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IRON OVERLOADING AND HEPATIC VULNERABILITY

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We adhere firmly to the conclusion, reached by Sheldon [1] in 1935, that tissue siderosis is not the cause of hemochromatosis but that this syndrome evolves from an inborn error of metabolism, both the tissue changes and the siderosis being directly attributable to this defect.

One experimental approach to this problem is the investigation of conditions leading to increased uptake of dietary iron. By this means, however, the degree of tissue siderosis which can be induced in the lifetime of a laboratory animal is limited. Furthermore, the addition of iron salts to the diet in large amounts brings about unsuspected complications [2] which may completely vitiate the conclusions drawn from such experiments. We have therefore investigated in the adult rat the coexistence of massive parenteral iron overload with a condition which predisposes to excessive iron absorption and the development of tissue siderosis. For this purpose a suitable choice is the antimetabolite DL-ethionine.[3] We have investigated the effect of prolonged oral administration of this agent at a low concentration in the diet concurrently with, and at varying periods after, gross overloading of the rat with parenteral iron. Bearing in mind the predominance of hemochromatosis in males and the often extreme degree of hypogonadism present, a study has also been made of the effects of gonadectomy. Finally, the effect of a diet low in protein and lacking vitamin E has been investigated.

These investigations have reaffirmed our previous view that severe iron overload *per se* does not appear to induce tissue damage. However, our further work now leads to the conclusion that a liver loaded with excessive quantities of iron is vulnerable to the action of toxic agents or deficient diets to a far greater degree than is the case with the normal liver.

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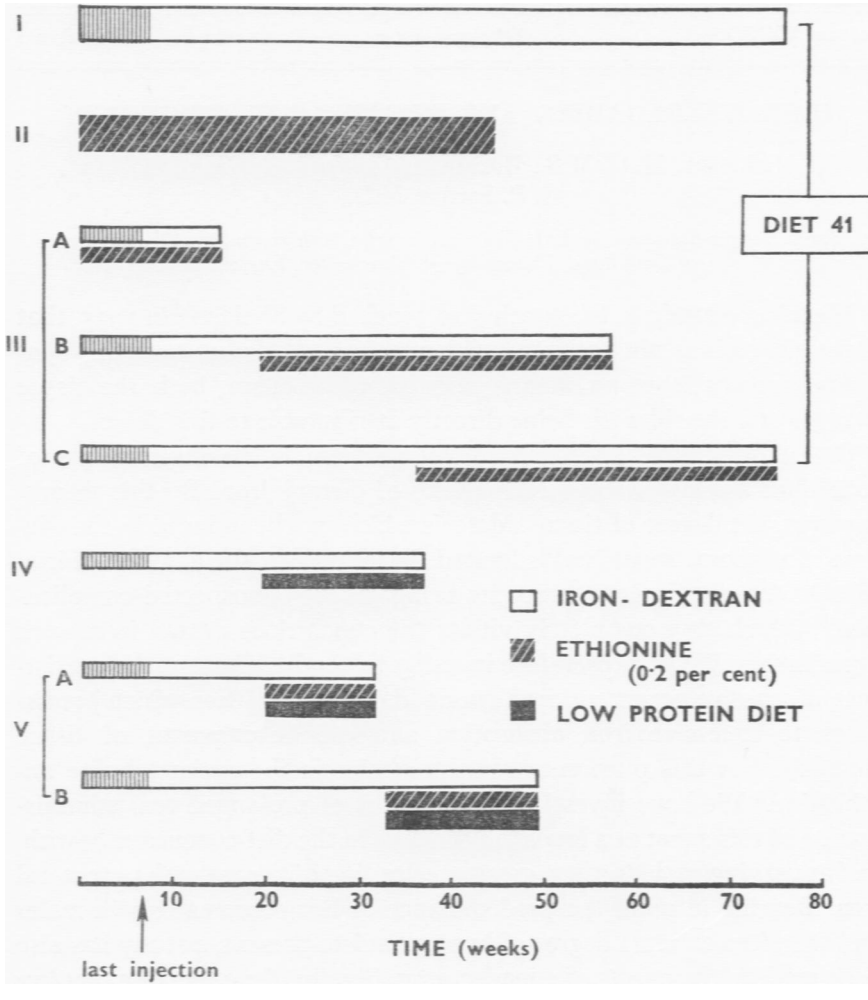
Material and Methods

Young adult albino rats of both sexes, weighing about 150 gm. (range, 135 to 180 gm. for males, and 115 to 160 gm. for females) were used. Twenty of the male and 54 of the female rats had been subjected to gonadectomy shortly after weaning. The experimental groups, diets and



Text-figure 1. Details of experimental groups, showing the nature and duration of treatments.

duration of treatment are summarized schematically in Text-figure 1. Of the 187 animals finally used, those included in the various groups were as follows (listed in the order: males, females, castrate males and females): Group I: 19, 21, 5, 15; Group II: 8, 7, 0, 3; Group III: 15, 17, 6, 20; Group IV: 5, 6, 4, 6; Group V: 7, 9, 5, 10.

The basal diet was Oxo 41.[4] When DL-ethionine was used, it was applied in solution to the pelleted diet to a final concentration of 0.2 per cent. The low protein diet had the following percentage composition: alcohol-extracted casein (Genatosan), 8; lard, 20; maize starch, 40; sucrose, 21; powdered cellulose, 5; salt mixture (U.S.P. XV, p. 883), 5; vitamin mixture, 0.5; choline chloride, 0.2. Each 5 gm. of the vitamin mixture contained the following: 0.05 gm. each of thiamine, riboflavin, pyridoxine hydrochloride, calcium pantothenate and ascorbic acid; 0.5 mg. each of folic acid and biotin; menaphthone, 0.01 gm.; nicotinic acid, 0.08 gm.; inositol, 0.15 gm.; and *p*-aminobenzoic acid, 0.35 gm.; sucrose to a total weight of 5 gm. Vitamins A and D (3,500 and 300 I.U. per 100 gm. of diet) were added separately in concentrated form immediately before use. It should be noted that no vitamin E was incorporated in the low protein diet.

The routine of iron overloading was the same as that described previously, consisting of 22 doses of 75 mg. of iron per kg. of body weight, administered intramuscularly into alternate hind limbs 3 times weekly.[2,4]

The animals were weighed once a week and finally killed with ether. The necropsy and histologic procedures have been described.[2,4]

RESULTS

Body Weight and Other Changes

The overall changes in body weight in relation to the initial weights are shown for each group in Text-figure 2. Some groups gained weight steadily, some gained initially but then lost weight, while others lost weight consistently. Loss of weight was for the most part gradual, followed by a sudden decrease which led to the sacrifice or death of the rat.

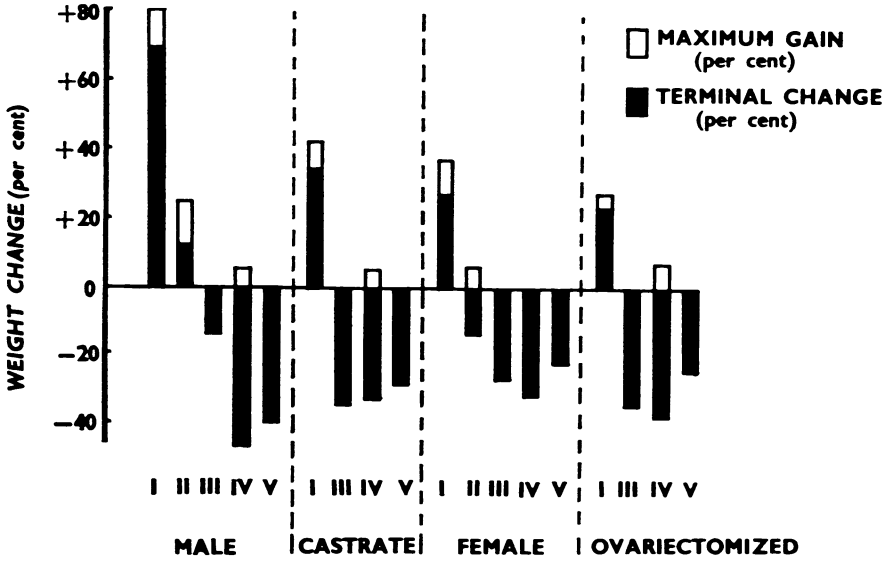
With ethionine (group II) there was inhibition of growth, particularly in females, but on concurrent iron loading (group III) weight loss also set in. By the time the animals were sacrificed they showed thinning of hair, perineal staining and the general unthrifty appearance characteristic of ethionine treatment. Surprisingly, several rats of groups II and III showed some of the alterations attributable to lack of vitamin E, including loss of pigmentation of the incisors with greatly shortened maxillary incisors, paresis of the hindquarters, ataxia and testicular atrophy as well as histologic changes described below.

Low protein diets which induce methionine deficiency rapidly lead to anorexia,[5] and ours was no exception. On the other hand, Kinney, Kaufman and Klavins[3] have shown that such reduction in food intake does not of itself influence the amount of iron in the liver. The deliberate exclusion of added vitamin E from the low protein diet rendered the rats liable to massive hepatic necrosis[6] but was considered necessary in order

to give free play to the "pro-oxidant" action of iron. In fact, at necropsy the animals of group IV and particularly those given ethionine as well (group V) displayed features suggestive of vitamin E deficiency.

Lesions in the Liver

These comprised iron and ceroid pigments in varying amount and distribution, fatty changes and necrosis of parenchymal cells, and the

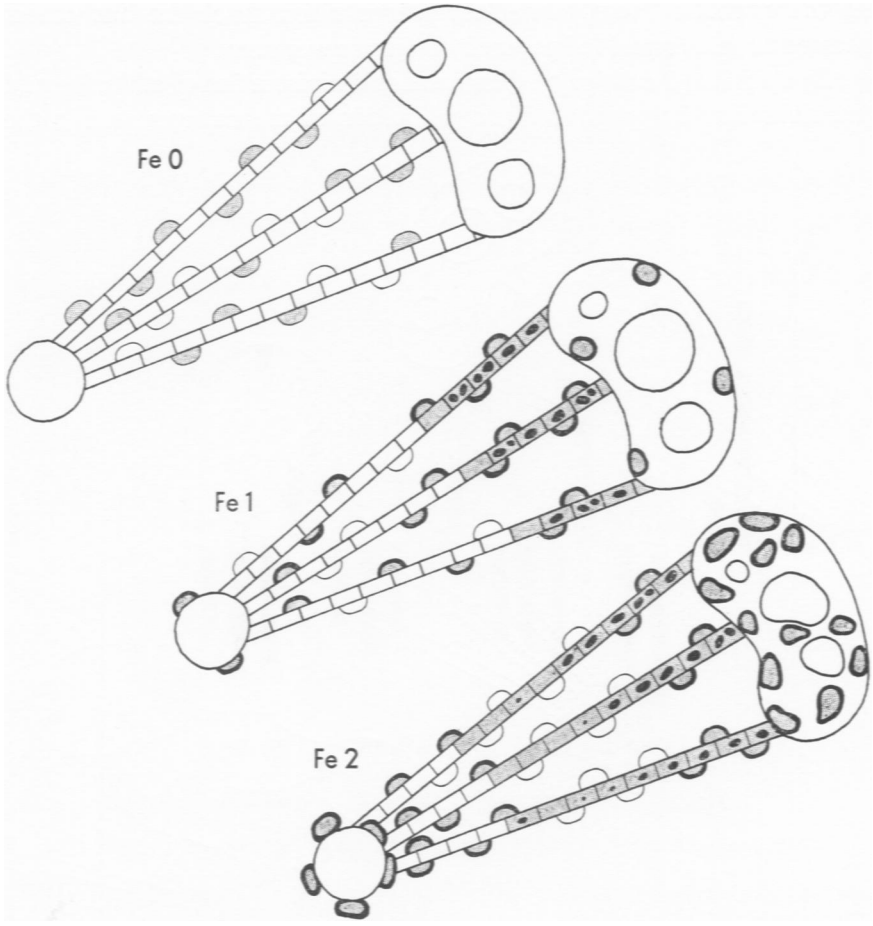


TEXT-FIGURE 2. Average weight changes in the different experimental groups.

eventual development of cirrhosis. The sequence in each experimental group has been divided into stages as follows.

Group I (*Text-fig. 3*). During the injection period and for some 15 weeks afterwards iron was found only in the Kupffer cells, many of which were enlarged (Stage Feo; Fig. 1). During following weeks a slow migration of Kupffer cells toward the portal tracts and central veins led to partial clearing of the sinusoids and the aggregation of small clusters of iron-laden cells in the portal tracts and around the veins. At the same time a little iron appeared in the periportal parenchyma and small amounts of ceroid polymer were found in many iron-laden cells (Stage Fe1; Fig. 2). A year or more after the injections, a more advanced development of these changes (Stage Fe2; Fig. 3) was seen. At no time were there significant fatty changes, and up to 80 weeks no signs of either necrosis or cirrhosis appeared.

Group II (*Text-fig. 4*). During the first months (Stage E1; Fig. 4) the livers of ethionine-fed rats showed some disintegration of parenchymal cells about the centrilobular veins and accumulation around



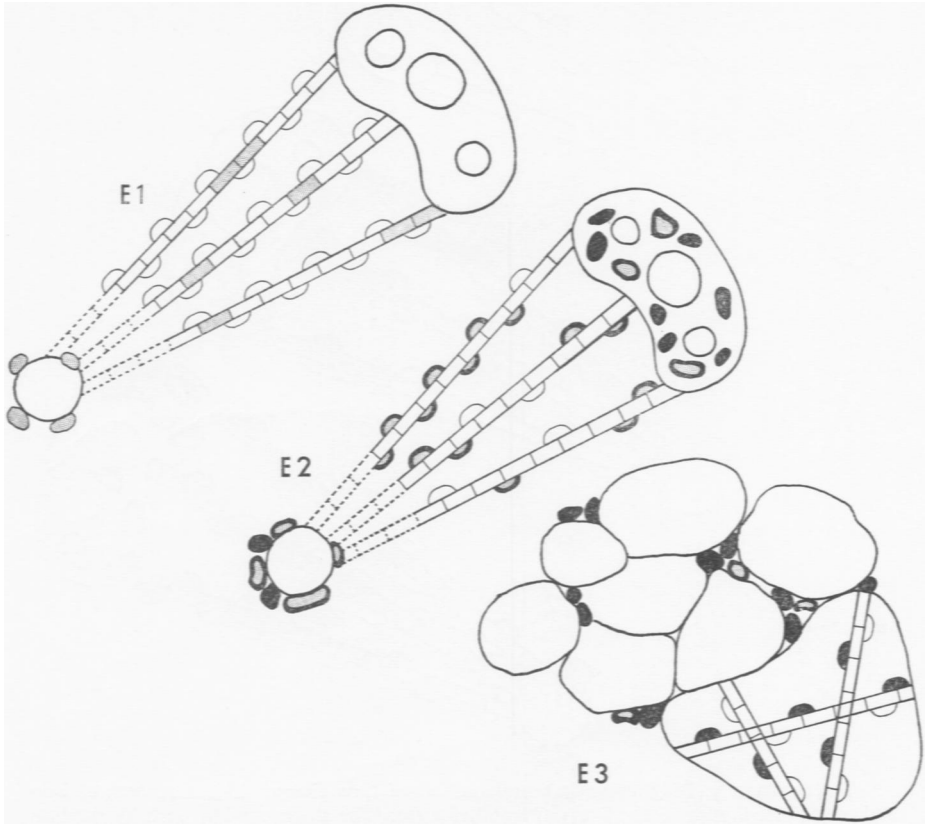
TEXT-FIGURE 3. Diagrammatic representation of time changes in the pattern of iron and ceroid distribution in the livers of iron-laden rats. The stages are indicated by symbols whose full significance is explained in the text. Stippled areas represent iron; solid black areas indicate ceroid.

these veins of iron-containing macrophages. A little iron was seen in some parenchymal cells, but there was no ceroid. At 16 weeks (Stage E_2; Fig. 5) much more iron was found in Kupffer cells and in siderophages around portal tracts and central veins; more striking was the presence in these sites of many large ceroid-laden macrophages. It is notable that there was no cirrhosis. All animals surviving 23 weeks showed well-developed cirrhosis (Stage E_3; Fig. 6) with small numbers of iron- and ceroid-containing cells in the fibrous septums and ceroid in the Kupffer cells of the new parenchyma. In all these livers there were nodules of bile duct proliferation characteristic of ethionine feeding.

Small amounts of fat, mainly as small droplets, appeared early in these livers, and the amount increased with time though it never became heavy.

In the cirrhotic livers, some lobules contained much fat but others showed none. Necrosis of parenchyma was never seen.

Group IIIA (*Text-fig. 5*). Concurrent administration of ethionine and intramuscular iron produced a combination of the changes seen in the



TEXT-FIGURE 4. Time changes in the pattern of iron and ceroid distribution, and the development of cirrhosis in rats given ethionine.

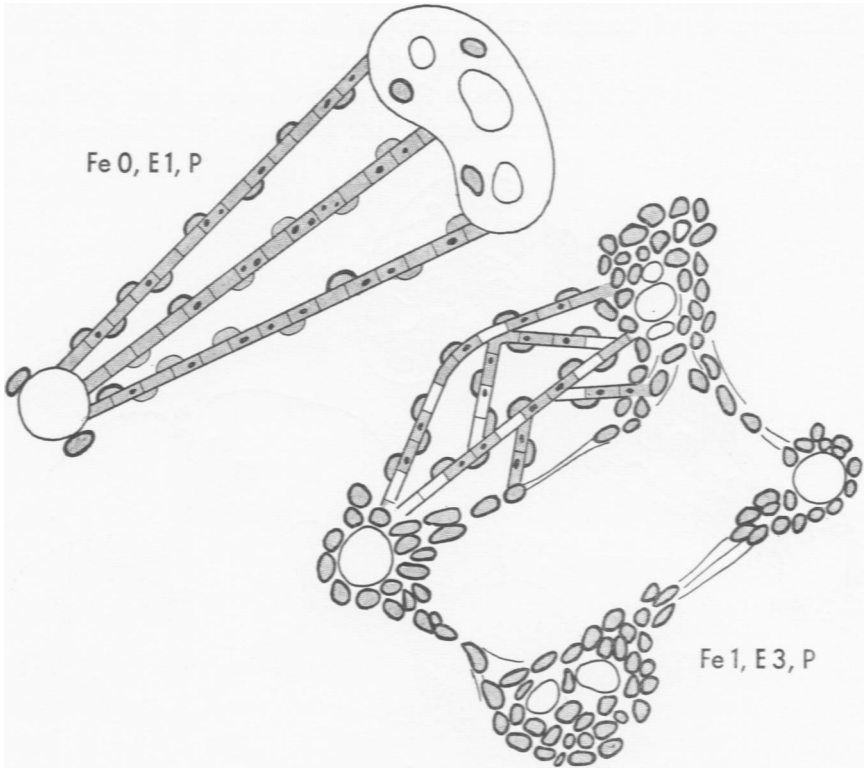
early stages when each was administered separately (Feo and E1). An additional effect was a very heavy, diffuse deposition of iron throughout the hepatic parenchyma represented hereafter by the symbol P. Small to moderate amounts of ceroid were found in most iron-containing cells. The overall picture may be summarized as Stage Feo, E1, P (Fig. 7).

Between 11 and 15 weeks from the beginning of the experiment, early cirrhotic changes were characterized by fine condensations of new reticulin fibers between portal tracts and central veins. Rapid clearing of Kupffer cells from the hepatic sinusoids was followed by their aggregation in large masses around the portal tracts. There was early disorganization of the pattern of the parenchyma: some cells, presumably the original cells, were full of iron, whereas others, presumably newly

formed parenchymal cells, contained no iron. This stage we have represented by the symbols Fe1, E3, P (Fig. 8), though the aggregates of iron were larger than those developing in animals treated with iron alone.

Fatty changes in the parenchyma were similar to those seen in animals of group II.

Group IIIB (*Text-fig. 6*). There was essentially no significant difference between the two separate subgroups with intervals of 12 and 29 weeks between iron-loading and feeding with ethionine. The first stage, 19 to 37 weeks after the first injection and 7 to 18 weeks on the diet, showed heavy centrilobular and periportal aggregations of macrophages full of iron and ceroid pigments. Similar pigments were found in most of



TEXT-FIGURE 5. Time changes in the pattern of iron and ceroid distribution, and the development of cirrhosis in rats treated concurrently with iron and ethionine.

the Kupffer cells and diffusely throughout the parenchyma (Fe2, E2, P; Fig. 9).

Some 15 to 20 weeks later an advanced cirrhosis (E4) was present, with very heavy siderophage aggregates—much heavier than could be accounted for by iron loading alone—in the stroma around the new liver lobules. In the new lobules, there was a little iron but no ceroid in many

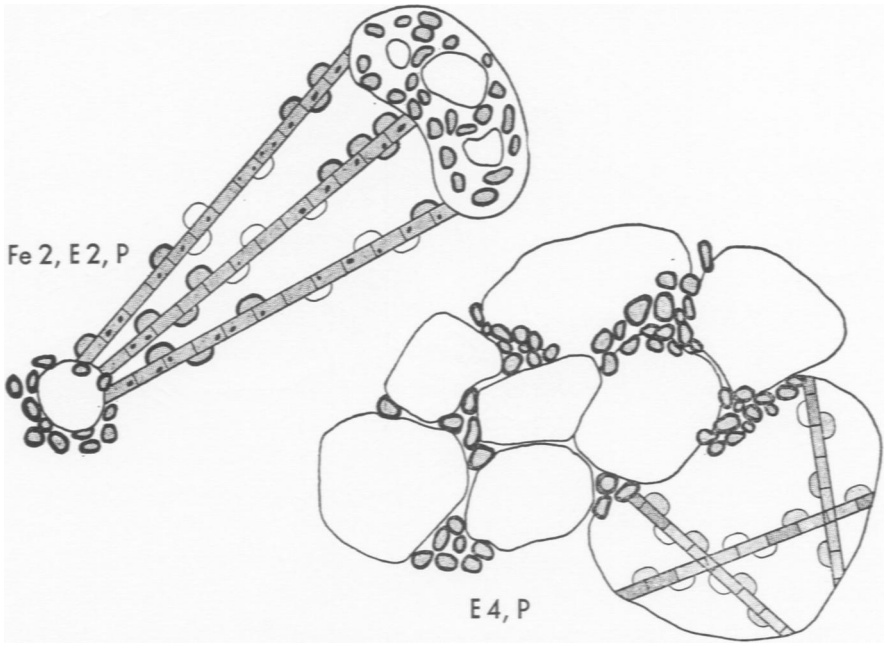
of the parenchymal and Kupffer cells (E_4, P; Fig. 10).

Fatty changes in the liver were similar to those in previous groups; in the early precirrhotic stages there was a scanty, very variable random scatter of fat; where cirrhosis was present, fat was heavy in some lobules and absent from others.

Group IV (*Text-fig. 7*). All the animals in this group died or had to be killed after only 9 to 18 weeks on the diet. All showed cirrhosis, varying from an early to an advanced lesion, according to the duration of the low protein diet. Very large aggregates of iron- and ceroid-containing macrophages accumulated in the interlobular stroma. A little iron and ceroid were present in some Kupffer cells of the new lobules, but there was no pigment in any parenchymal cells (E_4; Fig. 11).

There was much necrosis and atrophy of the livers of these animals and heavy fatty changes persisted throughout.

Group V (*Text-fig. 8*). Lesions in this group were the most rapid and severe. Within 6 weeks of commencing the low protein-ethionine diet,

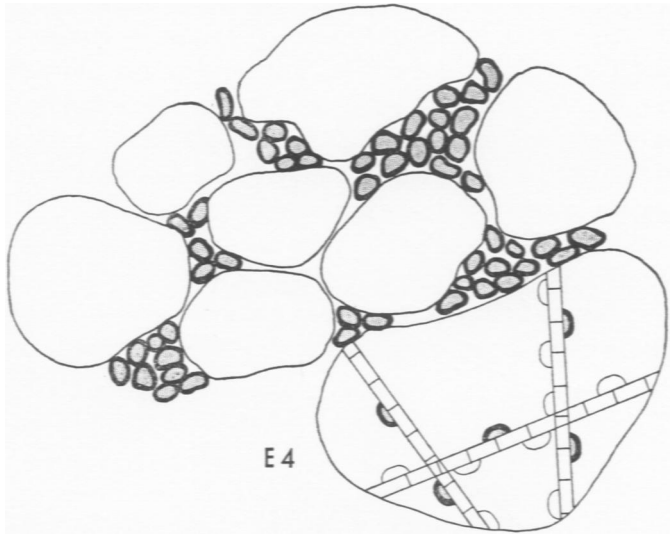


TEXT-FIGURE 6. Time changes in the pattern of iron and ceroid distribution, and the development of cirrhosis in rats treated with iron followed by ethionine after an interval of 20 weeks.

there were large central and periportal accumulations of siderophages and diffuse parenchymal iron, the appearances being similar to the early stage observed in group IIIB but more rapidly produced (Fe_2, E_2, P). In the next 6 weeks, during which all the animals were sacrificed, a more

advanced cirrhosis developed than was seen in previous groups; the newly formed parenchyma and Kupffer cells contained much iron and ceroid (E4, P and E5, P; Fig. 12).

Severe fatty changes and often extensive parenchymal necrosis were seen in most animals of this group.



TEXT-FIGURE 7. The pattern of iron and ceroid distribution, and cirrhosis in iron-laden rats placed on a low protein diet after an interval of 20 weeks.

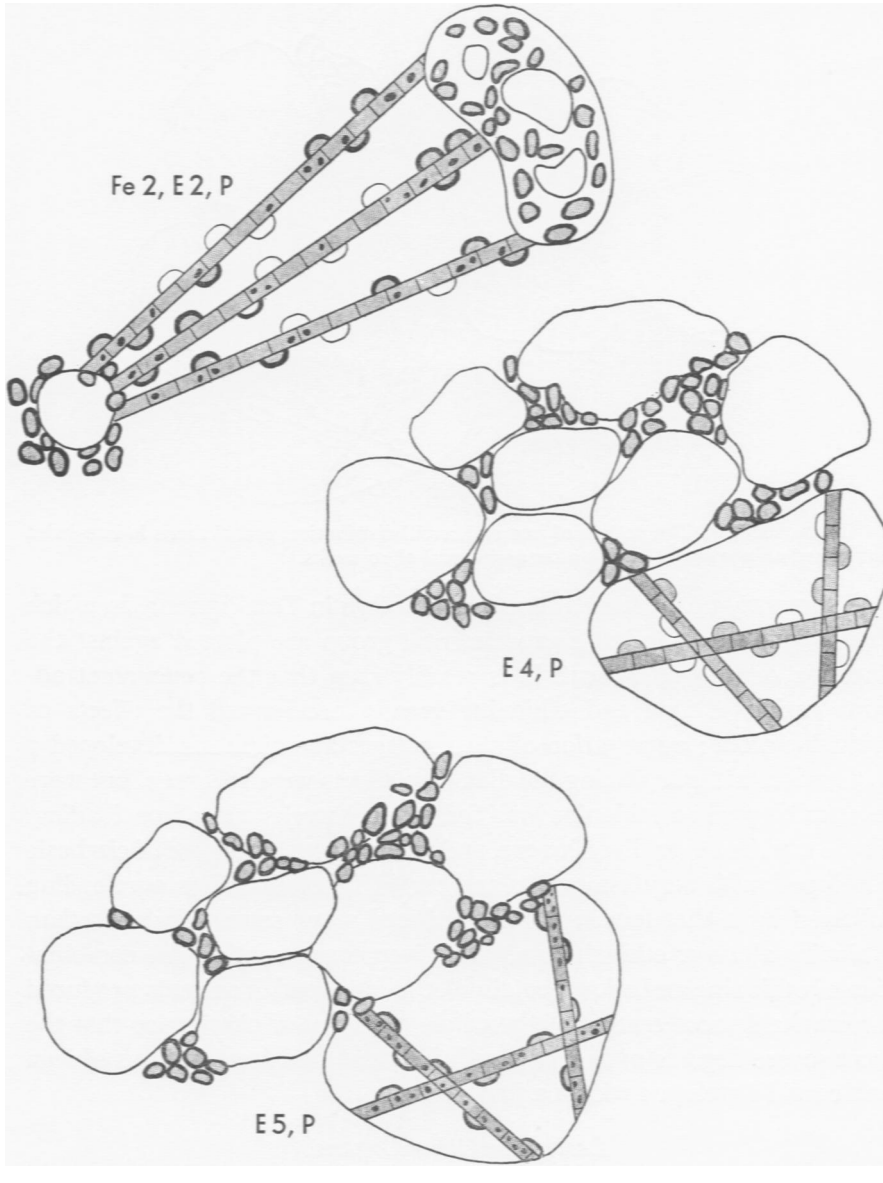
A summary of the liver alterations is shown in Text-figure 9, in which the various stages in each experimental group are plotted against the duration of the experiment. It is readily seen that the concurrent administration of iron and ethionine greatly accelerated the effects of ethionine alone; aggregation of siderophages and cirrhosis developed 7 to 12 weeks after beginning the diet. The effects were still seen, but were less striking when ethionine was fed several weeks after iron loading. These rats, however, lived longer, and an even greater degree of cirrhosis developed, with very heavy iron and ceroid deposition. Iron overloading followed by a diet low in protein induced more severe cirrhosis than ethionine alone or ethionine and iron given concurrently. The combination of ethionine and a low protein diet in iron-loaded animals produced a remarkable acceleration of the whole sequence of changes so that the most severe degrees of cirrhosis and the heaviest concentrations of iron and ceroid developed within a few weeks.

Lesions in Other Organs

The observations in organs other than the liver are summarized in Text-figure 10, in which an attempt has been made to show the relation-

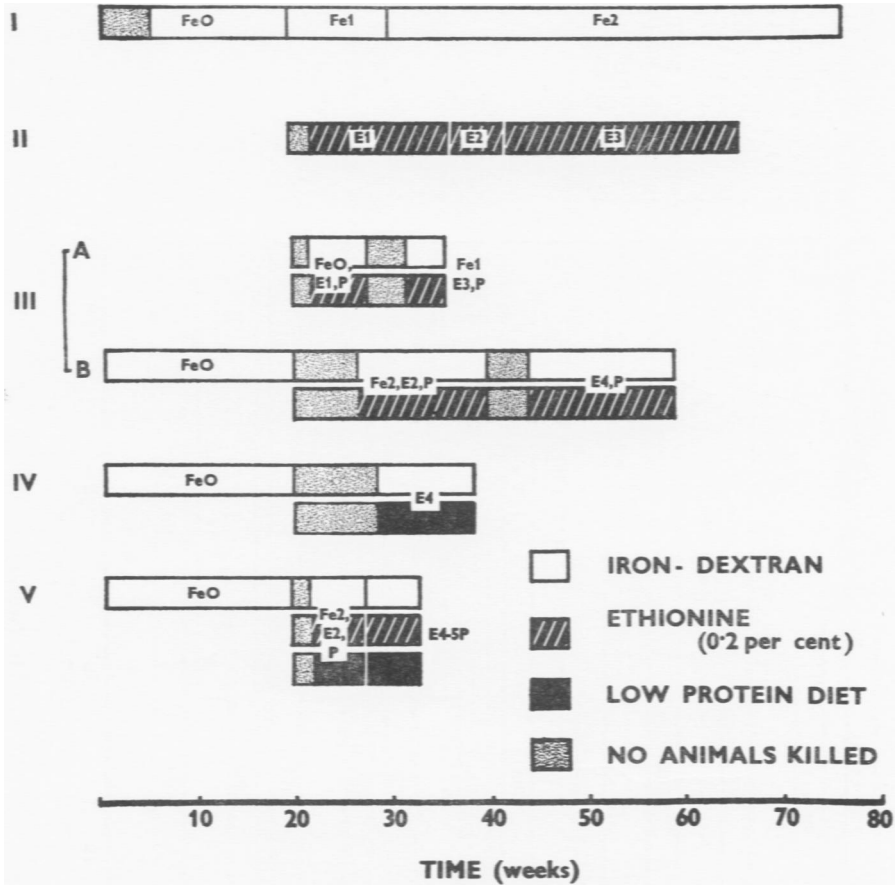
ship between the times of onset of some of the principal pathologic changes. Once again, because of the close similarity in groups IIIB and IIIC, and groups VA and VB, only the results for the two comparable groups, IIIB and VA are shown.

Pancreatic atrophy was induced by ethionine but was absent in iron-



TEXT-FIGURE 8. Time changes in the pattern of iron and ceroid distribution, and the development of cirrhosis in iron-laden rats placed on a low protein and ethionine diet after an interval of 20 weeks.

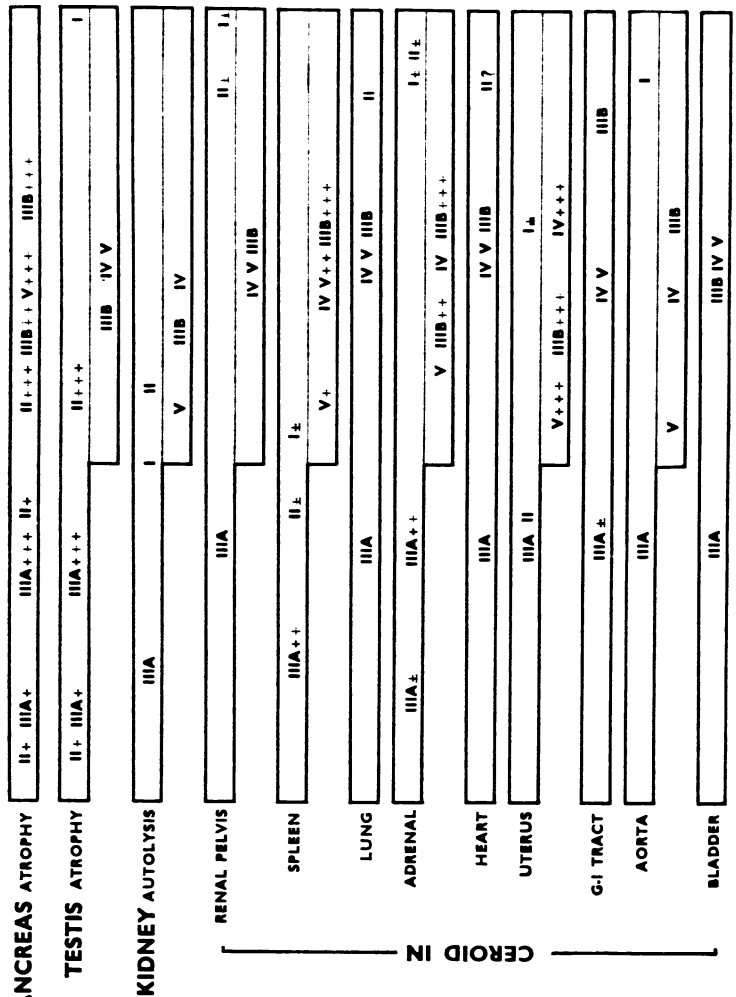
loaded rats on the basal or the low protein diets. Concurrent iron loading and ethionine (group IIIA) greatly hastened the speed with which atrophy developed, but this was not the case when a substantial interval (L-T) separated the start of the two treatments. The absence of pancreatic damage in the low protein group IV is not in keeping with the



TEXT-FIGURE 9. Summary of liver changes in the different experimental groups (groups IIIB and VA have been amalgamated with groups IIIC and VB, respectively).

statement by Kaufman, Klavins and Kinney [7] that "diets low in protein with or without high fat content produce pancreatic damage." In fact, our diet was similar to that used by György and Goldblatt,[8] who reported no abnormality in the pancreas. The lard content, 20 per cent, was much below that used by Kaufman and co-workers (60 per cent).

Testicular atrophy was infrequent in iron-loaded rats and was not seen at all in the males of group I (the time of 50 weeks shown in Text-figure 10 is based on previous results). With ethionine, the effect was manifest within 4 weeks, with complete atrophy at 23 weeks. Once again the



TEXT-FIGURE 10. Times of appearance of changes in other organs. L = start of iron loading (groups I and III to V) or of ethionine diet (groups II and IIIA). T = start of ethionine and/or low protein diet (groups IIIB, IV and VA). In the case of the pancreas, lung, etc., where a change is not observed in iron-laden rats of group I, the lower half of the time scale has been omitted since the interval between L and T is unlikely to be of importance.

change was accelerated in group IIIA. This striking result was matched by the observations on renal autolysis (the alteration seen when the excised rat kidney was kept at room temperature for 3 hours—a phenomenon hitherto considered characteristic of vitamin E deficiency in the rat). We have previously reported[4] that on diet 41 this alteration is first seen in the iron-loaded rat at about 9 weeks. It came as a surprise to find that ethionine was also capable of inducing this effect. Even more unexpectedly, in group IIIA renal autolysis was observed after only 7 weeks; on a vitamin E deficient diet the phenomenon was fully developed only after about 40 weeks.[9]

The formation of ceroid in various organs parallels its incidence in the liver. Without exception the ethionine diet with concurrent iron loading greatly hastened the appearance of ceroid. In some organs—lung, heart, gastrointestinal tract and bladder—no ceroid at all could be seen with iron loading alone. In others the scanty ceroid obtained with iron or ethionine alone was greatly augmented and appeared earlier as a consequence of the combined treatment. Where 12 or more weeks elapsed between the end of iron loading and the start of ethionine, the synergism was less obvious. Low protein diet in several instances displayed the same effect, and the combined low protein-ethionine diet lacking added vitamin E gave rise to ceroid in iron-treated animals within a few weeks.

The only distinct effect of sex was seen in group II, on ethionine diet only, where there was more ceroid in the kidney, spleen, lung and adrenal of females. On the whole, gonadectomy had little influence on the results.

Discussion

Liver Iron and Ceroid

A remarkable feature of the iron-loaded rat liver was the apparent migration of the iron-loaded Kupffer cells to form aggregates around the portal tracts and central veins and the late appearance of iron in the parenchyma. The first effect of ethionine was to enhance the deposition of parenchymal iron greatly and to accelerate considerably the migration of Kupffer cells especially when cirrhosis was developing. The aggregates were also much larger than those seen in purely iron-laden animals. In the cirrhotic livers there appeared to be two generations of cells in each aggregation of siderophages: (a) central cells heavy with iron and containing only a little ceroid, probably resulting from the injected iron; and (b) external to these, cells containing less iron but much more ceroid, perhaps stemming from destroyed parenchymal cells.

Before cirrhosis developed, all the parenchymal cells of iron-loaded

animals given ethionine contained a little ceroid and were heavily laden with iron. In the early development of cirrhosis seen in group IIIA, many parenchymal cells contained no iron; this was more apparent in the later stages of cirrhosis in group IIIB. We presume that these iron-free cells were newly formed. In the even later stages of cirrhosis in group V, the new parenchymal cells were taking up iron and had again begun to develop ceroid. It is evident from these changes in parenchymal iron and ceroid that a completely new generation of parenchymal cells developed in the cirrhotic livers of rats given ethionine.

Parenchymal ceroid was not more abundant in fatty cells, though Kupffer cells in areas of fatty parenchyma contained more ceroid than Kupffer cells in nonfatty parenchyma. However, it is known that as cirrhosis develops the fat tends to disappear,[5] and this may well be the case in the present groups. Taking the experiments as a whole, the development of ceroid was a clear example of synergy between the factors which provided conditions favorable for its formation; namely, (a) excess of intracellular fat, especially unsaturated fat; (b) excess of intracellular iron; (c) inadequacy or deficiency of tissue antioxidants such as vitamin E; (d) parenchymal, probably mitochondrial, damage.

The Relationship of Ethionine and Low Protein Diets to Vitamin E

Our results show that ethionine given alone over a long period could produce effects suggestive of vitamin E deficiency. These included depigmentation of the incisors, enhanced renal antolysis, and the ceroid-laden "brown" uterus. Such changes were never seen in control animals. To what extent the testicular atrophy due to ethionine is preventable by vitamin E remains to be ascertained.

Much work points to a close relationship between vitamin E and the sulfur amino-acids (summarized by Beckmann[10]). More generally, the protective action of tocopherol in liver damage caused by various low protein diets has been fully established. The metabolic relationship between methionine and vitamin E has been attributed to a synergistic protection of labile sulfhydryl groups such as those of coenzyme A[11] or of glutathione.[12] It has been claimed that synergistic action of methionine will more than triple the antioxidant activity of α-tocopherol in stabilizing animal fats.[13] The "pro-oxidant" property of iron and the antagonism of methionine by ethionine would thus lead one to expect that iron and ethionine would each reinforce the anti-tocopherol effect of the other. Our evidence that such is the case indicates that the methionine-vitamin E relationship must rest on some basis other than antioxidant activity: there is no reason why ethionine should not be as good a "co-antioxidant" as methionine.

Ethionine reduces the methionine supply of methyl groups required for choline synthesis.[14] The striking effect on hepatic microsomal enzyme systems in the ethionine treated rat [15] and particularly the inhibition of intracellular protein synthesis and degradation [16,17] readily explain why protein depletion enhances ethionine-induced pancreatic damage.[18] In view of the intimate relationship existing between protein and vitamin E,[19,20] it is apparent that ethionine in a low protein diet, with unsaturated fat and no added vitamin E, may be expected to provide powerful support for the action of heavy stores of iron.

The Hepatotoxicity of Excessive Amounts of Stored Iron

The clinical improvement brought about by repeated venesection in patients with hemochromatosis has made it clear that excessive amounts of stored iron exercise some sort of toxic action. Throughout our studies we have been impressed by the dynamic character of stored iron. This fact must be taken in conjunction with the difficulty of detecting iron histochemically until appreciable amounts of intracellular iron are present. Together, they render superfluous any discussion of parenchymal and mesenchymal iron as separate and distinct entities. This is an important point in considering the histogenesis of hepatic fibrosis which has been thought to result from stored iron. A primary reaction of reticuloendothelial cells, with a large increase in reticulin and ultimately of collagen, is considered by Popper and his colleagues [21] to be the outcome of extracellular iron storage in portal tracts. But as Walters and Waterlow [22] have pointed out, portal reticulosis alone is an extremely common reaction. The progression to frank fibrosis requires the intervention of a second factor, which the Gillmans [23] have postulated to be a persistent depression of oxidation. We have failed to find any depression of respiration in slices of iron-loaded rat liver and feel that the second factor is unconnected with the iron *per se*. The "second factor" may be endogenous damage to the liver associated with the cause of the siderosis —as in severe hemolytic anemia. Or it may arise from an exogenous dietary or toxic factor. Our experiments illustrate vividly the interaction of hepatic siderosis with "second factors" of this kind.

The apparently normal respiration of iron-loaded rat liver constitutes a phenomenon which, for lack of a better term, we propose to call "equilibrated swamping" of the body with iron. There can be but few instances—in fact we know of no other—in which a major bodily constituent can be retained in amounts as great as 300 times or more the normal total body content of that material without lethal effects. Even more surprising is the ability of the animal to live its normal span, apparently in good health. We have shown, however, by our earlier work

on the development of certain features suggestive of vitamin E deficiency and by our present experiments, that the iron-loaded rat exists in a condition of "equilibrated swamping." While under optimal circumstances this state is compatible with health and prolonged survival, it renders the animal particularly susceptible to nutritional, toxic and other metabolic hazards. This is but one more instance of the general rule that the metabolic state of an organism determines, in part at least, the manner in which it reacts to a defined stimulus.

Nothing can be said at present concerning the precise nature of the metabolic state of the iron-loaded liver. Bound ferric iron may be expected to display nonspecific activity in direct oxidations of sulfhydryl groups, tocopherol or adrenalin, just as it is claimed to do with toxins.[24] It might well act indirectly by bringing about peroxidation of unsaturated fatty acids and thus lead to inhibition of oxidative mitochondrial enzymes [25] possibly by slow reaction with sulfhydryl groups. The various possibilities are certainly not mutually exclusive but may be coupled; for instance, sulfhydryl or other reduction of ferric to ferrous iron may lead to release of the latter for other functions, such as its role in specific enzyme systems, especially DPN-cytochrome *c* reductase, of which α-tocopherol has now been found to be a cofactor.

The Final Common Pathway

Matet, Matet and Friedenson [26] considered the protective action of tocopherol against liver necrosis to be nonspecific. Himsworth [27] dismissed the presence of ceroid as "an incidental result of the diets used." The possibility exists that these are all aspects of one and the same phenomenon, so that ceroid may be regarded as a pointer to one final common pathway taken by disturbances of liver metabolism with a wide diversity of origin. It is conceivable that the normal antioxidant protection of the unsaturated lipids which are bound up with the mitochondrion is adversely affected in the course of the mitochondrial changes through which the action of hepatotoxic agents is mediated.[28,29] As previously mentioned, damage of this kind may involve the accumulation of iron. Given sufficient time, this would prepare the ground for ceroid synthesis. When the affected cell comes to be replaced, the ceroid is carried by macrophages to lodge in centrilobular or periportal mesenchymal tissue.

It must be remembered that histochemical methods are insensitive in demonstrating intracellular iron and fail to reveal iron in a cell with increased ferritin content. Equally, when ceroid is seen, it represents a stage of polymerization whose earlier forms are difficult to distinguish with certainty. For this reason there is nothing unusual in finding hepatic hemosiderin without demonstrable ceroid or the reverse. In this way both

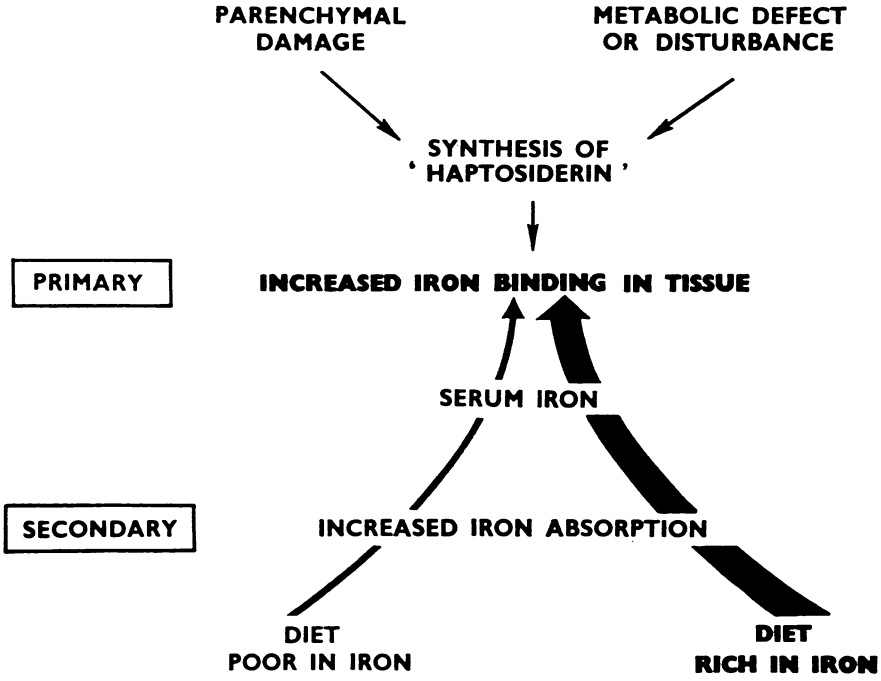
have come to be regarded as incidental findings and their true importance as twin indicators of potential or actual cellular damage overlooked. In the present experiments the stage has been set by providing the substrate (unsaturated fat) and the catalyst (iron) while the protective agent (tocopherol) has been eliminated, partly by omission from the diet and partly by the action of the stored iron. But these players alone do not constitute a cast, as can be seen by the vast increase in ceroid, as well as its rapidity of formation, when a nutritional or toxic factor is added.

Relevance of These Observations to Human Siderosis and the Problem of Hemochromatosis

Much of the difficulty and controversy which has arisen in the field of iron storage syndromes has its origin in the single-minded concentration on excessive intestinal absorption of iron. It is much more profitable to consider the question as primarily one of excessive iron-binding in the liver, from which the increased intestinal absorption follows secondarily. Even a cursory survey reveals that a wide variety of conditions not involving hemolysis or marrow hypoplasia can result in hepatic siderosis. By and large, such conditions have one factor in common: actual or potential liver damage, the latter taking the form of disordered metabolism of the hepatic cell. The suggestion is now put forward that such liver damage involves the synthesis of excessive amounts of an iron-binding material for which we suggest the name "haptosiderin." "Haptosiderin" is in all probability akin to the organic matrix of hemosiderin, recently shown by Bielig and Wöhler [30] to contain a deoxyribonucleotide. Or it may be similar to the copper-binding polypeptides isolated by Uzman, Iber, Chalmers and Knowlton [31] from the liver in Wilson's disease. In any case it is likely to be capable of binding other heavy metals which in turn are displaced by iron.[32] Thus, in rats poisoned chronically with nickel or cobalt,[33] it is hemosiderin which accumulates in the liver, while in human hepatic iron accumulation there is also excessive storage of lead, molybdenum and copper.[34]

A possible relationship of "haptosiderin" synthesis in excessive amounts and siderosis of the liver is illustrated in Text-figure 11. By this means, for example, the apparently conflicting views with regard to the etiology of Bantu siderosis are readily reconciled: Gillman and Gillman [23] point to disordered metabolism of the hepatic cell; Walker and Arvidsson [35] draw attention to the heavy level of dietary iron. Both are partially correct. The amount of iron present in the liver is far too great to have arisen solely from disrupted mitochondria as the Gillmans assert, unless these mitochondria were loaded with iron in the first place [36]; on the other hand, the dietary iron alone cannot adequately account for the

enormously enhanced absorption from the intestine. If, in fact, hepatic derangement as a primary influence is responsible for increased iron uptake, all the known facts fall into place. Where either the hepatic disorder or the excess of dietary iron is lacking, siderosis is not seen—for instance, elsewhere in Africa or among other poorly nourished peoples. Similarly with experimental observations such as siderosis in ethionine, carbon tetrachloride and other intoxications. Kinney and co-workers[3] have sought to attribute the ethionine effect to pancreatic damage, thus



TEXT-FIGURE 11. Suggested role of "haptosiderin" in iron storage syndromes.

bringing it into line with the results of Taylor, Stiven and Reid[37] on hepatic siderosis after pancreatic duct ligation in the cat. On a low protein, 60 per cent lard diet, Kaufman and his colleagues[7] induced pancreatic damage as well as hepatic siderosis. There is ample evidence[38] to indicate that pancreatic damage is not uncommonly associated with hepatic derangement, which would thus adequately account for the observed siderosis.

Of the factors which may be concerned in Bantu siderosis, a low protein diet suggests itself. In idiopathic hemochromatosis, "haptosiderin" in excessive amounts might be attributable to some inborn error of metabolism which has far-reaching consequences throughout the body. "Haptosiderin" may also arise in chronic refractory anemias which

are treated with multiple transfusions. In this way one could account for the fact that occasionally far more iron is found in the liver than can be accounted for by the transfusions. Moreover, the degree of terminal liver damage is often unrelated to the amount of iron present.

Summary

A gross degree of iron overload has been induced in rats. The siderotic but otherwise apparently normal liver displays an enhanced susceptibility to the action of dietary ethionine, or of a diet poor in protein and vitamin E, or more especially to the two factors acting together. Under this influence, hepatic cirrhosis, which does not supervene in the iron-loaded rat even after two years, makes its appearance within a few weeks, side by side with the massive aggregation of siderophages in the interlobular septums.

In other organs, lesions, some associated with lack of vitamin E, are correspondingly accelerated. The most striking of all these instances of synergy is the extensive development of ceroid throughout the body.

It is suggested that in the presence of tissue siderosis of such degree there exists a state of "equilibrated swamping" with regard to the iron, the equilibrium being precarious and, in the case of the liver, vulnerable to a host of adverse circumstances. An attempt is made to correlate the various human iron storage syndromes through a common mechanism of origin or development.

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[*Illustrations follow*]

Legends for Figures

Figures 1 to 3 represent the livers of rats sacrificed at varying times after the start of injections totaling 1,650 mg. of iron per kg. of body weight.

Fig. 1. After 9 weeks. Iron is confined to the Kupffer cells, many of which are enlarged. Prussian blue stain, neutral red counterstain. × 150.

Fig. 2. After 24 weeks. Small aggregations of Kupffer cells in portal tracts, with a little iron in the periportal parenchyma. Prussian blue stain, neutral red counterstain. × 150.

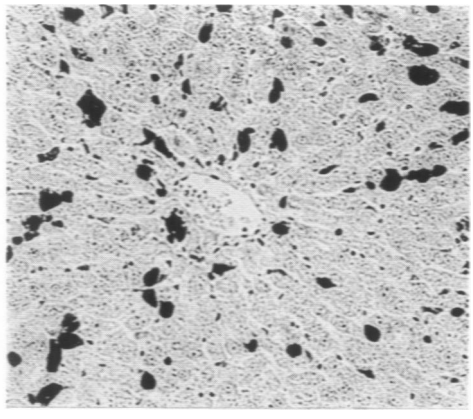
Fig. 3. After 76 weeks. Larger periportal and centrilobular siderophage accumulations with disappearance of iron from sinusoidal Kupffer cells and moderate amounts of iron in the periportal parenchyma. Prussian blue stain, neutral red counterstain. × 55.

Figures 4 to 6 represent the livers of rats sacrificed at varying times after commencement of the ethionine diet.

Fig. 4. After 15 weeks. Centrilobular accumulation of iron-containing macrophages. Prussian blue stain, neutral red counterstain. × 200.

Fig. 5. After 22 weeks. Ceroid pigment in large macrophages in the periportal stroma and in a few parenchymal cells. Ziehl-Neelsen stain. × 510.

Fig. 6. After 39 weeks. Fully developed cirrhosis with small aggregates of siderophages in the fibrous septums. Prussian blue stain, neutral red counterstain. × 55.



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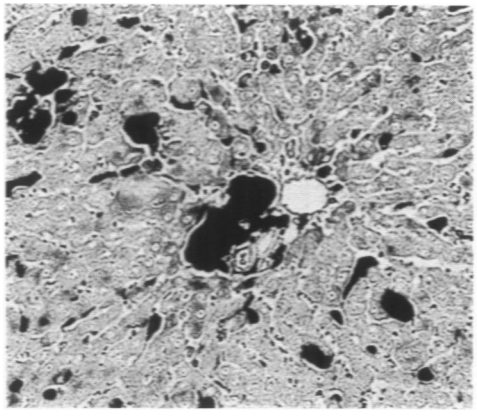

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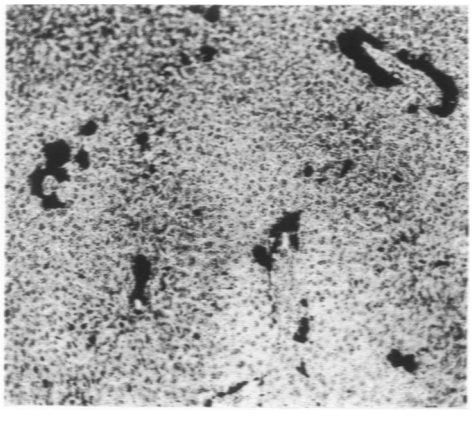

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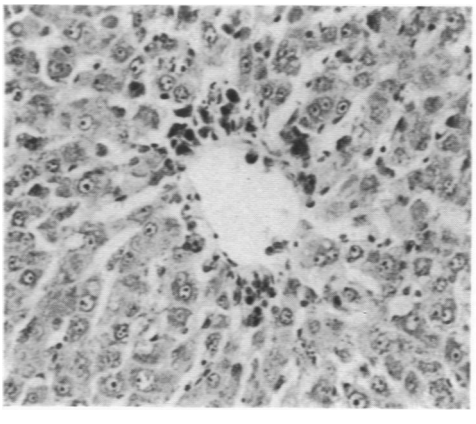

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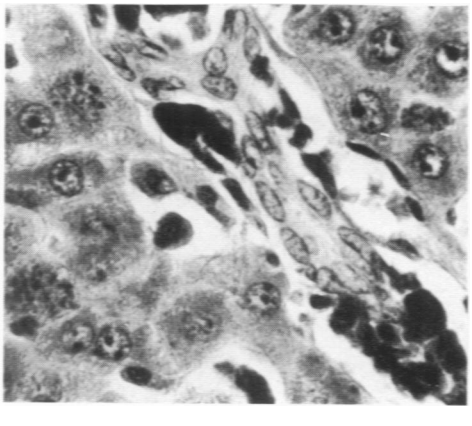

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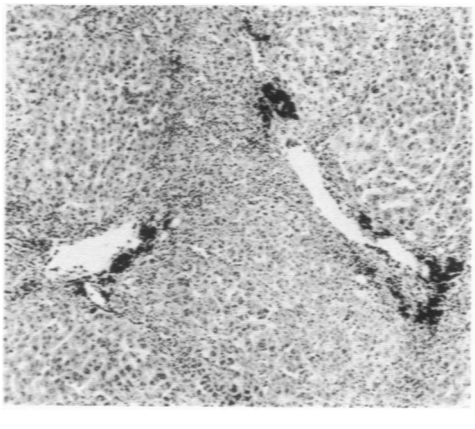

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Figures 7 and 8 represent livers of rats sacrificed after commencement of concurrent treatment with iron and ethionine.

FIG. 7. After 5 weeks. Uniformly heavy iron deposition in Kupffer cells and throughout the parenchyma. Prussian blue stain, neutral red counterstain. × 150.

FIG. 8. After 14 weeks. Early monolobular cirrhosis with very heavy iron deposits in the fibrous septums and, irregularly, in the sinusoidal Kupffer cells and parenchyma. Prussian blue stain, neutral red counterstain. × 55.

Figures 9 and 10 are sections from the livers of rats treated with iron followed by ethionine after an interval of 20 weeks.

FIG. 9. After a total of 32 weeks. Centrilobular and periportal aggregates of siderophages, with heavy diffuse parenchymal iron. Prussian blue stain, neutral red counterstain. × 55.

FIG. 10. After a total of 56 weeks. Advanced cirrhosis, with heavy iron in the fibrous septums. Prussian blue stain, neutral red counterstain. × 55.

FIG. 11. Liver of a rat on a low protein diet for 12 weeks. The diet was commenced after an interval of 20 weeks following iron loading. A fine cirrhotic pattern with heavy stromal iron and complete absence of iron from parenchymal cells. Prussian blue stain, neutral red counterstain. × 55.

FIG. 12. Liver of a rat after a total of 32 weeks; a low protein and ethionine diet had been fed after an interval of 20 weeks following iron loading. Severe cirrhosis with heavy stromal iron and iron in new parenchymal and Kupffer cells. Prussian blue stain, neutral red counterstain. × 55.

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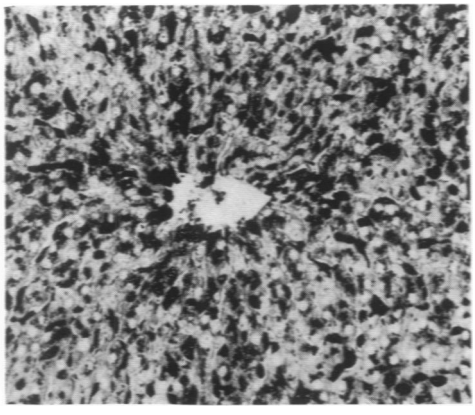

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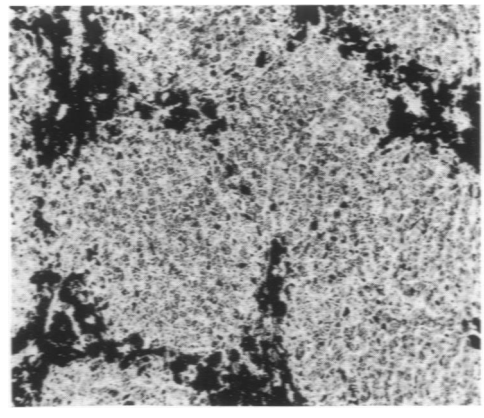

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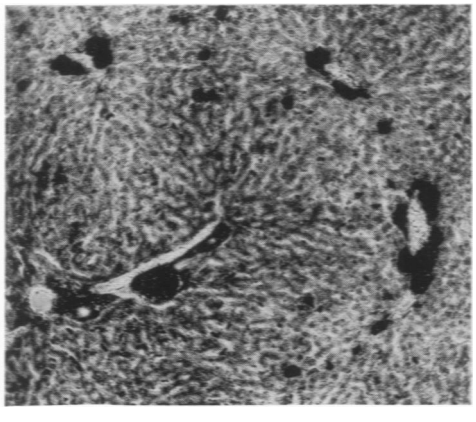

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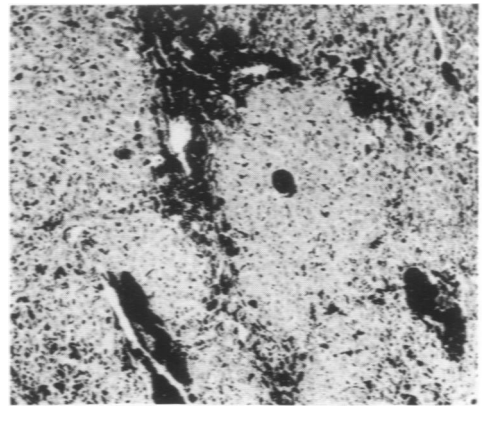

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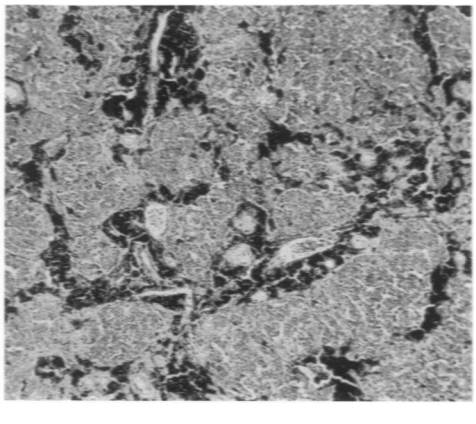

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