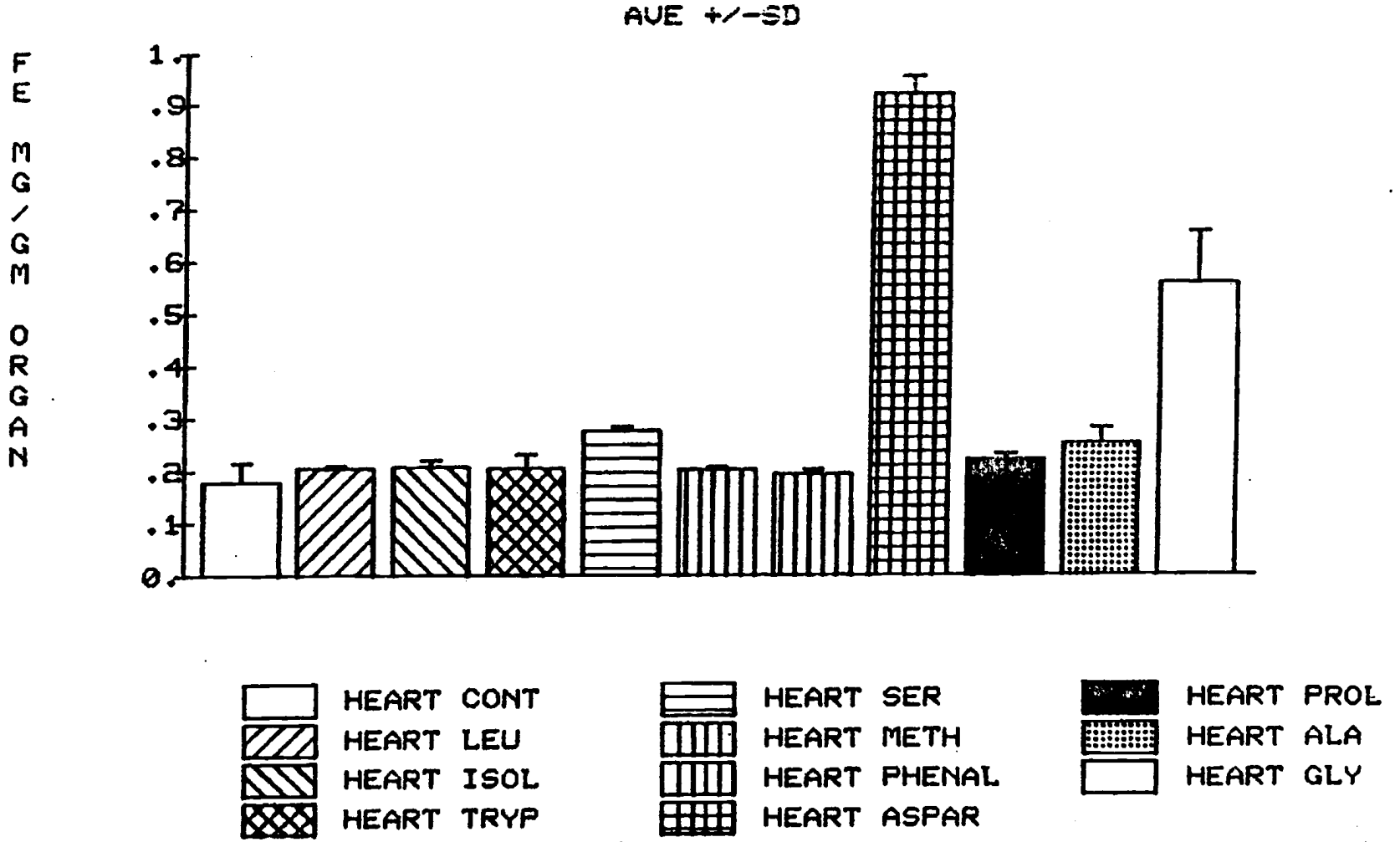

Figure 7. Comparison of iron concentration per 1 gm of heart, four hours after the oral administration of 0.2 M ferrous sulphate when it was given as control and when it was administered with 0.4 M of various amino acids.

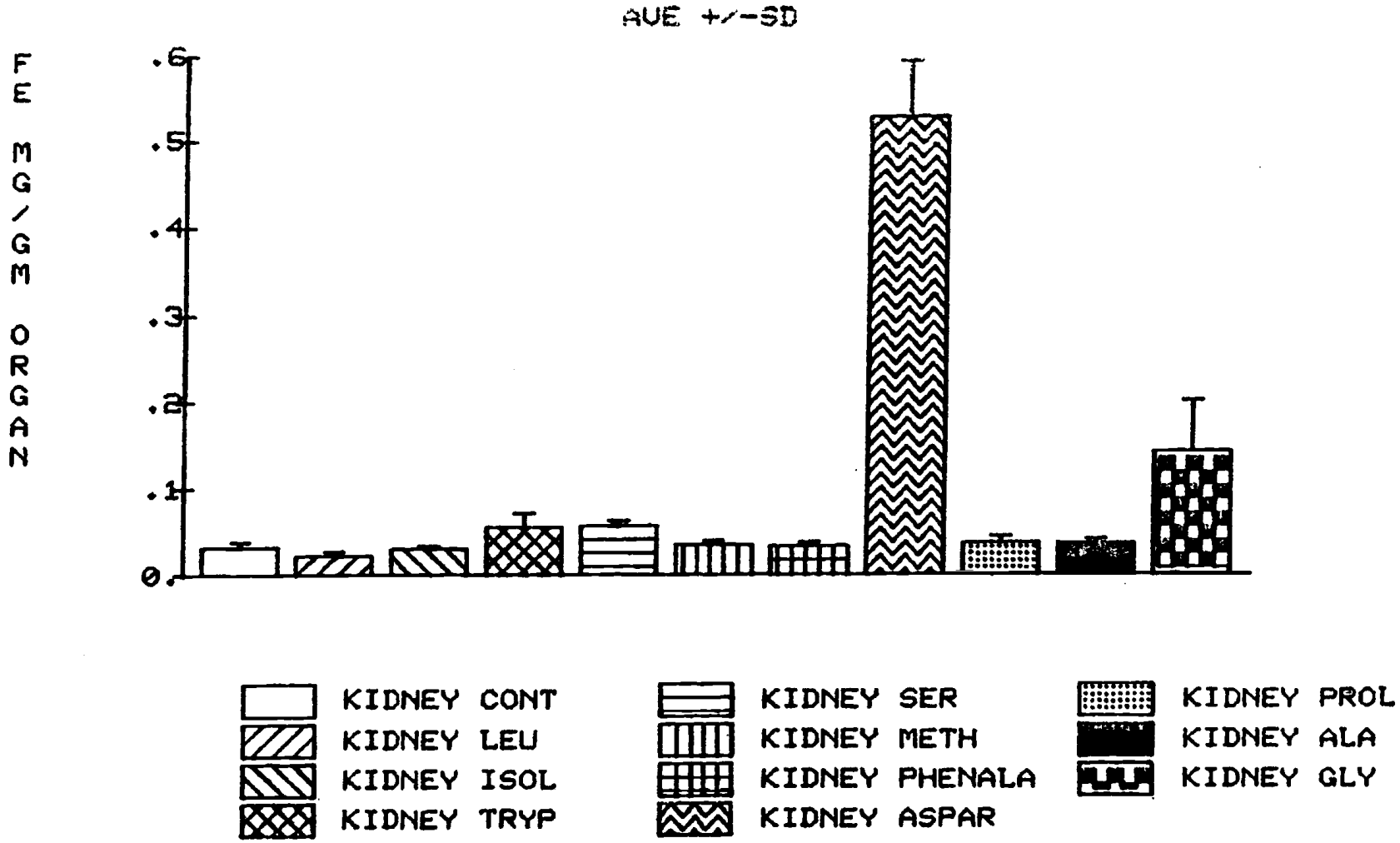

Figure 8. Comparison of iron concentration per 1 gm of kidney, four hours after the oral administration of 0.2 M ferrous sulphate when it was given as control and when it was administered with each of the amino acids.

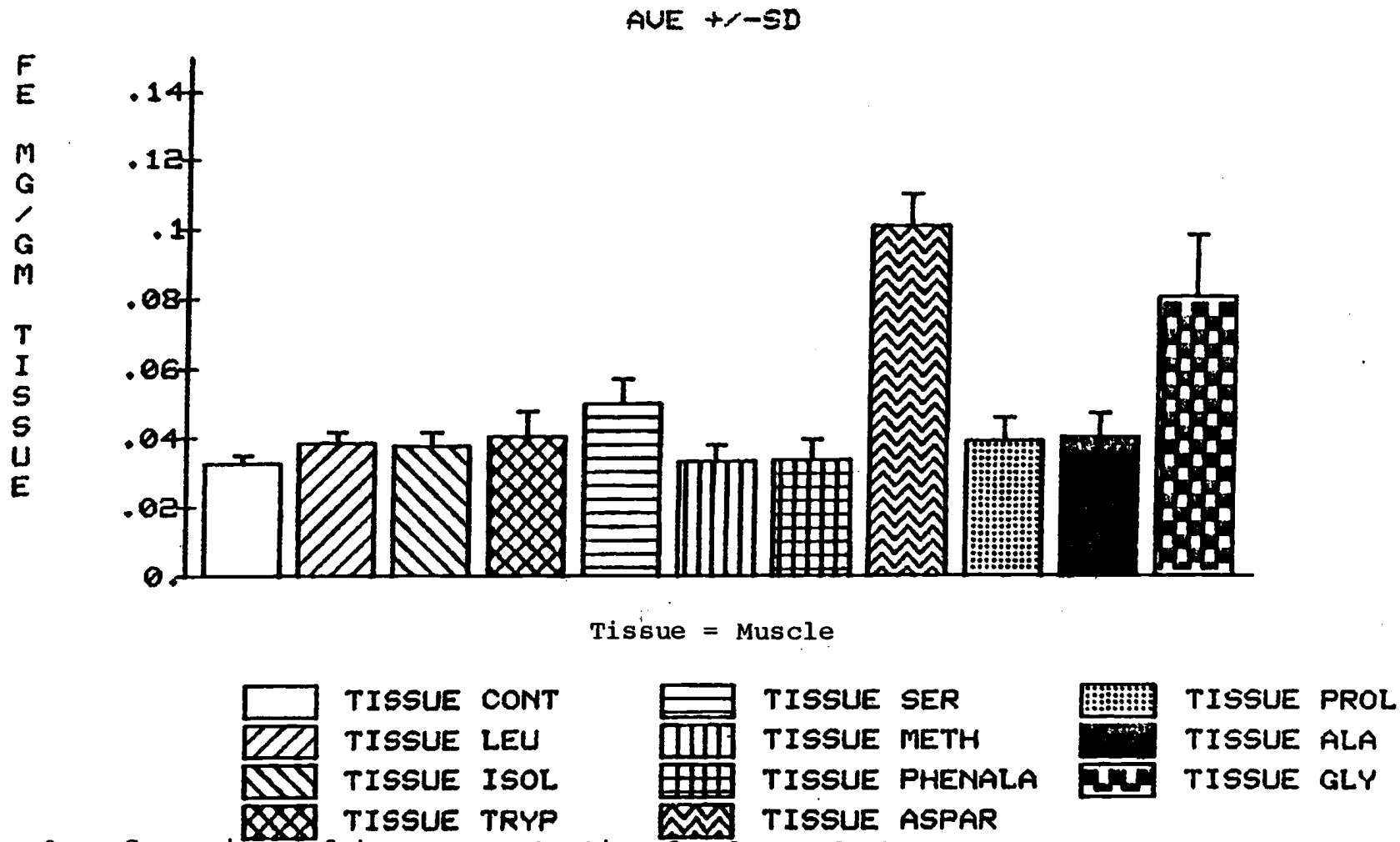

Figure 9. Comparison of iron concentration for 1 gm of tissue, four hours after the oral administration of 0.2 M ferrous sulphate when it was given as control and when it was administered with each of the amino acids.

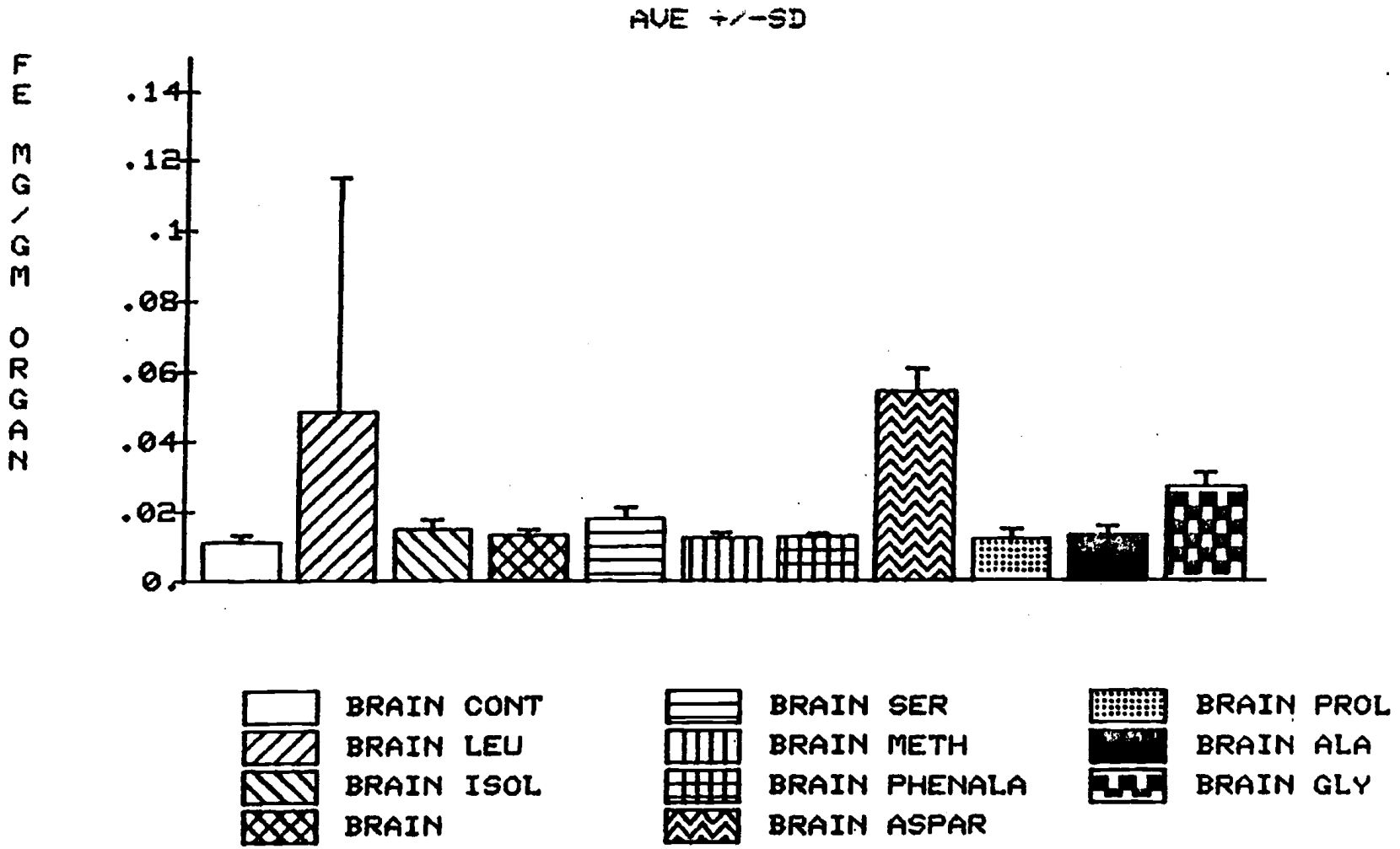

Figure 10. Comparison of iron concentration per 1 gm of brain, four hours after the oral administration of 0.2 M ferrous sulphate when it was given as control and when it was administered with each of the amino acids.

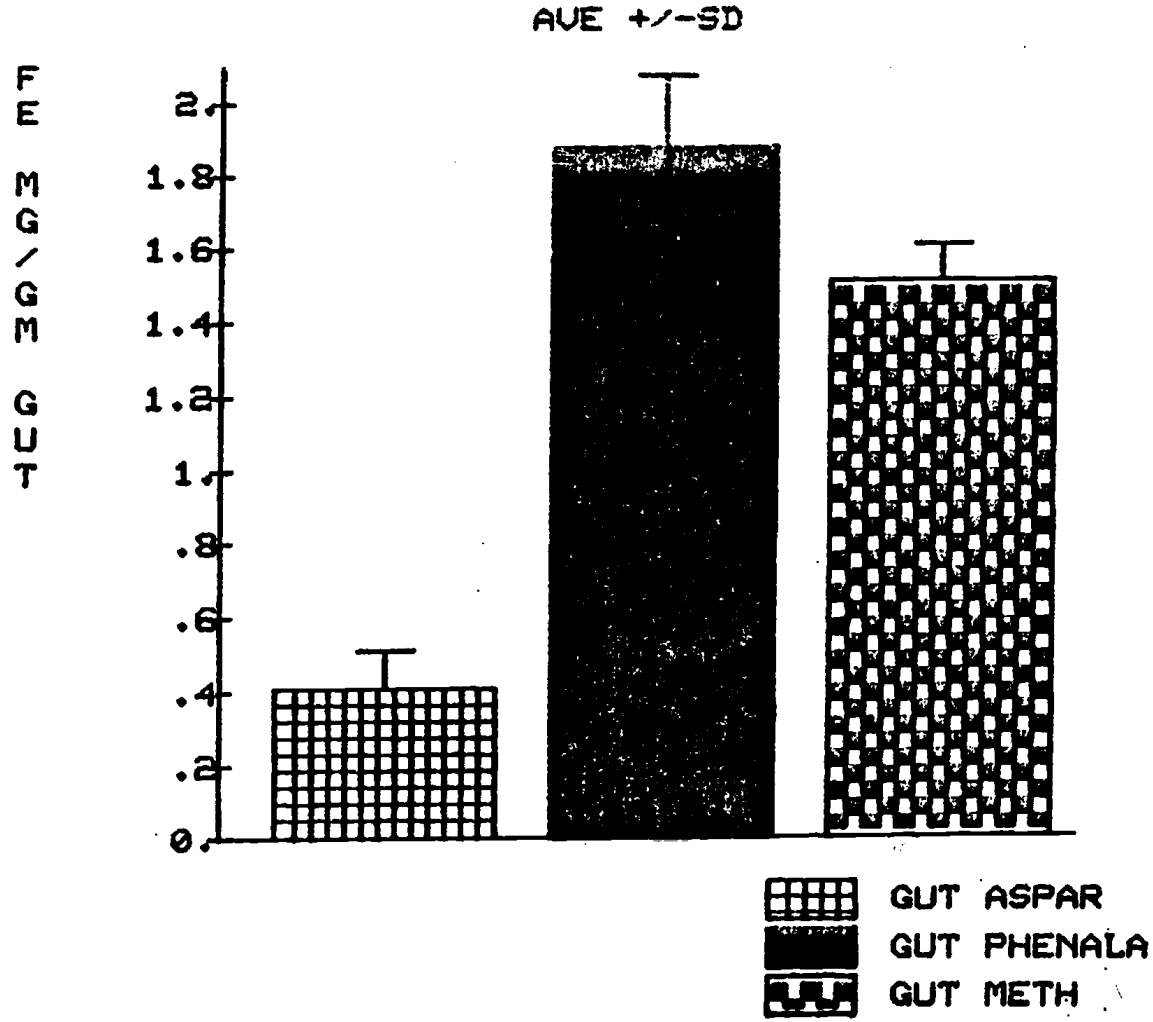

Figure 11. Comparison of iron concentration per 1 gm of gut, four hours after the oral administration of 0.2 M ferrous sulphate when it was given with 0.4 M of asparagine, phenyl alanine and methionine.

Table 27. Stability constants, initial absorption rates (mg/ml) and area under the curve (mg. min/ml) for each amino acid tested (47).

	Log K stability		Absorption rate (mg/ml)	Area under the curve (mg. min/ml)
	Experimental value	Literature value		
1. Asparagine	6.4	6.5	.0017	63.5928
2. Glycine	7.6	7.8	.00457	56.5052
3. Serine	7.0	7.0	.0021	37.3125
4. Leucine	7.0	6.9	.00202	22.5997
5. Methionine	6.6	6.7	.00199	21.7053
6. Isoleucine	6.9	7.1	.00193	23.475
7. Tryptophan	7.4	7.6	.0019	21.0493
8. Alanine	7.1	7.3	.00187	28.6359
9. Phenyl alanine	6.2	6.3	.0017	20.2352
10. Proline	8.2	8.3	.00162	25.2161

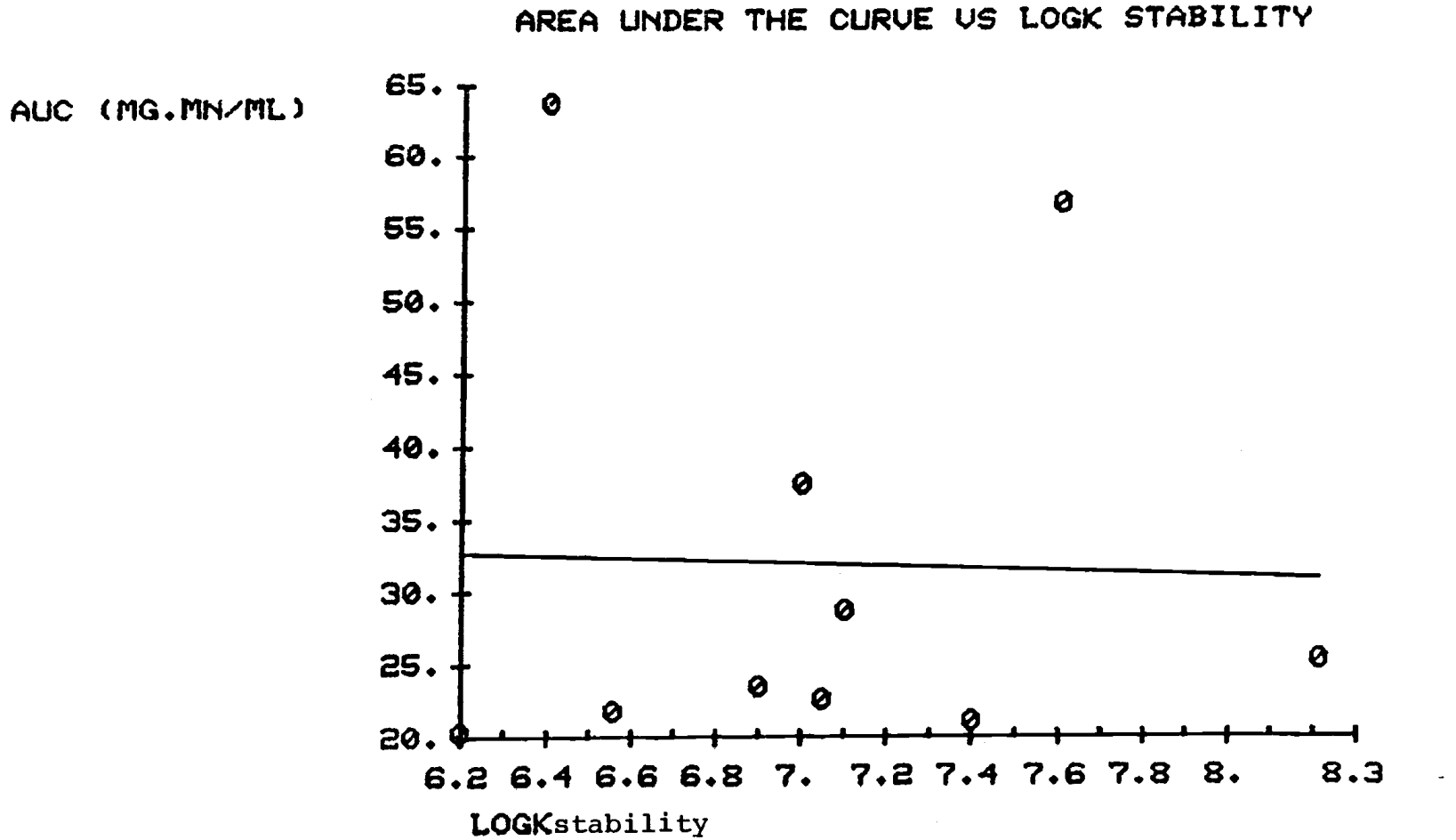

Figure 12. Area under the curve (mg, min/ml) versus log K_s for each of the amino acids tested.

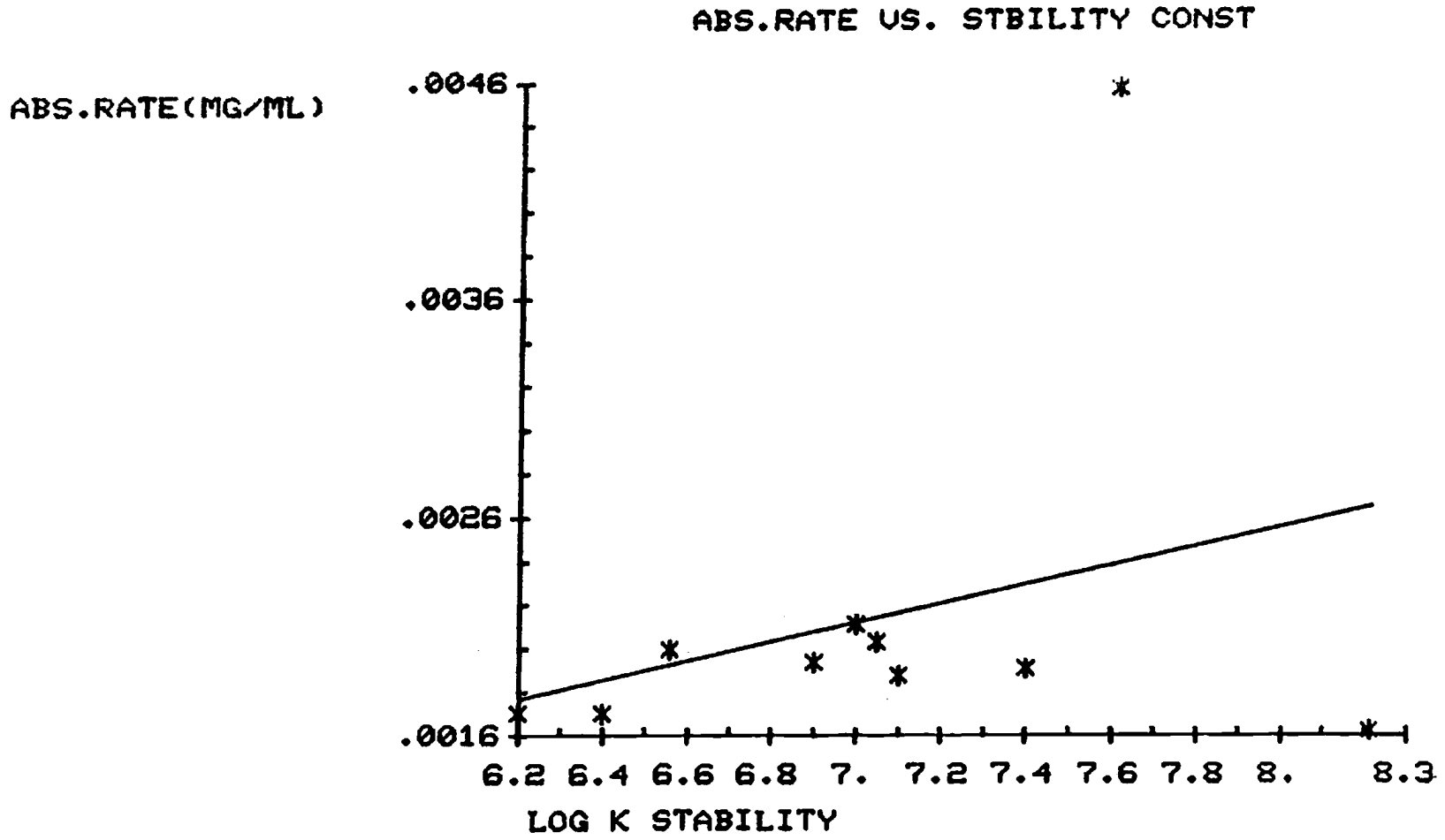

Figure 13. Initial rate of absorption (mg/ml) versus log K_s for each of the amino acids tested.

Table 28. Iron concentration in blood following oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.5 M ascorbic acid (80.0 mg/ml).

Sample time (min)	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 25 µl of blood	Average amount of iron (mg/ml of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	706	520	605	673	565	606	612	0.0489	0.0049	10.0204
10	680	533	412	361	289	273	425	0.0339	0.0114	33.6283
15	694	525	453	304	419	439	472	0.0377	0.0095	25.1989
20	306	413	512	553	688	471	491	0.0392	0.0094	23.9795
30	522	573	647	694	762	682	647	0.0517	0.0063	12.1856
45	653	731	782	844	889	762	777	0.0639	0.0067	10.4851
60	621	659	605	747	785	735	692	0.0553	0.0053	9.5840
90	771	863	933	969	1041	902	913	0.0854	0.0292	34.1920
120	1361	1474	1563	1632	1678	1542	1542	0.1233	0.0082	6.6504
150	1319	1382	1473	1517	1591	1423	1451	0.1160	0.0070	6.0344
180	1346	1454	1544	1564	1647	1484	1507	0.1205	0.0075	6.2240
210	1306	1388	1497	1551	1636	1449	1471	0.1176	0.0085	7.2278
240	1247	1294	1364	1418	1463	1267	1342	0.1073	0.0063	5.8713

[1]Each R represents a different rat.

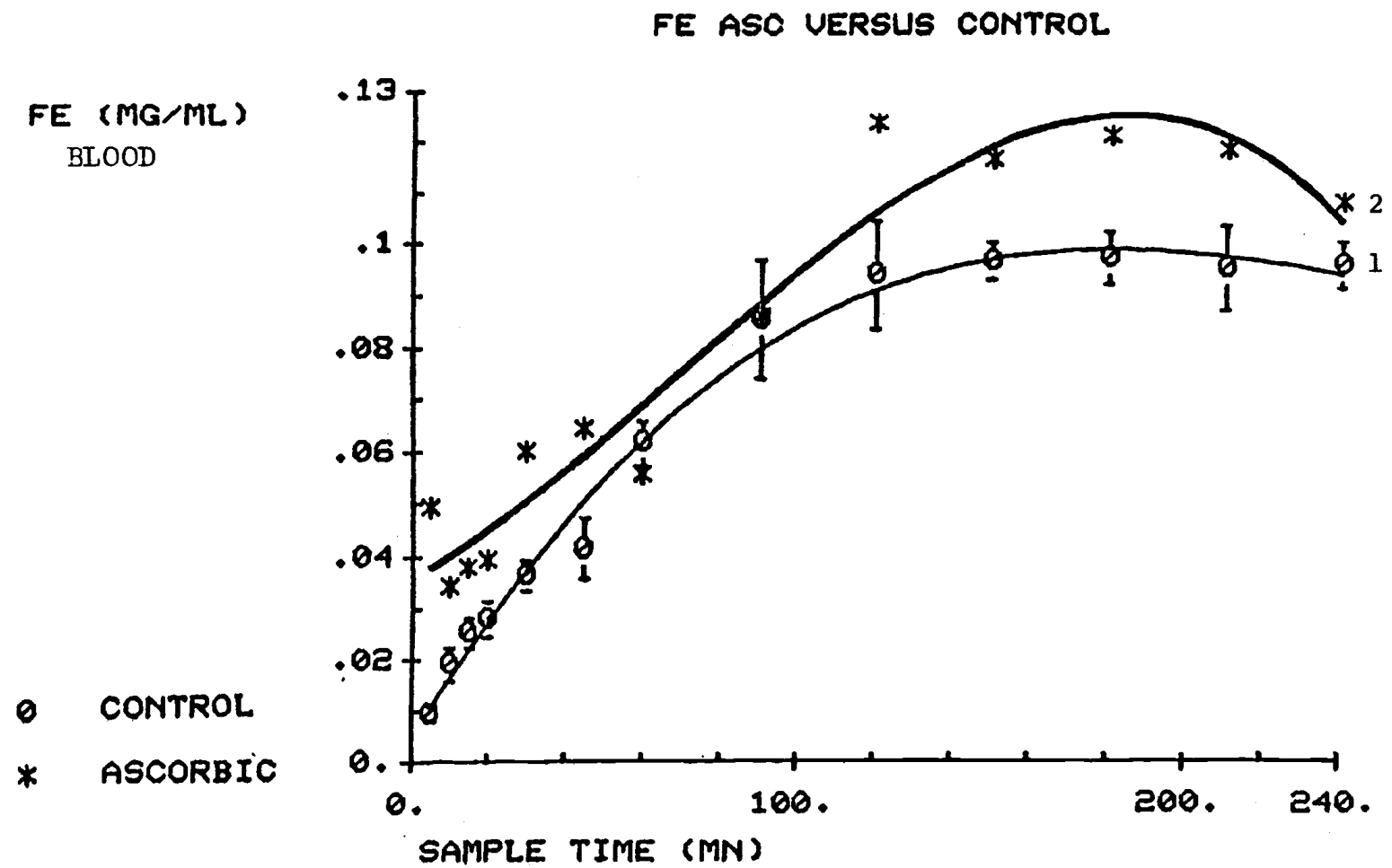

Figure 14. Comparison of blood iron concentration when 0.2 M ferrous sulphate (55.6 mg/ml) was given as (1) control and (2) with 0.5 M ascorbic acid (80 mg/ml).

Table 29. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.5 M ascorbic acid (80.0 mg/ml).

Organ	R_1[1]	R_2	R_3	R_4	R_5	R_6	Average counts/10 min. in 1 gm organ	Average amount of iron (mg/1 gm organ)	SD for iron (mg/gm)	CV% for iron (mg/gm)
Liver	2230	2365	1950	1895	2164	2322	0.1723	0.1723	0.0154	8.9378
Heart	2667	2732	2531	2455	2679	2581	0.2085	0.2085	0.0083	3.9808
Kidney	625	733	495	565	706	674	0.0506	0.0506	0.0071	14.0316
Muscle	511	569	709	461	499	506	0.0433	0.0433	0.0071	16.397
Brain	171	201	235	185	164	245	0.0159	0.0159	0.0026	16.352

[1]Each R represents a different rat.

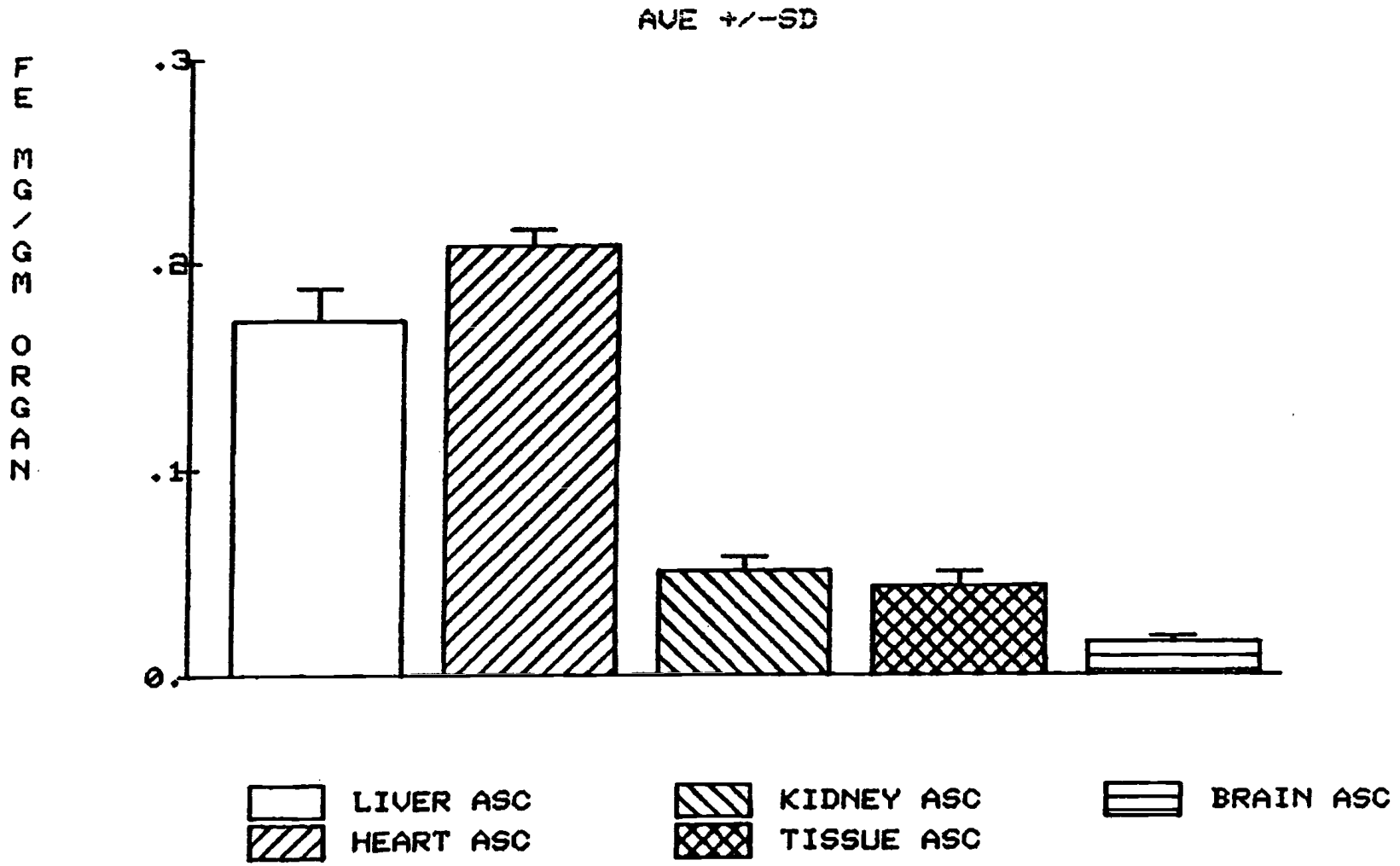

Figure 15. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.5 M ascorbic acid (80.0 mg/ml).

Table 30. Iron concentration in blood following oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M glycine (30.0 mg/ml) in the absence of buffer.

Sample time (min)	R_1[1]	R_2	R_3	Average counts/10 min. in 25 µl of blood	Average amount of iron (mg/ml of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	98	187	235	173	0.0138	0.0055	39.8550
10	133	244	382	253	0.0202	0.0099	49.0099
15	312	519	604	478	0.0382	0.0120	31.4136
20	662	694	823	726	0.0580	0.0068	11.7241
30	1004	1180	1243	1142	0.0913	0.0099	10.8433
45	1572	1744	1823	1713	0.1370	0.0102	7.4452
60	1964	2231	2319	2171	0.1736	0.0147	8.4677
90	2522	2604	2783	2636	0.2108	0.0106	5.0284
120	2911	3120	3234	3088	0.2470	0.0131	5.3036
150	3044	3117	3453	3205	0.2563	0.0174	6.7889
180	3089	3288	3450	3276	0.2620	0.0144	5.4961
210	3075	3267	3481	3274	0.2619	0.0162	6.1855
240	3131	3331	3494	3319	0.2654	0.0145	5.4634

[1]Each R represents a different rat.

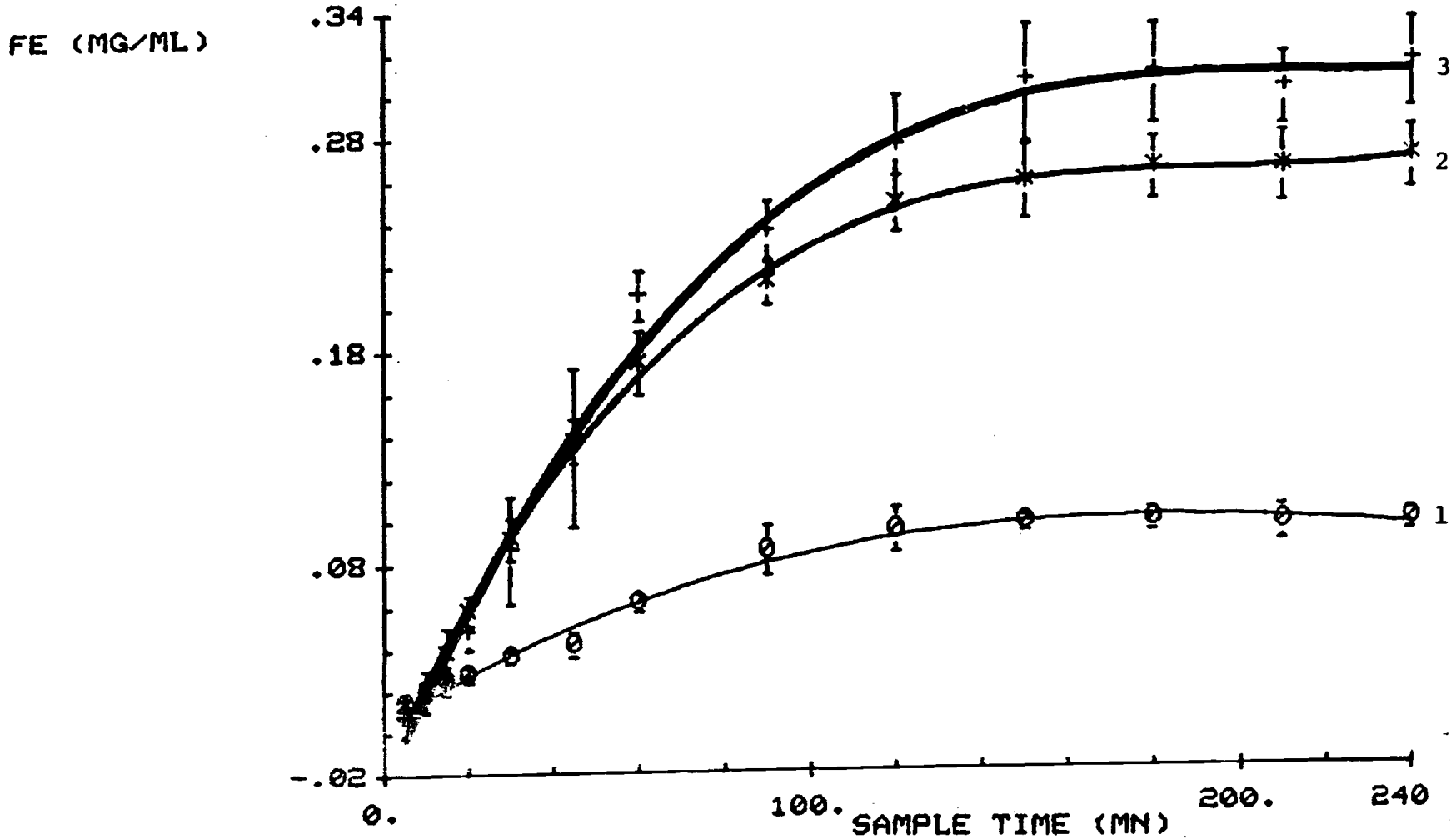

Figure 16. Comparison of blood iron concentration when 0.2 M ferrous sulphate (55.6 mg/ml) was given as (1) control and with 0.4 M glycine (30.0 mg/ml), (2) in the absence of buffer, (3) in the presence of buffer.

Table 31. Iron concentration in blood following oral administration of four tablets containing 192.92 mg of ferrous chloride.

Sample time (min)	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Average counts/4000 sec. in 100 µl of blood	Average amount of iron (mg/ml of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	30	14	14	18	16	0.0013	0.0005	38.4653
10	28	17	23	20	22	0.0015	0.0002	13.3333
15	32	20	24	22	25	0.0016	0.0003	18.7500
20	34	22	23	--	26	0.0017	0.0003	17.6470
30	43	25	29	26	31	0.0021	0.0005	23.8095
45	70	33	31	41	44	0.0030	0.0012	40.0000
60	163	68	55	118	101	0.0069	0.0033	47.8260
90	241	148	91	230	178	0.0121	0.0048	39.6694
120	300	194	110	293	224	0.0153	0.0062	40.5228
150	345	248	197	341	283	0.0193	0.0049	25.3886
180	374	302	259	358	323	0.0221	0.0036	16.2895
210	367	292	284	358	325	0.0222	0.0029	13.1818
240	361	297	273	349	320	0.0219	0.9028	12.7852

Table 32. Iron concentration in organs four hours after the oral administration of four tablets containing 192.92 mg of ferrous chloride.

Sample Time (min)	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Average counts/4000 sec. in 1 gm organ	Average amount of iron (mg/gm of organ)	SD for iron (mg/ml)	CV% for iron (mg/ml)
Liver	471	520	425	568	496	0.0339	0.0042	12.3893
Heart	522	633	475	505	534	0.0365	0.0047	12.8767
Kidney	125	160	105	143	133	0.0089	0.0015	16.8539
Muscle	120	119	142	130	126	0.0086	0.0008	9.3023

Table 33. Iron concentration in blood following oral administration of four tablets containing 197.64 mg of ferrous chloride and 158.12 mg of glycine.

Sample time (min)	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Average counts/4000 sec. in 100 µl of blood	Average amount of iron (mg/ml of blood)	SD for iron (mg/ml)	CV% for iron (mg/ml)
5	18	22	32	28	25	0.0017	0.0004	23.5294
10	21	31	48	39	35	0.0023	0.0008	34.7826
15	24	39	54	47	41	0.0028	0.0008	28.5714
20	26	45	66	52	47	0.0032	0.0011	34.3750
30	31	47	68	64	53	0.0036	0.0011	30.5555
45	38	59	92	104	73	0.0050	0.0020	40.0000
60	64	112	182	197	139	0.0095	0.0042	44.2105
90	212	247	338	374	293	0.0200	0.0052	26.0000
120	298	444	464	596	451	0.0308	0.0083	26.9480
150	529	562	642	671	601	0.0411	0.0045	10.9498
180	598	663	735	774	693	0.0474	0.0053	11.1814
210	624	681	728	768	700	0.0482	0.0046	9.5435
240	631	687	742	758	705	0.0482	0.0039	8.0912

Table 34. Iron concentration in organs four hours after the oral administration of four tablets containing 197.64 mg of ferrous chloride and 158.12 mg of glycine.

Organ	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Average counts/4000 sec. in 1 gm organ	Average amount of iron (mg/gm of organ)	SD for SD for iron (mg/ml)	CV% for iron (mg/ml)
Liver	1648	1723	1698	1642	1678	0.1149	0.0026	2.2628
Heart	1295	1345	1422	1123	1296	0.0887	0.0086	9.6956
Kidney	368	423	298	320	352	0.0241	0.-038	15.7676
Muscle	165	222	140	245	193	0.0132	0.0033	25.0000

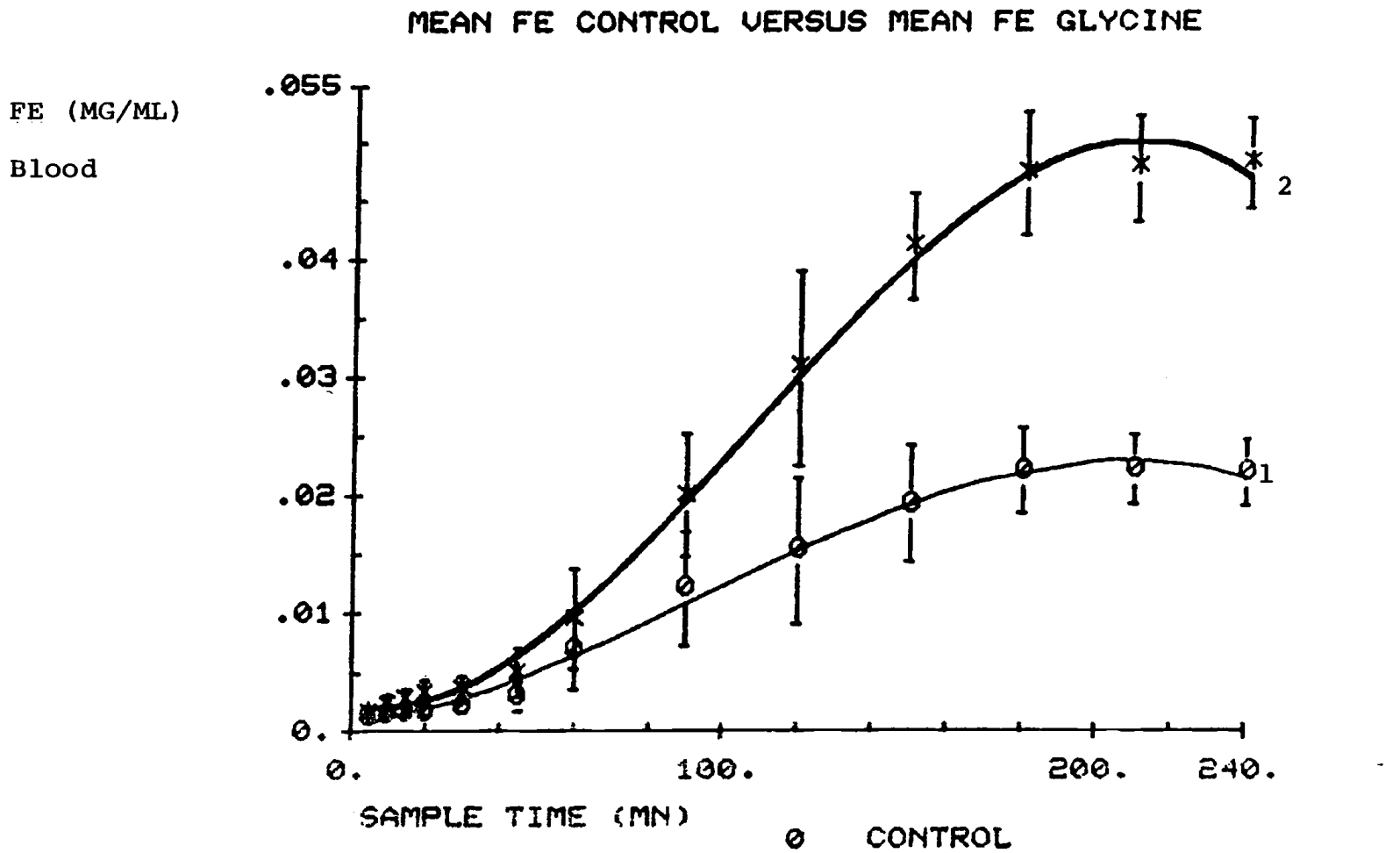

Figure 17. Comparison of blood iron concentrations following oral administration of (1) four tablets containing 192.92 mg of ferrous chloride and (2) four tablets containing 197.64 mg of ferrous chloride and 158.12 mg of glycine.

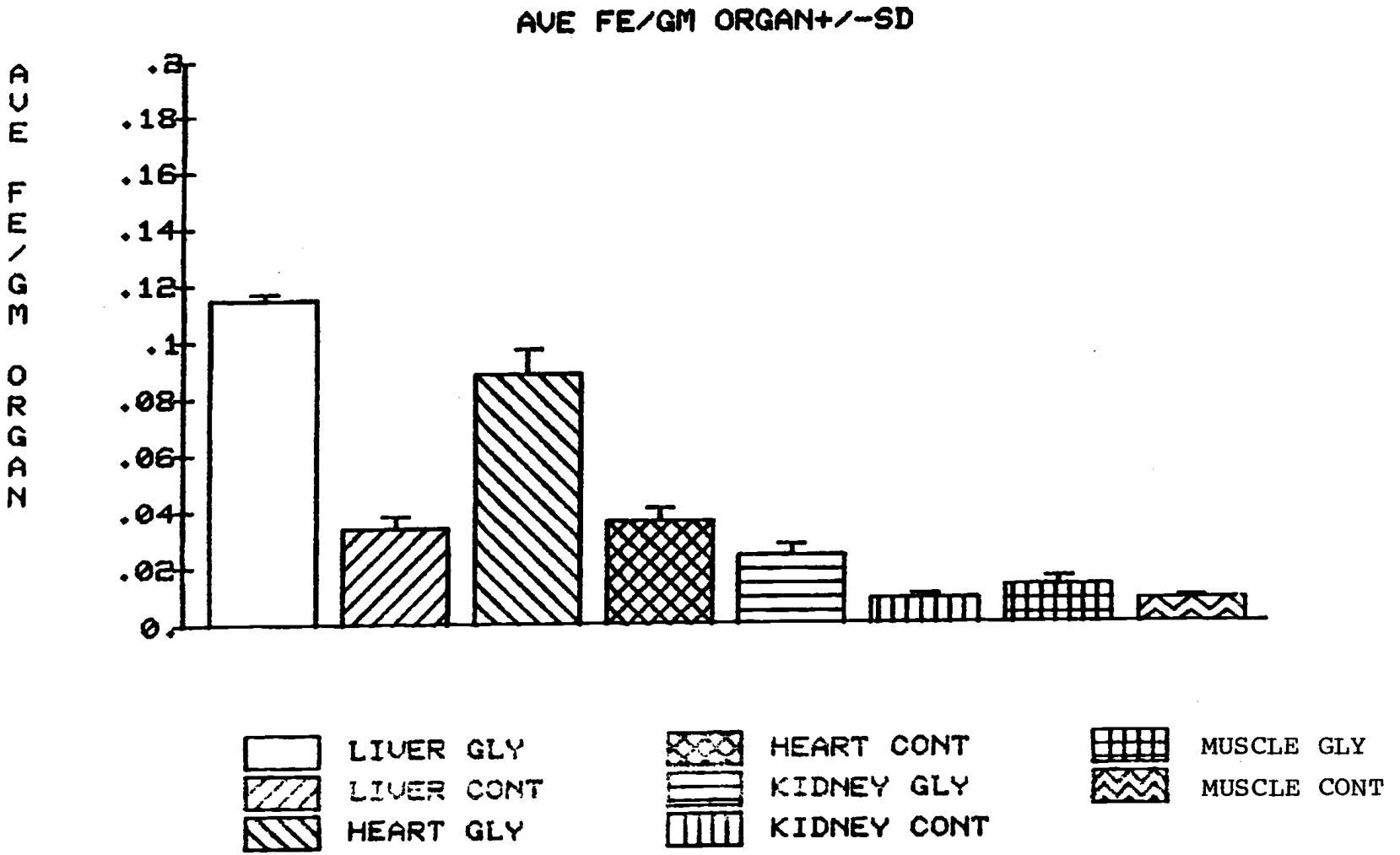

Figure 18. Comparison of iron concentration in organs four hours after the oral administration of (1) four tablets containing 192.92 mg of ferrous chloride (control) and (2) four tablets containing 197.64 mg of ferrous chloride and 158.12 mg of glycine.

V. CONCLUSIONS

1. The effect of ten amino acids on iron absorption was studied. Area under the iron concentration curves (time 0-240 min) for all amino acid iron complexes increased over the AUC (time 0-240 min) of the control. The increase in the area under the curve was most notable in asparagine and glycine where the increase was 265.22% and 224.52% respectively. The concentration of iron in the tissues of animals receiving amino acid-iron complexes was consistent with iron levels in the blood. Iron concentration was highest in the tissues of animals receiving asparagine-iron and glycine-iron complexes, while it was lower in the tissues of animals receiving the other amino acid-iron complexes.
2. No correlation was found between stability constants of each amino acid-iron complex and initial rate of absorption or area under the iron concentration curve (0-4 hr). This suggests that there are substances in the animals that compete with the amino acid ligands for iron. Conversely, various ions in the animal might compete with iron for combination with the amino acids.
3. It was found that amino acids increase iron absorption much more than ascorbic acid. Asparagine and glycine increase the area under the iron concentration curve (time zero to four hours) by 265.22% and 224.52%, while

in the case of ascorbic acid the AUC (time 0-4 hours) increases by only 31.59%.

4. A new technique using neutron activation analysis was introduced to test tablets having metals in vivo. This method is highly sensitive, safe, economical, fast, practical in elemental analysis and non-destructive.
5. A new tablet formulation containing iron was prepared that was tested after activation on groups of rabbits. Results showed that iron absorption was more when the tablets had glycine than control tablets without glycine.

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APPENDICES

Table A-1. Polynomial fit to curve 1 of Figure 5.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 1 OF CONTG

NUMBER OF DATA POINTS = 13

MULTIPLE R = .9944077 MULTIPLE R-SQUARED = .9888467
STANDARD DEVIATION OF REGRESSION = .004214122

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	.004644617	.003129636	1.484076	.1719
B1	.001236442	.0001453254	8.508093	.0001
B2	-5.02358×10^{-6}	1.480218×10^{-6}	-3.393812	.008
B3	5.903016×10^{-9}	4.056813×10^{-9}	1.455087	.1796

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.01417045	3.	.004723483	265.9795	.0001
RESIDUAL	.0001598294	9.	1.775882×10^{-5}		

Table A-2. Polynomial fit to curve 10 of Figure 5.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 1 OF GLG

NUMBER OF DATA POINTS = 13

MULTIPLE R = .9959737 MULTIPLE R-SQUARED = .9919636
STANDARD DEVIATION OF REGRESSION = .01263741

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	-.02519923	.009385229	-2.684988	.025
B1	.004569094	.0004358055	10.48425	.0001
B2	-2.083466×10^{-5}	4.438913×10^{-6}	-4.693639	.0011
B3	3.144448×10^{-8}	1.216567×10^{-8}	2.58469	.0295

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.1774153	3.	.05913842	370.2997	.0001
RESIDUAL	.001437338	9.	.0001597042		

Table A-3. Polynomial fit to curve 5 of Figure 5.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 1 OF LEUG

NUMBER OF DATA POINTS = 13

MULTIPLE R = .9987674 MULTIPLE R-SQUARED = .9975363
STANDARD DEVIATION OF REGRESSION = .002333204

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	.003640676	.001732764	2.10108	.065
B1	.002024378	8.046135×10^{-5}	25.15963	.0001
B2	-1.19016×10^{-5}	8.19542×10^{-7}	-14.52225	.0001
B3	2.288284×10^{-8}	2.246108×10^{-9}	10.18777	.0001

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.01983724	3.	.006612412	1214.659	.0001
RESIDUAL	4.899458×10^{-5}	9.	5.443843×10^{-6}		

Table A-4. Polynomial fit to curve 3 of Figure 5.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 1 OF TRYG

NUMBER OF DATA POINTS = 13

MULTIPLE R = .9987584 MULTIPLE R-SQUARED = .9975183
STANDARD DEVIATION OF REGRESSION = .002148696

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	.0004415403	.001595738	.2766997	.7883
B1	.001903719	7.409851×10^{-5}	25.69173	.0001
B2	-1.043605×10^{-5}	7.547331×10^{-7}	-13.82748	.0001
B3	1.725599×10^{-8}	2.068487×10^{-9}	8.342323	.0001

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.01670201	3.	.005567337	1205.862	.0001
RESIDUAL	4.155205×10^{-5}	9.	4.616894×10^{-6}		

Table A-5. Polynomial fit to curve 11 of Figure 5.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 1 OF ASPG

NUMBER OF DATA POINTS = 13

MULTIPLE R = .9995473 MULTIPLE R-SQUARED = .9990948
STANDARD DEVIATION OF REGRESSION = .004025085

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	.006949271	.002989247	2.324757	.0451
B1	.005208382	.0001388064	37.52263	.0001
B2	-2.775215×10^{-5}	1.413818×10^{-6}	-19.62922	.0001
B3	4.829427×10^{-8}	3.874833×10^{-9}	12.46358	.0001

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.1609369	3.	.05364564	3311.191	.0001
RESIDUAL	.0001458118	9.	1.620131×10^{-5}		

Table A-6. Polynomial fit to curve 9 of Figure 5.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 1 OF SERG

NUMBER OF DATA POINTS = 13

MULTIPLE R = .9561936 MULTIPLE R-SQUARED = .9143062
STANDARD DEVIATION OF REGRESSION = .01074904

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	.07888952	.007982819	9.882413	.0001
B1	.002104708	.0003706842	5.677901	.0003
B2	-1.520692×10^{-5}	3.775618×10^{-6}	-4.027665	.003
B3	3.372199×10^{-8}	1.034779×10^{-8}	3.25886	.0099

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.01109492	3.	.003698305	32.00837	.001
RESIDUAL	.001039876	9.	.0001155418		

Table A-7. Polynomial fit to curve 7 of Figure 5.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 1 OF PROG

NUMBER OF DATA POINTS = 13

MULTIPLE R = .9932002 MULTIPLE R-SQUARED = .9864466
STANDARD DEVIATION OF REGRESSION = .004975968

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	.0261566	.003695424	7.078106	.0001
B1	.001620292	.0001715979	9.442375	.0001
B2	-8.706661×10^{-6}	1.747817×10^{-6}	-4.981449	.0008
B3	1.564654×10^{-8}	4.79022×10^{-9}	3.266351	.0097

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.01621898	3.	.005406327	218.347	.0001
RESIDUAL	.0002228423	9.	2.476026×10^{-5}		

Table A-8. Polynomial fit to curve 4 of Figure 5.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 1 OF METHG

NUMBER OF DATA POINTS = 13

MULTIPLE R = .9928447 MULTIPLE R-SQUARED = .9857405
STANDARD DEVIATION OF REGRESSION = .005294602

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	.0021042	.003932059	.5351395	.6055
B1	.001991511	.0001825861	10.90724	.0001
B2	-1.237026×10^{-5}	1.859738×10^{-6}	-6.651614	.0001
B3	2.518624×10^{-8}	5.09696×10^{-9}	4.941424	.0008

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.0174409	3.	.005813632	207.3868	.0001
RESIDUAL	.0002522952	9.	2.803281×10^{-5}		

Table A-9. Polynomial fit to curve 8 of Figure 5.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 1 OF ALAG

NUMBER OF DATA POINTS = 13

MULTIPLE R = .9984386 MULTIPLE R-SQUARED = .9968795
STANDARD DEVIATION OF REGRESSION = .003303989

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	.008383797	.002453722	3.416768	.0077
B1	.001866659	.0001139392	16.38294	.0001
B2	-8.357286×10^{-6}	1.160532×10^{-6}	-7.201255	.0001
B3	1.255072×10^{-8}	3.180655×10^{-9}	3.945955	.0034

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.03138654	3.	.01046218	958.3959	.0001
RESIDUAL	9.82471×10^{-5}	9.	1.091634×10^{-5}		

Table A-10. Polynomial fit to curve 6 of Figure 5.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 1 OF ISOLG

NUMBER OF DATA POINTS = 13

MULTIPLE R = .9979399 MULTIPLE R-SQUARED = .9958841
STANDARD DEVIATION OF REGRESSION = .002966654

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	.009984061	.002203198	4.531622	.0014
B1	.00192643	.0001023061	18.83007	.0001
B2	-1.104578×10^{-5}	1.042042×10^{-6}	-10.60012	.0001
B3	2.080909×10^{-8}	2.855911×10^{-9}	7.286323	.0001

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.01916525	3.	.006388418	725.8713	.0001
RESIDUAL	7.92093×10^{-5}	9.	8.801034×10^{-6}		

Table A-11. Polynomial fit to curve 2 of Figure 5.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 1 OF PHALG

NUMBER OF DATA POINTS = 13

MULTIPLE R = .9991288 MULTIPLE R-SQUARED = .9982584
STANDARD DEVIATION OF REGRESSION = .001862824

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	-.001763901	.001383434	-1.275016	.2342
B1	.001696855	6.424012×10^{-5}	26.41425	.0001
B2	-8.913282×10^{-6}	6.543201×10^{-7}	-13.6222	.0001
B3	1.53962×10^{-8}	1.793287×10^{-9}	8.585467	.0001

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.01790113	3.	.005967042	1719.552	.0001
RESIDUAL	3.123102×10^{-5}	9.	3.470114×10^{-6}		

Table A-12. Linear fit to curve 1 of Figure 12.

WOULD YOU LIKE TO ADD THE FITTED LINE AS A NEW CURVE TO AUCG?

LINEAR FIT TO:
CURVE 1 OF AUCG
NUMBER OF DATA POINTS = 10
CORRELATION COEFFICIENT R = -.03419588 R-SQUARED = .001169358
STANDARD DEVIATION OF REGRESSION = 16.60921

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
INTERCEPT	38.37763	65.77277	.5834881	.5756
SLOPE	-.9010126	9.310242	-.0967765	.9253

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	2.58371	1.	2.58371	.009365818	.925
RESIDUAL	2206.927	8.	275.8659		

Table A-13. Linear fit to curve 1 of Figure 13.

WOULD YOU LIKE TO ADD THE FITTED LINE AS A NEW CURVE TO SEXG?

LINEAR FIT TO:
CURVE 1 OF SEXG
NUMBER OF DATA POINTS = 10
CORRELATION COEFFICIENT R = .3020884 R-SQUARED = .0912574
STANDARD DEVIATION OF REGRESSION = .0008773024

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
INTERCEPT	-.000963959	.003474133	-.2774675	.7885
SLOPE	.0004407781	.0004917692	.8963109	.3963

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	6.183236×10^{-7}	1.	6.183236×10^{-7}	.8033729	.396
RESIDUAL	6.157276×10^{-6}	8.	7.696596×10^{-7}		

Table A-14. Polynomial fit to curve 2 of Figure 14.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 3 OF
NUMBER OF DATA POINTS = 13
MULTIPLE R = .9701369 MULTIPLE R-SQUARED = .9411657
STANDARD DEVIATION OF REGRESSION = .009820092

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	.03603184	.007292933	4.940651	.0008
B1	.0003877375	.0003386492	1.144953	.2818
B2	3.692328×10^{-6}	3.449324×10^{-6}	1.07045	.3123
B3	-1.719482×10^{-8}	9.453518×10^{-9}	-1.818881	.1023

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.01388382	3.	.004627941	47.99065	.0001
RESIDUAL	.0008679079	9.	9.643421×10^{-5}		

Table A-15. Polynomial fit to curve 3 of Figure 16.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 1 OF NOBUFG
NUMBER OF DATA POINTS = 13
MULTIPLE R = .9944077 MULTIPLE R-SQUARED = .9888467
STANDARD DEVIATION OF REGRESSION = .004214122

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	.004644617	.003129636	1.484076	.1719
B1	.001236442	.0001453254	8.508093	.0001
B2	-5.02358×10^{-6}	1.480218×10^{-6}	-3.393812	.008
B3	5.903016×10^{-9}	4.056813×10^{-9}	1.455087	.1796

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.01417045	3.	.004723483	265.9795	.0001
RESIDUAL	.0001598294	9.	1.775882×10^{-5}		

Table A-16. Polynomial fit to curve 2 of Figure 17.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 1 OF CONGLYG
NUMBER OF DATA POINTS = 13
MULTIPLE R = .9969931 MULTIPLE R-SQUARED = .9939953
STANDARD DEVIATION OF REGRESSION = .0008020564

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	.0005633014	.0005956506	.945691	.369
B1	4.402799×10^{-5}	2.765919×10^{-5}	1.591804	.1459
B2	1.140758×10^{-6}	2.817237×10^{-7}	4.049208	.0029
B3	-4.008037×10^{-9}	7.721165×10^{-10}	-5.190975	.0006

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.0009583934	3.	.0003194645	496.6069	.0001
RESIDUAL	5.78965×10^{-6}	9.	6.432945×10^{-7}		

Table A-17. Polynomial fit to curve 1 of Figure 17.

POLYNOMIAL FIT OF DEGREE 3 TO:
CURVE 2 OF CONGLYG
NUMBER OF DATA POINTS = 13
MULTIPLE R = .9986439 MULTIPLE R-SQUARED = .9972896
STANDARD DEVIATION OF REGRESSION = .001191166

PARAMETER TABLE

PARAMETER	FITTED VALUE	STANDARD DEVIATION	T-VALUE	SIG. LEV.
B0	.00211107	.0008846243	2.386404	.0408
B1	-4.556326×10^{-5}	4.107775×10^{-5}	-1.109196	.2961
B2	3.681954×10^{-6}	4.18399×10^{-7}	8.800104	.0001
B3	-1.130373×10^{-8}	1.146701×10^{-9}	-9.857608	.0001

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F VALUE	SIG. LEV.
REGRESSION	.004698603	3.	.001566201	1103.832	.0001
RESIDUAL	1.276988×10^{-5}	9.	1.418876×10^{-6}		

APPENDIX B

Computer Predicted Figures for Each Amino Acid-Iron Complex

These figures include (a) blood iron concentration, and (b) iron concentration in organs following oral administration of amino acid-iron complexes.

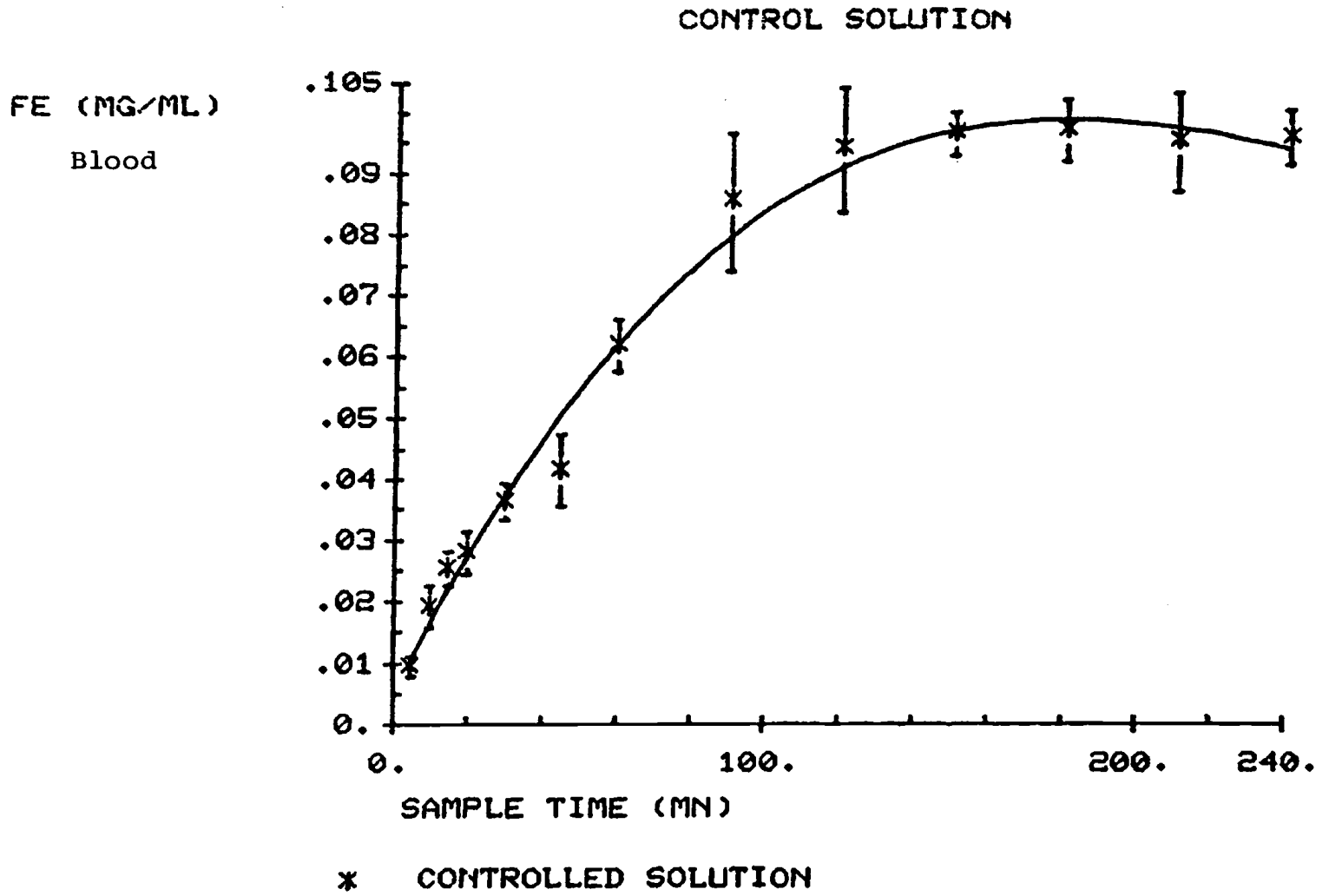

Figure B-1. Blood iron concentration following oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) given as control

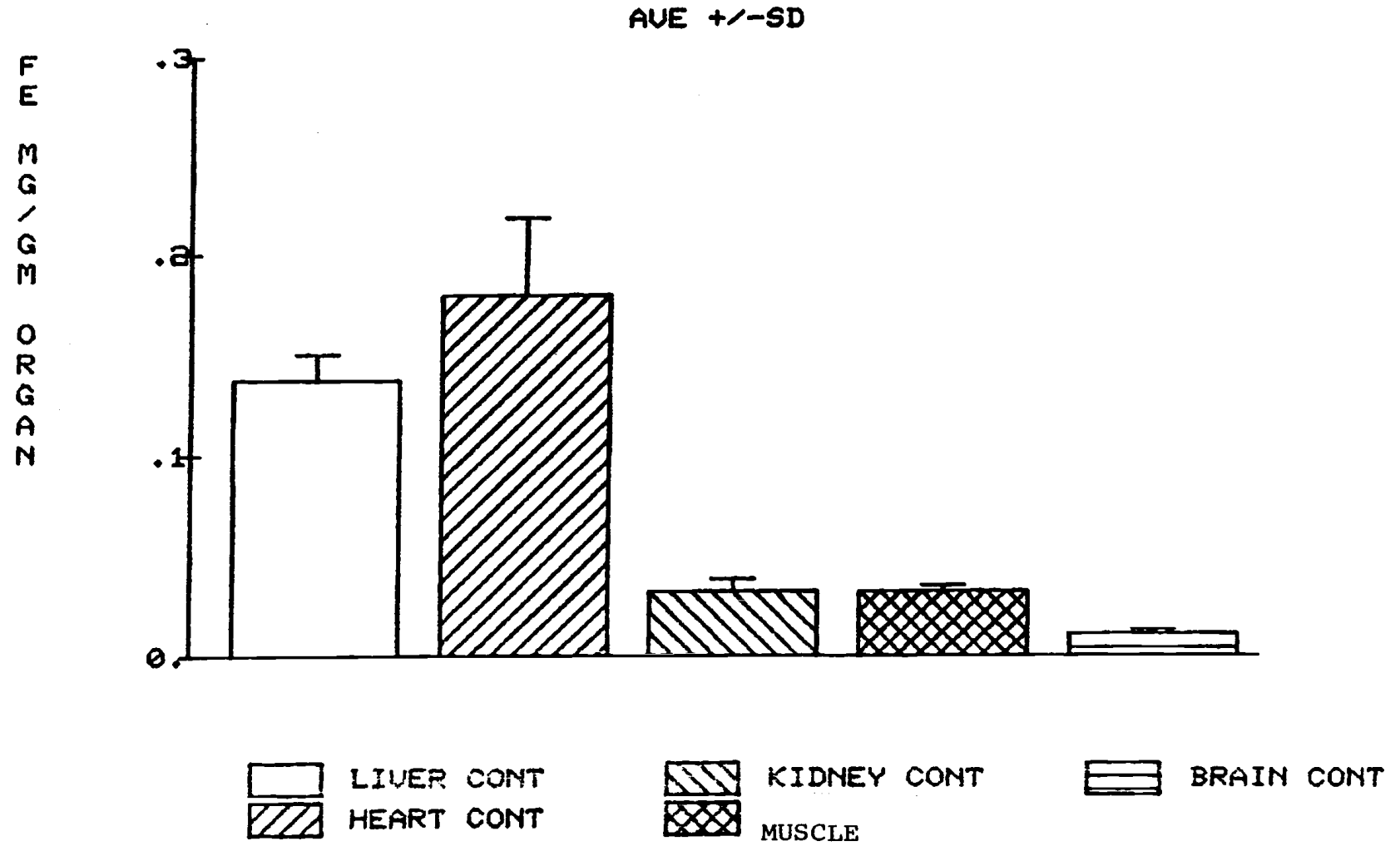

Figure B-2. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) given as control.

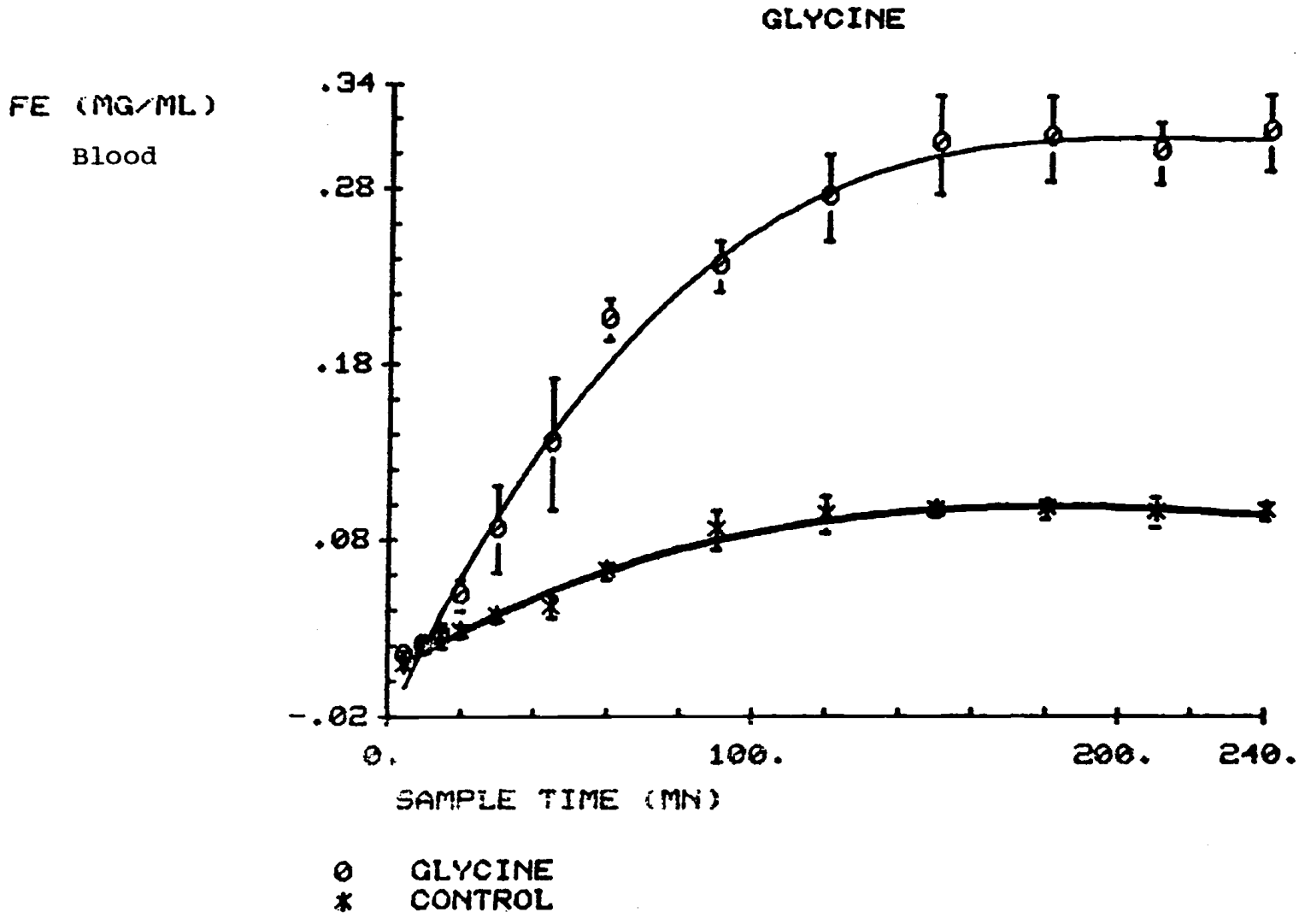

Figure B-3. Comparison of blood iron concentration when 0.2 M ferrous sulphate (55.6 mg/ml) given as (1) control and (2) with 0.4 M glycine (30.0 mg/ml).

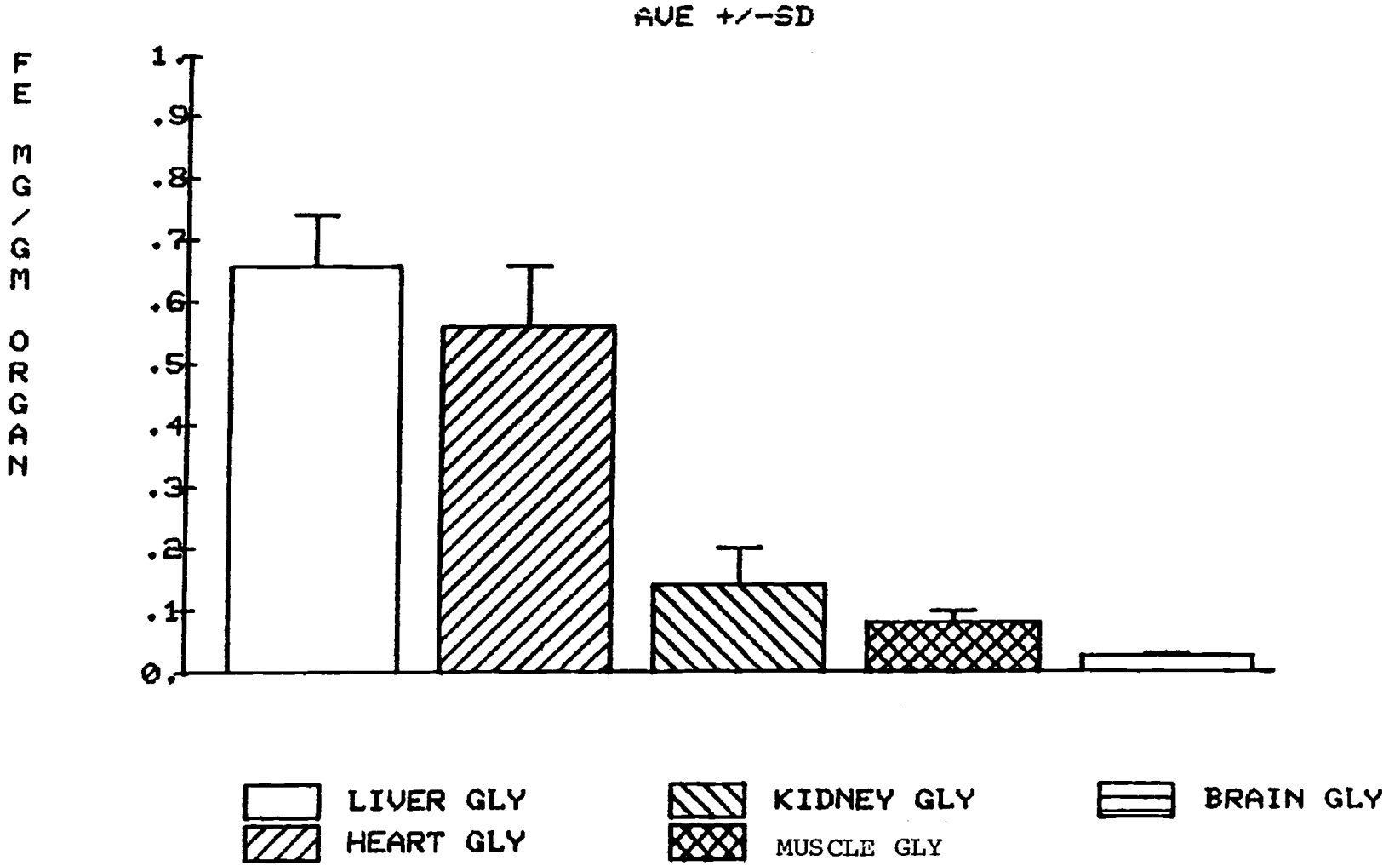

Figure B-4. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M glycine (30.0 mg/ml).

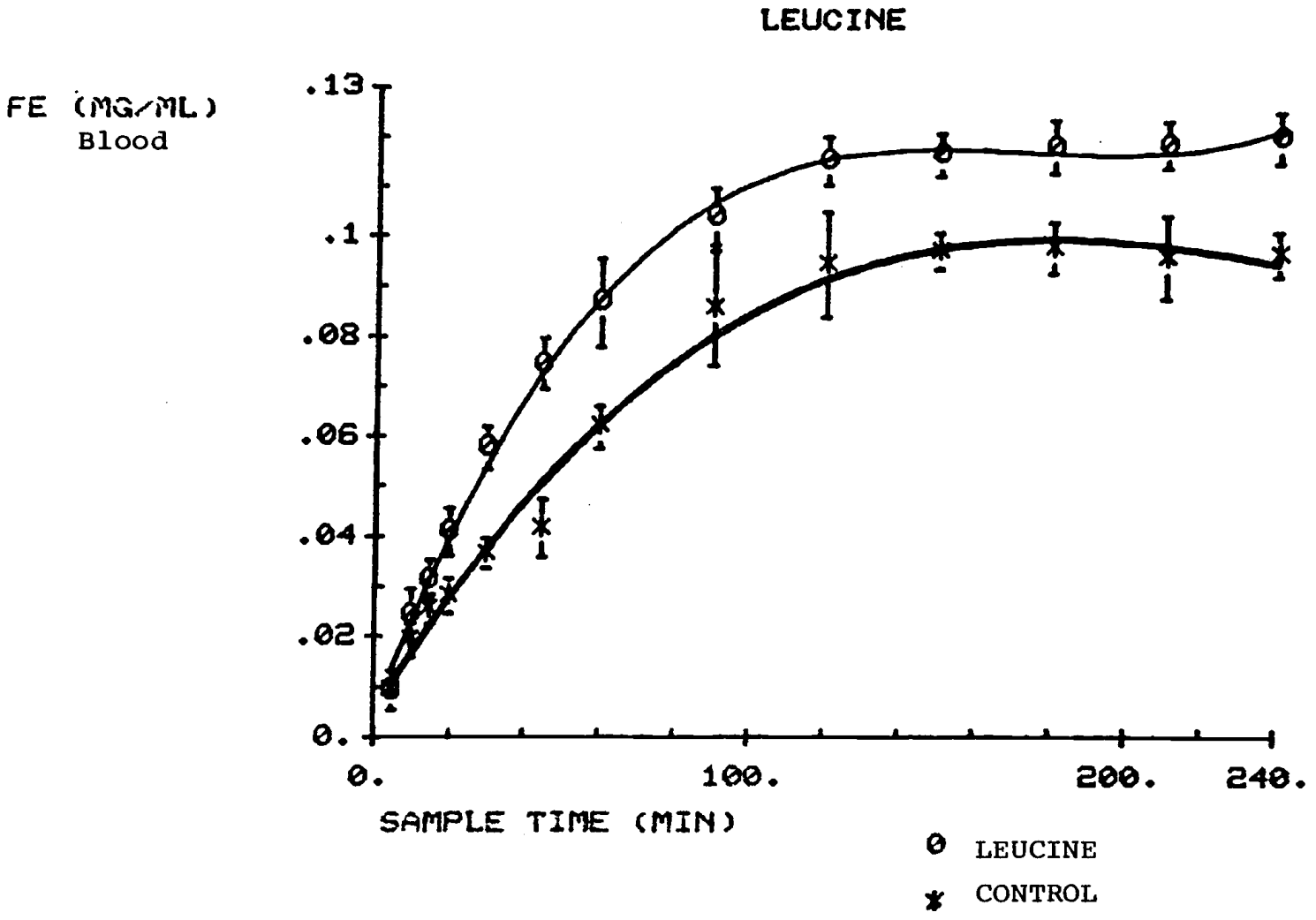

Figure B-5. Comparison of blood iron concentration when 0.2 M ferrous sulphate (55.6 mg/ml) was given as (1) control and (2) with 0.4 M leucine (52.4 mg/ml).

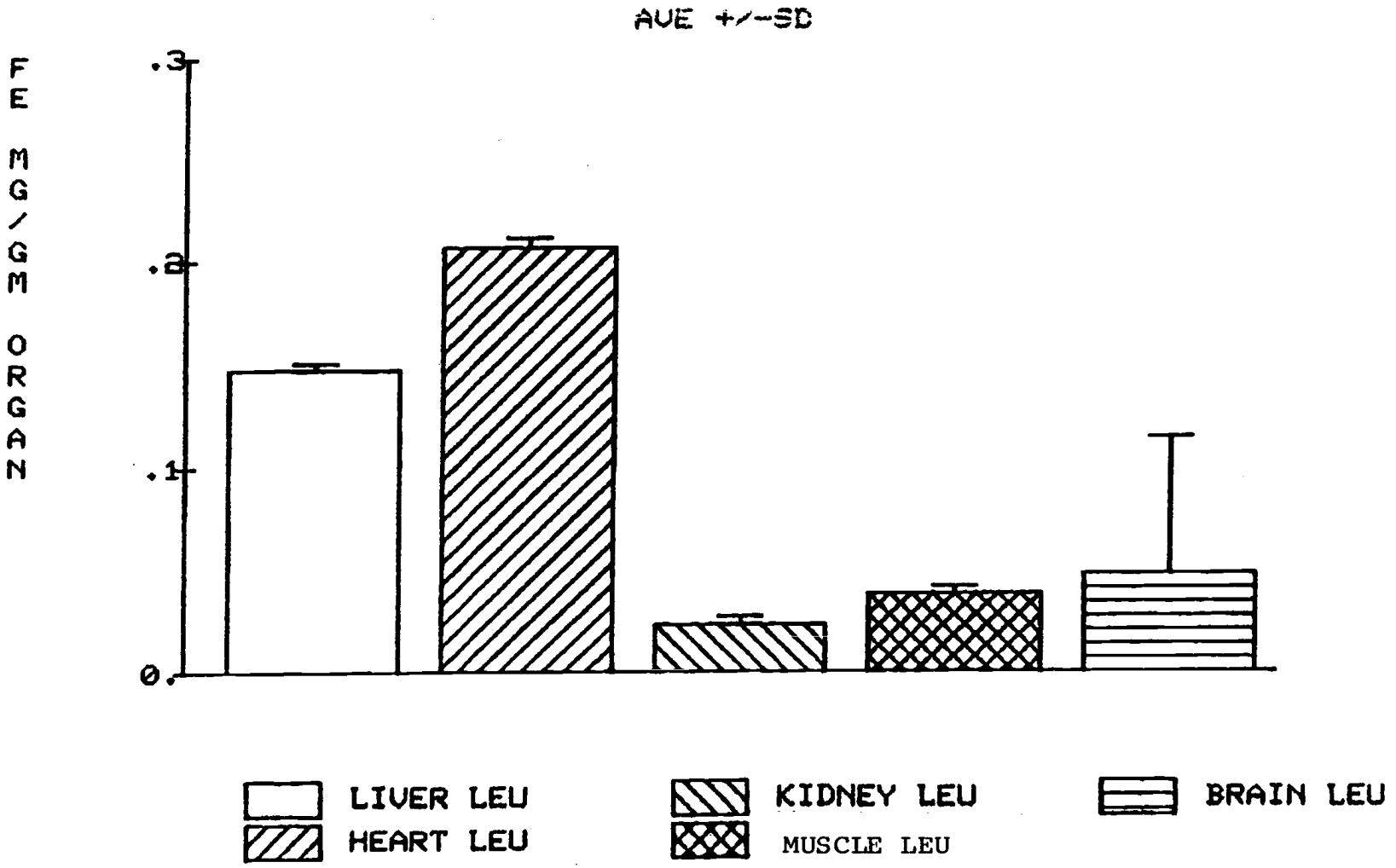

Figure B-6. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M leucine (52.4 mg/ml).

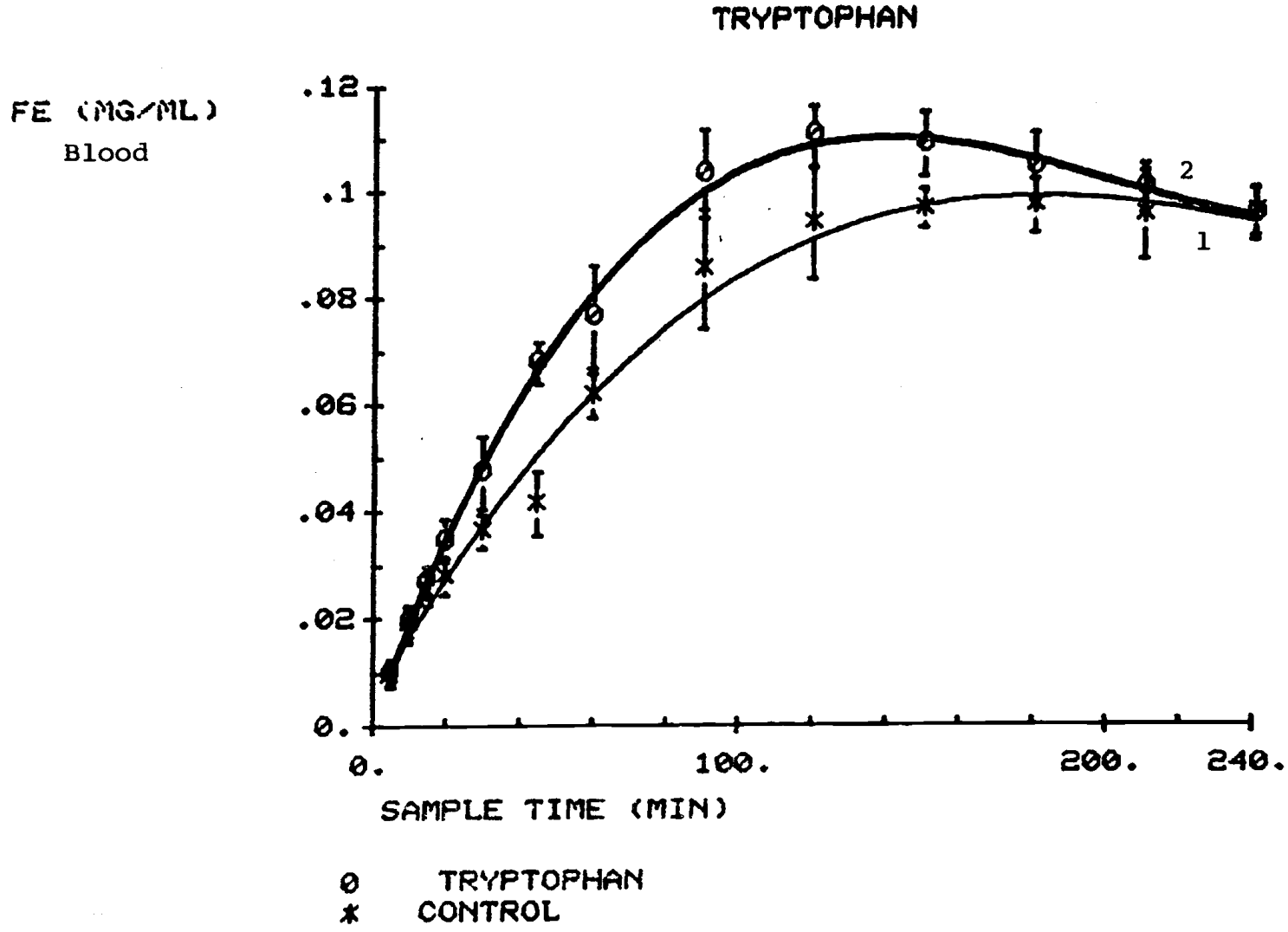

Figure B-7. Comparison of blood iron concentration when 0.2 M ferrous sulphate (55.6 mg/ml) was given as (1) control and (2) with 0.4 M tryptophan (81.2 mg/ml).

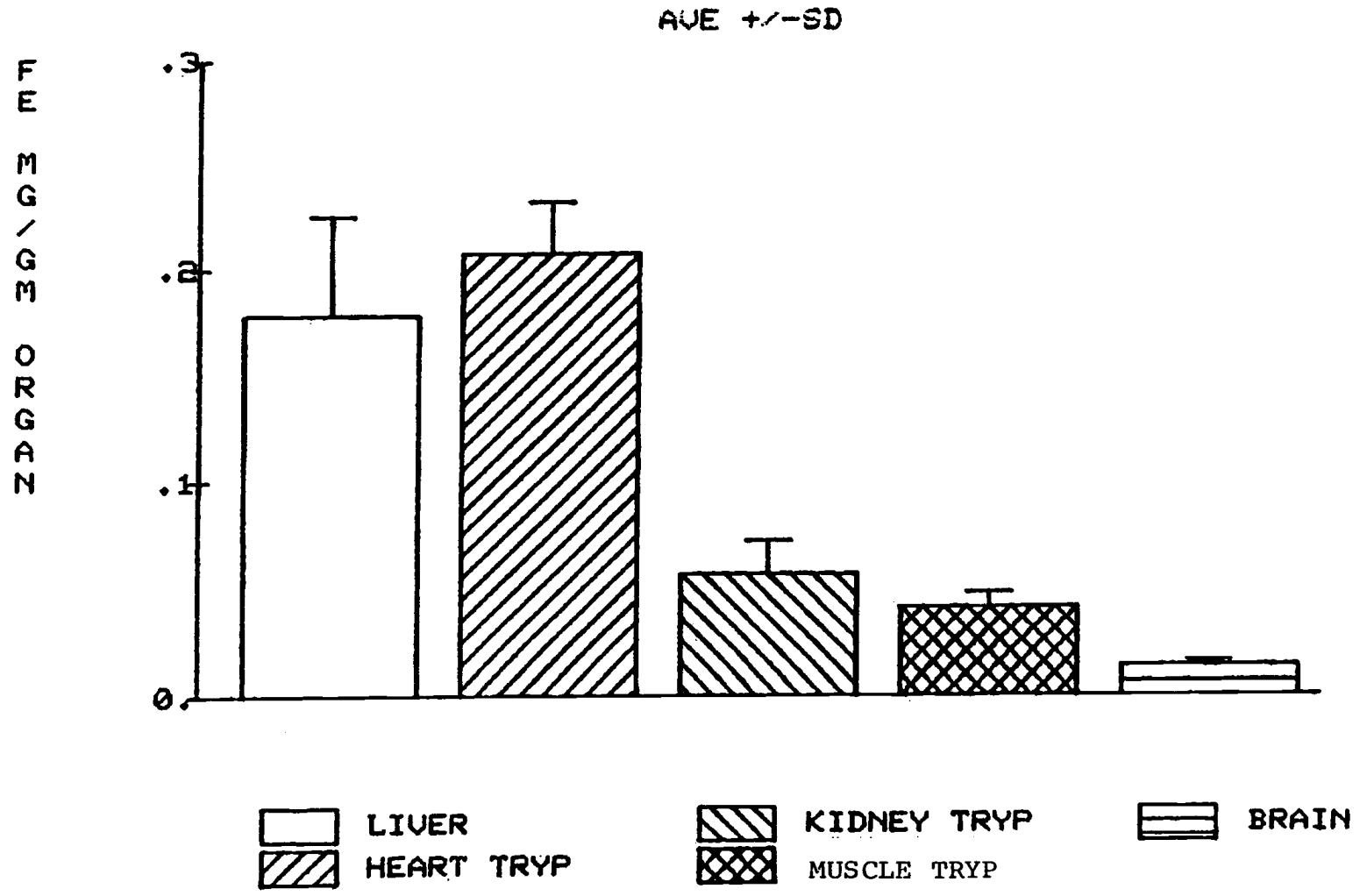

Figure B-8. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M tryptophan (81.2 mg/ml).

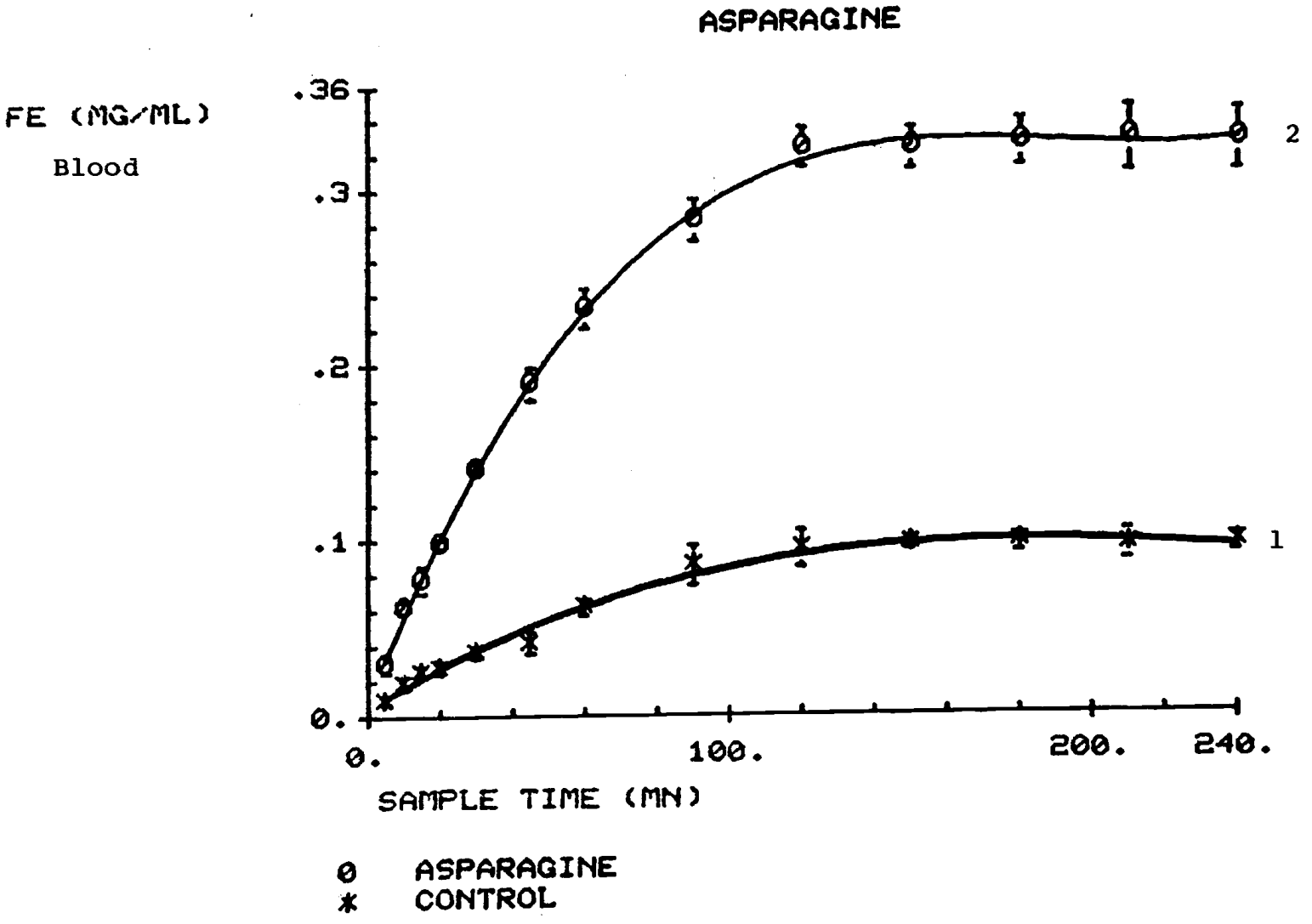

Figure B-9. Comparison of blood iron concentration when 0.2 M ferrous sulphage (55.6 mg/ml) was given as (1) control and (2) with 0.4 M asparagine (52.8 mg/ml).

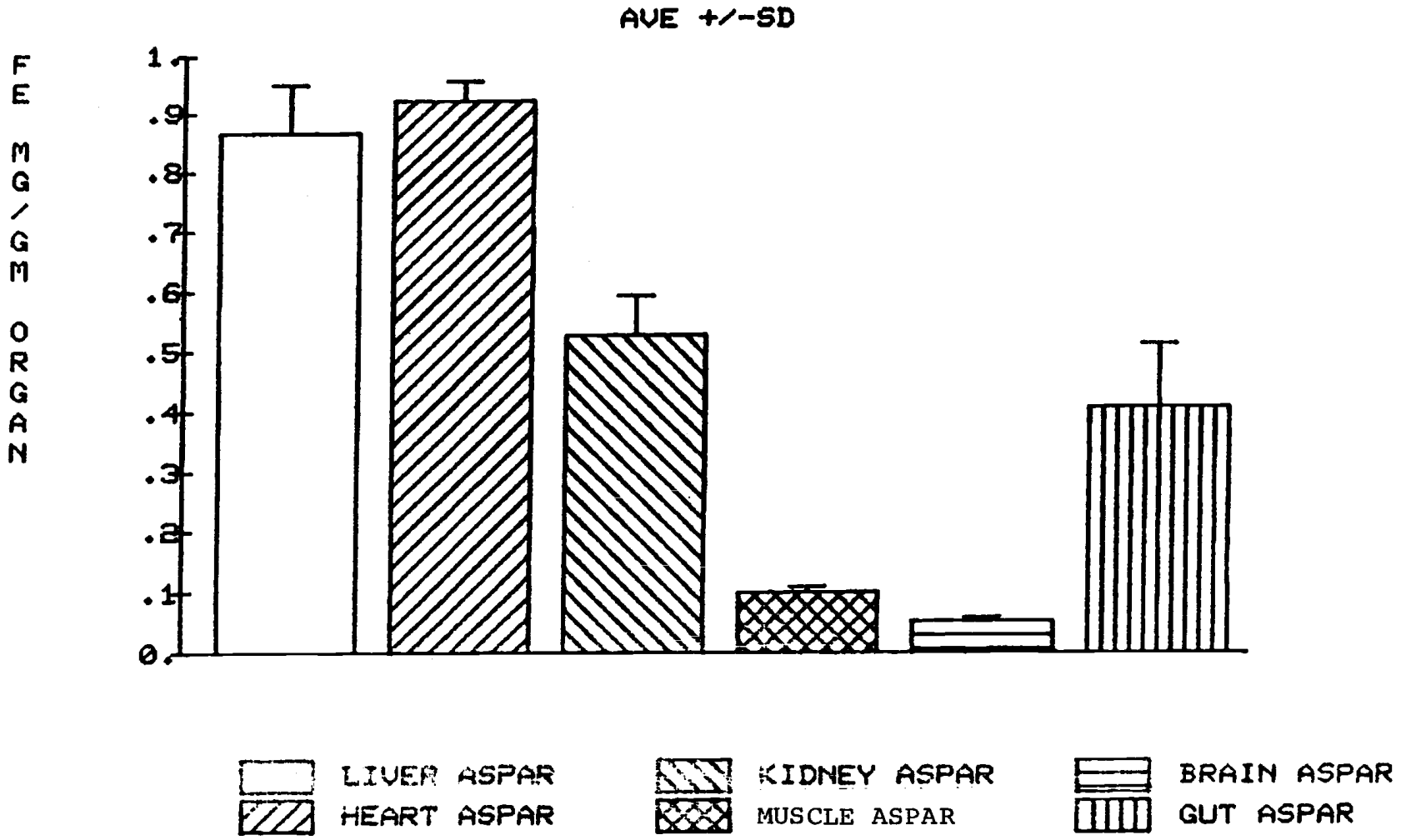

Figure B-10. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphage (55.6 mg/ml) and 0.4 M asparagine (52.8 mg/ml).

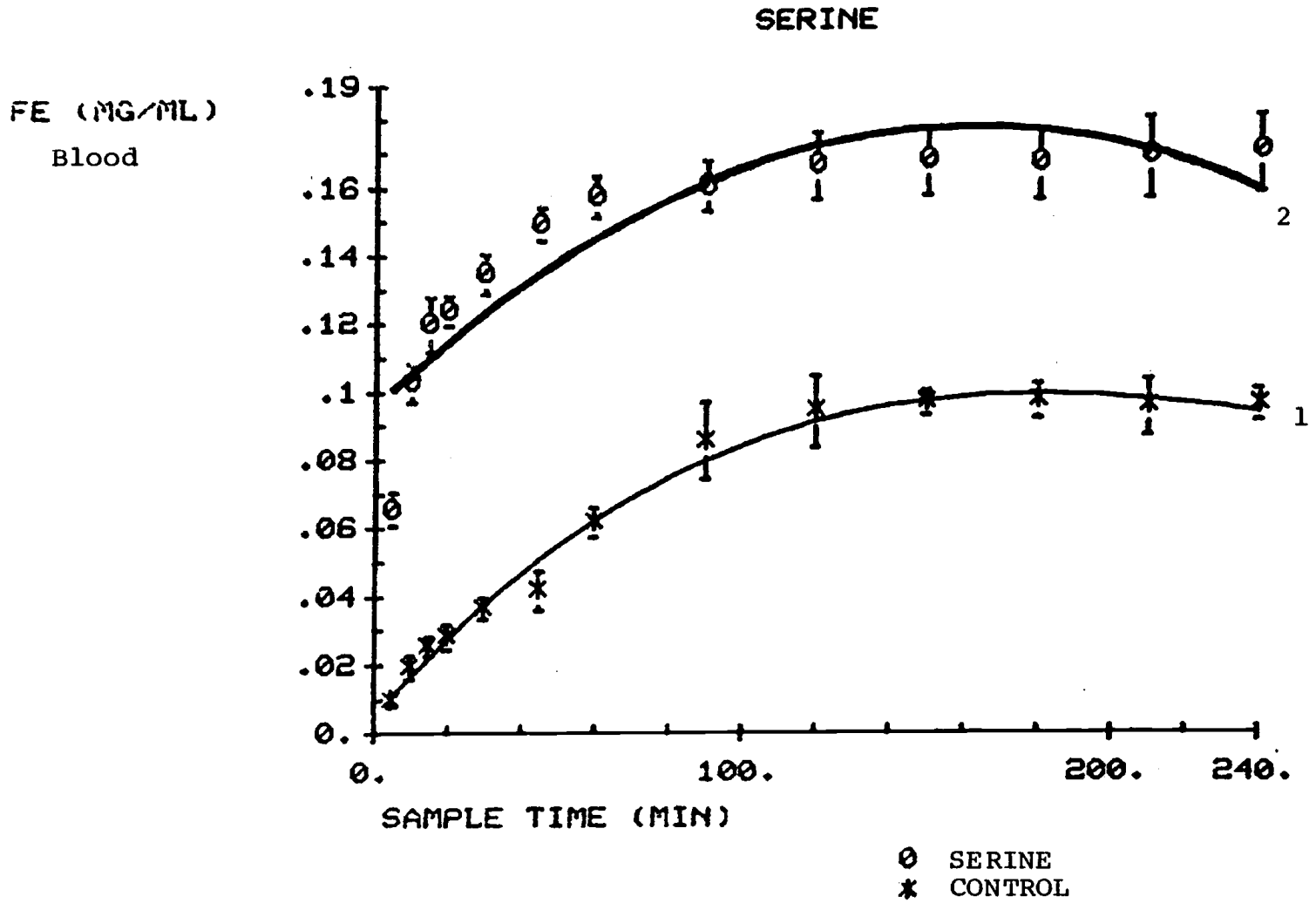

Figure B-11. Comparison of blood iron concentration when 0.2 M ferrous sulphate (55.6 mg/ml) was given as (1) control and (2) with 0.4 M serine (43.6 mg/ml).

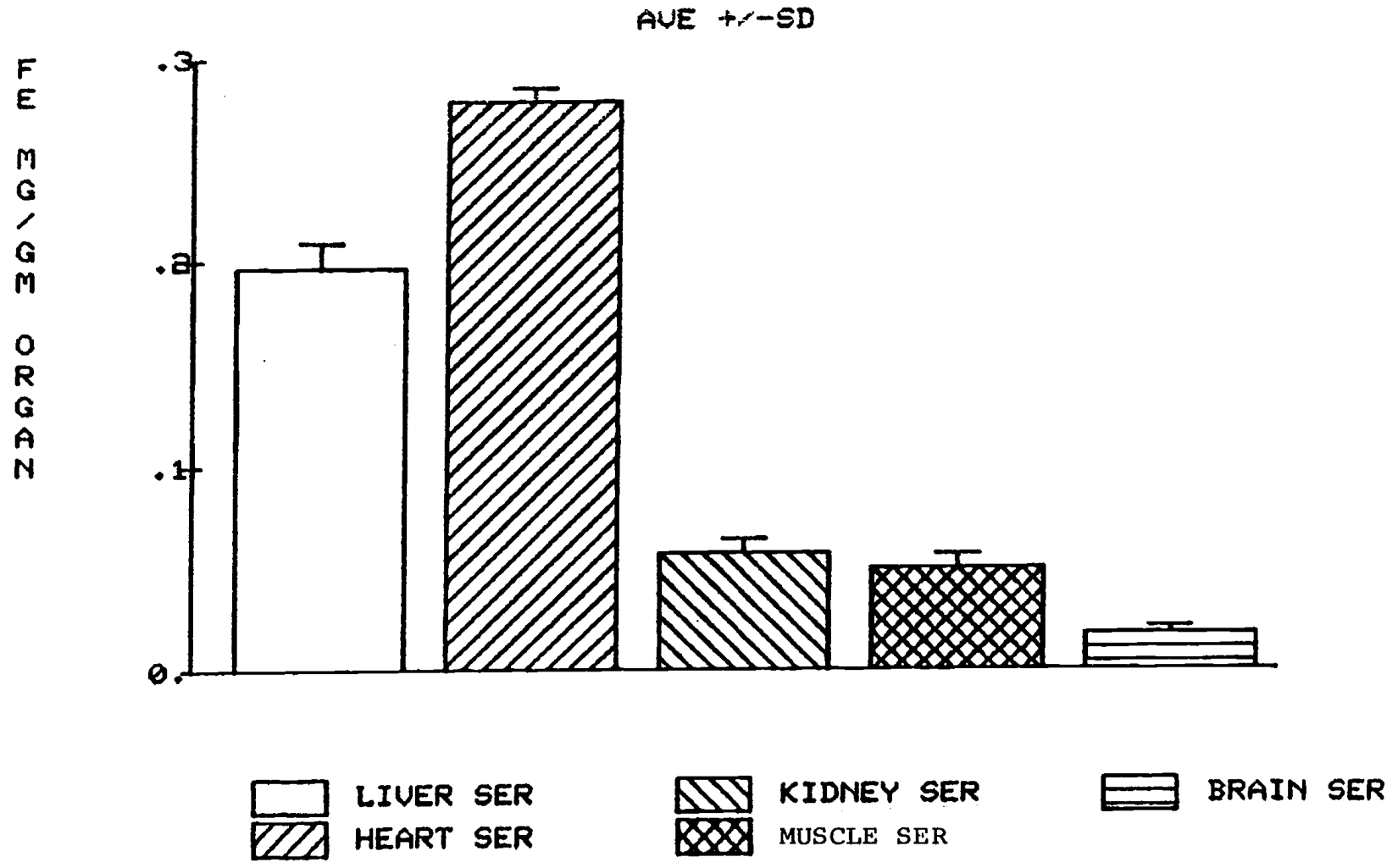

Figure B-12. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphage (55.6 mg/ml) and 0.4 M serine (43.6 mg/ml).

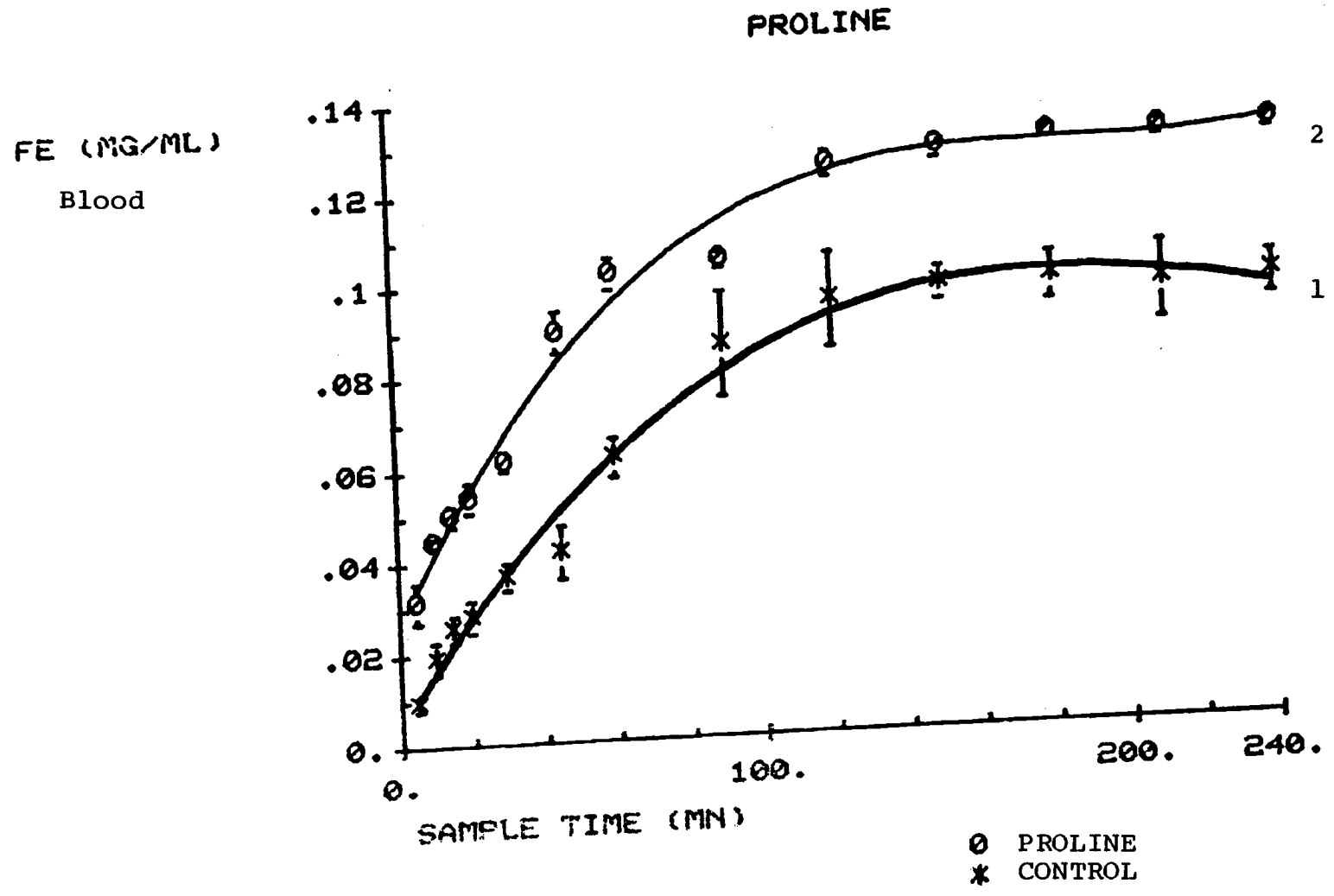

Figure B-13. Comparison of blood iron concentration when 0.2 M ferrous sulphate (55.6 mg/ml) was given as (1) control and (2) with 0.4 M proline (45.6 mg/ml).

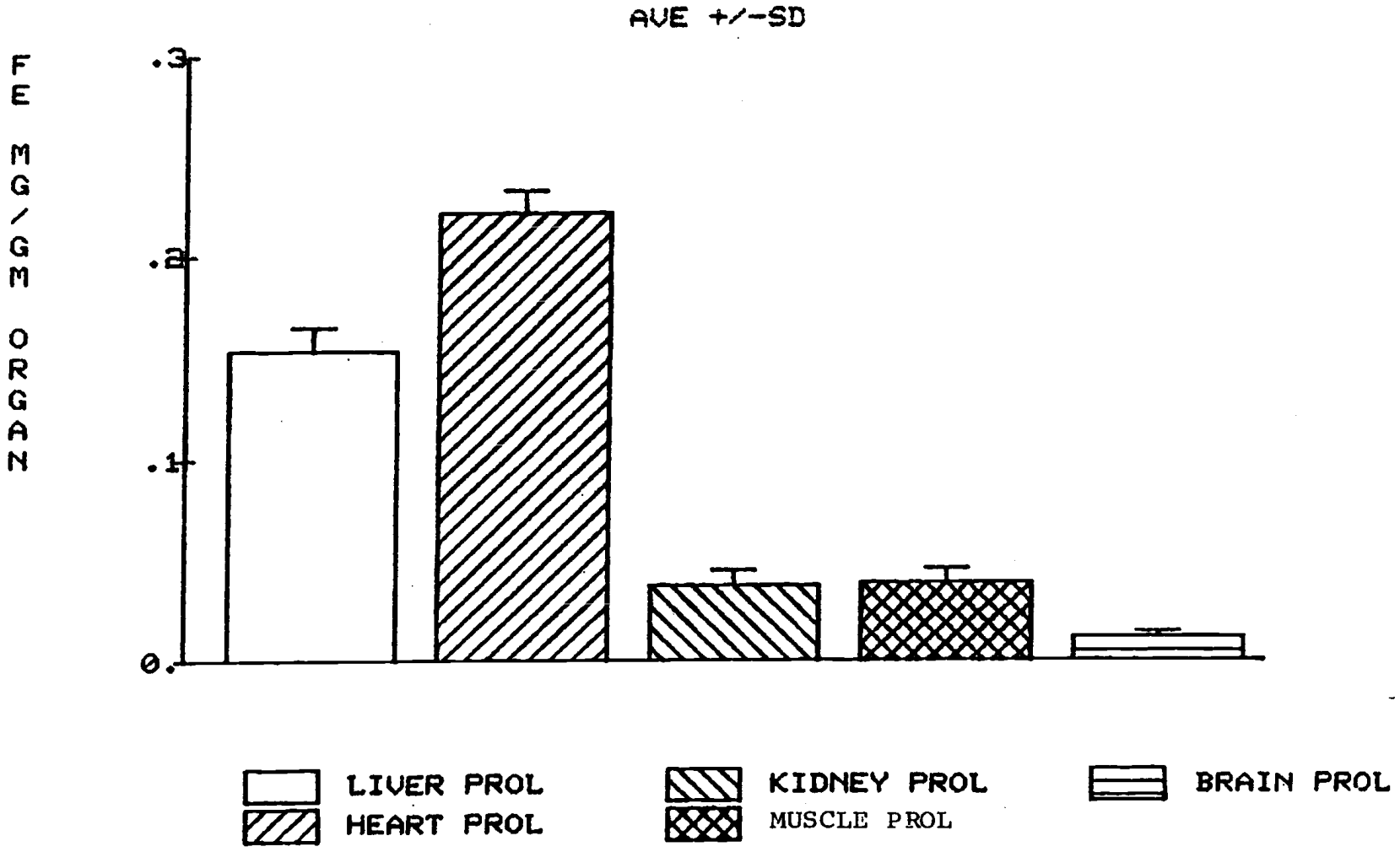

Figure B-14. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M proline (45.6 mg/ml).

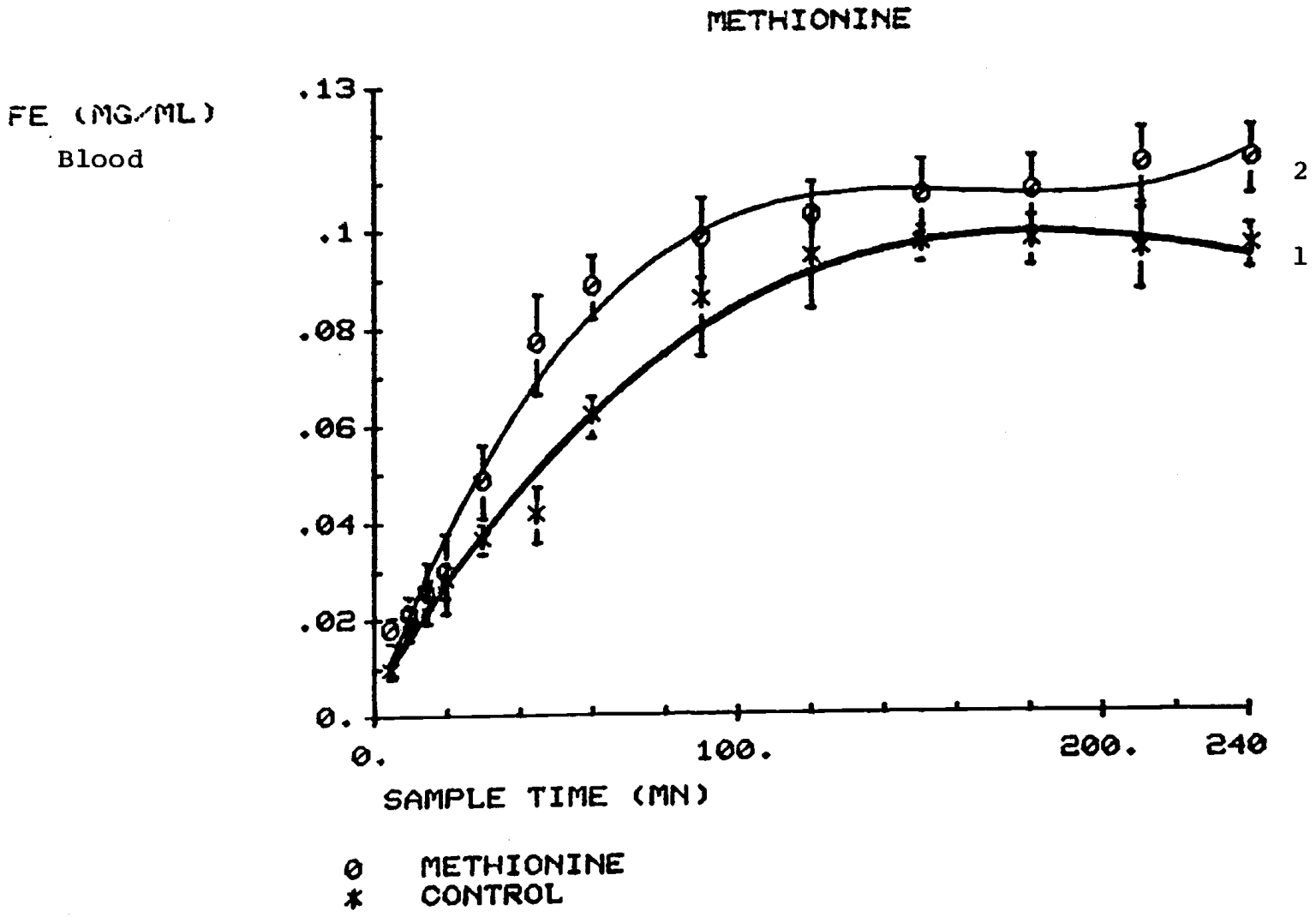

Figure B-15. Comparison of blood iron concentration when 0.2 M ferrous sulphate (55.6 mg/ml) was given as (1) control and (2) with 0.4 M methionine (59.6 mg/ml).

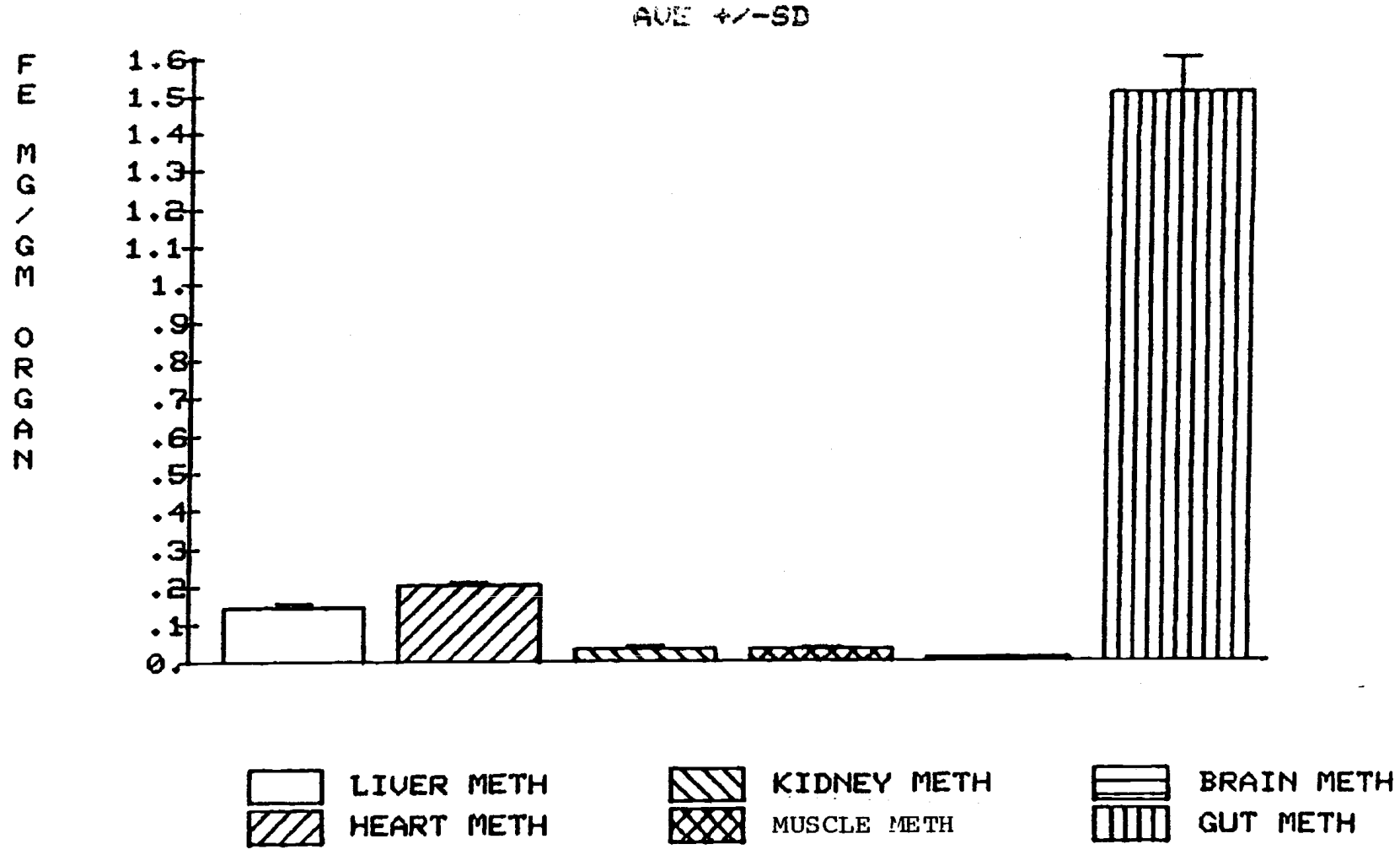

Figure B-16. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M methionine (59.6 mg/ml).

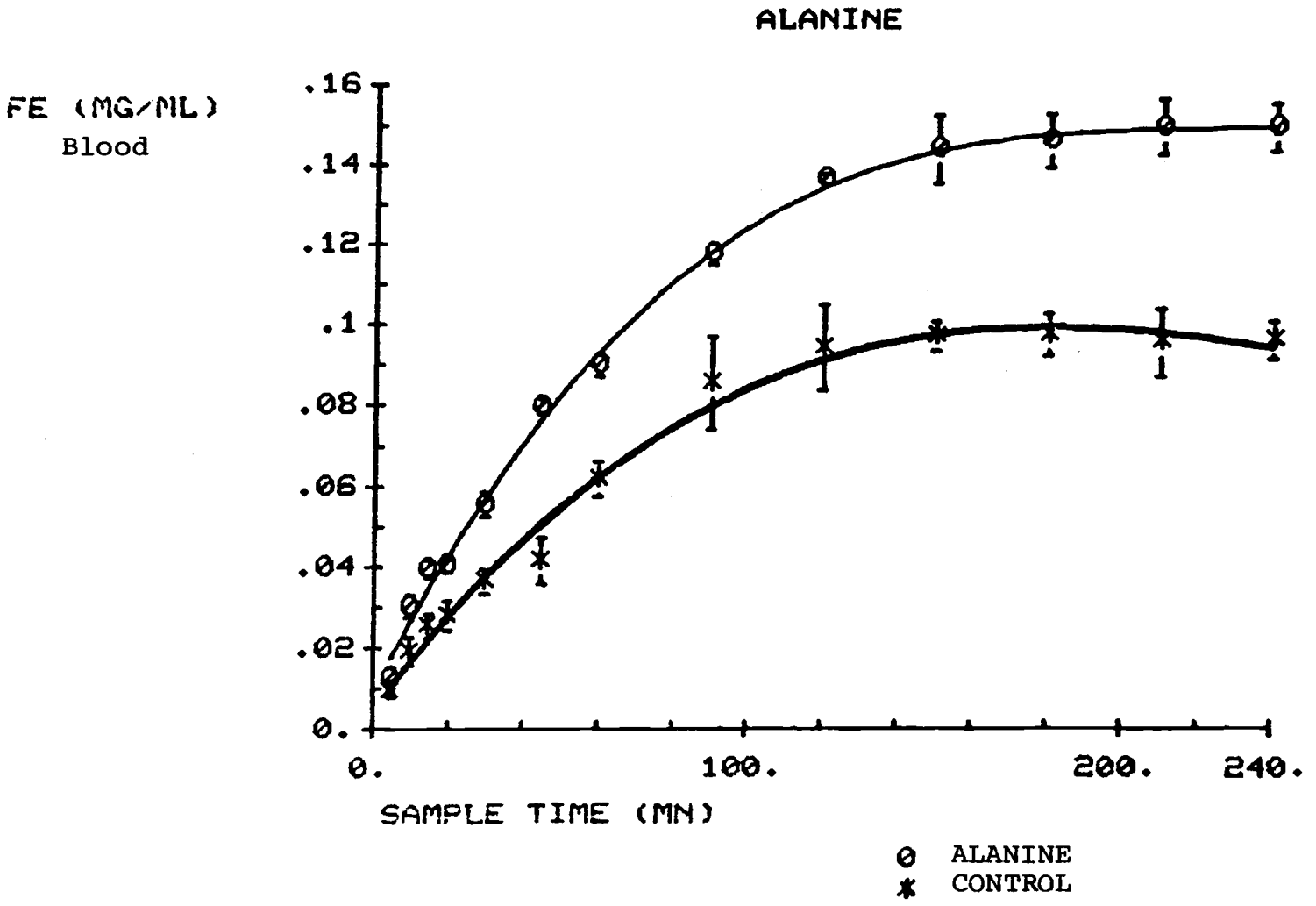

Figure B-17. Comparison of blood iron concentration when 0.2 M ferrous sulphate (55.6 mg/ml) was given as control and when it was administered with 0.4 M alanine (35.6 mg/ml).

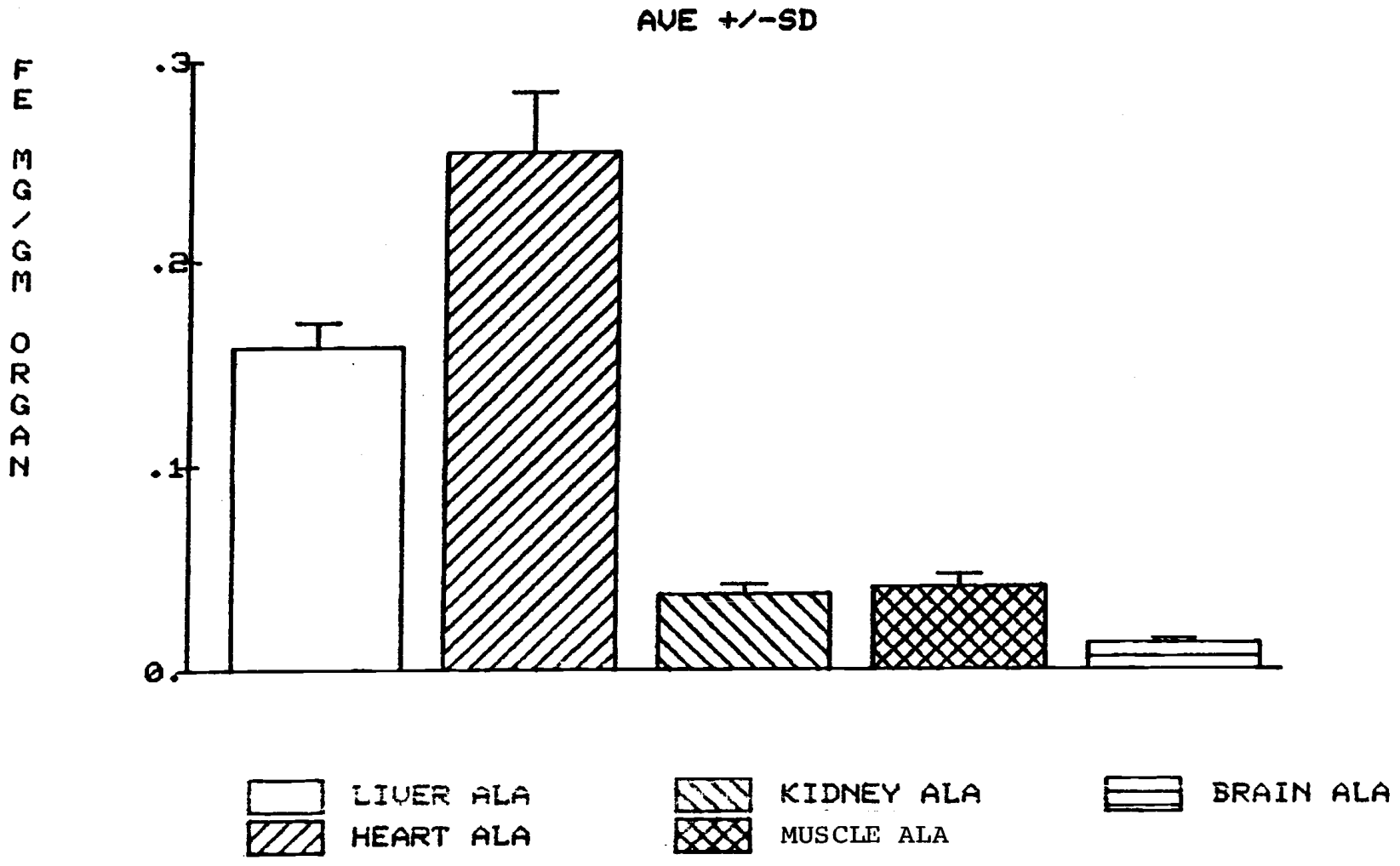

Figure B-18. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulfate (55.6 mg/ml) and 0.4 M alanine (35.6 mg/ml).

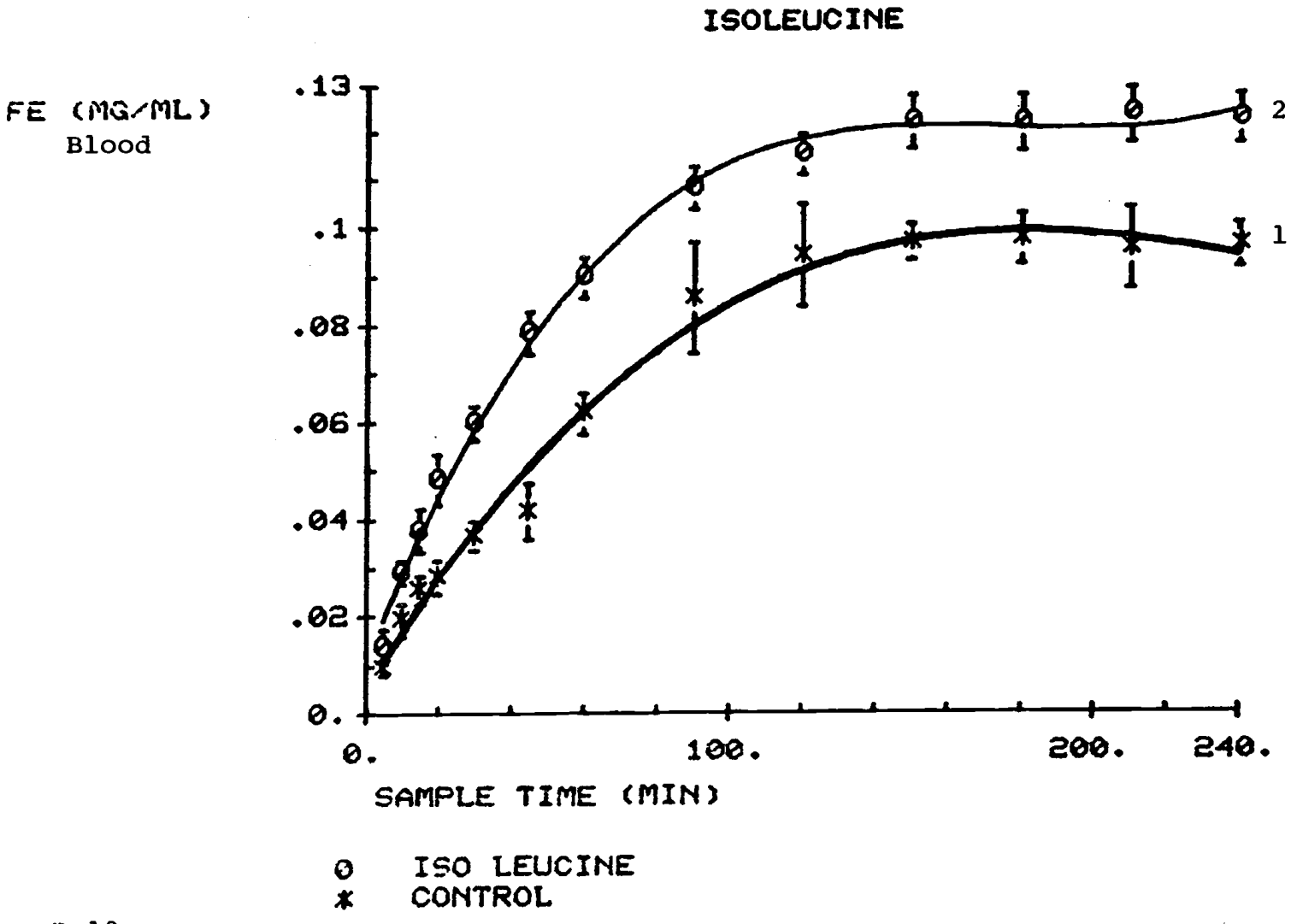

Figure B-19. Comparison of blood iron concentration when 0.2 M ferrous sulphate (55.6 mg/ml) was given as (1) control and (2) with 0.4 M isoleucine (52.4 mg/ml).

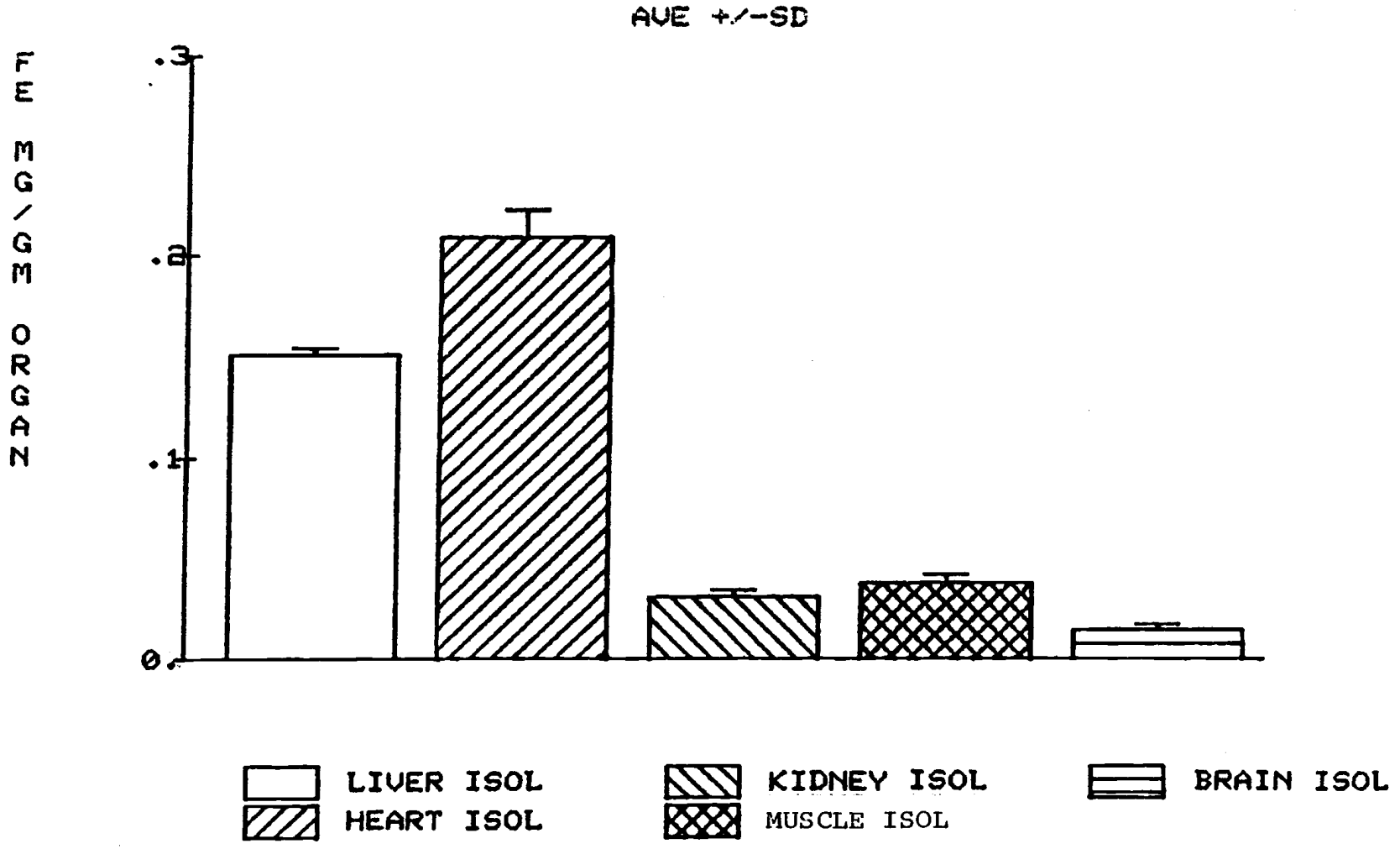

Figure B-20. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M isoleucine (52.4 mg/ml).

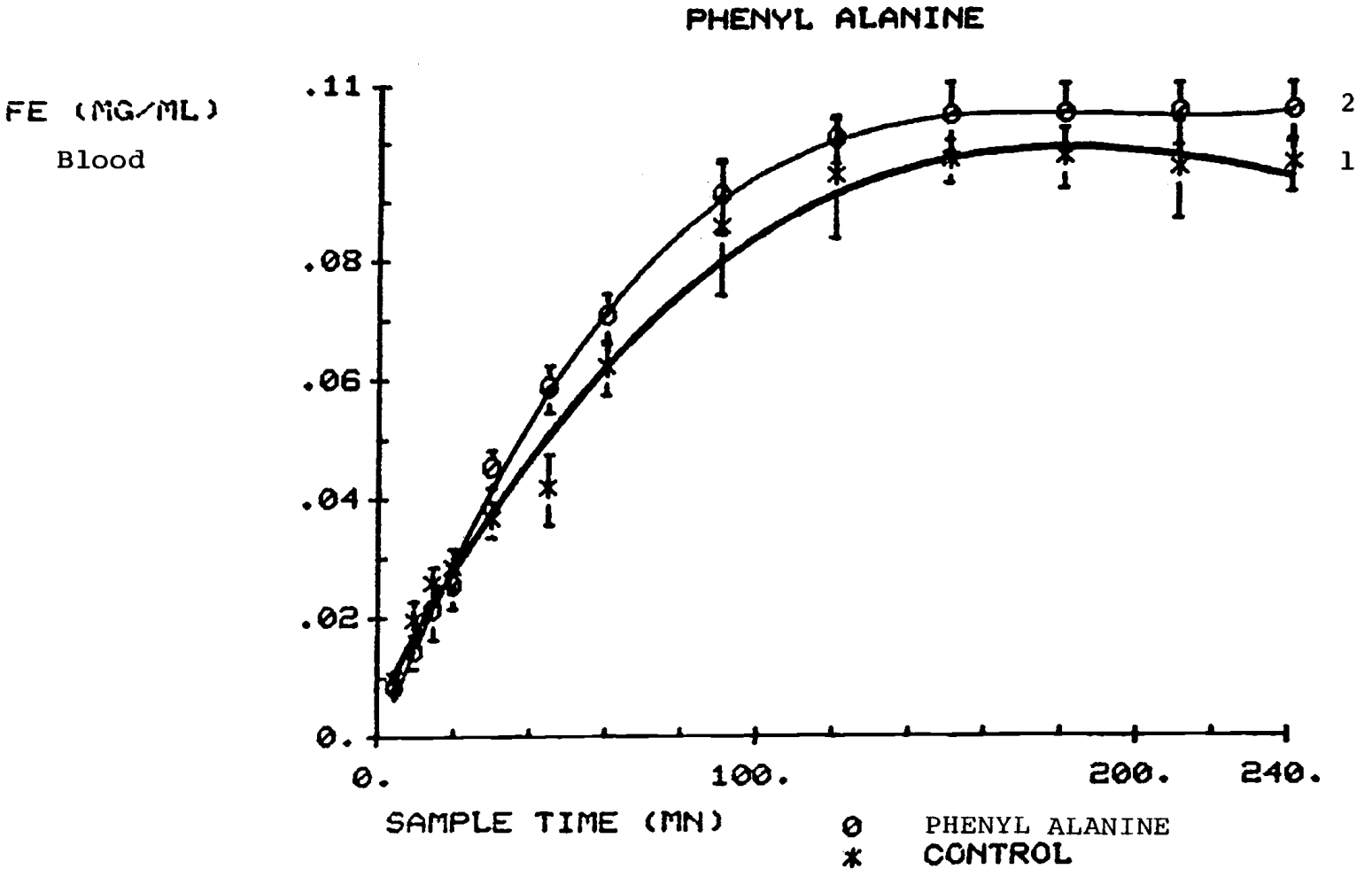

Figure B-21. Comparison of blood iron concentration when 0.2 M ferrous sulphate (55.6 mg/ml) was given as (1) control and (2) with 0.4 M phenylalanine (66.0 mg/ml).

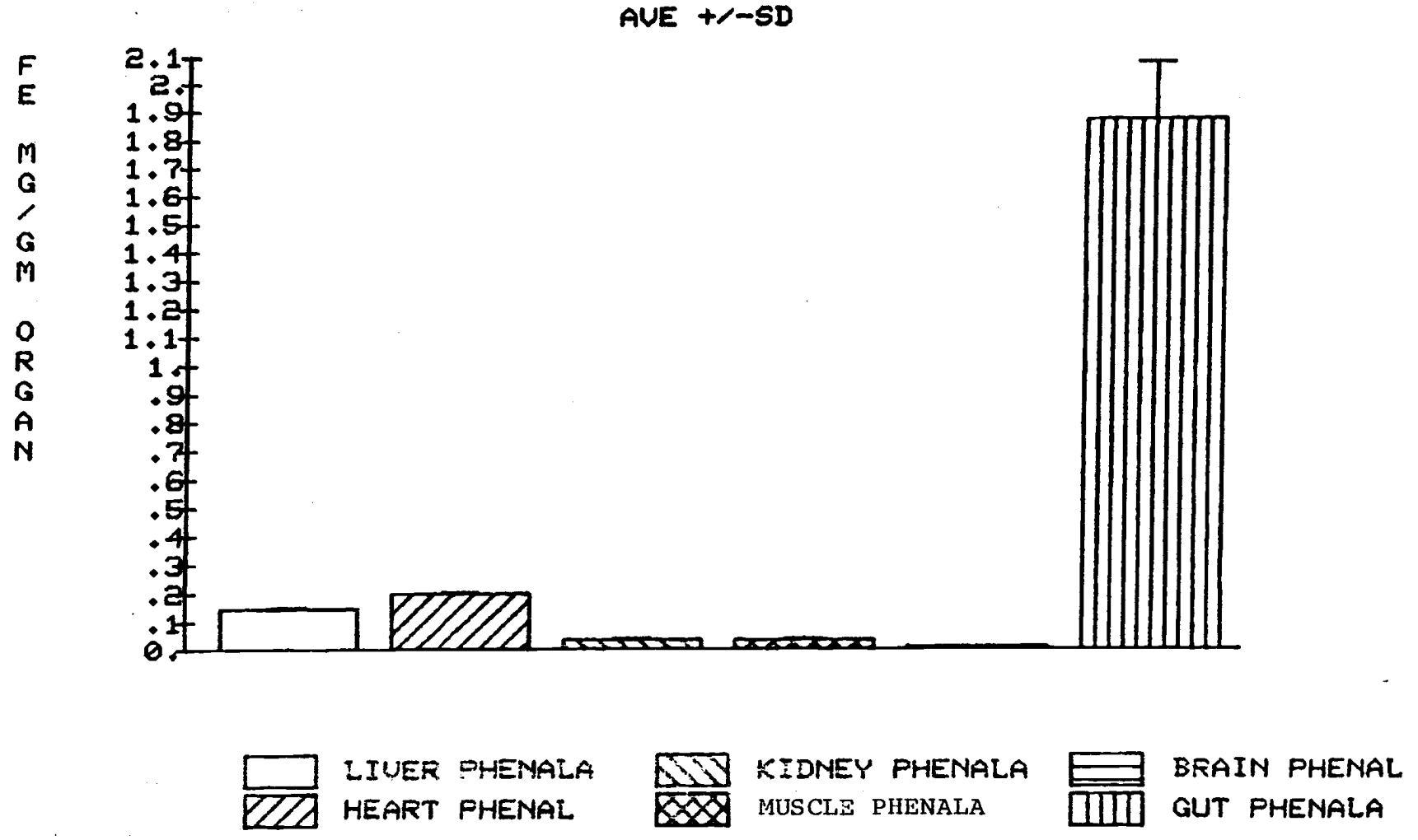

Figure B-22. Iron concentration in organs four hours after the oral administration of 0.2 M ferrous sulphate (55.6 mg/ml) and 0.4 M phenylalanine (66.0 mg/ml).

Appendix C

STABILITY CONSTANT EQUATIONS

1. $K_s = K_1 K_2$

2. $K_1 = \dfrac{\bar{n}}{(1-\bar{n})[Sc]}$

   Sc = the concentration of free chelating species

   $\bar{n}$ = the average number of molecules of complex forming agent bound by one atom of metal

3. $\text{Log}[Sc] = \log\{[HSc°] - [KOH] - [H^+] + [OH^-]\} - \log\{\dfrac{[H^+]}{Ka} + \dfrac{2[H^+]^2}{KaKa'}\}$

   [HSc°] = concentration of complex-forming agent (all species), before the metal was added.

   [KOH] = concentration of KOH which would be present if the complex forming agent and metal were absent

4. $\text{Log}[Sc] = (pH - pKa) + \log\{[HSc°] - [KOH]\}$

5. $\bar{n} = \dfrac{[HSc°] - \alpha[Sc]}{[M°]}$

   [M°] = total concentration of the metal (free or combined).

6. $\alpha = \dfrac{[H^+]}{Ka} + 1$

   $\alpha = \dfrac{[H^+]}{Ka} + \dfrac{[H^+]^2}{KaKa'} + 1$

7. $\bar{n} = \frac{2[KOH]}{[HSc^{\circ}]}$ (simplified)

8. $Ks = \frac{1}{[Sc]^2}$ valid only when $\bar{n} = 1$

i.e., $\log Ks = -2 \log[Sc]$

Sample Calculation

Iron-glycine complex

When adding 0.25 ml of KOH for example, the pH was 7.17

$$\log[Sc] = (7.17 - 9.17) + \log[(5\times10^{-4}) - (0.2621\times10^{-4})] = -5.85$$

when n = 1 $\log[Sc] = -3.8$

$\therefore Sc = 1.58\times10^{-4}$

$$K_s = \frac{1}{[Sc]^2} = \frac{1}{(1.58\times10^{-4})^2} = 0.4\times10^{8}$$

$\therefore \log K_s = 7.60$

Appendix D

POLYVIALS CLEANING PROCEDURE

1. Wash polyvials with 2N nitric acid for 10 minutes using "ultrasonic" cleaner.[12]
2. Rinse polyvials with distilled water for several times.
3. Wash polyvials with absolute (95%) ethanol in "Ultrasonic" for about 10 minutes.
4. Drain the alcohol and wash with acetone for 10 minutes.
5. Air dry polyvials on a clean sheet in "clean" room equipped with laminar flow filter.

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[12]Manufactured by Branson Cleaning Equipment Co., Shelton, Connecticut, U.S.A.