EXPERIMENTAL PIGMENT CIRRHOSIS DUE TO COPPER AND ITS RELATION TO HEMOCHROMATOSIS *

F. B. MALLORY, M.D., FREDERIC PARKER, JR., M.D., AND ROBERT N. NYE, M.D.

(From the Pathological Laboratory of the Boston City Hospital)

Synopsis:

INTRODUCTION

Hemochromatosis is a chronic disease characterized by the presence of two pigments in the cells of various organs and tissues of the body. One of the pigments, hemosiderin, gives the reactions for iron; the other, hemofuscin, does not. The accumulation of these pigments in certain cells leads eventually to three characteristic clinical signs, cirrhosis of the liver, sclerosis of the pancreas with resulting diabetes mellitus, and pigmentation of the skin. It is on the simultaneous presence of these three features in a typical case that a positive diagnosis of the disease is based during the life of a patient.

Well marked cases of hemochromatosis are rare; less than a hundred are on record in the literature at the present time. On the other hand the milder or earlier type of the condition, recognized by the pathologist as hepatic hemosiderosis, or pigment cirrhosis, in which the deposition of pigment is limited entirely or chiefly to the liver, is much more common than is

* Received for publication October 20, 1921.

generally appreciated, but there is nothing to call attention to it clinically.

The following statistics from the Pathological Laboratory of the Boston City Hospital are of interest in this connection:

Total number of autopsies during the past 25 years....	4507	
Total number of cases of well marked cirrhosis of the liver	224 —	5.08%

They are classified as follows:

1. Alcoholic cirrhosis (hyalin).....	104 —	46.42%
2. Pigment cirrhosis (hemosiderin).....	16 —	7.14%
1, 2. Alcoholic and pigment cirrhosis combined (hyalin and hemosiderin).....	50 —	22.32%
3. Syphilis.....	14 —	6.25%
4. Infectious (colon bacillus).....	10 —	4.46%
5. Acute yellow atrophy type.....	8 —	3.57%

In this series fourteen cases of central (sometimes called cardiac) sclerosis and one of chronic perihepatitis are not included.

These additional statistics showing the further occurrence of alcoholic hyalin and of hemosiderin are of value.

	Slight cirrhosis	No cirrhosis
1. Alcoholic hyalin.....	44	10
2. Hemosiderin.....	8	1
1, 2. Alcoholic hyalin and hemosiderin.....	13	6

In other words the hyalin characteristic of so-called alcoholic cirrhosis was present alone or associated with hemosiderin in 227 of the 4507 livers — 5.14%; while hemosiderin was found likewise alone or associated with alcoholic hyalin in 94 livers — 2.08%.

These additional cases listed above represent the early stages of lesions which if continued would eventually have terminated in typical examples of cirrhosis.

One point must be borne in mind in connection with these statistics. Of the 4507 autopsies 1057 were made on children from the infectious wards (scarlet fever, diphtheria, etc.). If they are excluded the percentages of livers showing the hyalin and hemosiderin which are characteristic of alcoholic and pigment cirrhosis are considerably larger, namely 6.57% and 2.72%.

The amount of alcoholic hyalin and of hemosiderin present in the livers classified here varied greatly, from a slight to an extreme amount. Of the cases of pigment cirrhosis four showed very general distribution of the hemosiderin so that they deserve to be included under the term hemochromatosis. One case was pure in type, two showed only a very slight amount of alcoholic hyalin and the fourth a marked degree of it.

Hemochromatosis has excited much scientific curiosity since the condition was first recognized. The earliest cases on record were reported by Trousseau (early in the eighteenth century); Troisier, 1871; Hanot and Chauffard, 1882; Hanot and Schachmann, 1886. The name and the first adequate description of the disease are due to von Recklinghausen, 1889, who noted the presence of the two different kinds of pigment and devised the word hemofuscin for the one which does not give the iron reaction. The French had already employed the term diabète bronzé, bronzed diabetes, for the same condition and this is still in common use.

The best known papers on the subject in English are by Maude E. Abbott, Opie and Sprunt. They consist, like most of the others, of case reports with gross and microscopic descriptions of the lesions found post mortem, references to the literature and discussions of the pathogenesis of the condition.

THE PRIMARY CELL LESION IN HEMOCHROMATOSIS

In order to attempt to produce hemochromatosis experimentally in animals it is obviously important to have if possible a perfectly clear understanding of the nature of the lesion as it occurs in man.

The study of a considerable series of cases shows definitely that the process of pigmentation makes its appearance first in the liver; then much later it appears more slowly and in varying degrees of intensity but more or less synchronously in the pancreas, heart, kidney, adrenal glands, mesenteric lymph nodes, gastrointestinal tract, skin and other organs and tissues. The lesion develops so slowly that it is difficult to follow every detail of the changes produced but enough is evident

for a full comprehension of the process. The liver is the best organ in which to study the lesion because owing to active and recurrent regeneration of the liver cells all stages of the effect produced by the deposition of the pigment can be followed.

(a). *Liver.* In the liver the pigment is deposited first in the endothelial cells lining the sinusoids and in the liver cells; then in the epithelial cells of the smallest bile ducts and in some of the fibroblasts of the stroma. Later and much more slowly it appears in the smooth muscle cells of the blood vessels, in the fibroblasts around the larger vessels and ducts and finally in the epithelial cells lining the large bile ducts.

The pigment granules are located at first in the cytoplasm of the liver cells close to the bile capillaries but later may fill the whole cell. One point is quite noticeable; in the early cases the pigment is deposited uniformly throughout the liver in all the liver cells, but as a rule more abundantly at the peripheries of the lobules than in the centers.

After the accumulation of pigment has reached a certain degree of intensity in the liver cells they begin to undergo necrosis singly here and there or in small groups. The necrotic cytoplasm is dissolved by the action of leukocytes and then the pigment granules are taken up in great numbers by endothelial leukocytes which tend to migrate with their contents to the periportal connective tissue where they accumulate in the lymph vessels and between the connective tissue cells of the stroma and attempt to digest the pigment.

The number of endothelial leukocytes filled with pigment which are present in the stroma varies greatly in the different cases. Sometimes they are few in number, at other times very abundant. The number seems to depend on the severity and duration of the process. In early active progressing lesions they are numerous. In cases of long duration where the process has been slight or intermittent the endothelial leukocytes have had time to digest and remove the hemosiderin they contained and to disappear themselves.

As the liver cells die off, regeneration takes place, often in the form of islands of new cells which may attain a size of one

to two millimeters or even more. These new cells at first are not pigmented. Then granules of light yellow pigment appear which do not give the iron reaction but stain more or less intensely with basic aniline dyes, especially with methylene blue, fuchsin and safranin. At a later stage the granules gradually change so that they no longer stain with the aniline dyes but give the iron reactions with ammonium sulphide and with ferrocyanide of potassium and hydrochloric acid. It is often possible to find in the comparatively young regenerated liver cells the two kinds of pigment granules in adjoining cells or mixed in various proportions in the same cells. The same is true of the liver cells in early cases of hemochromatosis. In other words the regenerating foci of liver cells show definitely that hemofuscin is deposited first and is then gradually transformed by the metabolic activity of the cells so that the hemoglobin compound is broken down and hemosiderin is formed, rendering possible the iron reactions. The presence of the two kinds of pigment granules in the liver cells of early cases of hemochromatosis strengthens this interpretation.

It is this primary pigment, hemofuscin, which is deposited much more slowly and hence relatively much later in the smooth muscle cells of the blood vessels, in the fibroblasts around the large vessels and ducts, and in the epithelial cells lining the large bile ducts. All these cells reduce the hemofuscin with great difficulty so that hemosiderin is formed only here and there. A possible explanation is the relatively greater distance of all these cells from the active circulation; their metabolic activity is probably less.

One other thing can be learned from the lesion in the liver. The islands of regenerating liver cells have one point in common with tumors; as they grow they are able to obtain by proliferation from the old stroma a new stroma of connective tissue and sinusoids of their own. In this way the amount of connective tissue in the liver is gradually increased. At the same time, as the old pigmented liver cells die off their stroma is left behind to shrink and coalesce. In this way the apparent increase of connective tissue in foci, that is, the scleroses in hemochromatosis (and also in the alcoholic type of cirrhosis)

are formed, not because of any direct proliferation of fibroblasts to take the place of the liver cells which have disappeared as is so generally taught.

There is no evidence of necrosis of fibroblasts owing to the deposition of pigment in them, or of regeneration of them in consequence.

(b). *Pancreas.* The lesion in the pancreas is much like that in the liver. Hemofuscin is deposited fairly readily in the cells of the glands and of the islands, but much later in the epithelium lining the ducts. It is easily changed to hemosiderin and leads eventually to necrosis of the cells followed by regeneration. In one case mitosis of island cells was of fairly common occurrence and was present also occasionally in the gland epithelium. The pigment from the necrotic cells is taken up by endothelial leukocytes which migrate with it to the stroma, especially around the larger blood vessels, where they accumulate in considerable numbers. As the gland cells are killed off the stroma which is left behind shrinks and condenses so that foci of compact connective tissue, scleroses, are formed. Hemofuscin is found in slight amount chiefly in fibroblasts in the stroma.

As is well recognized, the pigmentation and sclerosis in the pancreas lead eventually, if the patient lives long enough, to diabetes mellitus. This outcome is of much scientific importance because hemochromatosis is the one definite process which produces this condition. It is to be noted that the glands are much more affected than the islands.

No evidence of fibrosis or of disappearance of the islands can be found. In advanced cases of hemochromatosis, however, when diabetes has occurred, some of the islands present the typical hyaline transformation. This lesion in the islands suggests strongly that the hyaline change is the result, not the cause, of the diabetes.

(c). *Heart.* The pigment deposit takes place in the endothelial cells of the capillaries and in the muscle fibers. In the latter it is most abundant at first in the cytoplasm at the ends of the nuclei but later appears distributed often very uniformly between the striations throughout many of the fibers. When

the pigmentation has reached a certain degree of intensity the portion of a fiber most involved undergoes necrosis and the pigment granules are taken up by endothelial leukocytes which after a time migrate with them to the stroma and there remain. No regeneration of muscle takes place. In time the stroma shrinks, the capillaries disappear to some extent at least, and foci of sclerosis which may be fairly extensive are formed by the contraction of the old stroma.

It is doubtful if the lesion which is always a late one causes any clinical symptoms. The scleroses are like those occurring as the result of impaired nutrition due to sclerosis of the coronary vessels generally included under the term chronic myocarditis.

(d). *Adrenal glands.* The pigment is deposited almost exclusively in the epithelial cells in the outer layer of the cortex, that is, in the zona glomerulosa. Whether or not actual necrosis of the cells is produced cannot be determined.

(e). *Kidney.* The pigment granules are found chiefly in the epithelial cells lining the distal convoluted tubule and the ascending limb of Henle's loop of each renal element. They also occur in a few of the endothelial cells lining the capillaries in the glomerular tuft.

The lesion in the kidney is never marked because the renal cells destroyed by the presence or action of the pigment desquamate and are replaced by regeneration. There is no destruction of tubules. The pigment escapes with the urine and does not have to be taken care of by endothelial leukocytes.

(f). *Retroperitoneal lymph nodes.* The pigment is brought to the lymph nodes chiefly through the lymphatics which drain from the liver and pancreas. It is carried in endothelial leukocytes which have obtained it through necrosis of hepatic and pancreatic cells. These leukocytes pass through the peripheral and other lymph sinuses and invade the lymphoid tissue where they often accumulate in great numbers, causing, apparently mechanically disappearance of the lymphoid cells and increase of connective tissue in the same way as is done by carbon carried to the peribronchial lymph nodes.

(g). *Skin.* Hemosiderin occurs in fibroblasts around the coil glands and occasionally elsewhere, in the fat cells of the corium, and rarely in the epithelial cells of the coil glands but not in the cells of the epidermis. Its presence in the skin is of much clinical importance because it renders possible a positive diagnosis during life. The chemical tests for iron can easily be made on sections of an excised bit of tissue preferably from a well pigmented site.

(h). The pigment in hemochromatosis is deposited in various other organs and tissues besides in those already described, but they can be summarized briefly under the different types of cells involved.

In severe cases, pigment is present, chiefly in the form of hemosiderin, in both types of epithelial cells of the stomach, in the thyroid and parathyroid, in the ependymal cells of the choroid plexus.

Fibroblasts quite commonly contain pigment; in some situations as around the coil glands the granules give the iron reaction; in other locations as in the capsules of organs and around large blood vessels hemofuscin is more often present.

The smooth muscle cells of blood vessels and of the gastrointestinal tract often contain much pigment; most of it is hemofuscin but in places the granules give a good iron reaction.

The skeletal striated muscle fibers sometimes contain pigment usually located close to the nuclei which often undergo direct division and multiply considerably. Rarely necrosis of fibers occurs and endothelial leukocytes take up the pigment.

Fat cells, very generally everywhere but especially perhaps in the subcutaneous tissues, contain pigment which is present in the form of hemosiderin.

Endothelial cells lining capillaries are the first almost everywhere to take up the pigment. It is always in the form of hemosiderin because the hemofuscin in these cells is so quickly broken down. The pigment appears first in the endothelial cells of the liver, later in those of the heart, spleen, bone marrow and other organs.

The endothelial leukocytes obtain their pigment almost entirely from the destruction of the epithelial and other cells

which were filled with it. Some of them, however, may arise from desquamated endothelial cells filled with the granules.

Hemosiderin also occurs in the ganglion cells of the central and sympathetic nervous systems and in combination with hemofuscin in the peripheral nerves.

Interpretation. The primary essential lesion of hemochromatosis is the deposition of a pigment of hemoglobin origin in various cells of the body beginning with those in the liver. The pigment is hemofuscin; it gives no iron reaction. In many kinds of cells (hepatic, pancreatic, endothelial, etc.) it is quickly broken down so that from it is formed hemosiderin which gives the iron reaction. In certain cells (fibroblasts in various locations, smooth muscle cells, epithelial cells lining the larger bile ducts) the primary pigment is broken down slowly or not at all and persists more or less indefinitely as hemofuscin.

When the deposit of pigment reaches a high degree it causes necrosis of certain parenchymatous cells (liver, pancreas, heart); the necrotic cells are invaded by endothelial leukocytes which take up the pigment in great quantities. Following necrosis, regeneration of cells occurs in some of the organs (liver, pancreas, kidney).

A second result is scleroses which are formed in the liver, pancreas and heart owing to the disappearance of the parenchymatous cells in places and the subsequent shrinkage and coalescence of the stroma. These secondary lesions are recognized as cirrhosis of the liver which may cause ascites and jaundice; sclerosis of the pancreas which if severe enough always produces diabetes mellitus; and sclerosis of the heart.

The third clinical sign, pigmentation of the skin, may be due to the primary lesion, i.e., the deposition of hemofuscin in certain cells in the corium, followed by its transformation to hemosiderin, or to increase of melanin in the epidermis owing to injury to the adrenal gland (deposition of pigment in the zona glomerulosa) or to the combination.

PATHOGENESIS

The etiology of hemochromatosis has always been a fertile field for discussion because nothing definite is known about it. The *first* theory advanced in explanation of its origin was that the condition was secondary to the diabetes, the *second* that it followed the cirrhosis of the liver. These two theories have been very generally discredited because plenty of instances of both types of lesions occur without any sign of pigmentation. Recently, however, Rous and Oliver in their paper on Experimental Hemochromatosis have again advocated a primary cirrhosis of the liver as the starting point in causing a failure of the organ to deal adequately with the iron-containing products of normal blood destruction so that they accumulate in the body and cause automatically wide spread pigmentation.

The *third* theory, first advanced by Marie, 1895, is that the lesions are due to some as yet unknown toxin which is responsible for the hemosiderosis. The deposit of pigment then secondarily produces the sclerosis of the liver and pancreas.

The *fourth* theory would explain the source of the pigment as due to a perverted iron metabolism as a result of which the cells of the various organs and tissues retain most of the iron which reaches them through the circulation. The deposition of the pigment is regarded here also as the primary lesion which in time leads to sclerosis of the liver and pancreas.

The importance of the third theory will appear in connection with the results obtained by experimental work to be presented later. The great objection to it has been the apparently necessary great destruction of red blood corpuscles to account for the large amount of iron deposited in the tissues; of this no evidence has ever been obtainable either clinically or post mortem.

TECHNICAL METHODS EMPLOYED

The histological study of the lesions described here has been based largely on paraffin sections of Zenker fixed tissues stained by the eosin-methylene blue method. The demonstration of the two kinds of pigment granules, hemofuscin and

hemosiderin, however, required special methods. Inasmuch as the chemical properties and staining reactions of these pigments are of much importance in the study of hemochromatosis a brief statement of them seems advisable.

Hemofuscin occurs in the form of pale yellow granules, of various sizes up to a limited maximum, which are soluble in alkalies and in peroxide of hydrogen but not in dilute acids. They stain more or less deeply with basic aniline dyes, especially methylene blue, fuchsin and safranin, but do not stain with alum hematoxylin. The method found most useful for demonstration is as follows:

Directions.

Fixation: Zenker's fluid, alcohol or formaldehyde.

1. Stain paraffin sections in alum hematoxylin until the nuclei stand out sharply defined.
2. Wash in water.
3. Stain for ten to thirty minutes in the following solution:

Fuchsin	0.5 g.
Alcohol, 95%	50.0 c.c.
Water	50.0 c.c.

4. Wash off in water.
5. Differentiate and dehydrate in 95% followed by absolute alcohol.
6. Xylol, xylol colophonium.

Nuclei blue, hemofuscin granules bright red, hemosiderin unstained.

The method can be used equally well on celloidin sections if oleum origani cretici is employed in place of xylol.

The stain sharply differentiates the granules of hemofuscin from those of hemosiderin but no other claim is made for it except that it does not stain melanin.

Hemosiderin occurs in the form of yellowish or orange colored granules which often have a brownish tint, especially when occurring in masses. They are soluble in dilute acids but are not affected by alkalies or by peroxide of hydrogen. They are not stained by aniline dyes or by alum hematoxylin. On the other hand they give the characteristic color reactions of iron with ammonium sulphide and with ferrocyanide of potassium and hydrochloric acid. Apparently the hemosiderin in hemochromatosis always occurs as a ferric salt.

The following methods will be found to give excellent results:

A. Reaction with ammonium sulphide.

Fixation in Zenker's fluid, alcohol or formaldehyde.

1. Stain paraffin sections in alum hematoxylin until the nuclei are of a deep blue color.
2. Wash in water.
3. Place in a mixture of one part of strong yellow ammonium sulphide to three parts of 95% alcohol for one to two hours or longer. Use a glass staining dish with tightly fitting cover to prevent evaporation of the ammonia.
4. Wash quickly but thoroughly in several changes of water.
5. Dehydrate in 95% and absolute alcohol.
6. Xylol, xylol colophonium.

Nuclei blue, hemosiderin granules of an intense black. The stain keeps well. The results after Zenker fixation are perfect. If the sections are kept in water or alcohol too long the black color fades as the sulphide changes to the hydroxide.

The drawback to the ammonium sulphide reaction for general purposes is of course the fact that other metals besides iron form black sulphides, but this point is negligible in hemochromatosis, and if necessary can always be controlled by the Berlin blue reaction. The resulting stain is exceedingly sharp even with tissues preserved for years in formaldehyde and is especially recommended for photographic purposes as it yields results which are like etchings.

B. Berlin blue: due to the action of ferrocyanide of potassium and hydrochloric acid on a ferric salt.

This is the classical diagnostic color reaction for iron due to the formation of Berlin blue. It has at least two drawbacks. Hemosiderin is soluble in the hydrochloric acid and the Berlin blue in the ferrocyanide of potassium. As a result the stain readily diffuses, the fine granules all disappear and the larger ones have blue halos around them. A fine precipitate may be formed and deposited on the surfaces of the sections.

There are various methods of performing the test. The following is recommended for ordinary purposes.

Fixation in alcohol or formaldehyde. After Zenker's fluid the hemosiderin dissolves much too easily in the hydrochloric acid so that only a diffuse stain results.

Directions.

1. Stain paraffin (or celloidin) sections for ten to twenty-five minutes in the following mixture;

Ferrocyanide of potassium, 2% aqueous solution, freshly prepared (not over one week old)..... 1 part
Hydrochloric acid, 1% aqueous solution..... 3 parts

Better results will be obtained if, following a suggestion by Maude E. Abbott, the reaction is hastened by high temperature. Heat the mixture in a test tube until beads of gas form on the inner surface of the glass (about 80–85°C.) and pour over the sections. The reaction takes place within 30 to 45 seconds.

2. Wash thoroughly in several changes of water.

3. Counterstain lightly in alum carmine or in a one tenth per cent aqueous solution of safranin for two to five minutes.

4. Wash off in water.

5. Differentiate and dehydrate in 95% followed by absolute alcohol.

6. Xylol, xylol colophonium.

Nuclei red, hemosiderin blue, hemofuscin pale red.

In the method recommended here the reaction takes place so completely owing to the excess of acid and so quickly owing to the heat that there is little time for diffusion. The stain is intense and sharp except for the finest granules.

C. Turnbull's blue; The second diagnostic stain for iron is due to the action of ferricyanide of potassium and hydrochloric acid on a ferrous salt.

When hemosiderin is treated with ammonium sulphide the finest granules and the surface of the coarse ones are changed to ferrous sulphide. In consequence of this transformation, sections first stained with ammonium sulphide may be treated with ferrocyanide of potassium and hydrochloric acid (MacCallum, Hall, Nishimura) and give Berlin blue, or with ferricyanide of potassium and hydrochloric acid (Tirmann) and give Turnbull's blue, but neither method can stain all the iron present. Moreover, for the reasons already stated, both these methods are faulty and imperfect on account of the diffusion of the blue color owing to the action of the hydrochloric acid. If, however, the acid is omitted in the second reaction the ferricyanide of potassium alone will give a perfect, deep blue stain after ammonium sulphide, without diffusion, provided its time of action is limited, which rivals the ammonium sulphide stain in sharpness and surpasses it in

color and diagnostic value. The intensity of the blue stain depends on the length of action of the ammonium sulphide. If it is short, only the surface of the coarse granules is transformed to ferrous sulphide. When the color is turned blue the yellow within shows through the blue surface stain making the larger masses appear dark greenish. Therefore, it is important to have the reaction with ammonium sulphide as complete as possible. All the hemosiderin so far as practicable, should be turned to a ferrous salt. The reaction is quicker and more effective and takes place even after Zenker fixation if five per cent of acetic acid, which seems to have no dissolving effect on either hemosiderin or Turnbull's blue, is added to the ferricyanide of potassium.

Directions.

Alcohol, formaldehyde or Zenker fixation.

1. Stain paraffin sections in

Strong yellow ammonium sulphide	1 part
Alcohol, 95%	3 parts

for one to two hours or longer (twelve to twenty-four hours for Zenker fixed tissue).

2. Wash off in water.

3. Place in a freshly prepared mixture of

Ferricyanide of potassium (2 % aqueous solution)	19 c.c.
Glacial acetic acid	1 c.c.

for ten to twenty minutes or longer (twelve to twenty-four hours for Zenker fixed tissues).

4. Wash thoroughly in several changes of water.

COUNTERSTAINS

(a). *For ordinary tissues where only the iron reaction is desired.* Lithium carmine, which is ordinarily recommended, is inadvisable because of the injurious effect of the alkali in it and of the acid following it. Use alum carmine or a dilute solution of safranin or fuchsin.

(b). *For hemochromatosis material in which it is desirable to stain both kinds of pigment.* Use the stain for hemofuscin, steps 3 to 6. The alum hematoxylin is omitted because it dulls the picture. Hemosiderin blue, hemofuscin brilliant red, nuclei red, especially after Zenker fixation.

PREVIOUS ATTEMPTS TO PRODUCE HEMOCHROMATOSIS

Various attempts to produce hemochromatosis experimentally all seem to have been based on the idea that the hemosiderin is derived from hemoglobin set free by an abnormal destruction of red blood corpuscles. Two lines of experiments

have been tried: (a) the injection of blood into the peritoneal cavity to represent hemorrhage, and (b) the use of poisons, such as toluylenediamine, which destroy the red blood corpuscles in the circulation.

The most promising work along this line has been reported recently by Rous and Oliver. They injected large amounts of rabbit's blood into the circulation of rabbits almost daily for many months and as a result obtained deposits of hemosiderin in the liver, pancreas, heart, spleen and other organs and tissues. They noted slight cirrhotic changes in the livers of two rabbits which had survived a long time, but interpreted them as of an intercurrent nature and not due to the siderosis. Their conclusion is that while their results do not indicate the ultimate cause of the disease, they are practically identical with the siderosis characterizing hemochromatosis and throw light on its various features and on its course.

EXPERIMENTAL PIGMENT CIRRHOSIS DUE TO CHRONIC POISONING WITH COPPER

The lesions to be described under this heading are an outcome of a number of years of experimental work with various chemical reagents which might possibly be the cause of the two chronic and in some ways closely related toxic conditions known as alcoholic cirrhosis and hemochromatosis. It was, and is, believed that there is a perfectly definite etiological agent for each of them.

If half a gram of acetate of copper is given by stomach tube to a rabbit weighing two to three kilos it will cause death in less than twenty-four hours. If the dose in the beginning is made 0.1 gram it can be gradually increased in the course of a week to 0.2 gram and carried along at this amount daily without causing the animal any discomfort. A simple way is to mix a solution of the chemical with the food. A rabbit will readily take about 0.1 gram a day in this way and this amount was found to be sufficient to cause cirrhosis of the liver and death in from six to twelve months. For more chronic lesions a smaller dose and longer time are required.

The following general description is based on a series of twenty-two rabbits which died or were killed at various periods of time from one week up to eleven and a half months. The primary lesion was the same in all of them, depending only on the size of the dose and the length of time the copper was administered.

In a short while — one to three weeks — small yellow pigment granules begin to appear in the endothelial cells lining the sinusoids and in the liver cells. In one to two months they are numerous, in three months abundant. All the liver cells contain them but especially those at the peripheries of the lobules. In four to five months the condition is still more marked. Many of the liver cells have been killed as the pigment has increased in amount and other liver cells have regenerated. When a cell undergoes necrosis it is invaded by polymorphonuclear leukocytes which remove the cytoplasm. The pigment granules are taken up by endothelial leukocytes which stuff themselves full and tend to migrate to the periportal connective tissue and collect there in large numbers in the lymph vessels and between the fibroblasts around the blood vessels and bile ducts. The regeneration of liver cells in time becomes very active. As many as five mitotic figures (monasters and diasters) have been found in one oil immersion field. Occasionally triasters occur. The newly formed cells usually contain little or no pigment while the old ones are full of it.

The first increase of connective tissue appears in the neighborhood of the portal vessels where the liver cells are most pigmented and in consequence necrose first. As the cells disappear the elements of the stroma left behind coalesce and form foci of sclerosis. Where liver cells regenerate new stroma is formed for them.

Occasionally pigment granules were found in fibroblasts but there was never any evidence that they caused necrosis of the cells.

The earliest case of definite cirrhosis occurred at the end of five and one half months. The liver was somewhat enlarged and noticeably dense to the touch. The surface was slightly

and finely granular. Pieces of the tissue fixed in alcohol stained it yellow indicating bile stasis and this was confirmed by microscopic examination.

The second case of cirrhosis came at the end of six and one half months and was accompanied by intense jaundice of all the organs and tissues of the body.

A third animal died at the end of eleven and a half months with cirrhosis and also with intense jaundice.

Microscopic examination of all three cases of cirrhosis showed well marked increase of connective tissue, not only around the portal vessels but also running in various directions within the lobules.

All the pigment granules in this series of twenty-two rabbits have the chemical properties and the staining reactions of hemofuscin. None of them has changed to hemosiderin. Their presence gives to the liver on fresh examination a chocolate brown color which is very pronounced after poisoning has lasted for several months except so far as it is modified to a brownish or greenish yellow in the cirrhotic cases by the presence of jaundice.

Outside of the liver, pigment granules were found in endothelial leukocytes in the mesenteric lymph nodes and to a slight extent in the spleen and bone marrow. A few were present in the endothelial cells lining the capillaries of the heart, but none in the cardiac muscle cells or in the epithelium of the pancreas.

The examination of the blood was at all times negative. There was no evidence of anemia such as occurs so characteristically in lead poisoning owing to destruction of the red blood corpuscles.

Feeding acetate of copper to other animals than rabbits gave the following results:

The deposition of pigment in the livers of guinea pigs takes place very much slower than in the rabbit. On this account work carried on about eight years ago with this same chemical but fed only to guinea pigs yielded no results. It requires over a year to produce a moderate degree of sclerosis.

White rats show pigment in the liver about as quickly as rabbits do but have not been carried far enough along to develop cirrhosis.

A ring tail monkey which died after five and a half months feeding of a small amount of acetate of copper on its food showed a fair degree of pigmentation of the liver cells. About half the cells stained like hemofuscin. The rest gave a distinct iron reaction. Apparently the liver cells of the monkey possess to a much greater extent than do those of the rabbit the power to break down the copper hemoglobin compound and form hemosiderin.

A second monkey (*rhesus macacus*), fed on smaller doses is still alive at the end of fifteen months.

Of the other metals which form hemol compounds with hemoglobin, zinc is the only one which causes a lesion closely resembling that produced by copper.

Two other lines of experimentation with copper were tried. Chloride of copper in small doses was injected directly into the circulation of a rabbit for a period of thirty-three days. The same result was obtained as when a copper salt was introduced into the gastro-intestinal tract, namely, pigmentation of the cells of the liver, but not of those of other organs. This experiment would seem to show definitely that liver cells take up the hemol compound more quickly than do other parenchymatous cells, not because it reaches them first, but because of some special attraction.

In like manner a compound of copper with hemoglobin forming cuprohemol was injected into the circulation of two rabbits. The only parenchymatous cells in which the pigment appeared were those of the liver. This line of experimental work was not continued because it did not seem promising and the animals were easily killed if a very small dosage was exceeded.

While acute poisoning with copper is well recognized, the general consensus of opinion is that chronic poisoning does not occur except occasionally among workers in this metal and even this, according to some observers, may be due to the zinc present in brass. The symptoms caused point chiefly to

the respiratory tract. Halsey in his translation of Meyer and Gottlieb's Pharmacology goes so far as to state in an insert — "The prohibition of copper as a coloring agent for foods is not justifiable from a hygienic standpoint."

On the other hand some investigators have looked on copper with suspicion and considerable experimental work on animals and even on man has been done with practically negative results.. The criticisms of the work recorded are two. The administration of the copper was not continued over a long enough time to obtain results and the work apparently was not carefully controlled by microscopic examination.

These criticisms apply only in part, however, to the experimental work of Baum and Seeliger. They poisoned with various salts of copper a number of animals of different kinds (goat, sheep, dog, cat) for varying periods up to almost a year and observed "hemoglobin derivatives (hematoidin and hemosiderin)" in liver cells. They also noted the frequent presence of masses of hemosiderin in the liver but failed to recognize the early stages of sclerosis. They ascribed the pigments to the poisonous effect of copper but made no attempt to explain how they got there and very evidently did not realize their significance, that is, their possible relation to hemochromatosis and to the cirrhosis occurring in that disease. The animals they worked with, like the monkey used by us, apparently are able to break down the cuprohemol compound so that hemosiderin is formed. The results obtained by them, therefore, still more strongly favor the view set forth by us that chronic poisoning by copper may be the cause of hemochromatosis.

If hemochromatosis is due to chronic poisoning with copper, as the experimental work detailed here strongly suggests, the important question is, how does it obtain entrance to the body? There are several sources.

The salts of copper have long been used in connection with certain foods on account of their coloring properties. "To copper" is to stain bright green with these substances. The foods most often colored are pickles, canned peas and beans. The old fashioned way was to use a copper kettle and a little vinegar, but the salts have often been added directly. In

France where hemochromatosis is relatively common they are often added to absinthe to improve its color.

The salts of copper are also used to some extent on account of their antiseptic properties. Thus they are added to bread, beer and wine to prevent souring and to fresh water to stop or limit the growth of algae during the summer months.

Still another and possibly the most important source of poisoning may be from distilled liquors. Copper or brass tubing is often used in the coils and pipes connected with the still instead of block tin. The chief acid in the mash is acetic. This passes over along with the other volatile substances and readily attacks copper as is easily demonstrated experimentally.

Alcohol is claimed by various investigators to bear an intimate relation to hemochromatosis.

DISCUSSION

Hemochromatosis and alcoholic cirrhosis. — In studying the gross and microscopic appearances of cirrhosis of the liver as found in hemochromatosis it is important to bear in mind that as already stated the condition is often complicated by the lesion of alcoholic cirrhosis and vice versa. Histologically the two types of lesions are entirely distinct; one is characterized by the presence of pigment in the liver cells; the other, alcoholic cirrhosis, by a fine to coarse hyaline meshwork in affected cells. Both types of lesions may occur not only in the same liver but sometimes in the same cells. For purposes of discussion or description livers with but a single type of lesion should be selected. The three cases described by Sprunt, from whom material was available for study, were all pure types of hemochromatosis.

The frequent association of the two types of lesions in the same liver suggests an analogous cause for each.

Hypertrophic and atrophic cirrhosis. — The size of the liver in hemochromatosis depends as in alcoholic cirrhosis on the intensity and duration of the process. If the lesion is active and early it may cause increase in the weight of the liver up to

2500 grams or over (3200 grams in one of Sprunt's cases) owing to deposit of pigment, inflammatory infiltration with leukocytes to remove necrotic liver cells and the pigment, regeneration of liver cells and formation of new stroma. The surface may be smooth, slightly irregular or granular, the edges rounded and the density more or less increased. The color is light to dark reddish brown, or black if affected by post mortem changes.

If the process has been mild or intermittent and has lasted a great many years, regeneration in time fails to equal the rate of destruction and the liver diminishes in size to below normal. It may weigh as low as 1020 grams. The surface is very irregular and nodular and presents the typical hobnail appearance so characteristic of alcoholic cirrhosis in its late stages. The granules and nodules represent foci of regeneration; the depressions, contracted bands of old connective tissue stroma. The density is greatly increased and the color is light to deep bronze, depending on the degree of pigmentation which may be only moderate because endothelial leukocytes may have digested and removed all the hemosiderin set free by the necrosis of liver cells.

Extracellular hemosiderin. — The statement is often made, in descriptions of the lesions of hemochromatosis, that more or less of the pigment is extracellular. Unna used to make the same claim about leprosy bacilli. The statement in both instances is probably incorrect.

Immediately following necrosis of parenchymatous cells filled with pigment the cytoplasm is dissolved and removed first. Then the hemosiderin, which exerts a strong chemotactic attraction on endothelial leukocytes, is immediately taken up by them in large quantities. Rarely two or more of the leukocytes fuse together to form foreign body giant cells. The pigment persists indefinitely in these cells until it is digested and returned in some form or other to the circulation to be disposed of.

Rounded and oval masses of pigment not in epithelial cells mean endothelial leukocytes filled with it although the cell

outlines and nuclei are often more or less completely obscured. Rows of granules that seem to be free are always contained in flattened endothelial cells or in the cytoplasmic processes of fibroblasts.

Source of pigment. — One of the puzzles connected with hemochromatosis has always been the origin of the pigment which is evidently derived from hemoglobin. It has been very generally ascribed to an increased destruction of red blood corpuscles, but no evidence of such destruction has ever been found clinically or post mortem, and the spleen and bone marrow do not present the changes which are characteristic of such destruction.

The same peculiar condition is true of animals poisoned with copper. Examination of the blood shows no destruction of red blood corpuscles. There is no enlargement of the spleen until after cirrhosis has occurred and no abnormal activity of the bone marrow.

What is the explanation? It is probably very simple, namely, that there is no abnormal destruction of red blood corpuscles.

According to the physiologists one twentieth to one twelfth of the red blood corpuscles in the body disintegrate daily under normal conditions. The hemoglobin set free in this way circulates in the blood and is eventually eliminated. Copper has a great affinity for free hemoglobin (Kobert) and unites with it to form a compound known as cuprohemol. Apparently in the circulating blood it does not unite with the hemoglobin within the red cells and therefore does not cause destruction of them. Cuprohemol or a derivative of it is deposited as a yellowish pigment in the cells of the liver and leads eventually, as already described, to cirrhosis.

Diminished elimination of iron. — It has been demonstrated by Howard and Stevens and confirmed by McClure that the elimination of iron in hemochromatosis is lowered. In other words, iron is retained in the body. This retention is readily explained on the supposition that the hemoglobin set free by the normal disintegration of red blood corpuscles unites

in the circulation with copper absorbed from the intestinal tract and is deposited as hemofuscin in the cells of the liver and other organs and tissues of the body. Most of the cells readily break down this primary pigment into hemosiderin.

Proof that hemochromatosis is due to chronic poisoning with copper. — The experimental work reported here suggests strongly that hemochromatosis may be due to chronic poisoning with copper. The proof that copper is the cause is quite another thing and proof is not easy to obtain.

The amount of hemofuscin present in the body at any one time in a case of hemochromatosis is very small, probably much less than one hundredth part of the iron; therefore the possible amount of copper presumably is very slight also.

An excess of copper in the body might be demonstrated in at least three different ways.

(a). Copper in hemofuscin. This would require showing the presence of copper in the hemofuscin granules in the cells by means of microchemical tests. This is no more possible than demonstrating the iron also present in the cuprohemol compound. Both the copper, if present, and the iron are so intimately bound up in the organic compound that no diagnostic reaction can be obtained.

(b). Copper in the liver. By using large amounts of liver tissue and digesting it with strong acids it would be possible to demonstrate copper in the same way that iron can be shown to exist in hemoglobin. The test, however, would require tissue in large amounts and this has not been available.

(c). Elimination of copper. It seems probable that copper if present in hemofuscin would be eliminated when that substance is broken down to form hemosiderin. This offers a third method, and perhaps the best, of seeking evidence whether or not hemochromatosis is due to chronic poisoning with copper.

We have tried the first and second methods without success. A favorable, that is, early active case of hemochromatosis in the hands of a good analytical chemist might throw light on the subject.

Increase of normal body pigments. — It is commonly stated that in hemochromatosis there is a general increase of pigments throughout the body in cells which normally contain it. Opie ascribes the pigmentation of the skin entirely to an increase of melanin in the epidermis. If this is true it is possible that it is caused by the injury to the outer layer of cortical cells, the zona glomerulosa, of the adrenal glands and that the skin lesion really is one variety of Addison's disease. In other words, pigmentation of the skin may follow injury to the adrenal glands, owing to the deposition of pigment, in the same way that ascites follows cirrhosis of the liver and diabetes mellitus the sclerosis of the pancreas.

In marked amyloid formation of the adrenal glands only the zona glomerulosa is spared. Practically all the rest of the parenchymatous tissue is destroyed by the pressure due to the amyloid and yet no pigmentation of the skin occurs.

Clinically it is well recognized that the pigmentation of the skin due to Addison's disease and to hemochromatosis cannot be positively differentiated from each other except by the aid of the existence of other signs (ascites and diabetes mellitus) or most conclusively by the demonstration of hemosiderin in the skin.

The hemofuscin deposited in cells of the corium, and the hemosiderin derived from it, may or may not play some part in the appearance of pigmentation. It is rather deeply placed to produce much effect, and yet the silver in argyria occupies about the same location. The question of pigmentation of the skin cannot be decided solely by post mortem examination. It is most important to know if the patient was originally of the blonde or brunette type.

Poisoning with iron. — Ferric chloride in 1 gram doses was administered to a rabbit daily by stomach tube for five and a half months. On post mortem examination the liver was of a bright reddish brown, rustlike color. Microscopically the tissue showed the deposition of much iron in the form of yellow granules. It was in endothelial and liver cells and occurred most abundantly at the peripheries of the lobules. Occasional

liver cells had undergone necrosis and the pigment had been taken up by endothelial leukocytes. It seems probable that if the administration of iron had been continued long enough cirrhosis would have resulted.

In the experimental work of Rous and Oliver, where rabbit's blood was injected into the circulation of rabbits for many months, two out of the three animals which survived longest showed moderate cirrhosis of the liver. The authors interpreted the condition as of an intercurrent nature. It is probable that it was produced by the deposition of hemosiderin and that the third rabbit in time would have shown the same lesion. Animals are not equally susceptible to injurious agents. The diagnosis of the cirrhosis should have been based on necrosis and regeneration of liver cells. If those two processes were in evidence the cirrhosis in two of the rabbits was due to the hemosiderin and in time would have occurred in the third.

The theory of Rous and Oliver that cirrhosis of the liver is the primary condition which leads to retention of iron-containing products of normal blood destruction so that they accumulate as pigment in the various organs and tissues of the body is untenable in view of the many cases of all degrees of cirrhosis of the liver of alcoholic and acute yellow atrophy types which occur without a trace of hemosiderin being found in the liver or elsewhere. They also fail to consider the acute early cases of hemosiderosis of the liver in which little or no cirrhosis has yet occurred.

Susceptibility. — The experimental work with copper has shown quite definitely that all rabbits are not equally susceptible to its poisonous effects. Even animals of the same litter failed to develop lesions with equal rapidity. On the same dose one rabbit died from cirrhosis in five and a half months, a second in six and a half months, and a third in eleven and a half months.

When we compare the susceptibility of different kinds of animals the difference is still more marked. Rabbits, white rats, and monkeys are all fairly susceptible to poisoning by copper; guinea pigs on the other hand are very slightly so.

Human beings may differ much in their reaction to copper just as, it is well recognized, they do to arsenic, lead and some other poisons.

Cause of necrosis of parenchymatous cells. — In hemochromatosis the parenchymatous cells which undergo necrosis are those which are overloaded with hemosiderin. Hemofuscin never seems to be present in sufficient quantity to cause injury. In the experimental animals on the other hand the cells which die are those filled with cuprohemol or a derivative of it. Only the more highly differentiated cells such as liver, pancreatic and cardiac muscle cells succumb. Fibroblasts and endothelial cells and leukocytes persist indefinitely filled with either kind of pigment.

Necrosis does not seem to be due to a chemical action on the part of either kind of pigment but rather owing to mechanical action interfering, perhaps, with the normal functions of the cells.

Primary carcinoma of the liver. — Primary cancer of the liver is comparatively rare. On this account its relatively frequent occurrence in combination with cirrhosis is of interest. In our two hundred and twenty-four cases of cirrhosis primary cancer of the liver was present four times, once associated with the hemochromatosis type of lesion, three times with the combined lesions of alcoholic cirrhosis and hemochromatosis. In addition specimens from two other cases of carcinoma of the liver combined with hemochromatosis have been sent to the laboratory during this past year. The tumors all seemed to be of the primary liver cell type although the cells in some of them were not fully differentiated. In one instance metastases to regional lymph nodes had taken place. One of the four cases of hemochromatosis recently reported by Blanton and Healy had a primary cancer of the liver.

The significance of these cases is that they would seem to show some definite relation between long continued active regeneration of liver cells and primary carcinoma of the liver.

Summary

Hemochromatosis is a chronic disease due to a definite toxic agent which unites with the hemoglobin set free by the normal disintegration of red blood corpuscles. From the compound thus formed hemofuscin is deposited in various cells throughout the body. It appears first and in largest quantities in the liver; later in the pancreas, heart, kidneys, adrenal glands, and other organs and tissues. Most cells (endothelial, hepatic, pancreatic, etc.) readily transform the hemofuscin so that hemosiderin results as can be readily demonstrated by the iron reactions. Other cells (epithelium in ducts, smooth muscle cells, fibroblasts in certain locations) have little or no effect on the hemofuscin so that it persists in that form more or less indefinitely.

The effect of the excessive deposit of pigment in parenchymatous cells (liver, pancreas, heart) is to cause necrosis of some of them followed by active regeneration by others in certain organs (liver, pancreas). The pigment set free by necrosis is taken up in large quantities by endothelial leukocytes which collect, sometimes in great numbers, everywhere in the stroma.

The connective tissue in some of these organs, liver for instance, is gradually increased owing to new formation of fibroblasts, as in tumors, to form stroma for the islands of regenerated cells. The sclerosis is due to contraction and coalescence of old stroma as the pigmented cells die off.

The final result of these processes is cirrhosis of the liver, sclerosis of the pancreas with consequent diabetes mellitus, sclerosis of the heart. The pigmentation of the skin may be due, in part at least, as in Addison's disease, to injury to the adrenal glands (zona glomerulosa).

The spleen and bone marrow show no marked changes of any sort and clinically there is no evidence of anemia.

Chronic poisoning with salts of copper produces in rabbits within six to twelve months well marked cirrhosis of the liver with intense jaundice followed quickly by death. The liver appears of a dark chocolate color owing to the deposit of great

numbers of yellow pigment granules in the liver and endothelial cells most marked at the peripheries of the lobules. Excessive deposit of pigment results in necrosis of liver cells followed by active regeneration.

In the rabbit the pigment, which has the same staining peculiarities as hemofuscin, does not undergo transformation into hemosiderin. In a monkey, however, which died after copper poisoning of five months duration over half of the pigment granules in the liver cells gave a definite iron reaction.

The only deposit of pigment outside of the liver, except for occasional pigmented endothelial leukocytes in the spleen and bone marrow, occurred in the kidney and in endothelial cells lining some of the capillaries of the heart.

The spleen and bone marrow showed practically no changes until after cirrhosis had occurred and examination of the blood for evidence of the destruction of red blood corpuscles was always negative.

Conclusions

It is impossible to produce in months, lesions for which nature requires years.

Chronic poisoning with salts of copper produces in the livers of rabbits in six months to a year a series of changes comparable in many ways with those found in the liver in a chronic disease in man known as hemochromatosis. Poisoning with smaller doses continued over a much longer time would probably cause lesions in other organs.

Proof that hemochromatosis is due to poisoning with copper would require the demonstration of copper either in hemofuscin, or in the liver in excess of the minute amount said to be normally present, or in excretions from the body.

If hemochromatosis is due to poisoning with copper there are several sources from which it may be derived:

1. From its use as a coloring reagent to "copper" vegetables (pickles, canned peas and beans) and to improve the color of absinthe.

2. From its use as an antiseptic to prevent fermentation (beer, wine) or to inhibit the growth of algae (drinking water).

3. From distilled liquors (action of acids, chiefly acetic, on copper and brass tubing used in connection with stills).

The present extensive use of crude distilling apparatus in consequence of prohibition is likely to lead to an increase in the number of cases of hemochromatosis if the disease is due to chronic poisoning with copper.

(NOTE. — The expenses of the work presented here have been paid in part from a research fund due to the generosity of Dr. and Mrs. Henry F. Sears.)

BIBLIOGRAPHY

Abbott, Maude E. Pigmentation Cirrhosis of the Liver in a Case of Hemochromatosis, J. Path. and Bact., 1901, vii, 55.

Opie, E. L. A Case of Hemochromatosis — The Relation of Hemochromatosis to Bronzed Diabetes. J. Experimental Medicine, 1899, iv, 279–306.

Sprunt, T. P. Hemochromatosis. Archives of Internal Medicine, 1911, viii, 75–129.

Rous, Peyton, and Oliver, Jean. Experimental Hemochromatosis. J. Experimental Medicine, 1918, xxviii, 629–644.

Howard, C. P. Hemochromatosis. Oxford Medicine, 1921, iv, 215–222.

Baum und Seeliger. Die chronische Kupfervergiftung. Archiv für Thierheilkunde, 1898, xxiv, 80–127.

An extensive bibliography will be found in each of the above papers.

Blanton, W. B. and Healy, W. Hemochromatosis. Report of four cases. Archives of Internal Medicine, 1921, xxvii, 306–420.

Marie, P. Sur un cas de diabète bronzé suivi d'autopsie. Semaine méd. 1895, xv, 229.

Howard, C. P. and Stevens, F. A. The Iron Metabolism of Hemochromatosis. Archives Internal Med., 1917, xx, 896.

McClure, C. W. Metabolism in a Case of Hemochromatosis. Arch. Internal Med., 1918, xxii, 610.

DESCRIPTION OF PLATES

(The drawings in the colored plate were made by Miss Etta R. Piotti.)

PLATE VI. Fig. 1. — Surface view of hemochromatosis liver on section showing islands of non-pigmented and slightly pigmented regenerated liver cells.

Fig. 2. — Section through an island of regenerated liver cells which are slightly pigmented. Adjoining liver cells contain much pigment. Many endothelial leukocytes filled with pigment in the sclerotic tissue.

Zenker fixation. Stain, alum hematoxylin and ammonium sulphide.

X 100

PLATE VII. Fig. 3. — The primary cell lesion of the liver in hemochromatosis: hemosiderin granules (derived from hemofuscin) packed in the liver cells close to the bile capillaries.
Zenker fixation. Stain, alum hematoxylin and ammonium sulphide. X 500

Fig. 4. — The cell lesion characteristic of so-called alcoholic cirrhosis: a hyaline meshwork, which stains intensely red by the eosin-methylene blue method after Zenker fixation, occurring in liver cells and gradually increasing in amount until the cells undergo necrosis. X 250

PLATE VIII. Fig. 5. — Liver of rabbit: early stage of chronic poisoning with copper acetate. Liver cells contain large numbers of pigment granules which do not give the reactions for iron..
Zenker fixation. Stain, alum hematoxylin and fuchsin. X 400

Fig. 6. — Detail from Fig. 5 showing pigment granules in liver cells and in endothelial leukocytes. X 1000

PLATE IX. Fig. 7. — Liver of rabbit: chronic poisoning with copper acetate. Diaster due to regeneration of liver cell. Pigment granules in liver cells and in mitotic figure.
Zenker fixation. Stain, alum hematoxylin and fuchsin. X 1000

Fig. 8. — A clump of endothelial leukocytes filled with pigment granules following necrosis of liver cells. Two leukocytes have fused to hold a mass of granules.
Zenker fixation. Stain, alum hematoxylin and fuchsin. X 1000

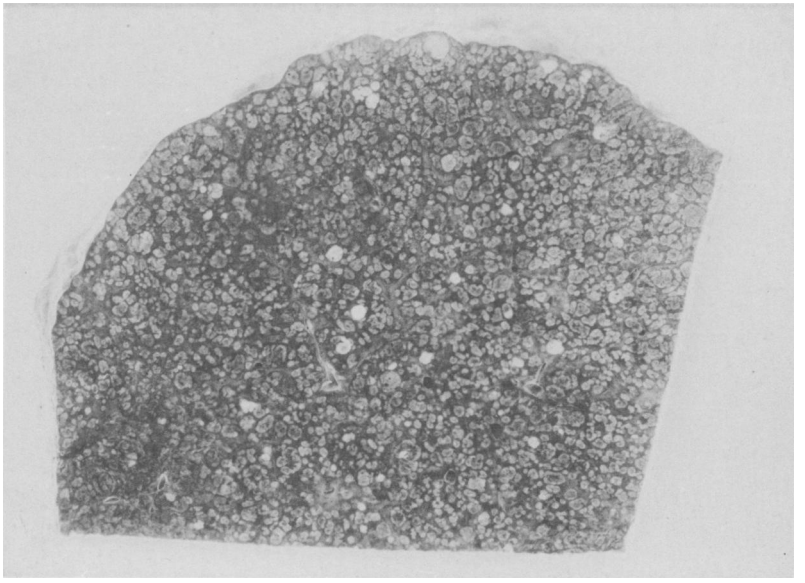
PLATE X. Fig. 9. — Liver of rabbit: a somewhat later stage of chronic poisoning with copper. A moderate number of endothelial leukocytes filled with pigment grouped around the portal vessels.
Zenker fixation. Stain, alum hematoxylin and fuchsin. X 250

Fig. 10. — Liver of rabbit: cirrhosis due to chronic poisoning with acetate of copper for eleven and a half months. Sclerosis confined chiefly to peripheries of lobules.
Zenker fixation. Stain, eosin-methylene blue. X 50

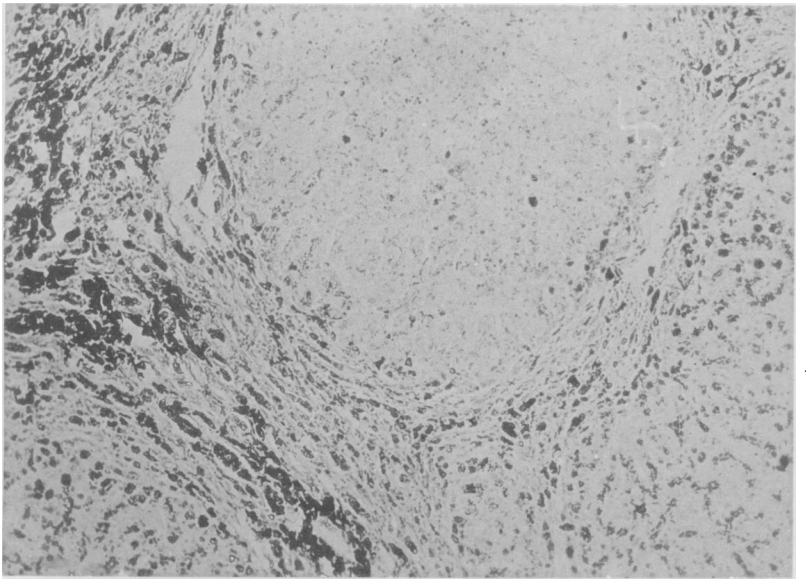
PLATE XI. Fig. 11. — From a hemochromatosis liver: a portion of the wall of an artery showing hemofuscin granules (stained red) in the fibroblasts of the adventitia and combined with hemosiderin (stained blue) in the smooth muscle cells of the media. X about 250
Zenker fixation. Stain, ammonium sulphide, ferricyanide of potassium, fuchsin.

Fig. 12. — From an island of regenerated cells in a hemochromatosis liver showing transformation of hemofuscin granules (stained red) to hemosiderin granules (stained blue).
Zenker fixation. Stain, ammonium sulphide, ferricyanide of potassium, fuchsin. X about 1000

Fig. 13. — Liver of rabbit: chronic poisoning with copper acetate: cells filled with pigment granules (stained red) which do not give the iron reactions but do stain like hemofuscin. One mitotic figure present.
Zenker fixation. Stain, alum hematoxylin, fuchsin. X about 1000

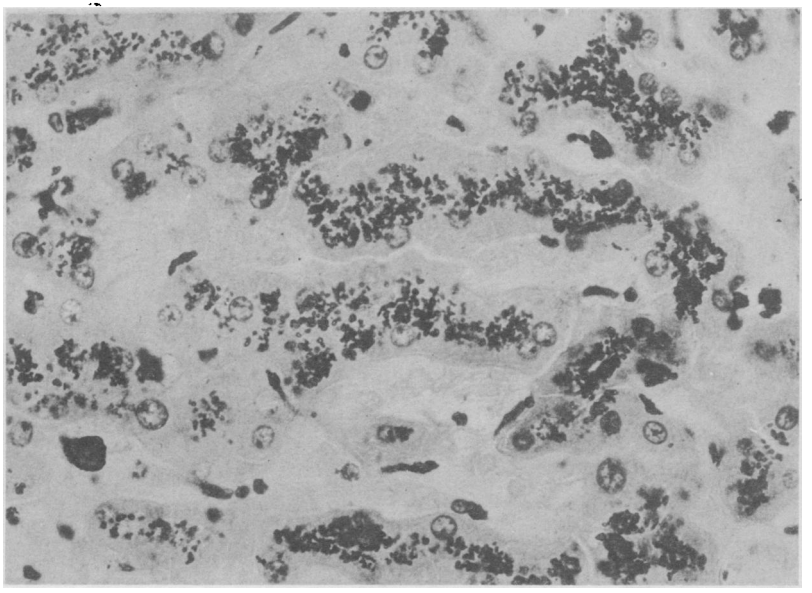


1

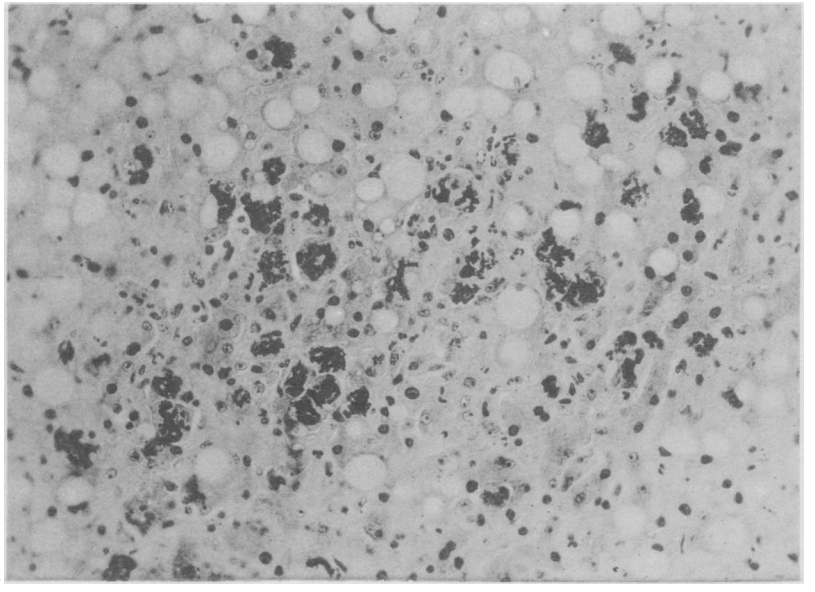


2

Mallory, Parker and Nye. Hemochromatosis.

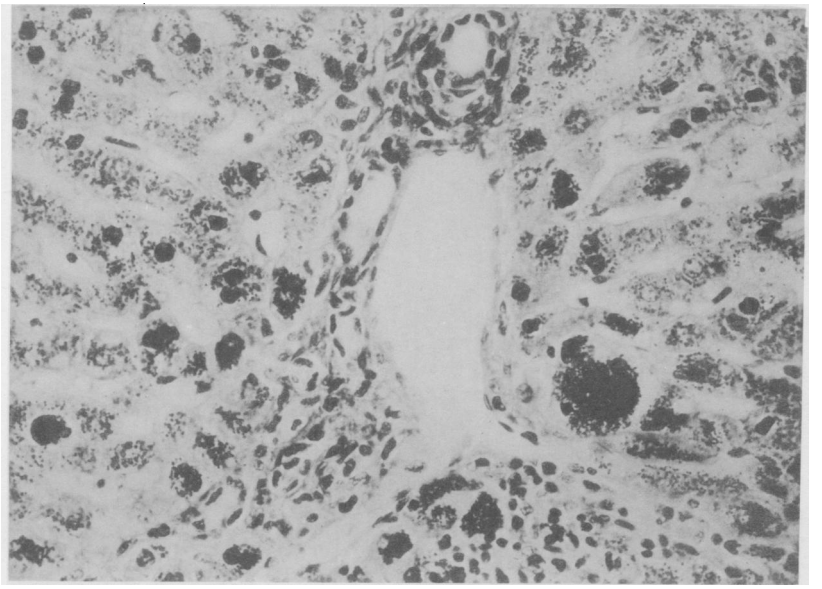


3

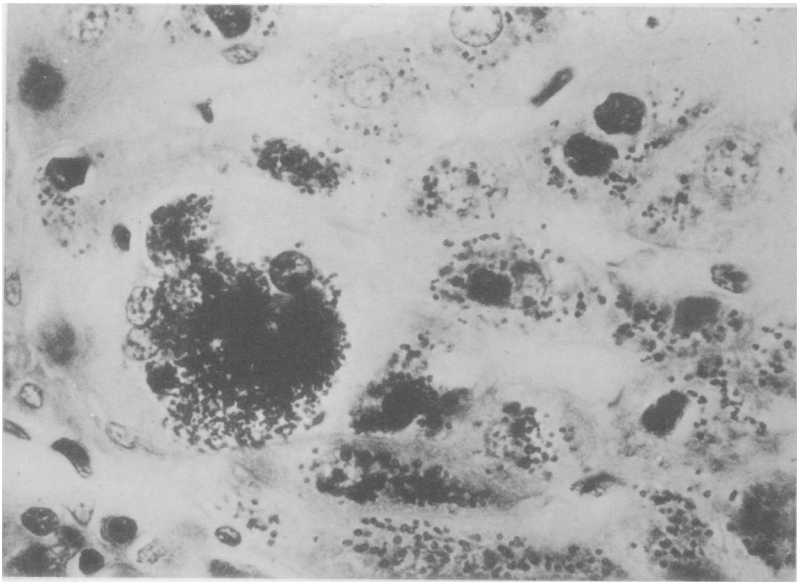


4

Mallory, Parker and Nye. Hemochromatosis.

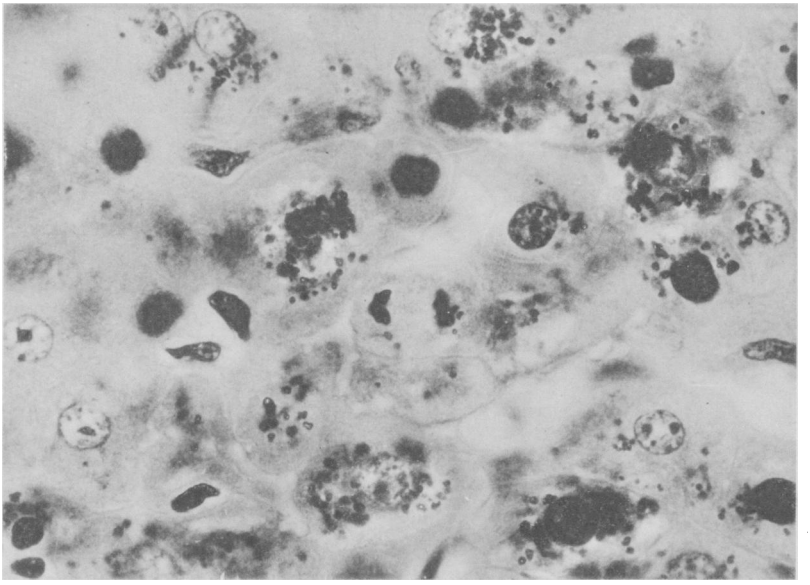


5

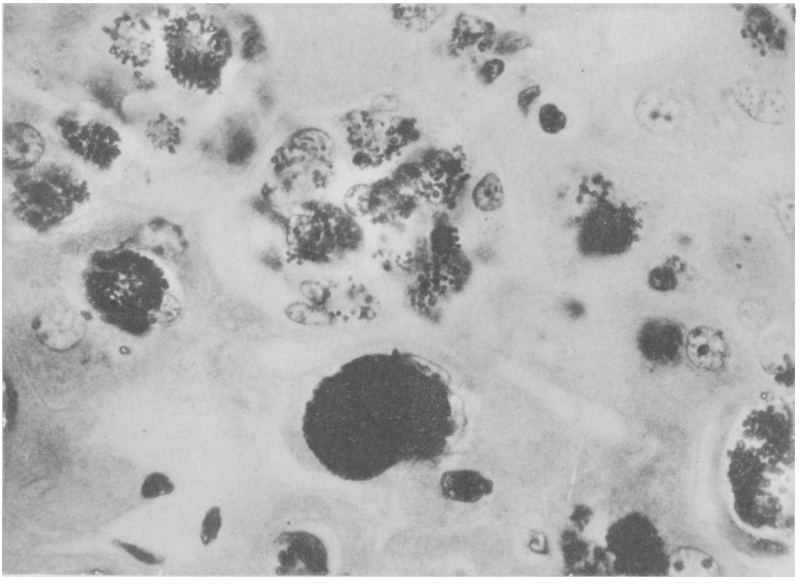


6

Mallory, Parker and Nye. Hemochromatosis.

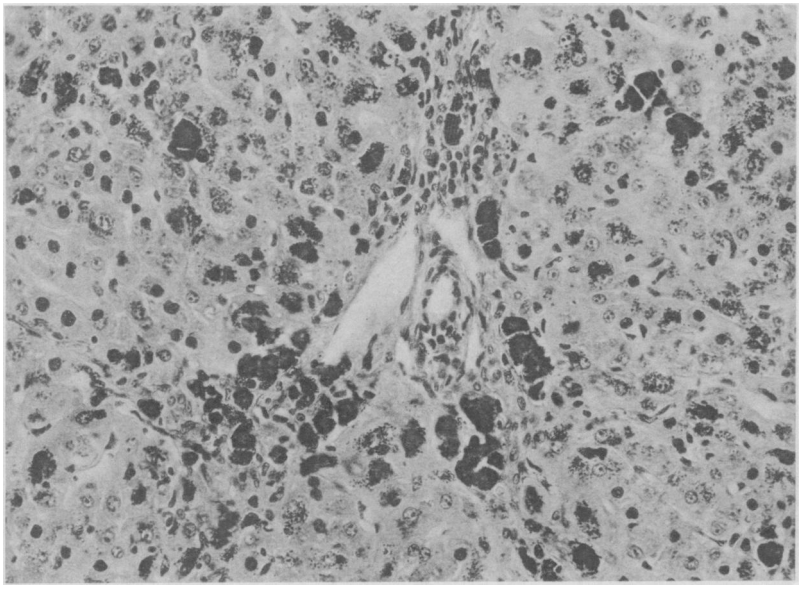


7

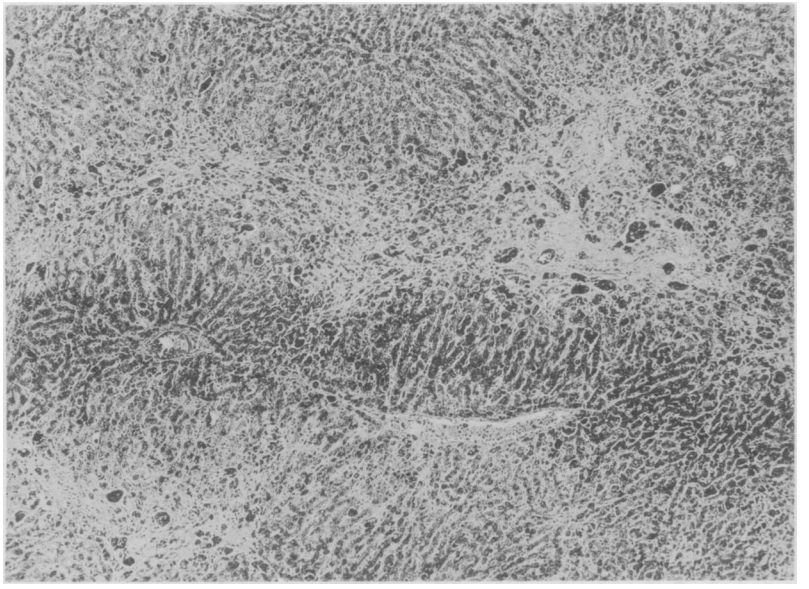


8

Mallory, Parker and Nye. Hemochromatosis.

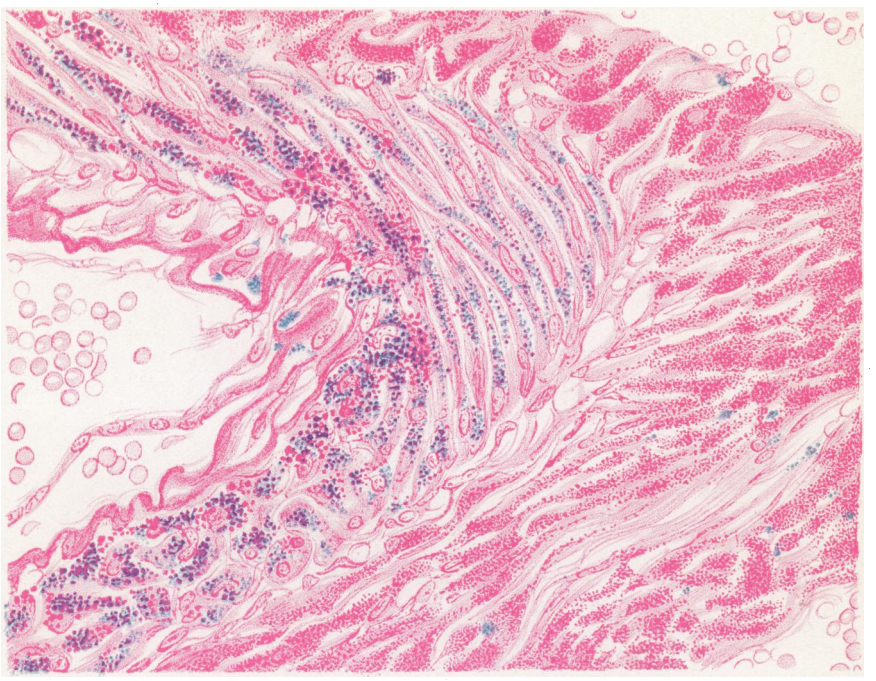


9

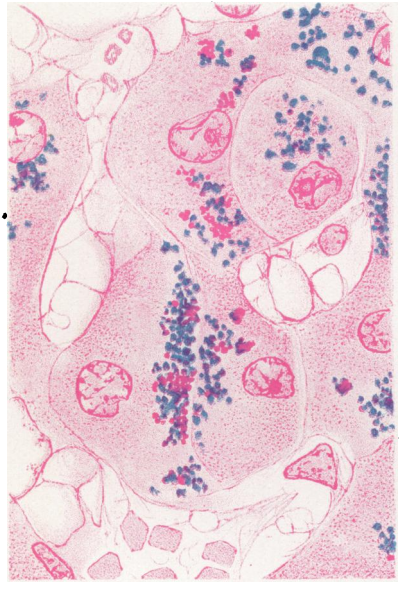


10

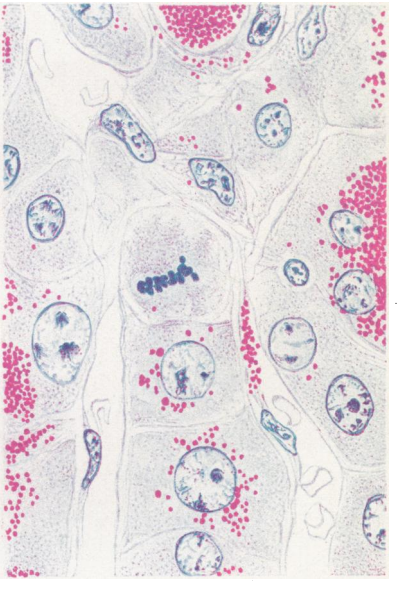
Mallory, Parker and Nye. Hemochromatosis.



11



12



13

Mallory, Parker and Nye. Hemochromatosis.