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Toxicity of parenteral iron : with special reference to ferric ethylenediaminetet raacetic acid (ferric fersenate)

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(Appendix I)

TOXICITY OF PARENTERAL IRON: WITH SPECIAL REFERENCE TO FERRIC ETHYLENEDIAMINETETRAACETIC ACID (FERRIC VERSENATE)

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INTRODUCTION

Parenteral iron investigation has gained much impetus in the last decade with the discovery and use of relatively non-toxic, non-ionic forms of injectable iron. The most widely studied aspects have been metabolism, hematologic response in orally refractive microcytic anemias, anemia of pregnancy, and infantile anemias. There have been an increasing number of studies on the topic of toxicity and relationship of iron to hemochromatosis.

The toxicity of intravenous iron salts has been known for many years. Nissim (1) studied the comparative effects on coagulation, capillary injury and hemolysis of iron salts and non-ionic forms. Hoppe, et al. (2, 3) presented an extensive review of the literature regarding the toxicity of iron salts in man and experimental animals and decided conclusively that such preparations are too toxic for parenteral use.

Polson (4, 5, 6) and Cappell (7) were the first to investigate the effect of colloidal iron and dialyzed iron administered via various parenteral routes. As earl as 1928 Polson (4) showed that intravenous colloidal iron was deposited in the lungs,

liver and spleen, producing emboli by intravascular precipitation and occluding especially the capillaries of the lungs. He also noted that the cells of the Reticuloendothelial System picked up the iron and later released the iron which entered into loose combination with blood proteins and subsequently the iron appeared in storage depots of the marrow, spleen, lymph nodes and parenchymal cells of the liver. Neither author produced any lesions resembling hemochromatosis n man, even though massive doses of iron were given by Cappell over a period of four years.

Since the first report of Nissim (8) in 1947 on toxic reactions with intravenous administration of Saccharated Iron Oxide, there have been many isolated reports and survey reports on parenteral iron toxicity (8-19) and several controlled animal studies on toxicity, distribution and metabolism, histological changes and excretion of iron (20-30).

The polyami o acid compounds known as Versenes have been used recently in cases of heavy metal poisonings because of their ability to form soluble non-ionic chelates with polyvalent metallic ions and increase their excretion (31). There is a variable stability between the chelate and the metallic ion with which it is combined. This preferential union

results in combinations with lead and some of the rarer heavy metals before other ions, however Calcium chelates preferentiall just below these ions and thus blood levels and body stores of calcium can be markedly reduced with the use of Versenes. The most commonly used Versene is Ethylenediaminetetraacetic Acid (hereafter referred to as EDTA).

It was found that ferric ions made combinations with EDTA and the excretion of iron was increased in the urine in cases of hemochromatosis treated with EDTA (32-34).

Since chelated compounds are water soluble, non-ionic and are probably more readily diffuseable, the possibility of using Ferric EDTA as a source of iron in the treatment of iron deficient anemias was considered. Will and Vilter (35) used Ferric EDTA orally in iron deficient patients with the results of no greater absorption of iron than with ferrous sulfate. They concluded that the chelate was split in the gastrointestinal tract liberating ferric iron. Feldman and Rummel (36) using the chelated compound ferro-glycine sulfate claimed excellent results orally with increased absorption of iron.

In the present study, Ferric EDTA was administered

intramuscularly and intravenously in rabbits and compared with intravenous Saccharated Iron Oxide and intramuscular Iron-Dextran.

MATERIALS AND METHODS

Animals and Dosage: The experiments were carried out using ten rabbit weighing 2.5 to 4.5 kilograms. The animals were caged separately and fed commercially prepared rabbit pellets (Nutrena). They were given supplemental feedings of lettuce about once a week. One rabbit received no form of parenteral iron and served as an overall control. A second received intramuscular Iron-Dextran (Imferon) and a third received intravenous Saccharated Iron Oxide (Proferrin) (SIO) for comparison with the remaining seven animals which received either intramuscular or intravenous Ferric Ethylenediamenetetraacetic Acid (Ferric Versenate or Ferric EDTA), pH 7.2. The animals received injections of 5 to 45 mgms. of elemental iron at about weekly intervals over a two to three month period. The smaller doses were given intravenously and comparative doses of Imferon and Proferrin were used. The injections were reduced to lower weekly doses because of early deaths in some of the animals receiving Ferric EDTA.

Investigations: The animals were weighed at the beginning and end of the experiment. At weekly intravals 15 cc of blood were withdrawn directly from the heart and laboratory determinations of hemaglobin, hematocrit, reticulocytes and serum iron were determined. Following withdrawal of the blood, the iron injections were given. Intravenous administrations were given in the marginal veins of the ear and intramuscular injections were given in the hips and thighs. Autopsy material was investigated grossly and microscopic sections were made of lungs, heart, liver, adrenals, kidneys, pancreas, spleen and ovaries or testes. These preparations were stained with hematoxylin and eosin and additional slides with Turnbull's Stain which stains hemosiderin blue (37).

RESULTS

Tabulated results of survival, iron dosage, weight changes and reticulocyte counts are shown in Table I.

Weight, Dosage and Survival: The control animal and the animals receiving Iron-Dextran and SIO all survived the experimental period and all showed weight gains ranging from 225 to 570 gms. Those receiving Ferric EDTA survived, 8, 10, 15, 49 and 97 days and two were alive at the end of the experimental period.

Those dying within a short period were given relatively small doses of iron, ranging from 25 to 67 mgms., and showed only a slight weight loss. Those surviving for a longer period or to termination showed greater weight losses; however one animal which had received 392 mgms. of Ferric EDTA intramuscularly showed a gain of 340 gms. The other two rabbits lost 255 gms. and 1250 gms.

Reaction to Injection: Ferric EDTA consistently produced more severe painful reactions in the experimental animals than the commercially available preparations. Intravenous Saccharated Iron Oxide produced considerable withdrawal whenever tissue leakage occurred. Intravenous Ferric EDTA resulted in marked vasospasm. Intramuscular Ferric EDTA produced squealing, involuntary defecation and micturation. The injected extremity often appeared flacid for several minutes following the injection. Intramuscular Iron-Dextran resulted in no apparent or slight discomfort to the animal.

Reticulocyte Counts: Reticulocyte counts were not increased appreciably in any of the animals with the small amount of blood withdrawn weekly and maximum

reticulocyte response showed values near 4% in the control and comparison animals. Those animals receiving Ferric EDTA and surviving a relatively short time had reticulocyte counts near normal, whereas the slightly higher values of 4% and 5% occurred in the longer survivors and approximated the controls.

Mean Corpuscular Hemaglobin Concentration: The control animal in which 145 cc of blood had been gradually removed over the three month period showed a decrease in the MCHbC from 31% to less than 29%. Intravenous administration of iron (figure I) produced less than 0.5% drop with Proferrin and the surviving animal receiving intravenous Ferric EDTA dropped about 2%. The three rabbits surviving for a shorter period showed no essential alteration in the MCHbC. Intramuscular Imferon resulted in a gradual decrease from 30.6% to 27.3% with a terminal rise to 31.2%. Both animals receiving intramuscular Ferric EDTA showed a general decrease in the MCHbC of about 2% (figure II).

Serum Iron: As seen in figures III and IV, serum iron determinations showed a gradual rise in the control rabbit for about 40 days, from 150 gamma to 270 gamma, then a gradual decline to 100 gamma after 100 days.

Rabbits on Iron-Dextran and Saccharated Iron Oxide showed consistently higher values of serum iron, whereas the experimental animals with Ferric EDTA showed a gradual decrease in serum iron and consistently lower values throughout the experimental period.

Tissue Examination

Site of Injection: The surviving animal receiving intravenous Ferric EDTA had marked thickening and thromboses of the marginal veins. No remarkable gross change was noted in the veins of the rabbit with Saccharated Iron Oxide. The hip and thigh muscles of the control rabbit and the rabbit with intramuscular Iron-Dextran appeared normal grossly, whereas muscles of the rabbits with repeated Ferric EDTA injections appeared very atrophic with areas of hemorrhage and a general grey appearance of the tissue. Microscopically, muscle injected with Iron-Dextran appeared normal, there being only deep iron staining of the connective tissue of the muscle. Intramuscular Ferric EDTA produced hemorrhagic areas in the muscle surrounded by necrotic muscle for several millimeters and round cell infiltration at the periphery. Most of the surrounding muscle bundles were very small and

some of the larger fibers showed typical "Zenkers" degeneration. There appeared to be a general increase in the fibrous connective tissue of the areas studied. No iron was seen in any of the muscle or connective tissue, however there were occasional histiocytes with small granules of stainable iron in their cytoplasm. One of the four rabbits which died relatively early in the experiment (34 days) after receiving 25 mgms. of intravenous Ferric EDTA showed generalized evidence of sepsis with the pleural, pericardial and peritoneal cavities filled with sero-sanguinous fluid. The lungs and adrenal glands appeared necrotic. This animal was not included in the pathological description of the tissues. The animal that died at 97 days also showed evidence of sepsis, however the tissue pathology is included below.

Liver: The rabbits used as control and comparisons (Iron-Dextran and Saccharated Iron Oxide) all showed marked swelling of the hepatic cells with obliteration of the sinusoids, granular cytoplasm and distinct cell membranes, all features characteristic of "clowdy swelling". They also showed perivascular infiltration of round cells in the portal areas. Grossly, the liver appeared normal in color, except for the animal

receiving IV SIC in which the liver appeared very dark red throughout. No hemosiderin was demonstrable in the control animal. Iron-Dextran administration produced fine blue granules in the hepatic cells with a blue staining cytoplasm seen mostly in the peripheral portion of the lobules. The Kupffer cells were filled with large dark staining hemosiderin grannules. With intravenous SIO hemosiderin was seen only as large grannules in the liver histiocytes.

The three animals which died shortly after beginning administration of Ferric EDTA all showed black areas on the surface of the liver, varying degrees of mid-zonal necrosis of the liver and dilated central veins. No hemosiderin was demonstrable. Animals surviving for longer periods showed no apparent gross abnormalities of the liver, but microscopically two showed central vein dilitation, marked congestion of the sinusoids and early "clowdy swelling" changes of the central cells. Sections from the third animal were not obtained. No hemosiderin was present.

Spleen: Grossly the spleen of the control, and the two comparison animals appeared normal and microscopically no abnormality was noted. Hemosiderin was seen in the macrophages of the control animal and in much

greater quantity in both the Iron-Dextran and SIO animals. In the latter two the macrophages had blue staining cytoplasm with larger dark blue granules and some free blue staining material outside the cells. No hemosiderin was seen in the lymphoid nodules.

Grossly, the spleens of two of the early death animals with Ferric EDTA showed black patches on the surface; the third was totally black. Microscopically the spleens showed decreased lymphoid nodules and increased red pulp filled with red corpuscles. No areas of infarction were seen. More hemosiderin was present in one animal than in the control; however two others showed less hemosiderin than the control. The iron present was confined to the macrophages.

The gross appearance of the spleen in two of the longer survivors did not appear unusual; however the spleen of the animal dying after 97 days was large and pale and showed mottled patches of darkness. The lymphoid follicles were decreased in number and size and the red pulp was increased with many erythrocytes in the sinusoids. Compared to the control, stainable iron was markedly decreased in two animals and increased in the rabbit with the enlarged pale spleen. This animal also had serosanguinous material in the

pleural cavities and diarrhea.

Heart: Grossly the hearts of all animals appeared fairly normal; however the weights ranged from 7 to 18 grams. Smaller hearts were found in the survivors (7 to 12 gms.) and the largest occurred in the three rabbits which died early (15 to 18 gms.). No histological abnormal es were seen other than that expected from repeated intracardiac punctures. Hemosiderin was seen only in the perivascular histiocytes of animals which received Iron-Dextran and SIO.

Adrenal Glands: The adrenals all weighed less than one gram and appeared normal grossly. No microscopic pathology was present and hemosiderin stain was found only in the rabbit which received Iron-Dextran. Here iron staining was present in the connective tissue and some of the cells of the cortex.

Lungs: Grossly and microscopically the lungs of the control and iron comparison animals were normal. Hemosiderin was seen only in the two comparison animals and then iron was confined to granules in the cytoplasm of small clumps of histiocytes. Of the animals which died early, one showed normal lung tissue and the other two showed small areas of consolidation with fluid n the alveoli, hemorrhage and

round cell infiltration in the interstitial tissue. No iron staining was present.

The lungs of the three rabbits which survived for longer periods of time on Ferric EDTA showed varying pathology. The rabbit which died at 97 days had pleural and pericardial fluid and the lungs weighed 33 gms, as compared to the lungs of the other two which weighed 13 and 14 gms. Histologically the lungs showed pneumonic consolidation with many polymorphonuclear cells and round cells. No hemosiderin was present. The two surviving rabbits exhibited no gross evidence of pneumonia; however in one there was interstitial thickening, fluid in some alveoli, and round cells infiltration. There was one area of hemorrhage in the lung of the third rabbit. Hemosiderin was seen only in one small clump of histiocytes.

Kidneys: Gross and histologic examination revealed normal kidneys in the control and comparison animals, except for occasional hyaline casts in the tubules of the rabbit which received Iron-Dextran. Hemosiderin was present in the glomeruli, proximal tubule cells and histiocytes in the interstitial spaces of the animal which received Iron-Dextran. The kidneys of early death animals with Ferric EDTA had normal

glomeruli, but there were many tubular degenerative changes and hyaline casts within the tubules. No stainable iron was seen.

The longer urvivors with Ferric EDTA had kidneys which varied from histologically normal, although they appeared "flea bitten" grossly, in the rabbit which received 120 mgms. intravenously to a marked increase in the interstitial tissue, round cell infiltration and hyaline casts in the two animals with higher intramuscular doses. The animal which died at 97 days also showed degenerative tubular changes. The glomeruli in all three apppared normal. Occasional histiocytes with hemosiderin granules were seen in the interstitial spaces in one of these latter two rabbits and blue staining material was seen in two capillaries.

Testes: The testes were examined only in the two rabbits which survived the test period. These rabbits received the largest doses of intramuscular Ferric EDTA and normal maturation was seen in both. No hemosiderin was demonstrable.

DISCUSSION

Toxic react ons to parenteral iron in humans have been considered under the headings of immediate toxic reactions, delayed toxic reactions, and the theoreti-

cal possibilities of exogenous hemochromatosis production. Investigation of iron toxicity in animals has been directed mainly along the lines of acute lethal dosages and histopathology associated with prolonged iron administration.

Toxic Reactions in Humans: Clinical use of Saccharated Iron Oxide and Iron-Dextran have not been without toxic reactions. In one reported instance a death occured following the use of 100 mgms. of SIO (13). Reactions to intravenous SIO have occured more frequently than with intramuscular iron and the former has been shown to be more toxic to laboratory animals (29, 30). Scott (17) estimated the incidence of reactions to intramuscular Iron-Dextran to be less than 0.5%. Nissim (28) in his review of toxic reactions to intravenous SIO found the incidence of reactions varying from less than 1% to as high as 70%, with an approximate average in almost 600 cases of 25%. Immediate Reaction: The early reactions as compiled by Nissim (28) included headache; pain in arms, shoulders, chest, lumbar area, sacral area and thighs; tachycardia; palpitation; faintness; pallor; feeling of heat and burning; sweating; lacrimation; nausea; coughing; bronchospasm; dyspnea; cyanosis and

hypotension. These immediate reactions generally followed relatively small doses of intravenous Saccharated Iron Oxide and occurred more often than the delayed type of reaction.

The cause of immediate toxic reactions has been ascribed to different mechanisms. Many investigators considered that toxic reactions to intravenous iron occurred when the iron binding capacity of the blood proteins was exceeded (12, 38, 39). Librach (12) further considered that when the plasma proteins could no longer bind with the excess iron the presence of this iron caused the release of histamine and the production of allergic manifestations. He also theo-rized that the reactions may simply have been an allergic respons to the iron protein complex or that the iron product may have acted as an enzyme inhibitor. Nissim (28) considered the early reactions to be a hypersensitivity phenomenon and symptoms arrose from ischaemia of poorly supplied visecera. These occurred more often in th elderly. Nissim's calculations suggested that the average adult iron binding capacity was exceeded with less than 16 mgms. of iron. Much higher doses of iron have been administered without reactions and thus he considered reactions not to have

been due to free ferrous or ferric ions circulating in the blood, but rather the toxicity was due to the properties of the whole molecule, in this case SIO. Immediate reactions which occurred after Iron-Dextran administration were similar to SIO, even though less frequent. These again occurred within a few minutes after injection and were associated with backache, tightness in the chest, tightness in throat, rapid pulse, flushing of the face, profuse prespiration, grey pallor, hypotension and unconsciousness (9,10,11,17,20). These reactions were considered to be produced by the same mechanism as with intravenous SIO, i.e. eschemia (12,20). Pain at the site of in-jection with Iron-Dextran has been one of the more common complaints of patients (10,17,20).

Martin, et al. (29) compared the effects of Iron-Dextran and SIO in several in vitro and in vivo studies. They found that neither preparation precipitated serum proteins nor was antigenicity produced following sensitizing doses in animals. There was no effect on coaguability of blood in rabbits with fairly large doses of Iron-Dextran; however in vitro SIO was shown to be more anti-coagulant than Iron-Dextran. Hemolytic activity

occurred with Iron-Dextran above concentrations of 0.5% and with SI above 0.125%. These concentrations have not been attained with clinical doses. Thus, in man the mechanisms of bleeding, hemolysis, sensitizations or plasm protein precipitation were probably not responsible for reactions which occurred (1,29).

Delayed Reactions: Delayed reactions have occurred with both Iron-Dextran and SIO, reactions to the latter again being the nore frequent (20,28). These reactions have generally followed larger doses and have been less common than immediate reactions.

Delayed symptoms have been vertigo; weakness; cold feeling; chillin ; pyrexia; sweating; nausea; vomiting; diarrhea; urticaria; tightness in chest; headache; tachycardia; gre. color; collapse and pain in abdomen, chest, lumbar area, arms, legs and feet.

Nissim (28) attributed these late reactions to gradual intravascular precipitation of fine particles with the production of multiple emboli. He based this conclusion on experimental work with laboratory animals in which vascular occlusion had been demonstrated repeatedly. He urther stated that in rabbits there was a threshold of 45 mgms./kilo. with SIO at which this gradual precipitation occurred and another

threshold at 180 mgms./kilo. in which there was an abrupt precipitation of SIO capable of producing immediate toxic eactions. Callender and Smith (11) regarded this delayed type of reaction to have been due to an allergic phenomenon since the symptoms respond to epinephrine. Patients who had a reaction following Iron-Dextran failed to react to Dextran alone or SIO; thus the toxic property probably rested with the whole molecule as suggested by Nissim (28). For this to have been purely allergic, there was implied a specific sensitization to the drug and since these reactions occurred often following the first injection a tissue type sensitization seemed unlikely. Also, as stated before, Martin, et al. (29) were not able to sensitize laboratory animals to Iron-Dextran.

From examin tion of the symptomatology produced in both immediate and delayed reactions it was seen that the symptoms were very similar, with the possible exceptions of pyrexia and urticaria which were generally mild and occurred as a delayed type reaction. Possibly this arbitrary division of immediate and delayed reactions was more apparent than real and the mechanism, whether precipitation, hypersensitivity, or allergy was the same.

Chronic Toxicity in Man: Most of this problem has been in the theoretical stage since no actual cases have been reported; however with the production of exogeneous hemachromatosis following multiple transfusions (40,41,42) the role of excess parenteral iron should be evaluated. Since there is no normal mechanism for the body to rid itself of excess iron, the accumulation of iron would seem to play a role in the production of hemochromatosis (43,44). Based on iron doses needed to produce histopathology in mice and rats, Nissim (26) pointed out that it would require over 60 gms. of iron administered parenterally in man. Lesions that have been produced in animals are not identical with those seen in human hemochromatosis and several authors have concluded that the mere presence of iron in tissue may not have been the only cause of hemochromatosis (4,6,7,23,24,26,30,44,45).

Animal Toxicity: The normal adult body contains about three to five gms. of elemental iron (38). Assuming the same rabbit-man ratio used by Pinniger and Hutt (30) of 1 to 30, then the total body iron of a normal rabbit should be between 100 and 200 mgms. In this study injections of between 120 and 450 mgms. of elemental iron substantially increased the iron stores,

assuming that the iron from Ferric EDTA was retained in the body.

Acute Toxicity: Ferrous sulfate toxicity in rabbits varied from 10-60 mgms./kilo. when administered intravenously and resulted in death within a few minutes with complete prostration, convulsions and respiratory failure (2,3). The two rabbits which died at 8 and 11 days following intravenous administration of Ferric EDTA were within the range of toxicity above; however since they did not die immediately, the mechanism of death was probably not the same.

Much larger doses of Iron-Dextran and SIO have been given to experimental animals without acute toxic reactions: Ellis (1956), 1 gm. of SIO intravenously in rabbits (22); Goldberg, et al. (1957) 1650 mgms. of Iron-Dextran intravenously in rats (24); Dubach & Moore (1956), 1 gram/kilo of intravenous iron in dogs (43); Nissim (1955), 500 mgms./kilo. of intramuscular Iron-Dextran in mice (27); and Pinniger and Hutt (1956), 1,200 mgms. of intravenous SIO and Iron-Dextran in rabbits (30). Martin, et al. (1955) found LD/50 of 1,013 mgms./kilo. of Iron-Dextran and 231 mgms./kilo. of SIO administered intravenously in mice. They found an LD/50 of 690 mgms./kilo. for Iron-

Dextran administered intramuscularly in rabbits (29). These extreme doses were much larger than used in this study and show the relatively low toxicity of these preparations. The acute deaths in laboratory animals from massive doses of these iron preparations were generally considered to be due to precipitation within the capillaries (30); however myocardial failure due to a direct action of the iron has been suggested (26).

Chronic Toxicity: The growth rate of rats has been inhibited by large doses of Iron-Dextran (23,24). In the present studies the growth rate was not checked because of the variable age of the rabbits, however Ferric EDTA administration was generally associated with weight loss, whereas the rabbits used as control and comparisons all showed weight gains. Prolonged iron administration produced no hematologic alteration in rats and rabbits in the study by Goldberg, et al. (23).

The Mean Corpuscular Hemaglobin Concentration values in this study showed a general decrease with time, although the decrease was greater in the rabbits with Ferric EDTA (figures I and II). The cause of this was not apparent nor was the rapid rise near 90

days. Similarly, the serum iron concentrations with Ferric EDTA were lower than the control and the animals receiving Iron-Dextran and SIO (figures III and IV). Following iron injection in rats and rabbits, the serum iron value found by Goldberg, et al. (23) were also higher than the controls. Chelates have been reported to change the physical and chemical reactions of the ions with which they are in combination (35). In order to determine whether the lower values of serum iron with Ferric EDTA were due to this property, the blood was examined for serum iron shortly after intravenous injection of 50 mgms. of Ferric EDTA. The color reaction was too intense to compare to the usual standards. This indicated that the iron in Ferric EDTA was measured and the values are probably a true representation of the iron level in the plasma.

Similar reticulocyte levels in the control, comparison and test animals indicated that there was no apparent depression of the hemopoetic system.

The functions of various organs under the influence of excess iron has been studied only on a limited basis. Rabbits have become azotemic with parenteral iron (22). Ellis (22) and Goldberg, et al. (23) have produced massive proteinuria, edema, and

diminished renal concentration power in rabbits with large doses of Iron-Dextran (22,23). Decreased testicular function associated with atrophy has been reported by Nissim (27).

The normal excretion of iron by the kidneys has been shown to be very slight (39) and only slightly increased in rabbits with massive doses of Iron-Dextran (23). Calcium EDTA has been shown to increase the excretion of iron in patients with hemochromatosis (32,33,34). Since serum iron determinations were lower in the rabbits with Ferric EDTA and since less iron was demonstrated n the tissues of these rabbits it was postulated that the iron in Ferric EDTA was not retained by the rabbit and probably was excreted via the kidneys. Turnbull's Hemosiderin stain does not react with iron in the ferric form, and this may account for the lack of stainable iron in the tissues of the rabbits receiving Ferric EDTA. For the time of this study, the resence of ferric ions in the body should have resulted in production of ferrous ions via metabolic pathways. This in turn should have increased the deposition of hemosiderin if the ferric iron was retained in the body.

Histological Pathology

Muscle: The site of injection with various iron preparations has been investigated by several authors. Martin, et al. (29) stated that with poorly absorbed preparations such as SIO there was a brownish discoloration of the muscle; whereas the muscle appeared normal with Iron-Dextran. In both of the comparison animals in the study the muscle fibers appeared normal microscopically and iron was limited to the connective tissue. Grossly the muscle of the rabbit which received Iron-Dextran in this study was similar to the above description. Nissim (26) also found the same histology in muscle following intravenous injection of SIO. A controlled study by Beresford, et al. (21) showed that there developed an acute inflammatory reaction with local degenerative changes in the muscle following intramuscular injections of Iron-Dextrans and SIO preparations. He stated that most of the iron was absorbed within three days from the injection site and that complete regeneration of muscle occurred rapidly. Most of the iron which remained at the injection site was found within macrophages and was still present after three months and he concluded that this iron may be perma-

nently fixed.

The painful reaction of rabbits to the injection of Ferric EDTA along with the grey appearance and microscopic degenerative changes of the muscle indicated that this drug may possess histotoxic properties. The lack of iron in the muscles following Ferric EDTA injection may have been the result of failure of the ferric iron to stain or it may have been removed from the tissue at the time of fixation or dehydration because of its solubility. Studies with Calcium EDTA have shown that over 90 percent of the dose is excreted via filtration and tubular excretion by the kidneys in less than seven hours (46). It was also shown in th same study with radioactive C-14 labeled Calcium EDTA that the drug was rapidly removed from the site of injection. This rapid excretion may have occurred with Ferric EDTA also and explain the lack of stainable iron in the muscle and apparent lack of storage iron in the animals.

Liver: Polson (4,6) and Cappell (7) were the first to describe the deposition of iron in the Kupffer Cells of the liver and then the appearance somewhat later of iron staining material in the liver parenchyma. Neither author was able to produce any pathology other

than the tissue siderosis. Nissim (26) also noted that the parenchymal cells were affected first near the central part of the lobule and at the periphery before the mid-zonal areas were affected. He also described infiltration of large masses of round lymphoid like cells and destruction of large portions of the hepatic parenchymal cells with complete de-struction of whole areas of lobules; however the characteristic f.brosis of hemochromatosis was lack-ing. Martin's (29) results were similar to the earlier work. Pinniger and Hutt (30) found that iron concen-tration in parenchymal cells occurred first in the mid-zonal areas of the liver lobules and later spread to the periportal areas. In none of their rabbits, which were injected with Iron-Dextran and SIO, was necrosis or fibrosis noted; however they used smaller doses of iron than did Nissim.

The presence of "cloudy swelling" of the parenchymal cells of the control, Iron-Dextran and SIO rabbits in this study probably was the result of hepatitis and limited the value of the histological features. There was noted round cell infiltration in the periportal areas in all three and this was probably associated with the "hepatitis". Iron was present in

the Kupffer Cells and parenchymal cells as noted in the work above; however it seemed to be more abundant in the periportal areas. The absence of iron in the control was as expected and the rabbits receiving Ferric EDTA all showed more of a congestive phe-nomenon with dil ted central veins, congestion of the sinusoids with blood and varying degrees of central necrosis. Some cells showed "clowdy swelling" changes in the central areas. There were no fibrosis or stainable iron noted in these animals. It appeared that the li ver changes were due to cardiac failure and/or lung damage. The kidneys may also have attributed to the congestive state.

Spleen: Iron deposition in the spleen has been similar in all previous works (4,6,7,26,29,30). In these studies the iron was found in heavy concentration in histiocytes of the red pulp and in the connective tissue, but only with very large doses was iron seen in the lymphoid nodules. The lymphoid tissue was also reduced with very large doses (30). The above findings were the same in the two rabbits on Iron-Dextran and SIO in the present study. All of the spleens in rabbits administered Ferric EDTA showed decreased iron stores and decreased lymphoid tissue with increased

red pulp. This was probably due to congestion as noted in the liver of these rabbits. The one rabbit which died at 97 days with evidence of infection showed more iron to be present than in the control and this probably represented a hemolytic phenomenon and not due to the iron from the Ferric EDTA. Since the spleen was the site of large amount of iron stores in the normal animal and in those following Iron-Dextran and SIO administration, the absence of staina-ble iron following Ferric EDTA emphasizes the postu-late that the iron from this source was probably not retained in the rabbit's body or not utilizable. Heart: Large do es of SIO in animals resulted in the deposition of iron in fibers of the myocardium and there was some apparent damage to the fibers. This was found only in patches and nearer the surfaces of the heart (26). Lower doses of iron have not resulted in iron deposition in the myocardium, but rather iron was occasionally found in the histiocytes and connective tissue of blood vessels. Large hearts in the two rabbits which died at 8 and 11 days following 45 mgms. of Ferric EDTA added emphasis to the possibility of cardiac toxicity failure in these rabbits. Fragmentation of some of the heart fibers

was seen in one of the rabbits. Cardiac failure may have been the re ult of a direct toxic reaction of Ferric EDTA on the myocardium or secondary to renal failure.

Adrenal Glands: No pathology was produced in the Adrenal Glands of animals injected with large doses of SIO by Nissim (26) even though iron was found in the cortical cells especially in the zona reticularis and zona glomerulosa. No iron was found in the adrenal medulla. Iron was seen only in the rabbit receiving Iron-Dextran in this study and then it was confined to histiocytes and occasional cortical cells. No pathology was noted in any animals in which the adrenals were examined.

Lungs: The main lethal and toxic effect of high doses of parenteral iron, especially SIO, have been attributed to precipitation within the capillaries of the lungs (22,26,28). Pinniger and Hutt (36) failed to produce demonstr ble lesions in the lungs as described by the above. With the smaller quantities of iron used in the present study, no iron was seen in the lungs of animals on Iron-Dextran or SIO, except in occasional clumps of histolcytes. The lung architecture was normal. Nissim (26) produced a derangement

of the lung his ology with prolonged large doses of SIO due to many iron filled histiocytes filling the interstitial space and infiltration with small round cells. Most of the pathology which occurred in rabbit lungs following Ferric EDTA can be explained on a congestive failure basis, with alveolar septal thickening and congestion, fluid in the alveoli and some hemorrhage. The rabbit which died at 97 days with gross evidence of sepsis also showed microscopic evidence of pneumonia. It should be noted that one of the rabbits which died early had essentially normal lung tissue and one of the rabbits which was sacrificed at the end of the experiment after receiving almost 400 mgms. of Ferric EDTA had essentially normal lungs microscopically. This suggested that the toxic effect of Ferric EDTA was not associated with damage primarily to the lungs. Iron was characteristically absent in the lungs of the Ferric EDTA animals, except for one small clump of histocytes just under the pleura in the surviving animal mentioned above.

Kidneys: Pinniger and Hutt (30), with large doses of iron, produced shrinkage of the glomaruli, tubular dilitation and colloid casts. Ellis (22) produced the Nephrotic Syndrome in rabbits with large doses of SIO

with proliferation of the glomerular cells, fibrosis and finally atrophy of the glomeruli. Tubular damage was also reporte . He attributed this to capillary damage by the precipitation of iron. Goldberg, et al. (24) produced a massive proteinuria in rabbits with large doses of Iron-Dextran and seven died during the first five weeks. Blood urea nitrogen was elevated in all. These rabbits showed slight histological changes in the glomaruli and tubules. There were heavy deposits of iron in the endothelium of the glomeruli capillaries and some iron in the tubular cells. Thrombi rich in iron were also seen. Goldberg also noted more rapid autolysis of kidney tubule cells after death. Nissim (26) also demonstrated iron precipi-tation in the glomerular and tubular capillaries.
He noted that shortly after this the proximal tubule cells were stained diffusely with iron and this staining increased with repeated doses. In those animals which died there were seen degenerative changes in the proximal convoluted tubules and many epi-thelial casts were present in the tubules. Iron was found in the tubular filtrate.

Except for occasional hyaline casts and stainable iron in the glomeruli and proximal convoluted tubule

cells, the kidneys of the animals which received Iron-Dextran and SIO appeared normal. The hyaline casts may have represented protein loss through glomeruli and tubular damage.

Renal tubular toxicity has been reported in man with large doses of Calcium EDTA (32,47). The tubular change produced by Ferric EDTA in the rabbits which died during the experiment may have represented post-mortum autolysis or may have been caused by the drug itself. The latter was doubtful since kidneys which were fixed soon after death showed nearly normal tubular cells. There was no apparent abnormality of the glomeruli in the longer surviving rabbits; however the rabbit which died at 97 days with evidence of sepsis showed marked interstitial infiltration of the kidney parenchyma. This was probably due to an infective process. The rabbit which received 120 mgms. of intravenous Ferric EDTA had normal kidneys, suggesting that nephrotoxicity was not too prominent. The rabbit which received almost 400 mgms. of intramuscular Ferric EDTA showed some hyaline casts and increased interstitial tissue. Stainable iron was seen in two capillaries and in occasional histiocytes. If Ferric EDTA was rapidly excreted via the kidney,

as would be susp cted when compared with the excretion rate of Calcium EDTA, then some of the iron may have been separated from the EDTA and produced the stainable iron seen in thi animal.

Testes: No effe t on testicular function was apparent nor were iron deposits seen in the testes at the levels of iron administered to the animals in this study. Several authors have described in laboratory animals heavy deposition of iron in the testes with subsequent atrophy of the testes (23,24,25,27). The amount of iron needed to produce this was very large.

Other Tissues: Many other tissues have been examined following iron administration. When present, iron was contained in the connective tissue stroma and cells of the Reciculoendothelial System. Parenchymal cells stained with iron occurred only after massive doses and no histopathology was demonstrated.

SUMMARY

The present study consisted of a review of the literature concerning the toxicity of parenteral forms of iron in humans and laboratory animals. The toxic effects of parenteral Ferric Ethylenediamine-tetraacetic Acid (Ferric EDTA, Ferric Versenate) were compared with intramuscular Iron-Dextran (Imferon)

and intravenous Saccharacted Iron Oxide (Proferrin, SIO) in ten rabbits.

A total of 25 to 50 mgms. of elemental iron was injected in divided doses about once a week until the rabbits died or the experiment was terminated after three months. Blood withdrawn by cardiac puncture at weekly or bi-weekly intervals was checked for hemoglobin, hematocrit, rectiulocyte count and serum iron. Autopsy material was examined microscopically for pathological changes and the presence of iron in the tissues. Original and terminal weights were recorded.

The salts of iron ave been shown to be too toxic for clinical use 1,2,3 . With the advent of non-ionic forms of iron, which dissociated slowly in the body, there came a method of treatment for orally refractive microcytic anemias; however these were not without undesirable side reactions in some instances and one recorded death 8-19).

Immediate toxic reactions in humans occurred after both Iron-Dextran and SIO; however reactions were more frequent with the latter. These immediate reactions usually followed smaller doses of iron and too rapid adminisiration when given intravenously.

These reactions have been attributed to free iron causing the release of histamine when the iron binding capacity of the blood is exceeded, or hypersensitivity to the whole molecule resulting in eschemia of viscera (12,28,38,39). In vitro and in vivo studies in laboratory animals have failed to show that either preparation was capable of precipitating plasma proteins, altering coaguability of blood, producing hemolysis or sensitizing animals in quantities used clinically (1,29).

Delayed reactions in humans usually followed larger doses and occurred up to several hours after the injection (20,28). Symptoms and signs in both types of reactions were very similar, except that pyrexia and urticaria occurred most in the delayed reactions. Both immediate and delayed reactions were more common following the use of SIO than they were with Iron-Dextran. The chief complaint with Iron-Dextran was pain and discomfort at the site of injection and in some a temporary staining of the skin. Nissim (28) considered the cause of these delayed reactions to be due to intravascular precipitation with the production of multiple emboli. Others (11) regarded this type of reaction to be due to an allergic phenomenon.

Lethal doses of ferrous sulfate in rabbits were between 10 and 60 mgms./kilo. (2,3). SIO and Iron-Dextran have been given to laboratory animals in much larger amounts. Martin, et al. (29) found a LD/50 of 690 mgms./kilo. of Iron-Dextran in rabbits. Acute deaths were considered to be due to precipitation within capillaries (30 or myocardial failure (26). Lung pathology was often seen with hemorrhage and intravascular precipitation of iron staining material (28).

Chronic iron toxicity has produced Nephrotic Syndromes in rabbits and testicular atrophy (22,23,27).

Venous thrombosis occurred frequently with intravenous injections of SIO (8,14). Intramuscular injections of Iron-Dextran produced little or no permanent local pathology. There was an acute inflammatory reaction around the site of injection and deposits of iron in histiocytes locally (21,26). These iron laden histiocytes were considered as being fixed by one author. Intravenous injections of Ferric EDTA produced venospasm and many local thromboses. Intramuscular Ferric EDTA produced a marked painful, withdrawal response in the rabbits and the tissue appeared grey and hemorrhagic with considerable

degenerative changes and increased fibrosis of the muscle. No iron was seen locally following intramuscular injection one week earlier. It was felt that Ferric EDTA produces considerable local destruction of tissue.

In the liver and spleen, congestion was the main pathology noted following Ferric EDTA. Central zonal changes and hyperemia were seen in the liver and hyperemia and reduction of lymphocytic nodules were seen in the spleen. No iron was seen in the liver and stainable iron in the spleen with Ferric EDTA was generally less than that found in the control rabbit. With SIO and Iron-Dextran most authors have been unable to produce any pathologic change in the liver and spleen except for tissue siderosis (4,6,7,26,29,30). Nissim did produce lymphoid infiltration and destruction of hepatic lobules, but no fibrosis (26).

The main toxic effects, and presumably the cause of death, in animals with massive doses of SIO and Iron-Dextran were seen in the lungs (22,26,28); however Pinniger and Hutt (36) were unable to produce such lesions. Iron deposition with the above drugs was found mainly in histiocytes after the initial precipitation within the capillaries. Hyperemia,

alveolar wall thickening and fluid within the alveoli occurred in the rabbits with Ferric EDTA and pointed to passive congestion, presumably on the basis of myocardial failure.

Renal tubular damage and nephroses have been produced in lab animals with large doses of iron in the form of Iron-Dextran and SIO (22,24,26,30). This has been attributed to capillary precipitation. Calcium EDTA has been shown to produce tubular toxicity in man (32,47). Kidneys of rabbits following Ferric EDTA ranged from microscopically normal in those with lower doses to the presence of casts and increased interstitial tis ue with higher doses.

Other tissues have taken up the iron of Iron-Dextran and SIO n varying amounts with no apparent pathology except siderosis, with the exception of the testes in which atrophy has been reported with very large doses (23,24,25,27). Iron in most tissues of the body was pre ent within the cells of the Reticulo-endothelial System. With massive doses iron staining was seen in the endothelium of vessels and finally iron appeared in the parenchymal cells of tissues.

There was a general lack of stainable iron in the tissues of rabbits treated with Ferric EDTA.

Studies with other forms of EDTA have indicated that this drug was excreted rapidly and unchanged via filtration and tubular excretion in the kidneys (31,32, 33,34). This suggested that iron from Ferric EDTA was not available to the body because it was rapidly excreted. Urinary iron excretion would have been valuable in this study.

CONCLUSIONS

1. Iron salts were too toxic for parenteral use.
2. The mechanism or mechanisms of immediate and delayed reactions to intravenous SIO or intramuscular Iron-Dextran were not definitely known; however allergic reactions, intravascular precipitation with emboli and toxicity of ferric or ferrous ions when the iron binding capacity was exceeded have been suggested.
3. Lethal doses of SIO and Iron-Dextran in animals were high.
4. Acute deaths were considered to be due to massive precipitation within lung capillaries of heart failure.
5. Nephroses have been produced with large doses of Iron-Dextran and SIO in rabbits.
6. Ferric EDTA produced a very painful reaction to local injection in rabbits. Degenerative changes occurred in the muscle at the site of injection.

7. Acute lethal doses of Ferric EDTA were probably lower than Iron-Dextran and SIO in rabbits.

8. Iron from Ferric EDTA was not available to the body and was probably excreted intact and rapidly via the kidneys.

9. Chronic toxicity with Ferric EDTA resulted in passive congestion of the lungs, liver and spleen possibly by way of myocardial failure.

10. No lesions resembling hemochromatosis were produced in the present study with the comparitively small amounts of iron administered.

	Survival (days)	Blood Taken (cc)	Iron Dose (mgm.)	Weight Change (Gms.)	Maximum Retics. (%)
Control	term.	143	none	+ 450	4.7
Iron-Dextran	term	99	362	+ 570	4.0
SIO (IV)	term	101	135	+ 225	4.0
Ferric EDTA (IM)	15	45	67	-------	2.1
Ferric EDTA (IM)	97	138	446	-1250	5.3
Ferric EDTA (IM)	term	99	392	+ 340	4.2
Ferric EDTA (IV)	10	30	44	- 110	1.2
Ferric EDTA (IV)	8	30	44	- 50	2.4
Ferric EDTA (IV)	49	42	25	------	2.2
Ferric EDTA (IV)	term.	110	120	- 255	3.9

Survival time, Blood withdrawn, Total iron dosage, Weight alteration and Maximum Reticulocytosis with various forms of iron and routes of administration.
(IM - intramuscular)
(IV - intravenous)

table I

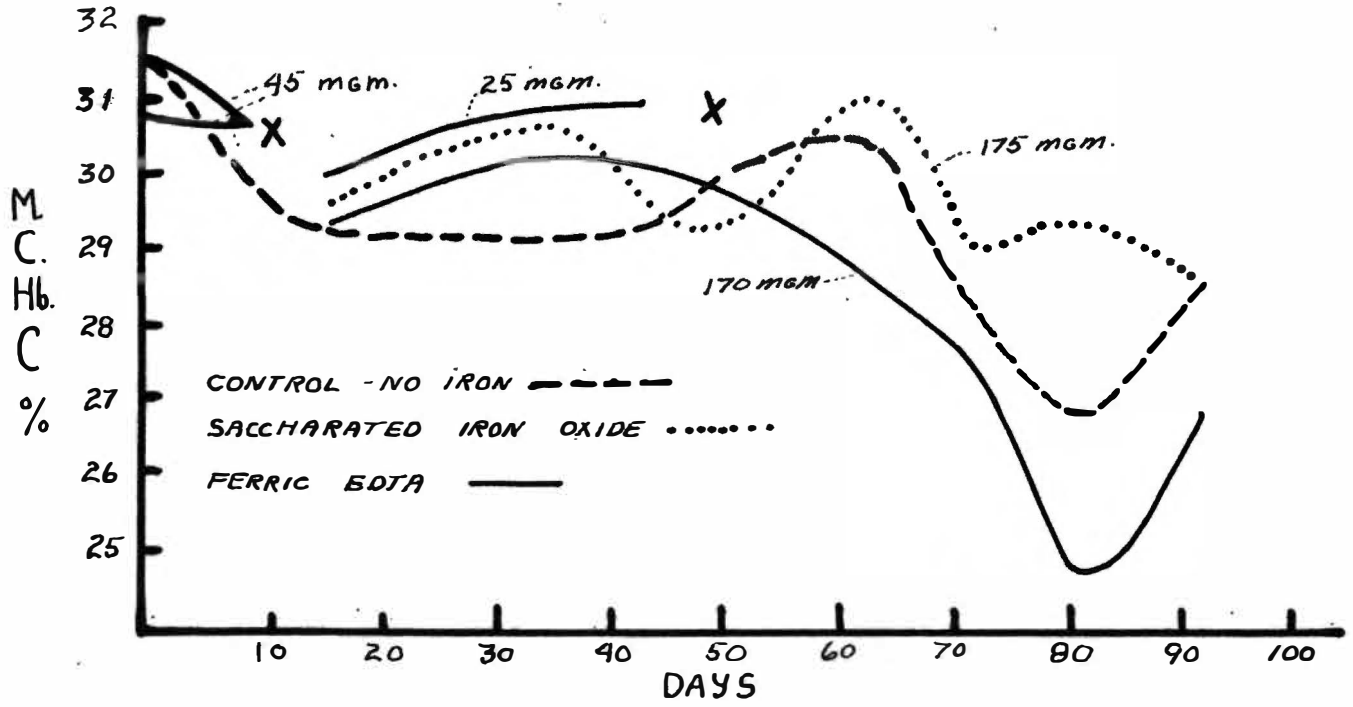

FIGURE I

MEAN CORPUSCULAR HEMAGLOBIN CONCENTRATION FOLLOWING INTRAVENOUS ADMINISTRATION OF IRON PREPARATIONS

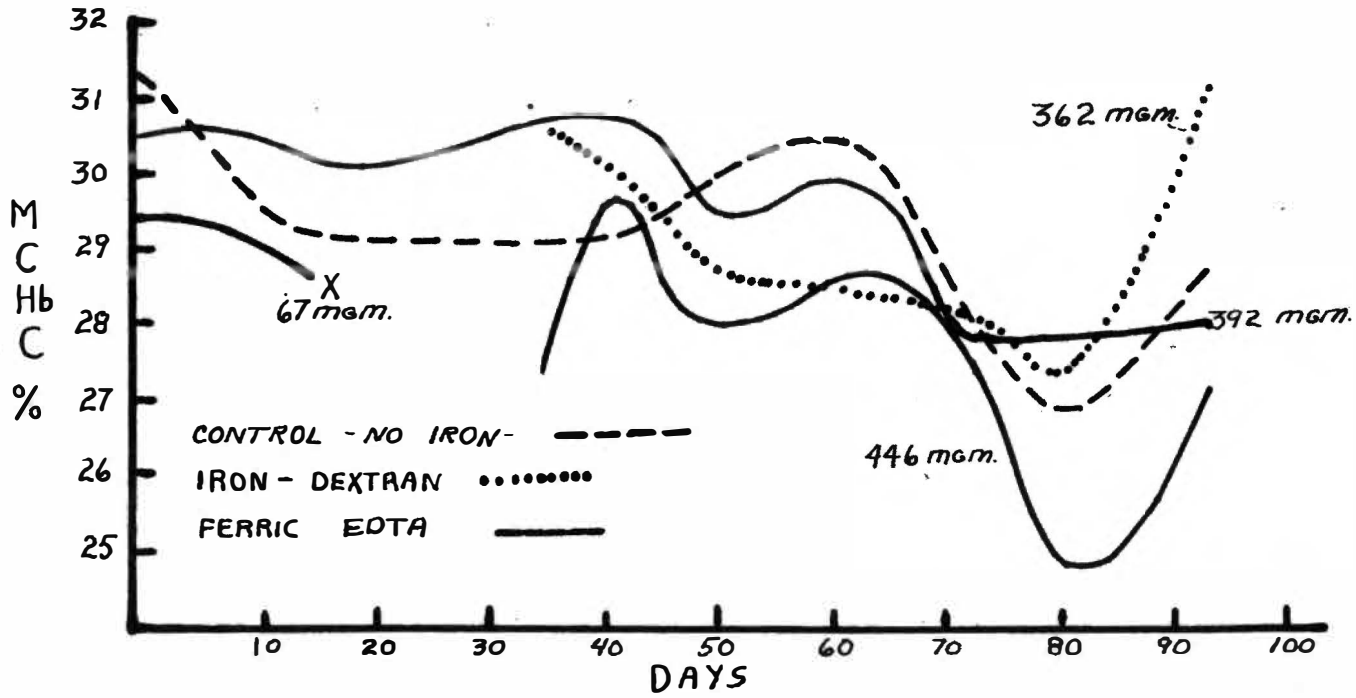

FIGURE II

MEAN CORPUSCULAR HEMAGLOBIN CONCENTRATION FOLLOWING INTRAMUSCULAR ADMINISTRATION OF IRON PREPARATIONS

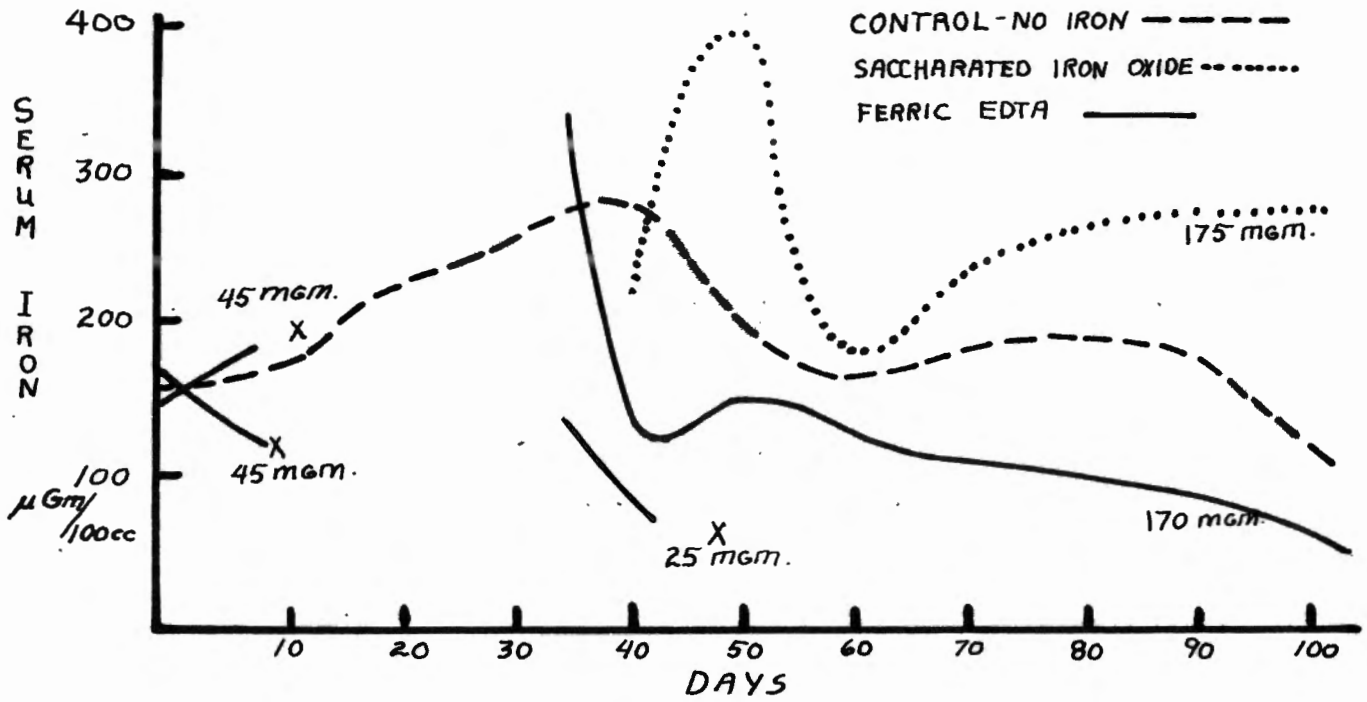

FIGURE III

SERUM IRON CONCENTRATION FOLLOWING INTRAVENOUS ADMINISTRATION OF IRON PREPARATIONS

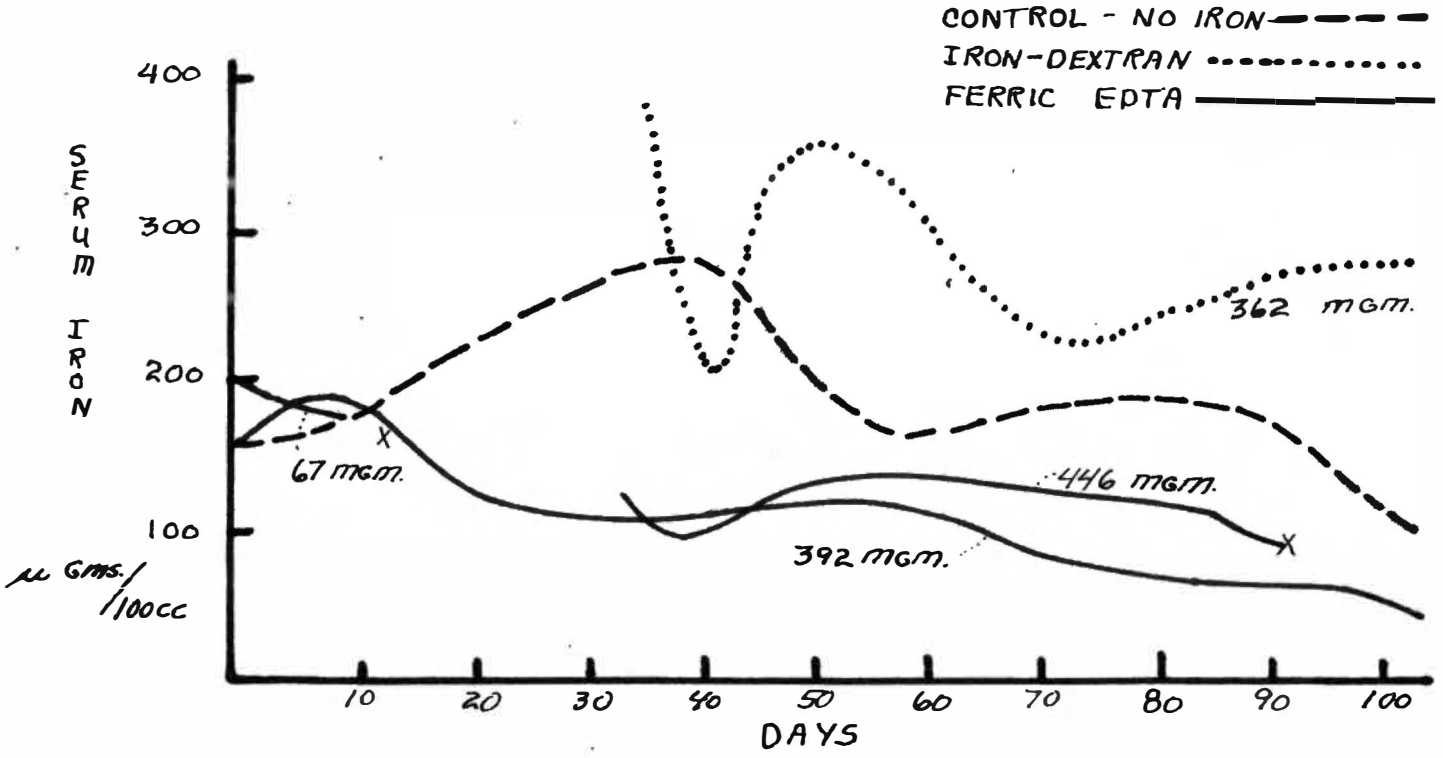

FIGURE IV

SERUM IRON CONCENTRATIONS FOLLOWING INTRAMUSCULAR ADMINISTRATION OF IRON PREPARATIONS

(Appendix IV)

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