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Results of prolonged intravenous administration of iron to normal and anemic rabbits

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BOSTON UNIVERSITY

GRADUATE SCHOOL

Dissertation

RESULTS OF PROLONGED INTRAVENOUS
ADMINISTRATION OF IRON TO NORMAL
AND ANEMIC RABBITS

by

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INTRODUCTION

With the great expansion of blood banking facilities and the availability of a relatively safe form of colloidal iron for intravenous administration, large amounts of iron are administered directly into the blood stream in the treatment of a large variety of anemias. The increasing incidence of systemic hemosiderosis after repeated blood transfusions and the prolonged oral administration of iron in anemic patients calls for certain precautions to avoid the possible tissue damage resulting from prolonged use of blood transfusion and intravenous injection of iron. Recently the syndrome of extensive systemic hemosiderosis with cirrhosis of liver in anemic patients who have received repeated blood transfusions or iron administration has been recognized as "exogenous hemochromatosis" in order to differentiate it from idiopathic hemochromatosis which is a rare disease of unknown etiology and is characterized by excessive hemosiderin pigmentation of the skin and viscera with associated fibrosis of the involved viscera.

The clinical and pathological findings of most of the reported cases of exogenous hemochromatosis strongly suggest that extensive deposition of iron is capable of inducing functional and morphological damage in the liver, spleen or pancreas resulting in cirrhosis and diabetes mellitus. The similarity of certain clinical and pathological changes between exogenous and idiopathic hemochromatosis has led to the thought that these two conditions may differ from each other quantitatively rather than qualitatively (11) and that the excessive absorption or storage of iron might be the chief etiological factor of idiopathic hemochromatosis. However, this concept is still highly controversial

and there is still much disagreement as to the etiologic role of the iron stores in the production of tissue damage. Furthermore the experimental evidence in support of such a theory is still lacking. The present status of the controversy is evidenced by the following three theories:

(A) The excessive absorption and storage of iron in the liver is the primary event giving rise to hepatic damage and consequent cirrhosis (32, 51, 60, 90). In addition to the occurrence of hepatic fibrosis following multiple blood transfusions and prolonged iron administration (9, 75, 85, 94, 98, 123, 143), this view is further supported by the fact that the deposition of iron usually precedes the fibrosis even in idiopathic hemochromatosis (6, 10, 51, 72, 77, 125, 142, 146). Moreover repeated phlebotomies with depletion of iron deposits have been noted to effect clinical improvement and amelioration of the cirrhosis on repeated liver biopsies in patients with idiopathic hemochromatosis (6, 21, 22, 71, 111, 136). On the other hand, this view is not favored by the absence of cirrhosis in cases of intense hemosiderosis in congenital hematoporphyrinuria (121), in pernicious anemia with multiple blood transfusion (74, 145) and in animal experiments producing an overloading of iron (3, 13, 93, 104, 115).

(B) Cirrhosis is the primary disorder and hemosiderosis is merely a secondary phenomenon (79, 97, 121). Muir and Dunn (95, 96) stated

"the fibrosis comes first certainly in the liver, because in many places in this disease (idiopathic hemochromatosis), there are abundant deposits of iron without signs of irritation and in ordinary cirrhosis the liver may contain more iron than usual." Roth (120) in 1915 stated that the increase of connective tissue was due to some derangement of liver function and not to the deposition of pigment. However, there is little evidence to

support this point of view save for the failure to observe cirrhosis in certain instances of marked hemosiderosis.

(C) The hemosiderosis and cirrhosis are two separate processes, caused by unknown primary disturbances which independently give rise to the abnormalities of iron metabolism and functional as well as structural changes in the liver, pancreas and other organs (26, 60). Sheldon (125) suggested that the two processes of pigmentation and cirrhosis were independent effects of a single cause, probably an inborn error of metabolism. He added, however, that when the pigmentation has reached a certain degree of intensity, it may by irritation accelerate the development of new fibrous tissue. Aufderheide (5) and Wyatt (73, 143, 144) and their associates suggested that the cirrhosis develops independently due to a local nutritional deficit, in which the intensity of the cirrhosis may depend upon the duration and severity of the anemia and not solely upon the excessive passage of altered hemoglobin or excessive iron into liver cells. The frequent occurrence of exogenous hemochromatosis in anemic patients also suggested that anemia might be an additional factor in the development of exogenous hemochromatosis. Herbut and his associates (65, 66), by reviewing necropsied cases and animal experiments, concluded that "in hemochromatosis, the cirrhosis and diabetes may be caused by a single substance, a ureide, and the cirrhosis further aggravated by dietary deficiencies and hyper-cholesterolemia." The hemosiderosis, he proposed, merely represented a separate disturbance following an abnormal retention of iron derived exogenously and perhaps to some extent endogenously.

No one of the above theories is well substantiated. Much attention has been directed, therefore to experimental studies aimed at establishing

the possible role of iron overload in the production of tissue damage. These experiments are summarized in Table 1 and will be reviewed later. As can be seen, no conclusive decision has been reached and further studies are indicated.

In the belief that the inconclusive experimental results may have been due to the use of inadequate amounts of iron as well as to insufficient periods of observation, the present experiment of prolonged intravenous administration of large amounts of iron was undertaken to answer the following questions:

- (1) What is the pattern of progressive experimental iron overload in rabbits?
- (2) Will prolonged and intensive administration of iron progress from simple iron pigmentation of tissues to structural changes such as cirrhosis?
- (3) Is the amount of iron deposit or the duration the more important factor in the development of cirrhosis?
- (4) Is anemia an additional factor in the development of tissue damage in iron overloading?

REVIEW OF LITERATURE

The effect of prolonged administration of iron can be best understood against a background of the normal metabolism of iron. Therefore, the literature is reviewed with respect to iron metabolism in the normal state and in conditions associated with the excessive storage of iron in the hope of clarifying some of the problems of iron overload.

I. Iron metabolism in the normal state.

Iron is one of the indispensable minerals in human metabolism. It is a component of hemoglobin as well as of such oxidizing enzymes as the cytochromes, catalase and peroxidase. Its insignificant total amount of 3-5 gm. in the human body is quite out of proportion to its great importance. About 55% of the total amount of iron in the human body is found in hemoglobin, and about 10% in muscle hemoglobin and heme enzymes; the remainder - some 30% to 35% - constitute the storage iron fraction, the major portion of which (some 0.8 gm.) is found in the liver, spleen and red bone marrow, largely in the form of non-heme iron compounds and ferritin, an iron protein complex (61). The human body handles iron very economically, using it over and over again; only a small amount (1-1.5 mg) is found in the bile, sweat and excreta each day (23, 28, 51, 82, 91, 106, 139, 140), as was confirmed by tracer studies with radioactive iron by Copp and Greenberg (17), Hahn et al (55) and Dubach et al (25). This small excretion cannot be appreciably increased by loading the body with iron by oral ingestion, by parenteral administration or by multiple blood transfusions (49, 54). The amount of iron in the body is mainly regulated by control of its absorption rather than by excretion. The regulation of iron absorption is in some way dependent upon the body needs of iron.

Iron is absorbed from the gastrointestinal tract in ferrous form. Owing to gastric acid and reducing agents such as -SH group of proteins and ascorbic acid in the food supply, the ferric iron of foodstuff is changed to ferrous iron before absorption. The ferrous iron is absorbed in the mucosal cells of the duodenum and jejunum and some of it combines with apoferritin to form ferritin (146, 48, 50). The mechanism of regulation of iron absorption in the gastrointestinal tract was recently suggested by Granick (51) and many other investigators as follows:

(1) Mucosal cells provide a one-way transfer of ferrous iron into these cells. Once iron gets into the organism it can no longer get out. Widdonson and McCance (139) demonstrated in 1937 that iron injected into the blood stream in larger dose was not excreted by way of the urine or feces.

(2) Normally, there is a resistance or mucosal block to iron absorption. Anemia can increase the absorption of iron into the blood stream. Hahn and Whipple (54, 58) have demonstrated this in dogs with radio-active iron as a tracer. Relatively little radioactive iron fed to a normal dog is absorbed, but a chronically anemic dog will absorb 5 to 20 times the amount absorbed by the normal dog. Dubach et al (24) demonstrated that patients with fever, untreated pernicious anemia and refractory anemia absorbed more radioactive iron than they used for hemoglobin and patients with hemolytic anemia may absorb more iron than can be recovered in the peripheral blood at any one time because isotopic hemoglobin is removed from circulation at a rapid rate. The absorbed iron is only used for erythropoiesis in anemia; once the demands of the bone marrow are met, the absorption stops (32).

(3) There is a relationship between this resistance or mucosal block

and the storage of iron. Hahn (57) demonstrated that it is the depletion of iron stores and not the anemia itself that stimulates iron deposition. When a well-nourished dog is bled of 60% of its blood volume and then fed iron, no more iron is absorbed than before hemorrhage; whereas after a week has elapsed, that is, after depletion of iron stores might be expected to have occurred, the absorption is increased. Granick (51) thought not only that the ferritin (the storage form of iron) was depleted in the liver, spleen and marrow, but also that the ferritin in the mucosal cells of the gastrointestinal tract was broken down.

(4) The presence of ferritin is in some way connected with the presence of mucosal block; only when ferritin in the mucosal cells is depleted can more ferrous iron be absorbed. That the level of ferritin in mucosal cells readily responds to the feeding of iron has been demonstrated in guinea pigs (51). In this animal ferritin is normally present only in traces in the mucosa. When high doses of ferrous iron are fed, ferritin is found to increase, especially in the duodenal region, and then after a few days to decrease.

The amount of iron absorbed is regulated by the amount of ferrous iron that the mucosal cells will tolerate. Ferrous iron passes from the intestinal lumen into the mucosal cells in one direction (51). Once the iron enters the cell, it never passes back again through the cell membrane. The ferrous iron of the mucosal cell is considered to be in equilibrium with the ferritin of these cells and with the plasma iron of the blood stream, governed in part by the oxidation-reduction potential of these cells. When iron absorption is active, the ferritin concentration of intestinal mucosal cells increases to a high concentration, and

further absorption of iron is blocked. The ferrous iron leaves the mucosa and proceeds to the blood stream where the oxygen tension is such as to auto-oxidize the ferrous iron to ferric iron. This ferric oxide becomes attached to the metal-combining globulin (Fraction IV-7) and is transported to the tissues of the body as a complex of ferric-hydroxide-protein. If plasma iron is lowered because of various conditions, the mucosal cells are depleted of their iron which then permits increased absorption of iron from the gastrointestinal tract. The plasma iron, in turn is in equilibrium with the ferrous iron and ferritin of the liver, spleen and bone marrow, and here, too, rapid exchanges of iron are possible (47, 56, 134). When in unusual situations, the rate or the quantity of iron deposition is too great to be handled by the ferritin mechanism, hemosiderin is formed in the tissues (136, 52).

In the bone marrow, not only is ferrous iron converted to ferritin, but also ferrous iron is incorporated into protoporphyrin to form heme which then combines with globin to form hemoglobin (31). This hemoglobin iron is constantly reutilized.

Degenerated red cells are destroyed by the phagocytes of the liver, spleen and bone marrow. This breakdown still leaves the iron intact, and this iron is again used by the body to build up hemoglobin and its various other iron compounds. Therefore the daily requirement of 5 mg iron is adequate for the human body even for the period of most rapid growth. Under normal conditions, the excretion and absorption of iron are very small in amount. Normally, a maximum of about 1.5 mg of iron per day is absorbed, and about 1.0^{mg}/or less is lost. The net gain of 0.5 mg per day is enough to make possible manufacture of 300 - 600 cc of blood per year.

II. Studies in iron storage diseases (hemochromatosis)

1. Idiopathic Hemochromatosis

Idiopathic hemochromatosis usually occurs in males past middle age with an occasional hereditary influence (125). It is characterized by excessive hemosiderin deposition in the skin and viscera, with associated fibrosis of some organs, particularly the liver and pancreas. The clinical and morphological features of this disease have been well known for a long time, but the knowledge of its pathogenesis is still far from clear. The following etiological factors, none of which are convincing, have been suggested: Inborn error in cellular metabolism; a disorder of metabolism of iron in the cytochrome system; some inability to utilize certain iron compounds; abnormally increased iron absorption in the intestinal tract over a long period owing to some congenital defect in the mucosal block; exogenous poisons or metallic poisons such as alcohol, copper, lead and zinc; protein lack in nutrition, infectious disease (hepatitis) and reduction of pancreatic secretion (64). There is still a good deal of controversy on this point. The clinical and experimental investigations of this disease can be summarized as follows:

(A) Clinical investigations

(1) Incidence of hemochromatosis: Hemochromatosis is rare in females, but when present, it occurs in the post-menopausal age. It has been suggested that its rarity in younger women is explained by the fact that they have menstruation and pregnancy as a means of excreting iron, mechanisms not present in the male. Houston (71) stated that during the 30 years of reproductive life of the female, the average loss of iron through menstruation and pregnancy is probably about 10-15 gm. In a

woman with the underlying metabolic defect of hemochromatosis, this loss might be sufficient to delay or suppress the clinical expression of the disease. His study of the age incidence of hemochromatosis lent some support to this hypothesis. In Sheldon's series (125) 277 cases in males were fairly evenly spread on each side of the peak incidence at 45-50 years. Only 2 of his 14 cases in females occurred before this age. According to Houston (71), Sheldon later stated that 1 of his 2 young females did not have proven hemochromatosis and was included in his chart of the age incidence by error; hence of his 13 female patients, only 1 developed the disease before the menopause. Houston himself further investigated the age incidence in 202 cases in males and 20 in females collected from the literature after 1935 and found only 2 females under 45 years of age. It was particularly interesting that 1 of the 2 young females had had amenorrhea for 13 years before dying of hemochromatosis at the age of 34. Houston concluded that hemochromatosis probably results from excessive absorption of iron owing either to an inborn error of iron metabolism or to multiple transfusions. Heilmeyers (64) also reported a series of 36 cases of hemochromatosis and stated that the highest incidence is between the ages of 50 and 59 and that all cases in females occurred after the menopausal age. Althausen (1) reported 3 cases in females among 23 patients with hemochromatosis and found that their menstrual history was so abnormal that considerably less blood was lost during the reproductive period.

(2) Studies on iron metabolism - Since the principal alteration in this disease is the excessive deposition of an iron-positive pigment (hemosiderin) in most of the organs of the body and the skin, much

much attention has been paid to the metabolism of iron. Chemical analyses have revealed a high iron content in blood and in all the organs varying from 4 to 40 times of normal value (1). For instance, normal liver contains 0.05 to 0.1% of iron, but in hemochromatosis the liver contains 1.15 - 3.65% of iron. Usually, a normal person has a total body iron of 3-5 gm, but in hemochromatosis the total value may increase up to 45 gm. Also, in patients with hemochromatosis, the total iron-binding capacity of serum is normal or low, but the saturation of the iron-binding protein is high (70). Studies with radio-active iron (30) have revealed a profound depression of utilization of the iron. Heilmeyer (64), analyzing a series of 36 cases of hemochromatosis, measured the absorption of iron from the gut in these cases by giving radioactive iron orally and found increased absorption.

(3) Effect of phlebotomy and BAL (British Anti-Lewisite; 2,3-dimercaptopropanol; dimercaprol) on hemochromatosis.

Granick was probably the first (1949) to suggest that repeated venesections might be beneficial to patients with hemochromatosis. Recently, there have been evidences that the increased iron content in hemochromatosis can be mobilized for synthesis of hemoglobin. Davis and Arrowsmith of Tulane University, (21, 22) treated 3 patients with hemochromatosis with repeated bleedings and found mobilization of the excessive iron manifested by a tremendous capacity to regenerate red blood cells and hemoglobin of up to 4-7 pints of blood per month for a period of 2 years. This amount of regenerated blood represents a negative iron balance of approximately 15-20 gm. per year. The patients also gave evidence of subjective improvement clinically, such as increased energy

and working ability, diminution in size of liver, and improvement in liver function and in carbohydrate metabolism. Repeated liver biopsies also showed a considerable reduction of the concentration of iron in the liver and improvement in the cirrhosis. Similar results were reported by Houston (71), Warthin (136), Peterson and Ettinger (111) and Beyers and Gitlow (6). Whether the bleeding will merely mobilize the iron or at the same time modify the hepatic fibrosis, cannot be answered now. However, mobilization of iron in hemochromatosis now seems well established. Finch and Haskins et al (29, 62) also noted the failure to develop anemia after repeated phlebotomies in patients with hemochromatosis who were on normal diets.

Ohlsson et al (110) reported a case of transfusion hemosiderosis after BAL with recovery. The pigmentation of the skin became fainter, the ascites disappeared, and the heart volume and liver function became normal. This may suggest that BAL also benefits the excretion of iron and thus improves the hemochromatosis.

(4) High frequency of hemochromatosis among South Africans.

The frequency of hemochromatosis in South Africa has also attracted a great deal of attention and has led to many studies. Gillman and Gillman (40) found that the diet of South Africans is mainly corn grits, with a deficiency of protein and a high iron content. South Africans also frequently use iron pots for cooking. It has been found that a typical diet cooked in the traditional way in an iron pot may contain 100-150 mg. of iron per day (133), in contrast to 10-15 mg. for people in other parts of the world. Squires (130) reported that abnormally high serum iron values are occasionally encountered in adults in South Africa. South Africans are also reported to develop hypochromic anemia very rarely.

Gerritsen and Walker (138) reported the abnormal deposition of iron in the tissues of an adult Bantu at autopsy. Gillman and Gillman (41) have therefore suggested that this particular type of chronic malnutrition affecting South Africans and the relatively large amounts of iron habitually ingested may be etiological factors in hemochromatosis. They thought the steady passage of relatively inert iron pigment of large molecular size into the circulation cannot be without consequence, preparing the background for development of premature arteriosclerosis, cirrhosis of the liver, keloid formation and reticulosis, all of which are known to be prevalent in Africans. Gillman and Gillman (39) by means of liver aspiration biopsy, showed that no less than 12% of 120 adult pellagrins were suffering from frank pigment cirrhosis and that an additional 18% were in the pre-cirrhotic stage. Consequently malnutrition can be regarded as a significant factor in the etiology of pigment cirrhosis in Africans. Gore, (44), who also described a white male of 64 who had pellagra associated with hemochromatosis, thought that the normal excretion of excess iron pigment through normal mucosa of the colon is defective in pellagra and that the excess depositions of this pigment represent a retention, the hemochromatosis in this case being secondary to pellagra.

(5) Copper in hemochromatosis. Copper content in tissues was reported to be higher than normal in hemochromatosis (87, 88). Althansen and Kerr (2) stated that in the Black Forest in Germany, where there is increased frequency of hemochromatosis, the wine makers spray grape vines with copper sulfate for protection against mold and that the inhabitants of the area habitually consume more alcohol. These facts led people to think

of the possibility of copper poisoning in hemochromatosis; the experimental studies along this line will be reviewed later.

(B) Experimental Studies

(1) Overloading animals with iron (Table 1). Because the absorption of iron is controlled by the intestinal mucosa and normally the excretion and absorption of iron are limited, when iron is given intravenously the mucosal control is by-passed and iron is almost completely retained by the body unless there is bleeding (83, 92). Since the early 19th century, investigators have given large amounts of blood or its components or iron intravenously to animals to ascertain whether prolonged administration of iron would produce hemochromatosis. Rous and Oliver (121) in 1918 transfused citrated whole blood from rabbits to other rabbits in amounts of 10 cc daily for 6 days out of 7 (about 195 mg per kilogram of body weight) for $3\text{--}6\frac{1}{2}$ months and found hemosiderosis of organs and tissues such as connective-tissue cells of lungs and choroid plexus, spleen, liver cells, Kupffer cells, adrenal glands, heart and lymph nodes and central atrophy of hepatic cells. Two of the most pigmented liver of 11 animals showed intralobular fibrosis. The authors explained the hepatic fibrosis as intercurrent.

Polson (112) gave large amount of iron (280 mg per kilogram of body weight) to rabbits in 1928 for 13 days and observed iron in reticulo-endothelial cells and pulmonary embolism by iron which was responsible for some deaths. He (113) also gave small amounts of iron to rabbits for as long as 14 months in 1929 and found 4 of 28 rabbits showing slight cirrhosis of liver. Furthermore, (115) he administered iron subcutaneously and intraperitoneally to rabbits for 1-4 years and in 1933

reported that liver cirrhosis and pancreatic damage were absent in all. He concluded: "It is unlikely that excess of iron is responsible for the hepatic and pancreatic lesions of human hemochromatosis." Cappell (13) in 1930, gave iron in amounts of 550 mg per kilogram of body weight to rats and mice up to 14 months and found hemosiderosis of organs but no cirrhosis.

In 1934 Menkin (93), after having injected diluted ferric chloride solution intravenously in rabbits for weeks, observed hemosiderosis of organs and fibrosis in peripheral portion of the Malpighian body of the spleen.

Andersson (3), in 1950 reported that large amounts of iron (267 mg per kilogram of body weight) given to rabbits for one year resulted in hemosiderosis of organs with no cirrhosis. He stated that his experimental study of the effect of iron administered parenterally to more animals and in greater amounts than those employed in any previous investigation had not borne out misgivings that parenteral therapy with colloidal iron might be well founded.

Two years later Nissim (104) gave iron in large doses to different species of animals for 5-6 months and observed hemosiderosis and damage of hepatic parenchyma, with atrophy of cytoplasm and nuclear pyknosis and karyolysis but with no true cirrhosis.

(2) Copper poisoning and iron feeding. Mallory et al (86) and Hall and Butt (59) claimed that large copper feedings or copper injections interfered with the excretion of iron or increased its absorption, resulting in deposition of iron in tissues. Necrosis and fibrosis were induced at the site of iron deposition in rabbits and sheep. Flinn and

TABLE I Experimental Studies on Iron Overloading Reported in the Literature

Writer	Date Reported	Animal Used	Approximately highest amount of elemental iron administered mg./kg.	Route of Administration	Duration of Experiment	Result
Rous & Oliver (121)	1918	rabbit	195	IV	3-6 $\frac{1}{2}$ months	Hemosiderosis of organs and central atrophy of hepatic cells. 2 of the most pigmented livers of 11 animals showed intra-lobular cirrhosis.
Polson (112)	1928	rabbits	280	IV	13 days	Iron in reticuloendothelial cells, coecum epithelium, thoracic and abdominal lymph glands and tubular epithelium of renal tubules. Iron also was present in vessels as emboli.
Polson (113)	1929	rabbit	112	IV	7 days - 14 months	4 out of 28 rabbits showed slight cirrhosis of liver.
Cappell (13)	1930	rats and mice	550	IV	14 months	Hemosiderosis of organs. No cirrhosis
Polson (115)	1933	rabbits	157	S.C. or I.P.	1-4 years	No liver cirrhosis or pancreatic damage.

TABLE I CONTINUED

Menkin (93)	1934	rabbits	*	I.V.	weeks	Hemosiderosis in all sections of bone marrow and half of liver. Fibrosis in peripheral portion of Malpighian follicles of spleen. No cirrhosis.
Andersson (3)	1950	rabbits	267	I.V.	1 year	Hemosiderosis of organs, no cirrhosis
Nissim (104)	1952	Mice Rats Rabbits Guinea pigs	2160	I.V. I.P. I.C.	5-6 months	Hemosiderosis of organs. Damage of hepatic parenchyma with atrophy of cytoplasm and nuclear pyknosis and karyolysis. No true cirrhosis. Acute hepatic necrosis in G.p. but without fibrosis.

* Diluted ferric chloride solution (0.25%). Amount not reported.

Von Glahn (33) and Polson (114) repeated the experiment and found no cirrhosis in experimental animals. Mallory (89) varied the experiment by subcutaneous injection of copper into rabbits, sheep, monkeys and guinea pigs in 1931 and explained that the different result obtained by other investigators might be due to different strains of rabbits. Wyatt and Howell (144) observed the effect of 0.5% copper acetate mixed with a diet containing corn grit and 2% ferric citrate on rats already suffering from iron overloading of the liver for a period of 72 days. Despite the addition of copper to the diet, no difference in the iron storage pattern was noted, and there was no evidence of fibrosis.

Chase et al (15) found that there is less absorption of iron from gastrointestinal tract of rats and swine deficient in copper than in animals supplied with copper. They thought the influence of copper on iron absorption is not due to the administration of copper simultaneously with iron, but appears to be correlated with the level of copper in the tissues (27).

Whether the copper poisoning has something to do with hemochromatosis is still undecided.

(3) Deficient diet and iron feeding. Hegsted et al (63, 77) produced marked hemosiderosis with corn grit and lard (which are deficient in protein) with excessive iron. They thought that the malnutrition can lower the intestinal barrier for absorption of iron and also that the low phosphorus content of iron-supplemented corn grit diet may be the primary cause of abnormal high iron absorption. They found that the absolute amount of iron and phosphorus in the diet as well as the iron-phosphorus ratio influences the amount of iron absorbed. The amount of

iron deposited in the liver was inversely related to the phosphorus content of the diet. Wyatt and Howell (144) also fed rats with corn grit and iron salts. They obtained dietary siderosis but no cirrhosis. They confirmed the finding that protein deficiency and iron-phosphorus imbalance lead to pathological excess of iron but believe that some factors other than excess of iron are required for the development of liver fibrosis.

(4) Ligation of the pancreatic duct or extirpation of the pancreas. In 1935 Taylor, Stiven and Reid (131) produced siderosis of the liver in cats by ligation of the pancreatic duct or extirpation of the pancreas. The degree of hemosiderosis was directly proportional to the amount of iron in the diet. They thought that cutting off the flow of pancreatic juice injured the epithelial cells at the tip of the villi of the small bowel and consequently weakened the barrier of iron absorption and allowed more iron to be absorbed from food.

(5) Alloxan poisoning. Herbert, Watson and Perkins (65) reported in 1946 that injection of Alloxan caused portal necrosis of the liver in 10 of 30 rabbits and diabetes and portal cirrhosis in 2 other rabbits; one of which developed interlobular and intralobular fibrosis of the pancreas. When a reduced form of iron was fed to the rabbits freely for only $2\frac{1}{2}$ weeks, a marked deposition of pigment in the spleen, intestinal submucosa, liver and renal tubules occurred. This led the authors to believe that in hemochromatosis the sequence of events may be as follows:

(a) Alloxan or an allied substance produces necrosis of the peripheral hepatic tissue and islets of Langerhans, resulting in cirrhosis of the liver and diabetes.

(b) Cirrhosis is further aggravated by dietary deficiency.

(c) Portal hypertension resulting from cirrhosis of the liver produces interlobular and intralobular fibrosis of the pancreas.

(d) Abnormal retention of iron derived from both exogenous and endogenous sources results in hemosiderosis.

Finally, a review of the clinical and experimental investigations indicates no single convincing explanation for pathogenesis of hemochromatosis; there is still much controversy.

2. Exogenous hemochromatosis resulted from prolonged iron therapy or repeated blood transfusion.

(a) In Iron Therapy.

Iron therapy has long been known to be effective in anemia (119). Intravenously administered iron has proved to be almost entirely (71.8-99.7%) used for the synthesis of hemoglobin (42, 137). For this reason, intravenous administration of iron appealed to physicians and pharmacologists for the emergency treatment of acute anemia and in cases in which oral administration is not tolerated, but on account of the toxicity of intravenous iron salts, the application of this therapy did not become clinically established until 1949, when Nissim (99,100,103) standardized a safe form of iron for intravenous use--saccharated iron oxide. Though immediate toxic reactions or ill effects such as bronchospasm and leukopenia resulting from this type of therapy have been reported from time to time (69, 81, 108, 138), their incidence and severity have not precluded its beneficial use in indicated cases (20, 34, 45, 53, 68, 76, 84, 99, 116, 124, 126, 127, 129). Saccharated iron oxide has been given intravenously to anemic patient in as many as 800 injections in 18 months (128) and in single doses as large as 1 gm. with no untoward side effect (80, 138).

However, 3 patients with chronic anemia not associated with blood loss receiving prolonged oral iron therapy developed so-called exogenous hemosiderosis (14, 43, 135). Attention has been called to the possible danger of insidious tissue damage such as fibrosis by excessive storage of iron. It is suggested to give iron intravenously in the minimum amount compatible with the patient's comfort and welfare (12, 117). The chronic iron poisoning or hemosiderosis resulting from intravenous administration is not distinctly harmful but is irreversible (16, 19).

(b) In Blood Transfusion.

Blood transfusion, which was first given to anemic patients at the beginning of this century after the discovery of the blood group in humans, reached its widest use from the early 1940's to the present, hundreds of thousands of bottles of blood being given as a general panacea for injuries of war with remarkably satisfactory effects. The first report of a transfusion with some claim to be considered beneficial to the patient pertained to a case of post-partum hemorrhage (8). Today blood transfusion is still believed to be the most dramatic, effective and simple therapy for acute hemorrhages. Administered blood persists in the blood stream far longer than any other transfused substance. Transfusion thus serves to produce a sustained increase in blood volume and also an increase in the oxygen-carrying power of blood. As a general rule, this method is applied to the treatment of anemia only when the anemia cannot be cured by the administration of iron, liver or other hematinics. Transfused blood supplies the recipient with a certain amount of iron, each 100 ml of blood containing some 50 mg., almost all of which is present in the form of hemoglobin. When the red blood cells break down,

this iron is retained in the body and can be used by the recipient for the formation of new hemoglobin. Recently, the reports on transfusional siderosis after multiple blood transfusions in anemic patients have been increasing in number (5, 7, 9, 35, 73, 85, 109, 110, 123, 142, 143, 146). Much attention has been paid to its possible damage to remote tissues.

The possible association of secondary hemochromatosis with multiple blood transfusions was first recognized in 1937 by Kark (75), whose patient had received more than 290 transfusions over a period of 9 years for aplastic anemia and had developed pigmentation of the skin, impotence and a large cirrhotic liver. He assumed that it was due to a failure of excretion of excess and nonutilized iron. Since then, many isolated cases and collected series of cases have been reported. Opinions differ about whether the hemochromatosis results from blood transfusions or from other mechanisms.

Bomford and Rhoads (9) in 1941, reported 3 cases of secondary hemochromatosis developing after 16 transfusions in $2\frac{1}{2}$ years, 12 transfusions in 2 years and 54 transfusions in 9 years respectively for refractory anemia. All 3 patients showed pigmentation of skin and lymph nodes and fibrosis and pigmentation of liver and pancreas. One of them had glycosuria.

In 1942 Mackey (85) reported a case of aplastic anemia with organ changes resembling hemochromatosis after transfusion of 39.8 liters of blood in $3\frac{1}{2}$ years. The amount of iron recovered from the patient's liver was directly related to the amount of hemoglobin administered in transfusions.

Six years later Schwartz and Blumenthal (123) reviewed 8 cases of exogenous hemochromatosis resulting from blood transfusions and reported

5 cases of their own. They thought that the hemochromatosis developing in these patients was the end result of the deposition and subsequently irritating action of the excess amounts of iron in the parenchymatous tissues, and that the underlying anemia and the not infrequent transfusion reactions acted as predisposing factors for the development of exogenous hemochromatosis.

Muirhead, Crass, Jones and Hill (98) in 1949, observed 5 cases of hemosiderosis aggravated by blood transfusions. One patient had cirrhosis of liver. The amount of iron in the liver in these cases was estimated and found to be approximately equal to the total amount of iron in the transfused blood.

In 1950 Wyatt, Mighton and Moragues (143) described 8 patients with severe anemia of diverse causes who were treated by multiple blood transfusions. Post-mortem observations revealed pronounced iron storage. Five of these cases showed granular livers; 2 gave chemical assays of iron comparable to the levels encountered in conventional hemochromatosis. The authors thought that since these anemic patients are kept alive by repeated blood transfusions, it is quite possible that cellular degenerative processes and local nutrient deficits are partially responsible for tissue siderosis and fibrosis.

Rath and Finch (118) in 1949, found that in 4 cases of transfusional hemosiderosis the serum iron concentration was greatly increased and the iron-binding capacity of the serum was fully saturated.

Four years later Aufderheide et al (5) reported 2 cases of exogenous hemochromatosis with diabetes mellitus after blood transfusions and postulated anemia as the basic etiologic factor in secondary hemochroma-

tosis by causing increased iron absorption; the iron introduced in the form of blood transfusion only accelerates a process already in progress.

Morningstar reported (94) in 1954, 3 cases of exogenous hemochromatosis. One patient who received 254 blood transfusions developed pigment cirrhosis of the liver, pigmentation of the skin, diabetes mellitus and testicular atrophy. The amount of pigmentation and fibrosis of the liver and pancreas correlated with the number of transfusions.

Hemochromatosis following homologous serum jaundice nine years before death was reported by Aquilina et al (4), in 1953. They thought that the attack of severe homologous serum jaundice may have initiated hemosiderin deposits in various organs of the body.

3. Relation between idiopathic hemochromatosis and exogenous hemochromatosis. There are two different main opinions regarding the relation between the two chief forms of hemochromatosis:

(A) There are morphological and clinical similarities between idiopathic and exogenous hemochromatosis. Brook and Hunter (11) thought that hemosiderosis and hemochromatosis are quantitatively rather than qualitatively different. Block, Bethard and Jacobson (7) reported a case of acute acquired hemolytic anemia and 3 cases of primary refractory anemia developing hemosiderosis, cirrhosis, auricular fibrillation, skin pigmentation, diabetes mellitus and decreased body hair after repeated blood transfusions. They found that at autopsy the tissues were practically identical with those of patients with idiopathic hemochromatosis. They thought that the idiopathic and exogenous forms of hemochromatosis are similar and possibly identical so far as the type and progression of pathological changes of tissues are concerned. The

major differences, however, are the extensive chronic anemia present in patients with secondary hemochromatosis and the inability to utilize iron for erythropoiesis in patients with hemochromatosis secondary to primary refractory anemia.

(B) The idiopathic and exogenous hemochromatosis are two different entities.

Zeltmacher and Bevans (146) described a patient with aplastic anemia associated with hemochromatosis receiving transfusion of 6.5 gm. Fe, but the liver was found to contain 29 gm. of iron. They considered hemochromatosis and hemosiderosis two separate entities and believed that transfusion in aplastic anemia is an unlikely cause of exogenous hemochromatosis.

Cottier, (18) from Switzerland, and Klechner, Baggenstoss and Weir, (78) from the Mayo Clinic, state that hemochromatosis and transfusion hemosiderosis are two distinct and separate pathologic entities, and that hemosiderosis was not converted into hemochromatosis because of the differences of distribution of iron in organs and the inconstant occurrence of cirrhosis of the liver, parenchymatous damage to the pancreas, pigmentation of the skin and deposits of iron in bile ducts in marked cases of hemosiderosis. They claimed that in idiopathic hemochromatosis, the liver is the organ containing the most iron pigment deposit, while in exogenous hemochromatosis the spleen is the organ with the most iron-pigment deposition. Cottier (18) even thought that the co-existence of cirrhosis in transfusional hemosiderosis is due to the pre-existing cirrhosis. Gaskell et al (37) observed that in idiopathic hemochromatosis the iron pigment is more marked in the ascending

limb of Henle and distal convoluted tubules, while in exogenous hemochromatosis the iron pigment is predominantly in the proximal convoluted tubules.

Thus, there are still many contradictory opinions about the possible relation of hemosiderosis to hemochromatosis. Though more people regard hemochromatosis as secondary to hemosiderosis, the lack of experimental evidence serves as a strong source of debate.

MATERIALS AND METHODS

A total of 103 young male rabbits and 9 young female rabbits weighing 3.5 to 5.0 Kg (average of 4 Kg) were used. All were kept in individual cages and fed with ordinary rabbit pellets, carrots, green vegetables and water ad libitum. They were divided into two groups, non-anemic and anemic rabbits.

A. Non-anemic Rabbits. This group consisted of 58 rabbits; 16 controls (males) received no treatment, and 42 (7 females and 35 males) were repeatedly injected intravenously through the ear veins with saccharated iron oxide (Feojection-Smith, Kline and French Laboratories). The dosages varied from 20 mg to 200 mg over intervals from 2 days to 2 months. The total dosages received by each rabbit ranged from 45 mg to 4100 mg of elemental iron. Injections were planned every other day to start with 20 mg per rabbit, with a 20-mg increase in each succeeding injection up to a maximum single dose of 200 mg of elemental iron. The longer intervals of injections were occasioned by the development of multiple phlebothrombosis of their superficial veins that took time to recover so as to become available again for the injection. The rabbits were sacrificed over a period of time varying from 4 days to 14 months.

B. Anemic Rabbits. 54 rabbits were rendered anemic by repeated bleedings from the ear veins to maintain a hemoglobin below 6.0 gm per 100 cc (normal value for rabbit is $13\frac{1}{2}$ 1.2 gm per 100 cc (141) before each injection. Seventeen males were used as anemic controls, and 37 (35 males and 2 females) were injected with iron following the same schedule as outlined for the non-anemic rabbits. The period of study ranged from 4 days to 12 months, with total corrected dosages of iron

ranging from 25 mg to 4040 mg. This dosage was corrected for the iron lost through bleeding by subtracting 50 mg iron for each 100 cc of blood lost.

Complete autopsies were performed on rabbits sacrificed at different intervals and tissues were fixed in 10% neutral formalin. Sections of the liver, spleen, adrenal, pituitary, thyroid with parathyroid, mesenteric lymph nodes, lung, pancreas, skin, kidney, stomach, small bowel, testes or ovary, heart and bone marrow (femoral or vertebral) were prepared with hemotoxylin-eosin stain and iron stain; aniline blue stain of sections of liver were done in selective cases.

The urines of rabbits that had received a total of more than 3000 mg of elemental iron were tested for sugar (Benedict qualitative method) together with blood glucose level determinations (modified Folin-Wu method).

RESULTS

Non-anemic Rabbits

I. Gross examination.

(1) Control group. Their organs were not remarkable.

(2) Iron-injected group. The spleens and livers of rabbits receiving a total of more than 1000 mg. of elemental iron were dark brown and enlarged up to twice the normal size and weight. (Average weight of spleen was 10 gm. and average weight of liver was 180 gm.). The livers of 2 rabbits that were given a total dosage of 3560 mg. (R467-male) and 4100 mg. (R257-female) of iron respectively showed brown color with irregular nodularity, increased consistency and positive iron stain and weighed 200 gm. and 270 gm. respectively (Fig. 1-4). The bone marrows of most of the animals having received more than 2000 mg. of iron totally were also brown in color.

II. Microscopic examination.

(A) Control group: None of the tissues examined in this group yielded evidence of the presence of iron-staining material. The liver of one rabbit (R 111) demonstrated portal fibrosis with proliferation of bile ducts.

(B) Iron-injected group: The organs in this group of rabbits are described in the order of the approximate amount of iron pigment found within the tissues. The occurrence of iron pigment in different organs is shown in table II.

(1) Spleen: The amount of iron present in the spleen appeared to bear a direct relationship to the total amounts of iron received by each rabbit. Iron-positive pigment granules were present in the phago-

cytes in the red pulp of every injected animal. In those receiving small amounts, the pigment appeared as fine, dustlike granules within phagocytes in the pulp. With gradually increasing total dosages, the pigment granules clumped together to produce large irregular masses found both within the phagocytes of the splenic pulp as well as in apparent extra-cellular situations, presumably occasioned by the death of the cells bearing such massive accumulations of pigment (Fig. 5). The connective tissue cells in the capsule and the trabeculae and the endothelial lining cells of the capillary and sinusoids contain similar granular golden pigment in those rabbits receiving large dosages (over 2000 milligrams of iron). The Malpighian corpuscles were well preserved and contained no pigment, and the normal architecture of the spleen was always retained. No fibrosis was observed.

(2) Liver: In all of the experimental animals, the liver contained large amounts of iron pigment, but apparently in amounts less than that present in the spleen. In rabbits receiving less than 200 milligrams of iron, the liver showed fine golden-yellow granular pigment in the Kupffer cells distributed throughout the hepatic lobules (Fig. 6 and Fig 7). With increasing amounts of iron, similar granules appeared in the parenchymal cells in the central regions of the lobules. With progressive increase in the total iron dose, the pigmentation extended toward the periphery of the hepatic lobules, and in those receiving the largest doses in the range of 4 grams of iron, large masses of pigment granules were present in the peripheral zones of the hepatic lobules (Fig. 8 and Fig. 9). In these last mentioned-animals, the total accumulation of pigment appeared to become more marked in the

periportal hepatic cells than in the central hepatic cells. Concomitant with this increase of hepatic parenchymal pigmentation, the Kupffer cells showed progressive accumulation of pigment granules as the total dose of iron increased (Fig. 10). In animals receiving these large doses, the connective tissue cells in the portal spaces and the endothelial cells of central veins also contained iron-positive granules. The epithelial cells of the bile ducts remained normal at all stages of iron dosage. In addition to the hemosiderosis described, 20 rabbits demonstrated various degrees of fibrosis in the periportal region (Fig. 11). In such animals, iron-positive granules were sometimes present within the fibrous tissue. However, the amount of fibrosis was less extensive than that observed in the single control animal demonstrating periportal fibrosis without apparent iron pigmentation. The presence of fibrosis of the liver is correlated with the total dosage of iron and duration of study in Table III and IV.

(3) Bone marrow: Pigment-laden macrophages were noted in the reticular stroma of the bone marrow. The amount of pigment increased commensurate with the dose of injected iron, but never reached the same degree of severity as that present in the liver or spleen.

(4) Mesenteric Lymph Node: Approximately two-thirds of the rabbits showed pigmented macrophages in the sinuses and pigmentation of the connective-tissue cells of the capsule and trabeculae. This pigmentation did not bear any apparent correlation with the dosage of the administered iron or the duration of the experimental period.

(5) Lung: Iron-positive granules were present in macrophages within the alveoli and in the endothelial lining cells of the capillaries

in all rabbits receiving more than 150 milligrams of total iron dosage (Fig. 12). No pigment was present in any of the alveolar epithelium, nor in the lining epithelial cells of the bronchi or bronchioles. No iron embolism was found in the vessels of any of the animals.

(6) Adrenal Gland: Iron pigment was present in almost all of the animals in the fibrocytes in the stroma of the adrenal cortex, and in the connective tissue of the medulla. It was also present in the cortical cells, particularly in the zona reticularis and inner half of the fasciculata in rabbits receiving doses of iron over 2 grams in total amount. Occasional animals demonstrated large, irregular masses of iron pigment in these cortical regions, similar to those observed in the spleen. This distribution of iron pigment agrees with the finding of Nissim (101) who observed a distribution of iron paralleling that of the ascorbic acid in the adrenal; but Nissim stated that the adrenal medulla was invariably free of iron in all of his experimental animals (105).

(7) Pituitary gland: Small amounts of fine granular iron pigment were present in the fibrous stromal cells of rabbits having received more than 300 milligrams of iron. No pigment was ever noted in the glandular cells of the anterior pituitary.

(8) Kidney: Fine granular iron pigment was present in the endothelial cells of the glomerular capillaries and in the tubular epithelial cells of the ascending limb of Henle's loops and collecting tubules. This distribution was most evident in animals receiving the larger doses of iron. The proximal convoluted tubules were never involved, and no iron-staining material was noted in the tubular lumens.

(9) Parathyroid gland: As in the pituitary, fine granular iron-positive pigment was present in the connective tissue stromal cells, but none in the epithelial cells of the glands.

(10) Ovary: Iron-positive pigment was present in the stromal cells and central scars of corpora lutea in rabbits that had received more than 2500 milligrams of iron. No such pigment was found in the follicles or germinal epithelium. This pattern of pigment distribution again agrees with the findings of Nissim (102), who observed the correlation between the distribution of iron and that of ascorbic acid in the ovary as well as in the adrenal. Nissim thought that a metabolic association between iron and ascorbic acid was possible.

(11) Testes: Scanty amounts of fine iron pigment granules were occasionally present in the interstitial tissue of testis in rabbits given more than 500 milligrams of iron and in all those having received more than 3500 milligrams of iron.

(12) Skin: Iron pigment was present in the hair follicles and some connective tissue cells in the corium of rabbits that had received more than 700 milligrams of iron. This finding was not constant, however, and iron pigment did not appear in all animals, even in those having received maximum total dosages. No iron pigment was present in the epidermal cells. The sweat glands were absent in all the sections of skin examined, so the distribution of iron pigment in sweat glands cannot be evaluated.

(13) Pancreas: Scanty amounts of fine iron granules were noted in some of the acinar cells and some of the islet cells in rabbits having received more than 400 milligrams of iron. This pigmentation, however, was not

constant and was not present in all animals, even in those with maximum dosage. Fibrosis was not noted in any of the rabbits, and no total destruction of islet cells was ever present.

(14) Thyroid: In a few rabbits having received more than 1400 milligrams of iron, occasional fine iron pigment granules were noted in the stromal cells. No pigment was present in the follicular cells in any animal.

(15) Heart: Iron pigment was present in the endothelial cells of the endocardium in scanty amount in all rabbits, and in the muscle fibers of the myocardium in only a very few animals, without apparent correlation with the total dosage of iron administered.

(16) Gastrointestinal tract: Iron pigment was present in the capillary endothelium of most of the rabbits. It appeared in the stromal cells of the mucosa in the stomach of one animal (R 726) and in the mucosal fibro-blasts in the small bowel of an additional animal (R 135). No iron pigment was ever noted in the mucosal epithelial cells of any animal.

TABLE II

OCCURENCE OF IRON PIGMENT IN VARIOUS ORGANS OF NON-ANEMIC EXPERIMENTAL RABBITS*

Rabbit No.	Iron injected (mg.)	Liver	Spleen	Adrenal	Pituitary	Parathyroid	Lymph node	Lung	Pancreas	Skin
R342	45	+	+	+	0	0	0	0	0
R138	80	+	+	+	0	0	0	0	0
R569	80	+	+	+	0	0	0	0	0
R114	105	+	+	+	0	+	0	0	0
R100	150	+	+	+	0	+	0	+	0	0
R 44	150	+	+	+	0	0	0	+	0	0
R384	200	+	+	+	0	+	0	+	0	0
R173	215	+	+	+	0	0	0	+	0	0
R 28	310	+	+	+	+	+	+	+	0	0
R539	365	+	+	+	+	+	+	+	0	0
R 54	385	+	+	+	+	+	+	+	0	0
R353	390	+	+	+	+	+	0	+	0	0
R128	395	+	+	+	+	+	0	+	0	0
R 84	440	+	+	+	+	+	+	0	0
R525	520	+	+	+	+	+	+	+	0	0
R247	800	+	+	+	+	0	+	0	0
R135	980	+	+	+	+	+	+	0	0
R150	1000	+	+	+	+	0	+	+	0	+
R562	1080	+	+	+	+	+	+	+	0
R873	1100	+	+	+	+	+	+	0	0
R869	1110	+	+	+	+	+	+	+	0	+
R717	1150	+	+	+	+	+	0	+	0	0
R732	1150	+	+	+	+	+	+	+	0	0
R165	1200	+	+	+	+	+	+	0	0
R476	1200	+	+	+	+	+	+	+	0	0
R651	1265	+	+	+	+	+	+	+	0	0
R600	1300	+	+	+	+	+	+	+	0	0
R722	1400	+	+	+	+	+	0	+	+	0
R 2	1430	+	+	+	+	+	+	+	0	0
R372	1460	+	+	+	+	+	+	+	+	0
R324	1650	+	+	+	+	+	+	+	0	0
R552	1700	+	+	+	+	+	+	+	0	+
R430	1810	+	+	+	+	0	0	+	0	0
R726	1900	+	+	+	+	+	+	0	0
R576	2115	+	+	+	+	+	0	+	+	0
R369	2125	+	+	+	+	+	+	+	+	+
R132	2945	+	+	+	+	+	+	+	+	0
R723	3000	+	+	+	+	0	+	+	0	0
R357	3060	+	+	+	+	+	+	+	+	+
R467	3560	+	+	+	+	+	+	+	+	+
R720	3960	+	+	+	+	+	+	+	+	+
R257	4100	+	+	+	+	+	+	+	+	0

* + - present; 0 - absent; - tissue was not examined.

TABLE II (Continued)

Rabbit No.	Iron injected (mg)	Kidney	Stomach	Bowel	Thyroid	Testis	Ovary	Myocardium	Bone Marrow
R342	45	0	0	0	0	0	0	/
R138	80	0	0	0	0	0	0	/
R569	80	0	0	0	0	0	0	/
R114	105	0	0	0	0	0	0	/
R100	150	0	0	0	0	0	0	/
R 44	150	0	0	0	0	0	0	/
R384	200	/	0	0	0	0	0	/
R173	215	/	0	0	0	0	0	/
R 28	310	/	0	0	0	0	0	/
R539	365	/	0	0	0	0	0	/
R 54	385	/	0	0	0	0	0	/
R353	390	0	0	0	0	0	0	/
R128	395	/	0	0	0	0	0	/
R 84	440	/	0	0	0	0	0	/
R525	520	0	0	0	0	/	0	/
R247	800	/	0	0	0	/	0	/
R135	980	/	0	/	0	0	0	/
R150	1000	0	0	0	0	0	0	/
R562	1080	0	0	0	0	/	0	/
R873	1100	/	0	0	0	0	0	/
R869	1100	/	0	0	0	/	0	/
R717	1150	/	0	0	0	/	0	/
R732	1150	0	0	0	0	0	0	/
R165	1200	0	0	0	0	0	0	/
R476	1200	/	0	0	0	0	0	/
R651	1265	/	0	0	0	0	0	/
R600	1300	/	0	0	0	0	0	/
R722	1400	/	0	0	/	0	0	/
R 2	1430	/	0	0	0	0	0	/
R372	1460	/	0	0	0	0	0	/
R324	1650	/	0	0	0	/	0	/
R552	1700	/	0	0	0	0	0	/
R430	1810	/	0	0	0	0	0	/
R726	1900	/	/	0	/	0	/	/
R576	2115	/	0	0	0	0	0	/
R369	2125	/	0	0	0	0	0	/
R132	2945	/	0	0	0	0	0	/
R723	3000	/	0	0	0	/	0	/
R357	3060	/	0	0	0	/	0	/
R467	3560	/	0	0	0	/	/	/
R720	3960	/	0	0	/	/	0	/
R257	4100	/	0	0	/	/	0	/

TABLE III
EXPERIMENTS ON NON-ANEMIC RABBITS

RABBIT	TOTAL DOSE OF ELEMENTAL IRON	TIME FROM FIRST INJECTION TO DEATH	FIBROSIS OF LIVER *
	mg.	month	
R342	45	1/6	0
R138	80	1/2	0
R569	80	1/3	0
R114	105	1/3	0
R100	150	1/2	0
R 44	150	1/2	0
R384	200	1/2	0
R173	215	1/2	0
R 28	310	1	0
R539	365	2	0
R 54	385	2	/
R353	390	2	0
R128	395	1	0
R 84	440	3	/
R525	520	1	0
R247	800	1	0
R135	980	1	0
R150	1000	2/3	0
R562	1080	1	/
R873	1100	2	0
R869	1110	2 2/3	/
R717	1150	3	/
R732	1150	1 1/3	/
R165	1200	1	/
R476	1200	3	/
R651	1265	1	/
R600	1300	5	/
R722	1400	3	0
R 2	1430	3 1/3	/
R372	1460	7	/
R324	1650	2 2/3	0
R552	1700	3	/
R430	1810	8	/
R726	1900	7	/
R576	2115	4	0
R369	2125	11 1/2	/
R132	2945	11 1/2	/
R723	3000	17	0
R357	3060	12	/
R467	3560	13	/
R720	3960	8 1/2	0
R257	4100	14	/

*

0 ---- absence of fibrosis

/ ---- presence of fibrosis

TABLE IV

Incidence of Hepatic Fibrosis Among the Total Experimental Non-anemic Rabbits.

TOTAL DOSAGE RANGE	NUMBER OF RABBITS INJECTED \bar{c} IRON	NUMBER OF RABBITS SHOWING HEPATIC FIBROSIS	% RABBITS WITH HEPATIC FIBROSIS AMONG THE TOTAL EXPERIMENTAL RABBITS
MG.			%
45 - 100	13	1	8
401 - 1000	5	1	20
1001 - 2000	16	13	81
2001 - 3000	4	2	50
3001 - 4100	4	3	75

Anemic Rabbits

I. Gross examination:

(1) Control group: Their organs were not remarkable except that the liver of one rabbit (R 40) showed slight nodularity and increased consistency, but no change in color.

(2) Iron-injected group: The organs were similar to those of the non-anemic rabbits except that none of livers showed gross nodularity.

II. Microscopic examination:

(1) Control groups: None of the tissues of this group of rabbits showed the presence of iron-staining material. Two rabbits (R 40 and R 103) showed marked portal fibrosis with proliferation of the bile ducts and disruption of the normal architecture of the liver. These hepatic changes appeared to be completely unrelated to the presence of any iron pigmentation.

(2) Iron-injected group: In general the distribution of iron pigment in this group of anemic rabbits paralleled that of the non-anemic rabbits. The tissue sections in general resembled almost precisely those described in the iron-injected group of non-anemic rabbits. The occurrence of iron pigment in different organs is shown in table V. Whereas 20 out of 42 non-anemic iron-injected rabbits showed some fibrosis of the liver, in this anemic group 8 rabbits out of 37 showed similar fibrosis, periportal in distribution, associated with the hemosiderosis. The correlation between fibrosis of the liver and the amount of iron injected and duration of experimentation are presented in tables VI and VII.

Glycosuria was never demonstrated in any of the experimental animals and the blood sugar levels of all rabbits, both anemic and non-

anemic, failed to disclose any hyperglycemia. All determinations fell within the normal range of 100-120 mg. of glucose per 100 c.c. of blood.

TABLE V

OCCURRENCE OF IRON PIGMENT IN VARIOUS ORGANS OF ANEMIC EXPERIMENTAL RABBITS*

Rabbit No.	Iron injected (mg.)	Liver	Spleen	Adrenal	Pituitary	Parathyroid	Lymph node	Lung	Pancreas	Skin
R179	25	/	/	/	0	0	0	0	0
R226	70	/	/	/	0	0	0	0	0
R283	75	/	/	/	0	0	0	0	0
R122	75	/	/	/	0	/	0	0	0
R293	80	/	/	/	0	0	0	0	0
R287	80	/	/	/	0	0	0	0	0	0
R277	95	/	/	/	0	0	0	0	0
R 27	150	/	/	/	0	0	0	/	0	0
R436	175	/	/	/	/	0	0	/	0	0
R202	185	/	/	/	0	0	/	/	0	0
R189	205	/	/	/	0	/	/	/	0	0
R234	210	/	/	/	/	/	/	0	0
R284	315	/	/	/	/	0	/	/	0	0
R300	330	/	/	/	/	0	0	/	0	0
R473	330	/	/	/	/	/	/	/	0	0
R292	385	/	/	/	/	0	/	0	0
R465	425	/	/	/	/	0	/	0	0
R286	435	/	/	/	/	0	/	0	0
R 89	470	/	/	/	/	/	/	/	/	0
R488	530	/	/	/	/	/	/	/	0	0
R444	700	/	/	/	/	/	/	/	0	/
R464	730	/	/	/	/	/	0	/	0	0
R315	790	/	/	/	/	/	/	0	/
R245	830	/	/	/	/	/	0	/	0	0
R288	1220	/	/	/	/	/	/	/	0	0
R232	1320	/	/	/	/	/	/	/	0	/
R304	1360	/	/	/	/	/	0	0	0
R294	1575	/	/	/	/	/	/	0	0
R587	1850	/	/	/	/	0	/	0	0
R714	2700	/	/	/	/	0	/	/	0	0
R224	2800	/	/	/	/	/	/	0	/
R374	2930	/	/	/	/	/	/	0	0
R278	3100	/	/	/	/	/	/	0	0	0
R727	3160	/	/	/	/	/	/	/	/	/
R323	3400	/	/	/	/	/	/	0	/
R715	4000	/	/	/	/	/	/	/	/	/
R441	4040	/	/	/	/	/	/	0

* / present; 0 - absent; - tissue not examined

TABLE IV (Continued)

Rabbit No.	Iron injected (mg.)	Kidney	Stomach	Bowel	Thyroid	Testis	Ovary	Myocardium	Bone marrow
R179	25	0	0	0	0	0	0	0
R226	70	0	0	0	0	0	0	/
R283	75	0	0	0	0	0	0	0
R122	75	0	0	0	0	0	0	/
R293	80	0	0	0	0	0	0	/
R287	80	0	0	0	0	0	0	/
R277	95	/	0	0	0	0	0	/
R 27	150	0	0	0	0	0	0	/
R436	175	0	0	0	0	0	0	/
R202	185	0	0	0	0	0	0	/
R189	205	/	0	0	0	0	0	/
R234	210	/	0	0	0	0	0	/
R284	315	0	0	0	0	0	0	/
R300	330	0	0	0	0	0	0	/
R473	330	/	0	0	0	0	0	/
R292	385	/	0	0	0	0	0	/
R465	425	/	0	0	0	0	0	/
R286	435	/	0	0	0	0	0	/
R 89	470	/	0	0	0	0	0	/
R488	530	/	0	0	0	/	0	/
R444	700	0	0	0	0	0	0	/
R464	730	0	0	0	0	/	0	/
R315	790	/	0	0	0	/	0	/
R245	830	/	0	0	0	0	0	/
R288	1220	/	0	0	0	0	0	/
R232	1320	/	0	0	0	/	0	/
R304	1360	0	0	0	0	/	0	/
R294	1575	0	0	0	0	/	0	/
R587	1850	/	0	0	0	0	0	/
R714	2700	/	0	0	0	/	0	/
R224	2800	/	0	0	0	/	0	/
R374	2930	/	0	0	0	0	0	/
R278	3100	/	0	0	/	0	0	/
R727	3160	/	0	0	0	/	0	/
R323	3400	/	0	0	0	/	/	/
R715	4000	/	0	0	/	/	0	/
R441	4040	/	0	0	/	/	/	/

TABLE VI
EXPERIMENTS ON ANEMIC RABBITS

RABBIT	TOTAL DOSE OF IRON INJECTED	IRON LOSS BY BLEEDING	CORRECTED TOTAL DOSE OF IRON	TIME FROM FIRST INJEC- TION TO DEATH	FIBROSIS OF LIVER *
	mg.	mg.	mg.	month	
R179	100	75	25	1/7	0
R226	120	50	70	1/3	0
R283	125	50	75	1/2	0
R122	225	150	75	2/3	0
R293	140	60	80	1/3	0
R287	260	180	80	1/5	0
R277	165	70	95	1/2	0
R 27	200	50	150	1/4	0
R436	215	40	175	1/2	0
R202	245	60	185	1/5	0
R189	330	125	205	1/2	0
R234	300	90	210	1	0
R284	465	150	315	1 1/2	0
R300	505	175	330	1 1/2	0
R473	400	70	330	1	0
R292	495	110	385	1	0
R465	490	65	425	1 1/2	0
R286	510	75	435	1	0
R89	539	69	470	1 1/2	/
R488	650	120	530	2	0
R444	825	125	700	2 1/2	/
R464	855	125	730	1 1/2	0
R315	985	195	790	2	0
R245	1005	175	830	2 1/2	0
R288	1720	500	1220	4	0
R232	1600	280	1320	6	0
R304	1582	222	1360	7	/
R294	1860	285	1575	7 1/2	/
R587	2050	200	1850	8	/
R714	3100	400	2700	7 1/2	0
R224	3300	500	2800	9	/
R374	3530	600	2930	11 1/2	0
R278	3416	316	3100	12	/
R727	3590	430	3160	7	/
R323	4100	700	3400	12	0
R715	4750	750	4000	8	0
R441	4790	750	4040	12	0

* 0 ---- absence of fibrosis

/ ---- presence of fibrosis

TABLE VII

Incidence of Hepatic Fibrosis Among the Total Anemic Experimental Rabbits

TOTAL DOSAGE RANGE OF IRON	NUMBER OF RABBITS INJECTED	NUMBER OF RABBITS SHOWING HEPATIC FIBROSIS	RABBITS WITH HEPATIC FIBROSIS AMONG THE TOTAL EXPERIMENTAL RABBITS
MG.			%
25 - 400	16	0	0
401 - 1000	8	2	25
1001 - 2000	5	3	60
2001 - 3000	3	1	34
3001 - 4040	5	2	40

DISCUSSION

These experimental observations permit certain conclusions with respect to the questions presented in the introductory section:

1) What is the pattern of progressive experimental iron overload in rabbits? In both the anemic and non-anemic rabbits, a fairly clearly-defined sequence of tissue changes is apparent. This pattern appeared to be directly related to the total amount of iron administered to each animal, and did not appear to be modified by the presence or absence of anemia. In animals receiving the smaller total dosages of iron, iron pigment identical with hemosiderin was first noted in the reticulo-endothelial cells of the body and in phagocytic macrophages. The most intense pigmentation affected the phagocytic endothelial cells of the splenic sinuses and Kupffer cells of the liver. Pigment was also present in reticulo-endothelial cells of the bone marrow and lymph nodes. With progressive increase in iron overload, masses of iron pigment were found in the splenic pulp, in apparently extracellular locations, suggesting that death of the phagocytic cells had occurred with deposition of the pigment accumulations into the intercellular spaces. In the liver, with progressive iron overload, after the Kupffer cells had become completely stuffed, iron pigment progressively accumulated in the hepatic cells. This pigment appeared first in the central zones of the hepatic lobules, but with the largest doses, the entire lobules soon became affected. In animals receiving approximately 4 grams of iron, large masses of pigment were found in the periportal regions. At these maximum dosage levels, the amount of iron pigment appeared to become more extensive in the peripheral regions of the hepatic lobules than in the central zones.

In certain animals, this intense hepatic pigmentation was associated with some increased periportal fibrous tissue, suggesting the development of a pigment cirrhosis. This will be discussed more fully a little later.

No pigmentation was present in the bile duct epithelium. These findings agree with those of previous investigators (13, 32, 104, 107, 112, 122, 132).

The pancreas in most animals was only minimally affected by the iron overload. In a few instances, iron pigment was noted in the acinar cells and islets of Langerhans. This finding, however, was quite inconstant and was never associated with any pancreatic fibrosis. No significant structural change was noted in these rabbits, and the intensity of pigmentation was of far less significance than that found in most clinical cases of well-advanced hemochromatosis. It is noteworthy that no abnormalities in carbohydrate metabolism were ever detected in any of the animals, however large the total dose of administered iron. Pigmentation was also noted in the stromal connective tissue cells of many of the glandular organs, such as the testes, thyroid, parathyroid and pituitary glands. In none of these situations was there significant pigmentation of the parenchymal cells. Iron pigment was noted in the hair follicles and in some connective tissue cells in the corium of rabbits receiving more than 700 milligrams of iron. This finding, however, was inconstant and did not appear to be related to either the total dosage of iron or the duration of the deposit.

These anatomic changes do not completely parallel those observed in the usual classical cases of clinical hemochromatosis. However, the progressive pattern of deposition affecting the reticulo-endothelial cells,

liver, glandular organs, and skin strongly suggests a resemblance to clinical hemochromatosis, particularly cases that have been described as exogenous hemochromatosis. It is possible that if the experiment had been carried further, both with respect to the total amount of iron administered and the duration of the study, the tissue changes may have progressed to a more close resemblance to clinical hemochromatosis.

2) Will prolonged and intensive administration of iron progress from simple iron pigmentation of tissues to structural changes, such as pigment cirrhosis? The experimental observations suggest that there is a relationship between intense hemosiderosis of the liver and the development of pigment cirrhosis. Periportal fibrosis was noted in 20 (47%) of the non-anemic rabbits and in 8 (22%) of the anemic rabbits. However, it must be noted that one control animal in the non-anemic group and two in the anemic control group showed a portal type cirrhosis unassociated with iron pigment - an incidence of 10% of the control animals in both groups. These cases of cirrhosis in the control animals are interpreted as incidental hepatic changes in the general population of this experimental animal. These incidental findings, however, do not invalidate the significantly higher incidence of cirrhosis found in the experimental animals. The results therefore strongly suggest that intense iron pigmentation may be causally related to the development of hepatic fibrosis and the development of an anatomic pattern resembling pigment cirrhosis in humans. These findings support the statement made by Brock and Hunter (11) that the difference between hemosiderosis and hemochromatosis is a quantitative rather than a qualitative one. The mechanisms by which the progressive hemosiderosis

produces such hepatic fibrosis are still not clear. Mallory (86) suggested that the hemosiderosis itself induces necrosis of liver cells and fibrosis of the hepatic parenchyma. Schwartz (123) supported this view by postulating that the macroparticles may cause damage to cells and create fibrous scarring. Granick (51) proposed that the fibrosis may be due to the improper storage of iron in individuals already suffering from some underlying hepatic infection. Himsworth (67) also considered the pigment to be causally related to the development of hepatic fibrosis. However, he favored the view that the overloading of the Kupffer cells may, by producing circulatory disturbances, result in hepatic damage and fibrosis. It is noteworthy that no direct evidence of hepatic cell necrosis was identified in the experimental animals, even in those that received maximum dosages and developed hepatic fibrosis. With respect to the liver, the conclusion cannot be escaped that the iron pigmentation was in some way causally related to the occurrence of a higher than anticipated incidence of hepatic fibrosis, suggesting a pigment cirrhosis. Significant structural changes were not associated with the iron pigmentation in other organs. This observation was not unanticipated, since in many clinical cases of hemochromatosis the pigmentation does not appear to give rise to or be associated with structural change.

3) Is the amount of iron deposit or the duration of the deposit the more important factor in the development of cirrhosis? The experimental results obtained do not permit any conclusive observation with respect to this question. It can be seen from Tables III and VI that the fibrosis occurred in a sporadic, haphazard pattern. Certain

animals receiving rather small amounts of iron developed hepatic fibrosis, whereas other animals receiving similar amounts over an identical period of time failed to disclose tissue changes. There was no clear correlation between the total dose of iron and the appearance of cirrhosis of the liver. In many animals receiving the maximum dosages, hepatic changes failed to develop, whereas cirrhosis was noted in animals with considerably smaller amounts of administered iron. In the anemic group of rabbits, the three animals receiving the largest amounts of iron failed to develop hepatic change. Neither was there any correlation with time, since in many rabbits cirrhosis appeared within 2 to 3 months, whereas longer term animals showed no apparent structural fibrosis. The experimental results therefore do not permit any conclusion as to the specific factors which may have been important in this random pattern of development of hepatic fibrosis.

The incidence of cirrhosis observed in the present experiment is somewhat higher than that reported by previous authors (see Table I). This observation suggests the possibility that earlier experiments may have failed to develop hepatic fibrous changes either because insufficient total dosages of iron were administered, or the rate of administration may have been too slow. It is of interest to compare the largest total dose of iron injected in this experiment, 4.1 gm, with the largest amount of iron reported in patient with hemochromatosis, 54 gm. The 4.1 gm of iron for a rabbit weighing 4 kg is comparable to the 72 gm in an average human weighing 70 kg. This experimental dose of 4.1 gm of iron is further equivalent to 288 transfusions (500 mg. of iron in 1000 c.c. of blood) given to a patient.

4) Is anemia an additional factor in the development of tissue damage in iron overloading? As can be seen from the experimental results presented, the presence of anemia did not appear to materially affect the development of structural changes in organs. The anemia did not appear to favor the development of hepatic fibrosis. Howell and Wyatt, (73) in discussing the development of pigmentary cirrhosis in Cooley's anemia, pointed out that the recurrent, nonresponsive bouts of anemia may, through oxygen deprivation, affect hepatic or other cells with a high metabolic level, inhibiting their enzyme system and resulting in degenerative cellular changes and consequent fibrosis. Schwartz and Blumenthal (123) also emphasized the contributing role of the anoxia due to anemia. Wyatt, Mighton and Moragues (143) considered a local nutritional deficit in anemia to be another factor in the development of parenchymal fibrosis. Thus, anemia exerts its effect mainly through tissue anoxia and the local nutritional deficit. This hypothetical possibility that tissue anoxia might potentiate the damaging effect of iron pigment was not borne out by this experimental observation. Also it is not unlikely that in the present experiment, the anemia caused by bleeding was always corrected within a short time by the iron injections. This gave no opportunity for the anemia to last for any significant period so as to produce sufficient tissue anoxia and local nutritional deficit and thus to favor the development of hepatic fibrosis.

SUMMARY AND CONCLUSION

Repeated intravenous injections of saccharated iron oxide were administered to 42 non-anemic rabbits and 37 rabbits rendered anemic by bleeding, up to a maximum dosage of 1025 milligrams per kg of body weight. The longest duration of experimental observation was 14 months. A progressive pattern of iron pigment deposit in the body was apparent, affecting first the reticulo-endothelial system and then particularly the hepatic parenchymal cells. Twenty of the 42 non-anemic rabbits and 8 of the 37 anemic rabbits developed varying degrees of portal fibrosis. This portal fibrosis was in all cases associated with intense deposits of iron pigment in the peripheral zones of the hepatic lobule. There was no clear evidence relating the occurrence of fibrosis to either the total amount of iron administered or the duration of the iron deposit. Certain anatomic resemblances between exogenous and endogenous hemochromatosis in humans were noted suggesting the possible relationship of progressive hemosiderosis in rabbits to the development of tissue changes resembling the clinical hemochromatosis.

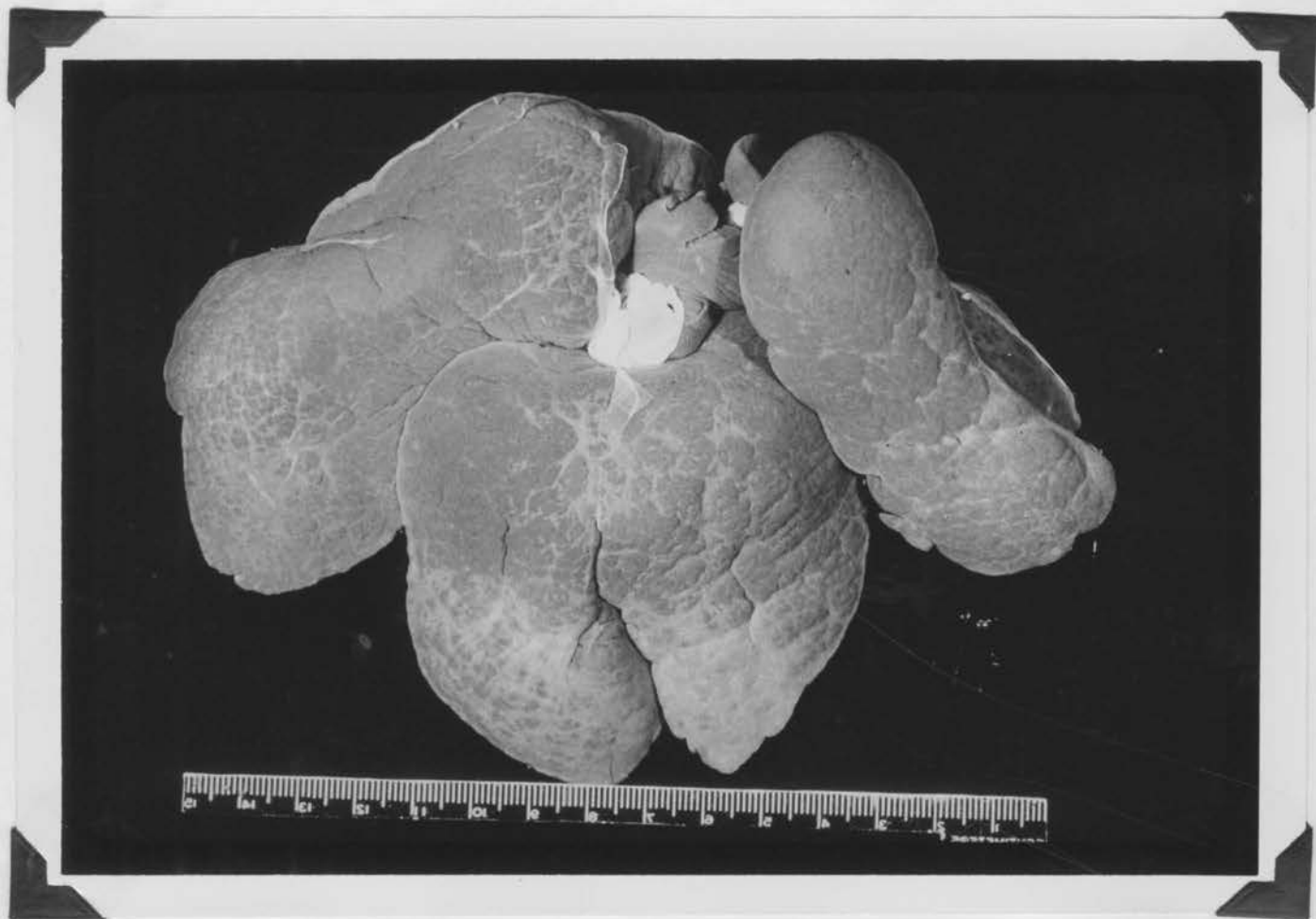
APPENDIX

Figure 1. LIVER OF RABBIT #467, SHOWING
IRREGULAR NODULAR SURFACE.

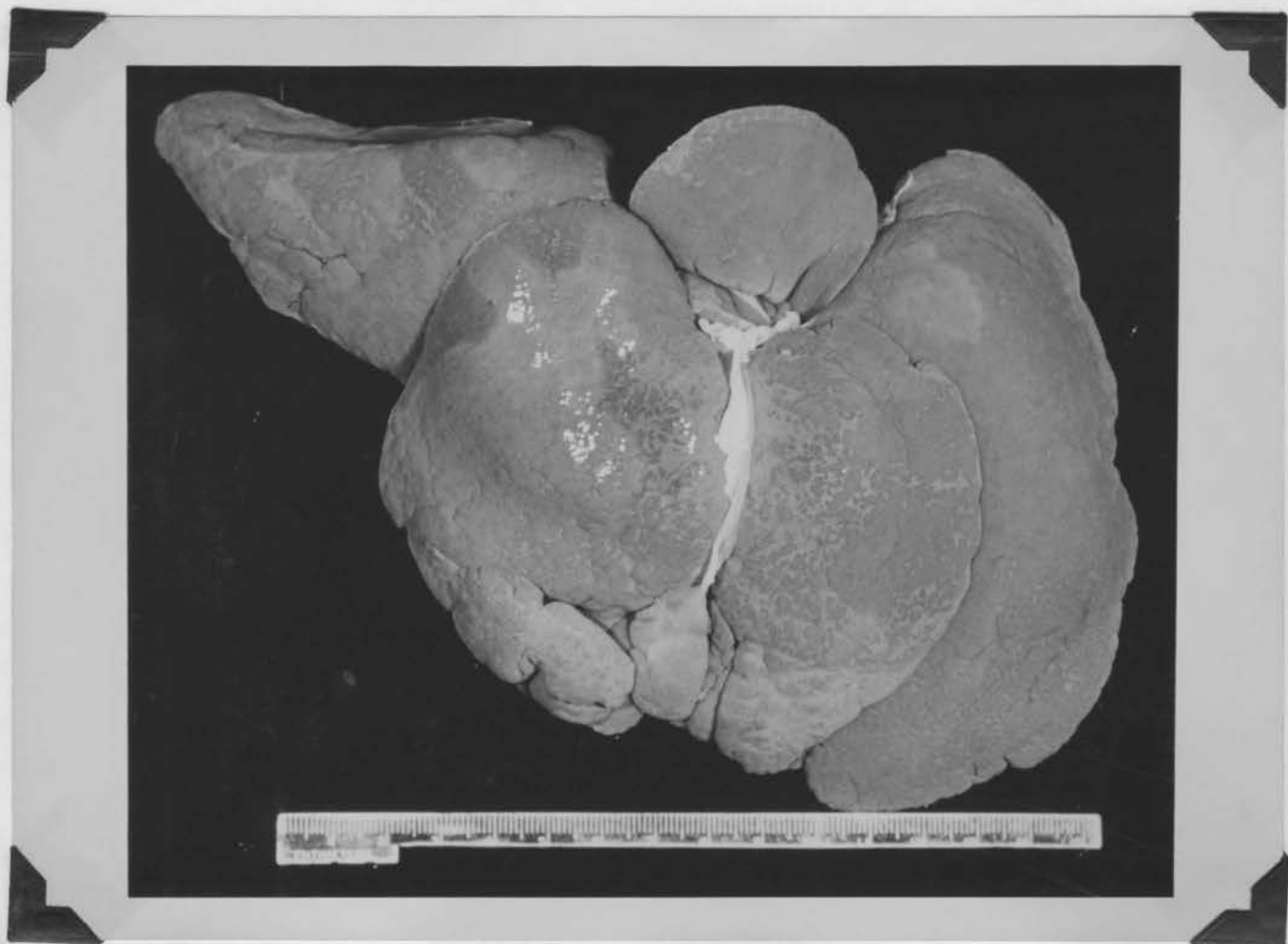


Figure 2. LIVER OF RABBIT #257, SHOWING
IRREGULAR NODULAR SURFACE.

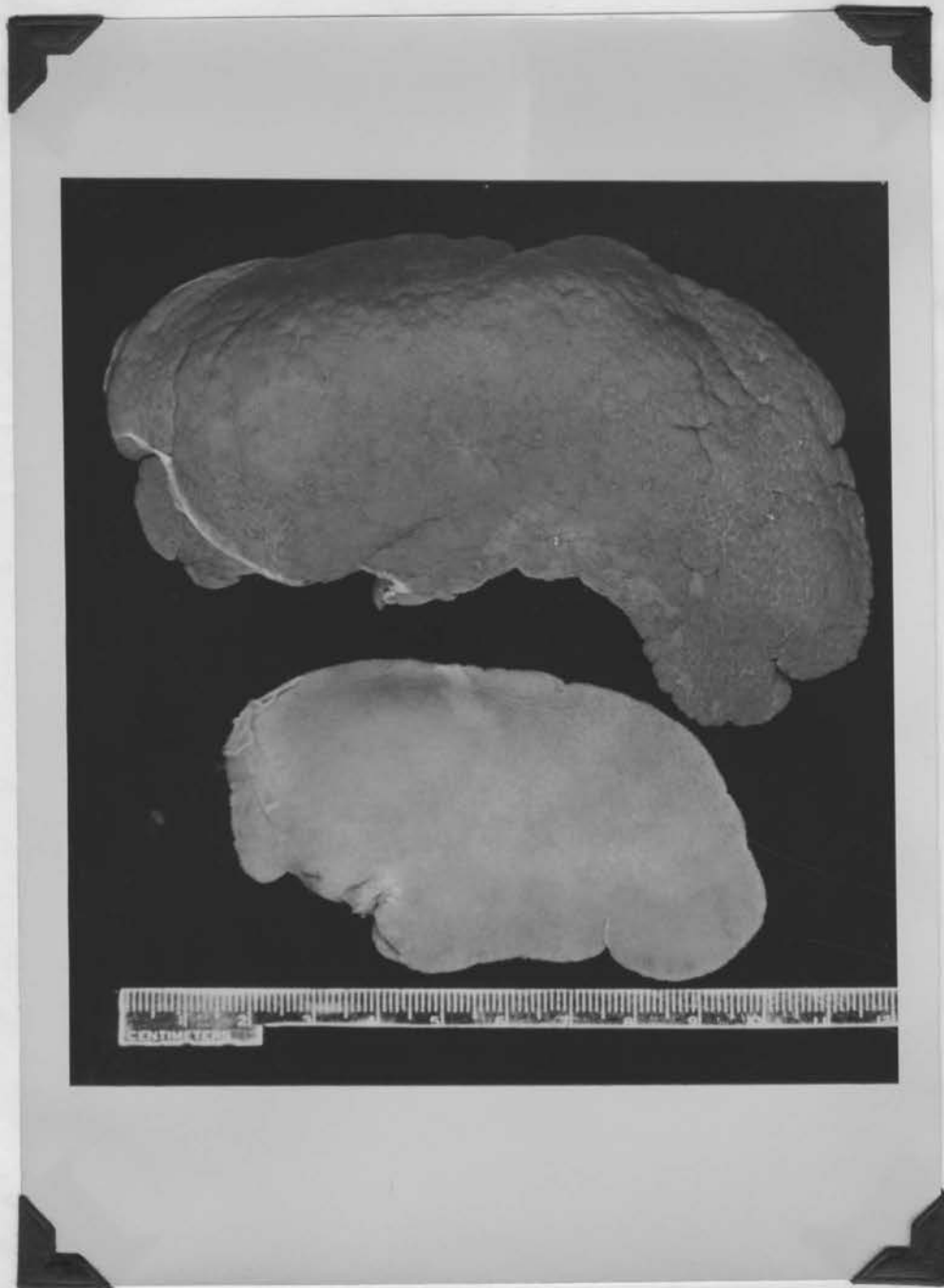


Figure 3. ABOVE: LEFT LOBE OF THE LIVER OF RABBIT #257.
BELOW: CORRESPONDING LOBE OF THE LIVER OF A
CONTROL RABBIT FOR COMPARISON OF SIZE,
COLOR AND SURFACE APPEARANCE.

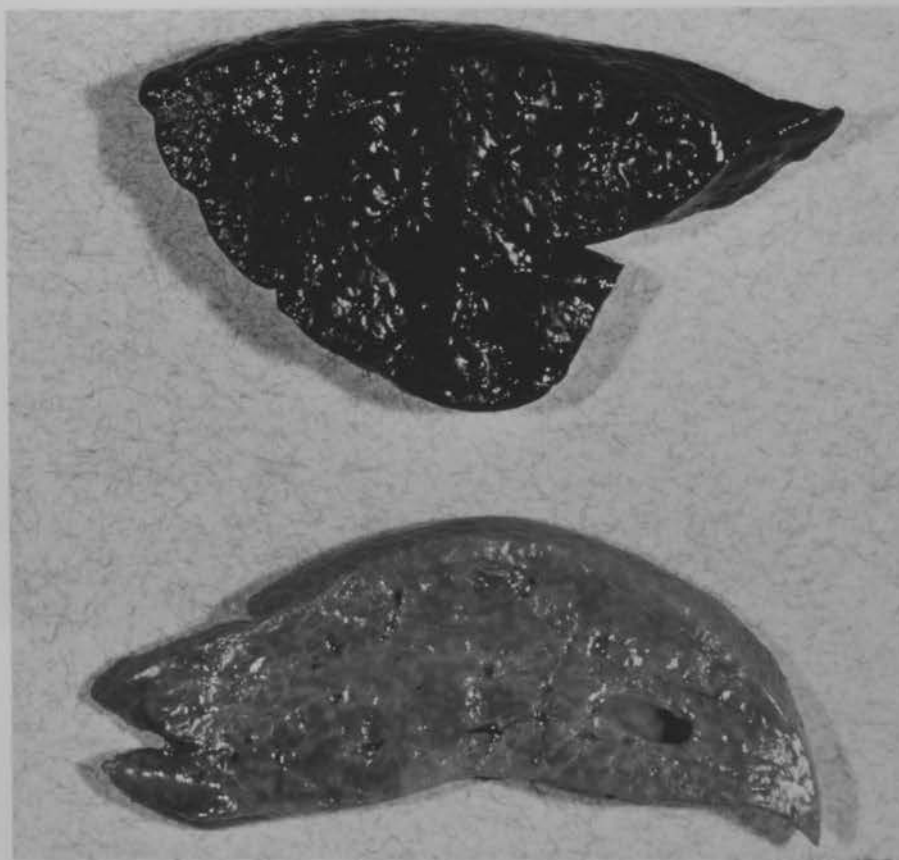


Figure 4. PARENCHYMAL SURFACES OF LIVERS STAINED BY
POTASSIUM FERROCYANIDE - HYDROCHLORIC ACID:
ABOVE: FROM RABBIT #257 SHOWING STRONGLY POSITIVE
IRON REACTION
BELOW: FROM A CONTROL RABBIT SHOWING NEGATIVE
IRON REACTION.

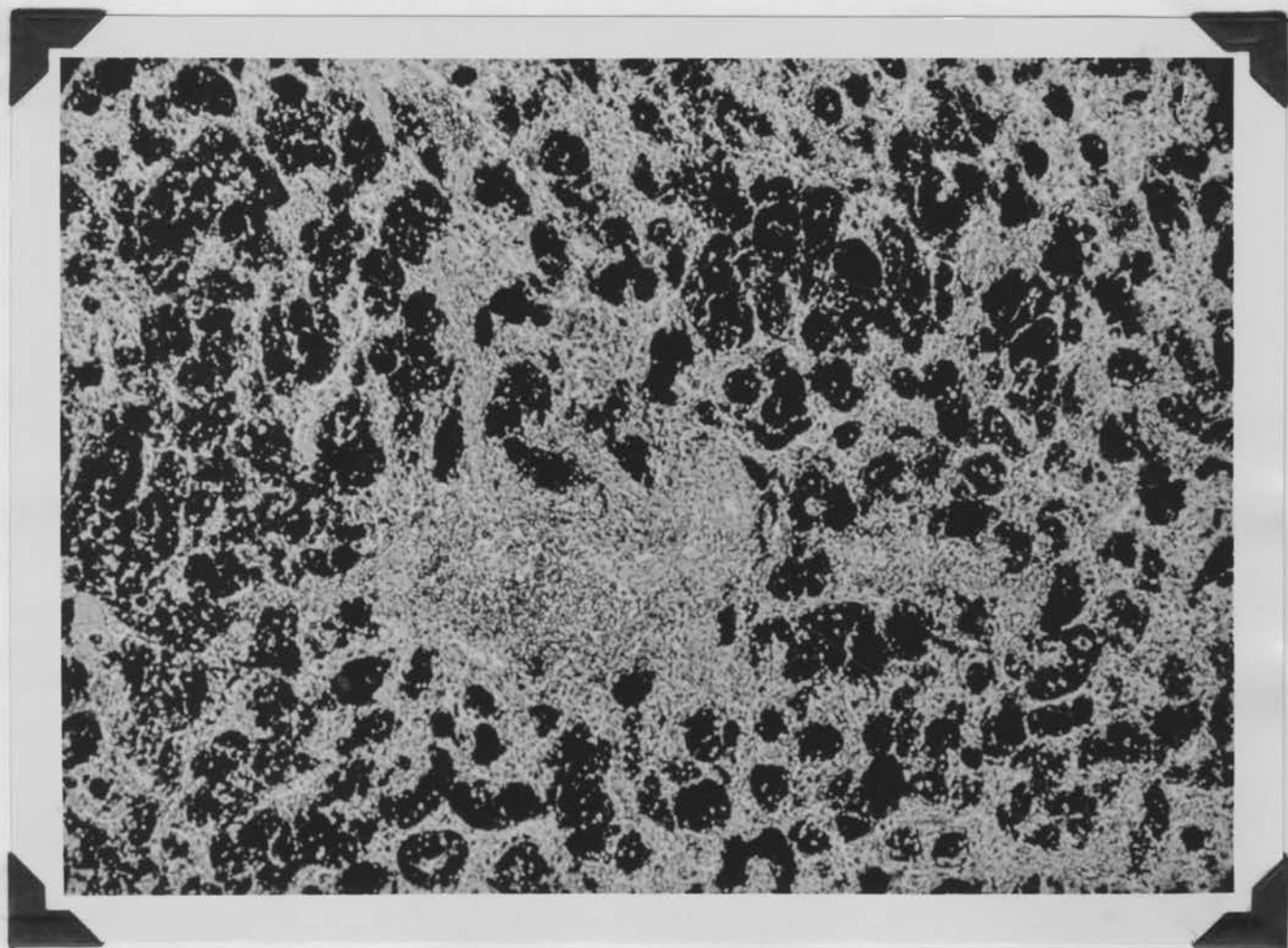


Figure 5. SECTION OF SPLEEN OF RABBIT #132. NOTE LARGE AND SMALL IRREGULAR MASSES OF PIGMENT GRANULES IN THE SPLENIC PULP AND THE WELL-PRESERVED MALPIGHIAN CORPUSCLE CONTAINING NO PIGMENT. HEMATOXYLIN AND EOSIN. LOW POWER.

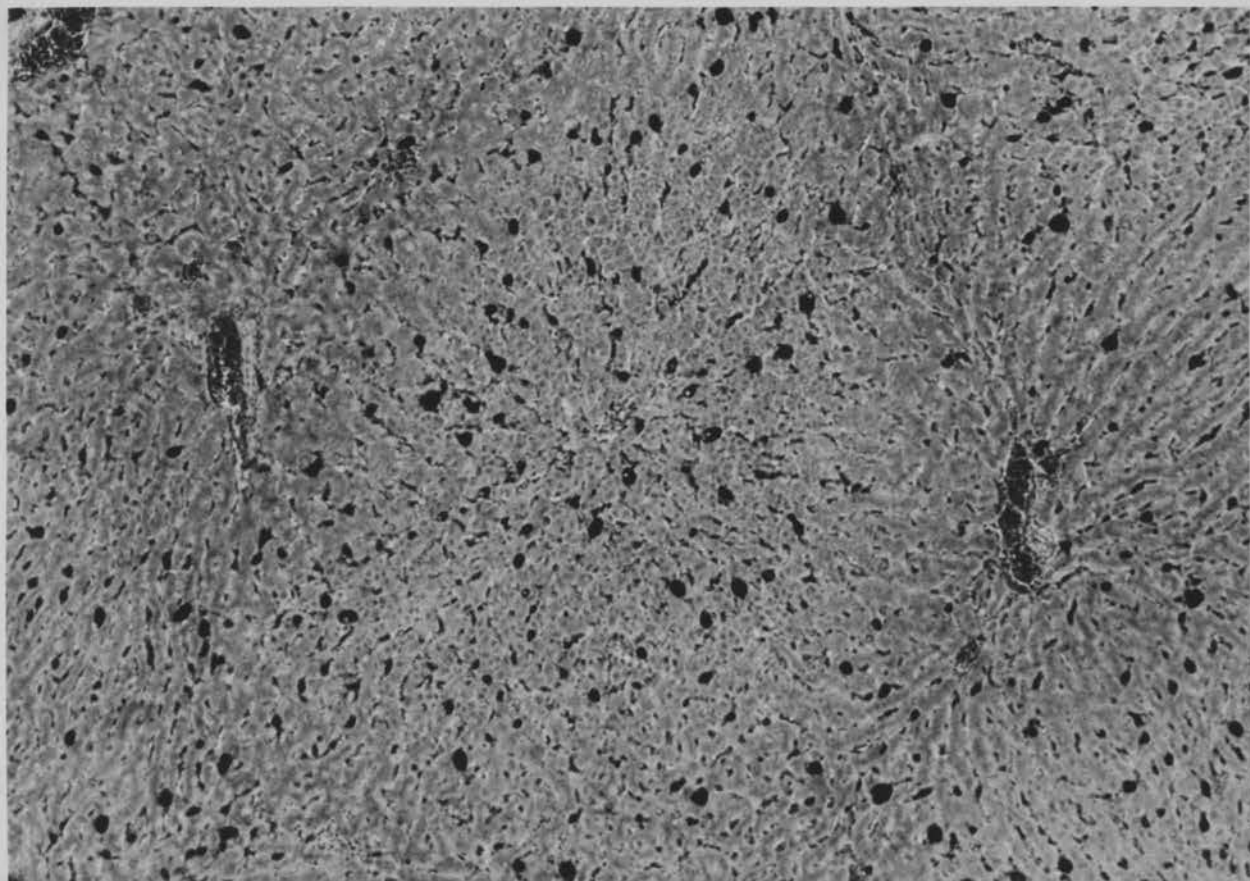


Figure 6. SECTION OF LIVER OF RABBIT #384. NOTE THE
IRON-POSITIVE GRANULES IN KUPFFER CELLS
THROUGHOUT THE HEPATIC LOBULES. IRON STAIN
LOW POWER.

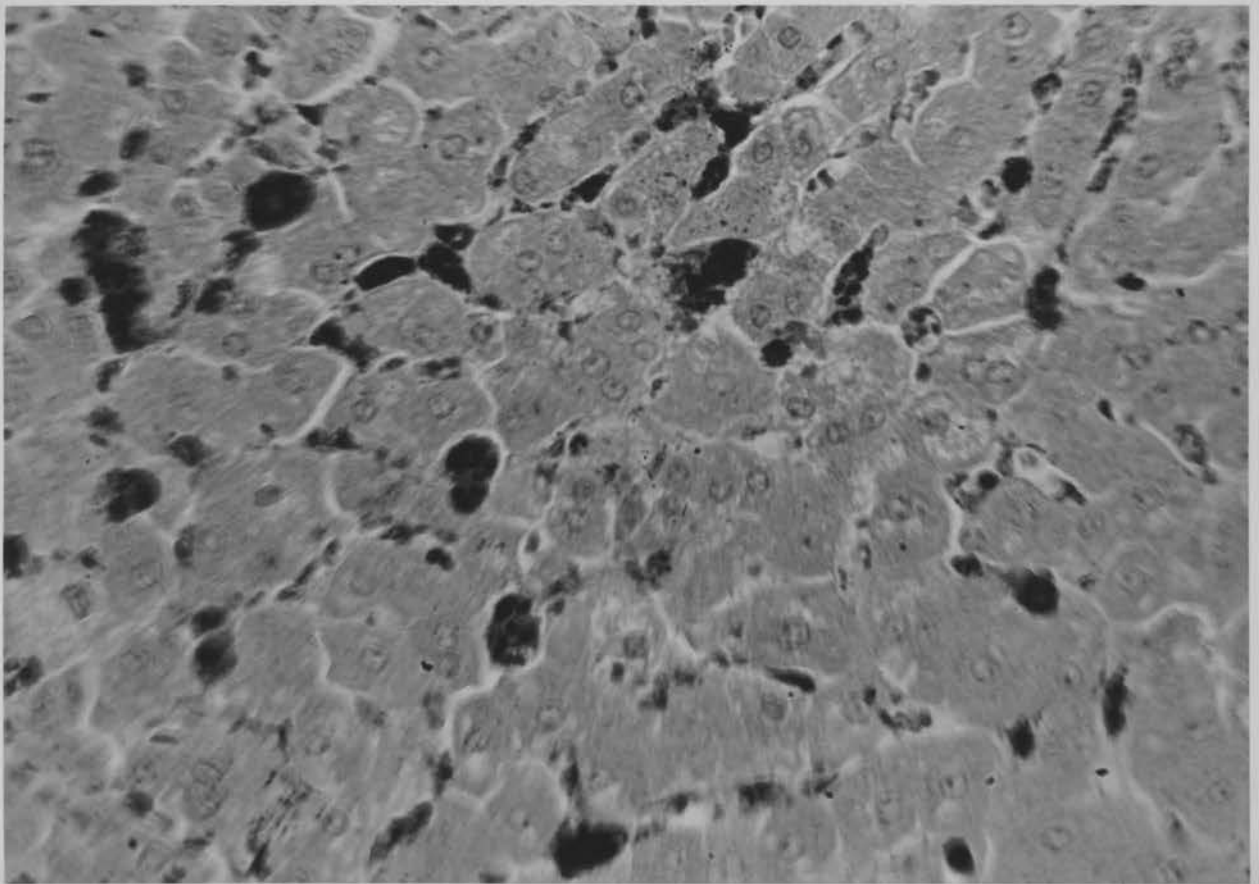


Figure 7. HIGHER MAGNIFICATION OF THE SAME SECTION OF
THE LIVER OF RABBIT #384 AS SHOWN IN FIG. 6.
IRON STAIN. HIGH POWER.

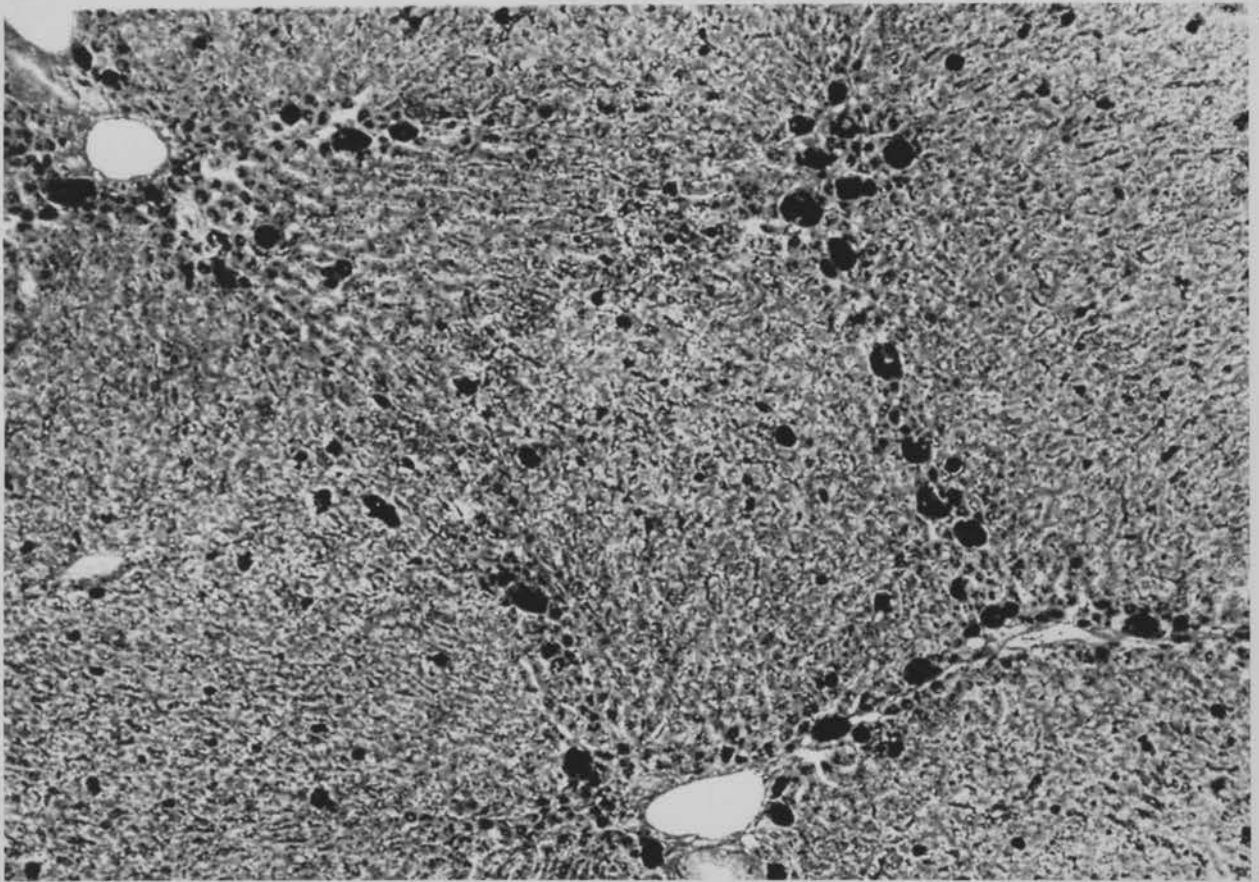


Figure 8. SECTION OF LIVER OF RABBIT #324. NOTE IRON-
POSITIVE GRANULES IN ALL HEPATIC CELLS WITH
GREATER AMOUNTS IN THE PERIportal HEPATIC
CELLS AS WELL AS IN KUPFFER CELLS. IRON
STAIN. LOW POWER.

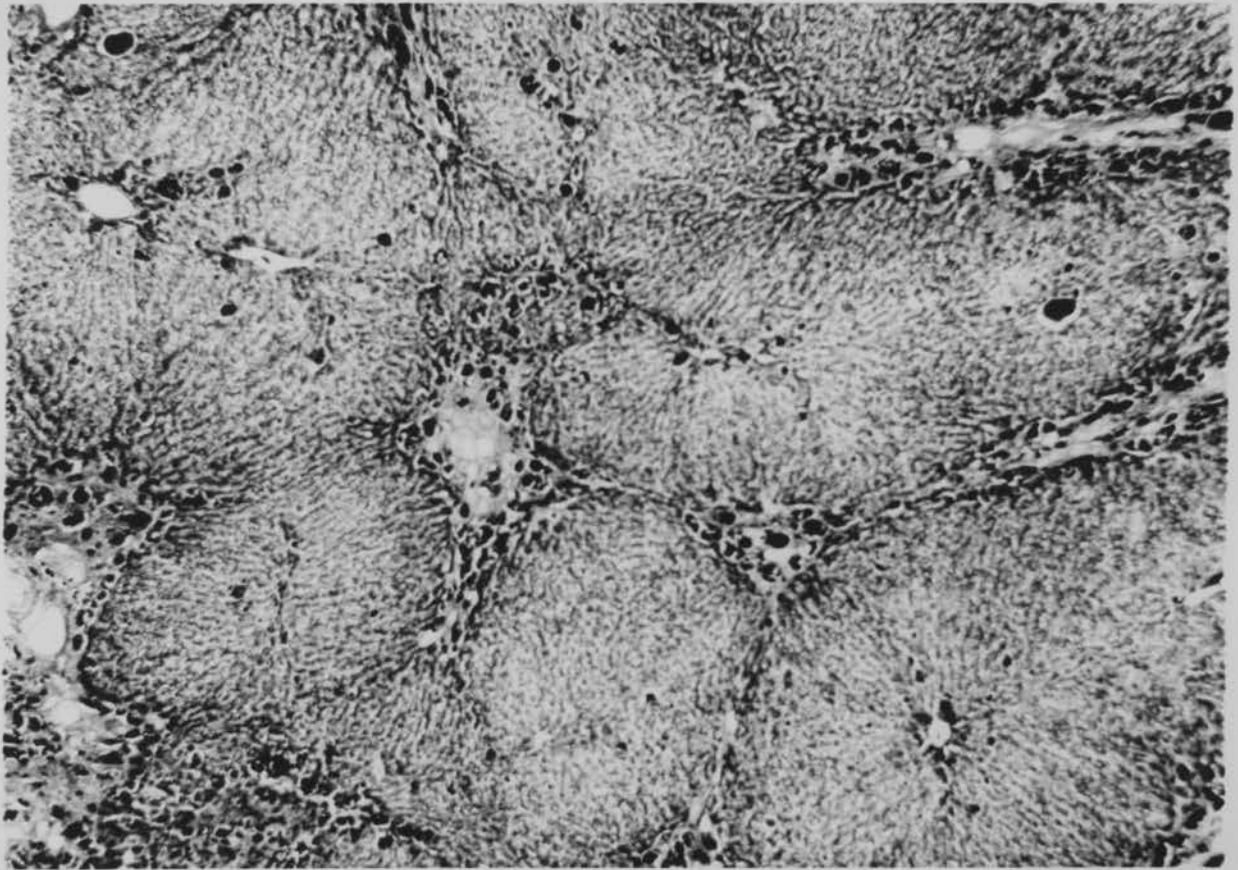


Figure 9. SECTION OF LIVER OF RABBIT #257. NOTE
IRON-POSITIVE GRANULES IN ALL THE HEPATIC
CELLS AS WELL AS IN THE KUPFFER CELLS AND
LARGE MASSES OF PIGMENT IN THE PERIPHERAL
ZONES OF THE HEPATIC LOBULES. THERE IS
ALSO SLIGHT DEGREE OF FIBROSIS IN THE
PERIportal AREAS. IRON STAIN. LOW POWER.

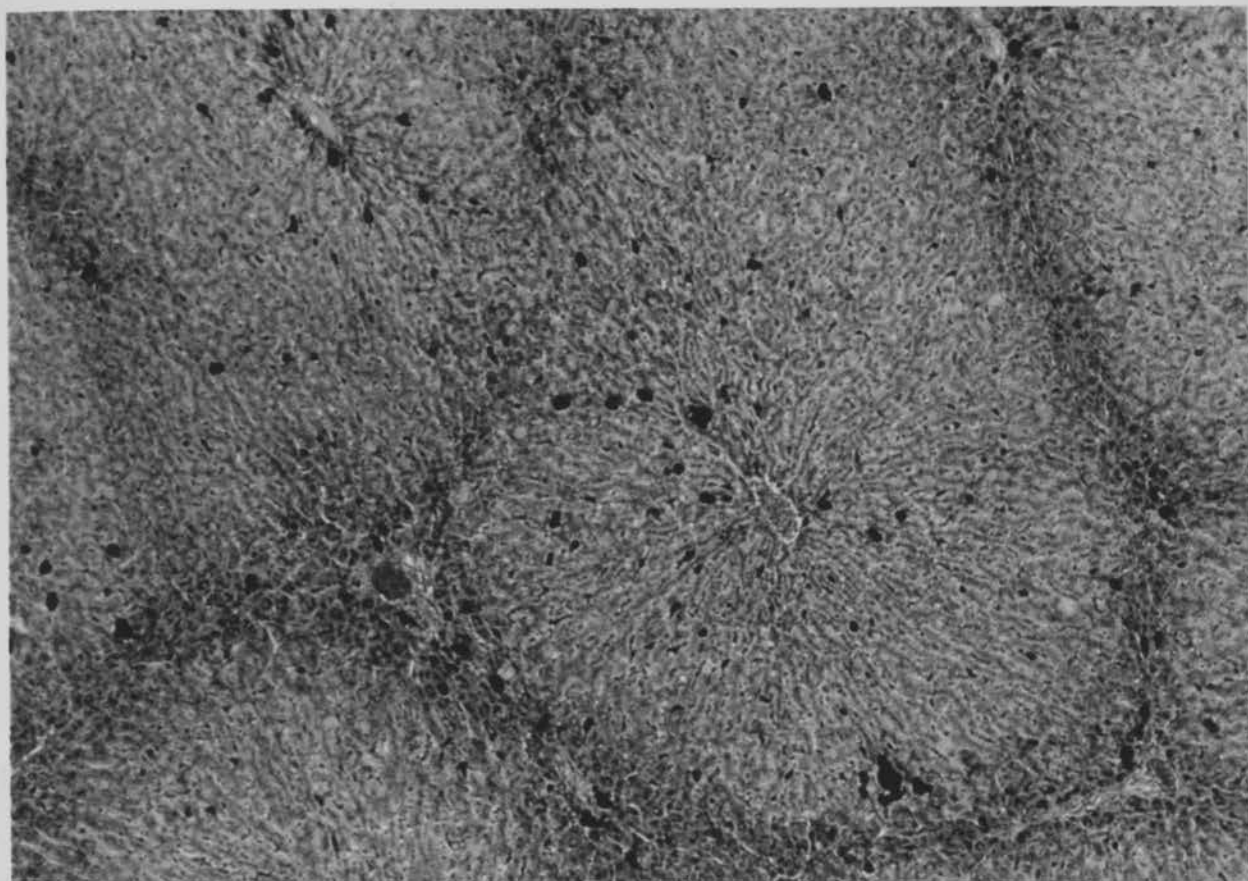


Figure 10. SECTION OF LIVER OF RABBIT #720. NOTE THE MARKED DEGREE OF HEPATIC PARENCHYMAL PIGMENTATION AND ACCUMULATION OF LARGE MASSES OF PIGMENT IN KUPFFER CELLS. IRON STAIN. LOW POWER.

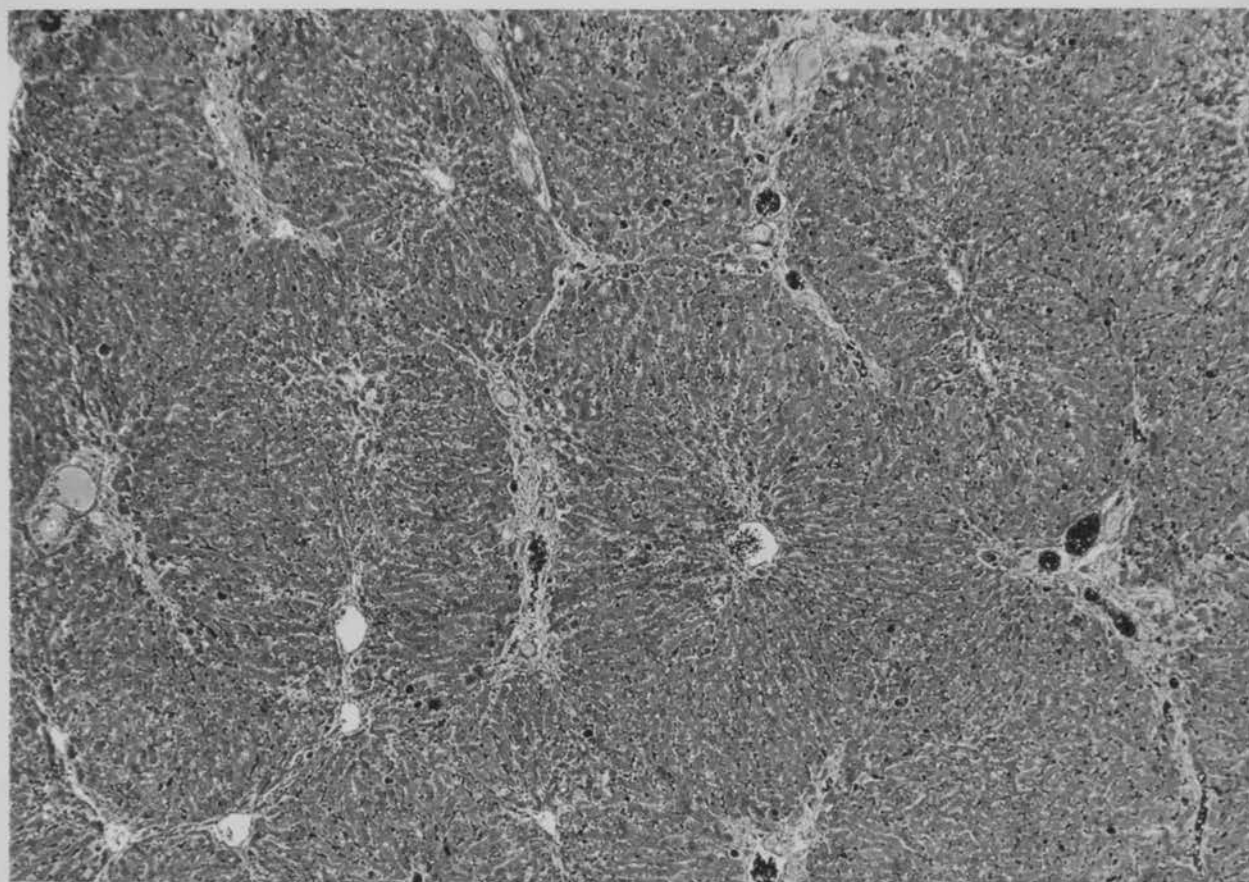


Figure 11. SECTION OF LIVER OF RABBIT #726. NOTE
VARIOUS DEGREES OF FIBROSIS IN THE PERI-
PORTAL REGIONS. ANILINE BLUE STAIN. LOW
POWER.

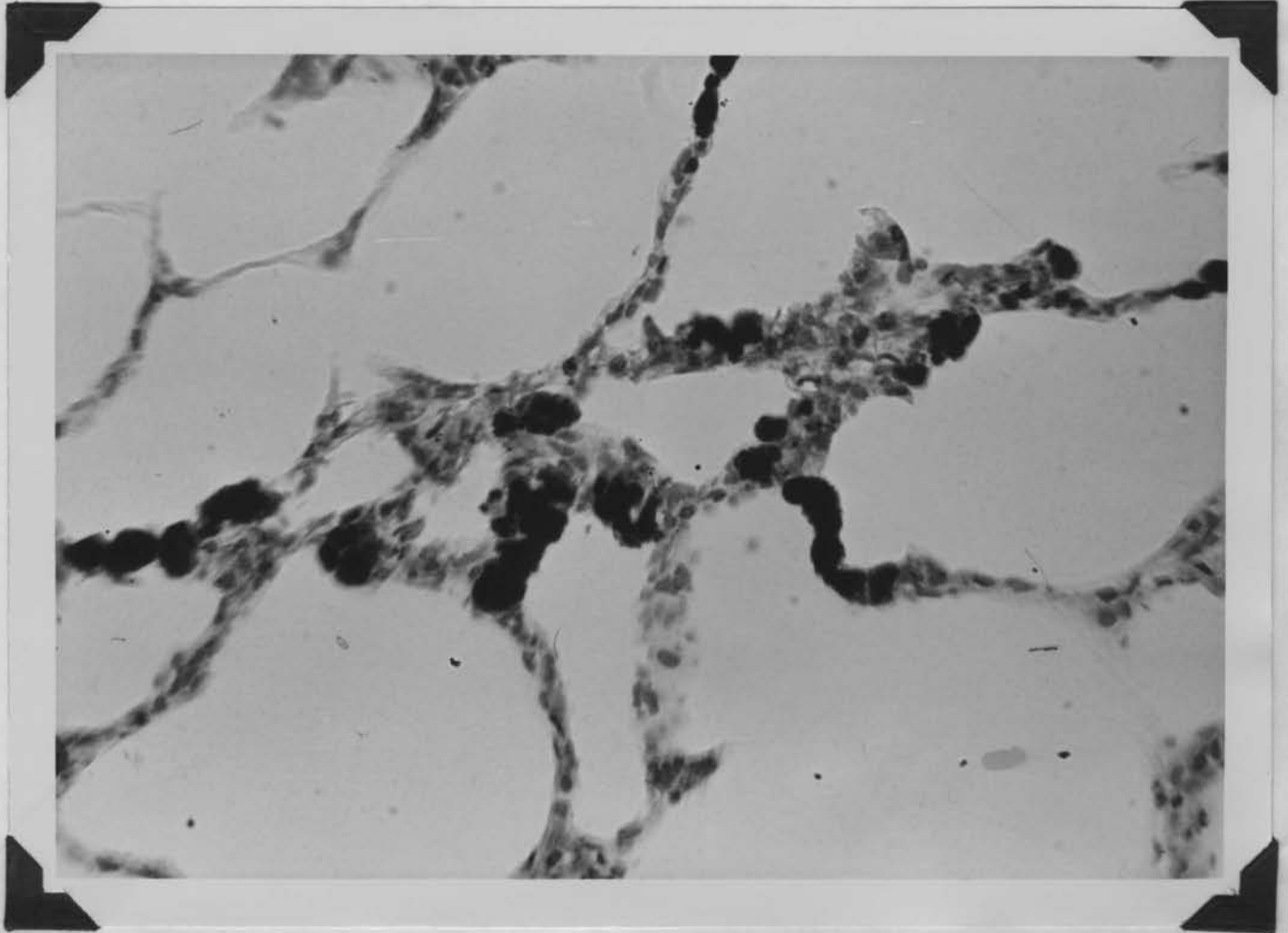


Figure 12. SECTION OF LUNG OF RABBIT #357. NOTE THE IRON-
POSITIVE GRANULES IN MACROPHAGES AND IN THE
ENDOTHELIAL LINING CELLS OF THE CAPILLARIES
BUT NO PIGMENT IN ANY OF THE ALVEOLAR
EPITHELIUM. IRON STAIN. LOW POWER.

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RESULTS OF PROLONGED INTRAVENOUS ADMINISTRATION OF IRON
TO NORMAL AND ANEMIC RABBITS

Abstract of a Dissertation

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The increasing incidence of transfusional hemochromatosis after repeated blood transfusions in anemic patients and the similarity of certain morphological changes between the transfusional and idiopathic hemochromatosis have suggested that the excessive storage of iron after transfusions might be the chief etiological factor of idiopathic hemochromatosis. However, the experimental evidence supporting such a possibility is still lacking. The purpose of this investigation has been to study the late morphological changes of organs of non-anemic and anemic rabbits after prolonged intravenous administration of saccharated iron oxide and to see whether prolonged administration of large amount of the iron will result in hemochromatosis.

A total of 103 young male rabbits and 9 young female rabbits weighing 3.5 to 5.0 kg., kept in individual cages and all fed with ordinary rabbit pellets, carrots, green vegetables and water ad libitum, were divided into two groups. The first group consisted of 58 non-anemic rabbits, 16 used as controls receiving no treatment and 42 treated with repeated intravenous injections of saccharated iron oxide (Feojectin-Smith, Kline and French Laboratories) with dosages gradually increasing from 20 to 200 mg. at intervals from 2 days to 2 months for a total period of 4 days to 14 months. The total dosages ranged from 45 to 4100 mg. of elemental iron per rabbit. The longer intervals of injections occurred in rabbits showing phlebothrombosis of the superficial veins that took time to recover so as to become available again for further injections. The second group consisted of 54 anemic rabbits rendered anemic by repeated bleeding from ear veins with hemoglobin maintained below 6.0 gm. per 100 c.c. Seventeen of them were used for

controls and 37 were injected with iron in a manner similar to that of the non-anemic injected rabbits for a period from 4 days to 12 months, with a total corrected dosage of iron varying from 25 to 4040 mg. The dosage was corrected for the iron lost through bleeding by subtraction of 50 mg. of iron for each 100 cc. of blood lost. The tissues of various organs of both groups were fixed in 10 per cent neutral formalin and then sectioned and prepared for microscopic examination with hemotoxylin and eosin stain and iron stain and aniline blue stains for liver in selected cases. The urines of rabbits having received a total of more than 3000 mg. of elemental iron and of the compatible controls were tested for sugar (Benedict qualitative method) and their bloods checked for level of glucose (modified Folin-Wu method).

In the control group of non-anemic rabbits none of the organs examined were grossly remarkable or showed the presence of iron-staining material microscopically. One rabbit, however, was found to have portal fibrosis with proliferation of bile ducts in liver. In the iron-injected group of non-anemic rabbits, the spleen and liver of those having received totally more than 1000 mg of elemental iron showed definite dark brown color and enlargement up to twice the normal size and weight. The livers of 2 rabbits receiving a total dosage of 3560 and 4100 mg. of iron respectively were brown, with irregular nodular surfaces and increase of consistency grossly. The bone marrow of most of the animals receiving a total of more than 2000 mg of iron was also brown. Microscopically, the spleen of every injected rabbit showed the heaviest amount of iron-positive pigment, mainly in the phagocytes in the red pulp. The pigment first appeared as fine, dust-like granules, then

gradually increased in size with increasing amounts of injected iron up to large irregular masses throughout the spleen except the Malpighian corpuscles. The connective-tissue cells in the capsule and the trabeculae and the endothelial lining of the capillaries and sinusoids all contained similar pigment toward the high dose of the injection. Extracellular pigment masses were also noted in red pulp after the tissue has been clogged with pigment macrophages. The normal architecture of the spleen was always well preserved. Fibrosis was never observed. The liver of rabbits that received less than 200 mg. of iron showed fine iron-positive pigment granules in all the Kupffer cells. With the increasing amount of iron injected, similar pigment granules gradually appeared in parenchymal cells in the central zones of lobules, then in all the parenchymal cells but still more in central zones, and finally there were marked large pigment masses in parenchymal cells in the peripheral and central zones and a lesser amount in the intermediate zones. At the later stage with more iron injected the connective-tissue cells in the portal spaces and the endothelial cells of central veins also showed iron-positive pigment granules, but the epithelial cells of the bile ducts never did so. In addition to the hemosiderosis, 20 injected normal rabbits showed in the portal spaces various degrees of fibrosis, with proliferation of fibroblasts and small amounts of scattered iron-positive granules. The normal architecture of the liver was distorted but much less than that in the few control rabbits with cirrhosis. Less pigment was noted in macrophages in the reticular stroma of bone marrow of every injected rabbit, in sinuses and in connective-tissue cells of capsules of the mesenteric lymph nodes of approximately two thirds of the injected rabbits

and in macrophages within the alveoli and in the endothelial lining of the capillaries and larger blood vessels of the lung in all the rabbits that received a total of more than 150 mg. of iron. The adrenal glands showed the pigment in reticular stroma cells of the cortex and the medullary sinuses of all the injected rabbits. The kidney presented moderate amounts of fine granules of the pigment in glomerular tufts and tubular epithelium of the ascending limb of Henle's loops and collecting tubules. The pituitary and parathyroid both showed small pigment granules in their stroma cells in rabbits having received comparatively large doses of iron. The skin showed pigment in the hair follicles and corium of some rabbits that received more than 700 mg. of iron. The amount of pigment identified in ovary, testes, pancreas, thyroid, heart and gastrointestinal tract was small and usually in rabbits that received more iron. Fibrosis of the pancreas was not noted in any rabbit.

In the control group of the anemic rabbits none of the organs were grossly remarkable except the liver of one rabbit, which showed slight nodularity and increase of consistency. Microscopically, none of the tissue examined contained iron-staining material. Two rabbits showed marked portal fibrosis, with proliferation of bile ducts and interruption of normal hepatic architecture. In the iron-injected group of anemic rabbits the findings were similar to those of the iron-injected normal rabbits, with no apparent qualitative difference. However, none disclosed gross nodularity of the liver. Microscopically, 8 rabbits also showed in the portal spaces various degrees of fibrosis with proliferation of bile ducts and small amounts of scattered iron-positive pigment granules.

Blood sugar levels of the rabbits having received more than 3000 mg.

of iron were in the same range as those of normal control rabbits.

Glycosuria was never demonstrated in any of the experimental animals tested.

This investigation was able to demonstrate the pattern of progressive iron-overloading - loading of iron in the reticuloendothelial system during the early stage followed by loading to lesser degrees in parenchymal tissue during later courses of iron injection. Storage of iron in the two major tissues, especially in the reticuloendothelial system of the liver, spleen and bone marrow, is stressed.

This investigation offers suggestive evidence that prolonged, intense administration of iron may result in progress from hemosiderosis to pigment fibrosis. The higher incidence and the milder degree of portal fibrosis in experimental rabbits than in incidental cirrhosis of control rabbits and the scanty pigment in the increased portal fibrous tissue of experimental animals suggest that the portal fibrosis is not pre-existing. This portal fibrosis was in all cases associated with intense deposits of iron pigment in the peripheral zones of the hepatic lobule. The hypotheses advanced by various authors regarding the hemosiderosis progressing to pigment cirrhosis are discussed. There was no clear evidence relating the occurrence of fibrosis to either the total amount of iron administered or the duration of the iron deposit. The failure of previous animal experiments to produce hepatic fibrosis by overloading of iron is probably due either to insufficient amount of iron as evidenced by the largest total iron dose of 1025 per kilogram of body weight in this investigation in comparison to 550 mg in the literature or the rate of administration may have been too slow. The largest total dose of 4.1 gm. for a rabbit weighing 4.0 kg. in this investigation is comparable to 72 gm. of iron

in a human body weighing 70 kg. and is equivalent to 288 transfusions of blood to a human body.

In this investigation the anemia caused by bleeding did not appear to favor the development of hepatic fibrosis. This may be explained on the basis of correction of the anemia within a short time by iron injections and the consequent lack of prolonged tissue anoxia and local nutritional deficit for initiation of the hepatic fibrosis if tissue anoxia does initiate the damaging effect of iron pigment.

In conclusion, this investigation has demonstrated that 8 of the 37 iron-injected anemic rabbits and 20 of the 42 iron-injected non-anemic rabbits showed various degrees of portal fibrosis in the liver. Also given in the presentation is a comprehensive review of the literature on iron metabolism, exogenous hemochromatosis and idiopathic hemochromatosis.

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