EXPERIMENTAL HEMOCHROMATOSIS.

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PLATES 64 TO 70.

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In pernicious anemia and hemochromatosis an iron-containing pigment, hemosiderin, ordinarily derived from hemoglobin, is deposited in organs free from such pigment in most conditions that involve blood destruction. When sudden hemolysis takes place in a previously healthy human being, as after cutaneous burns or the action of a "blood poison," hemosiderin granules appear in the spleen, the red marrow, and sometimes in certain lymph nodes, but as a rule in these organs only. A similar localization of the pigment is found in most diseases accompanied by a destruction of red cells,—acute malaria, for example; and it is regularly encountered in animals submitted to experiments or infections which, like trypanosomiasis, involve a breaking down of blood. Far different is the siderosis of pernicious anemia and hemochromatosis. The spleen and bone marrow are still pigmented, but to only a slight degree as compared with some of the other organs. In pernicious anemia most of the hemosiderin is found in the liver and much in the kidneys. In hemochromatosis the liver, kidneys, pancreas, heart, abdominal lymph nodes, and many other organs are heavily loaded with the pigment.

What is the cause for the difference thus outlined? Has the hemosiderin in hemochromatosis another source than the blood? These and other important questions suggest themselves. And numerous attempts to answer them through an experimental production of the significant lesions have already been made. Stadelmann,[1] Hunter,[2] Biondi,[3] and others obtained a siderosis of the liver parenchyma as the

[1] Stadelmann, E., *Arch. exp. Path. u. Pharmakol.*, 1887, xxiii, 427.

[2] Hunter, W., Pernicious anæmia, London, 1901, 182.

[3] Biondi, C., *Beitr. path. Anat. u. allg. Path.*, 1895, xviii, 176.

result of fatal doses of toluylenediamine. The drug injures the liver greatly. Schurig,[4] who injected pure hemoglobin into rabbits day after day for several weeks found some hemosiderin in the liver and kidneys, in addition to the usual abundance in spleen and marrow. Muir and Dunn,[5] producing an acute hemolytic anemia in rabbits by means of a specific hemolysin, noted a little granular hemosiderin in the liver and a diffuse Prussian blue reaction in the kidney parenchyma of animals surviving the destruction in the course of 3 days of more than half their total blood. Other less successful results might be quoted. The general findings warrant the conclusion that under most circumstances of blood destruction the spleen and red marrow act as depots for hemosiderin and more than suffice for the purpose; but when the breaking down of red cells is fulminant, or large quantities of blood pigment are furnished the organism for a considerable period, the buffer activity of these organs is overcome, and some little hemosiderin is laid down elsewhere. There are several obstacles to obtaining a great deposition of it by experimental means. Free hemoglobin injected in considerable amount is for the most part rapidly lost through the kidneys; blood cells that are damaged tend to accumulate in the capillaries and cause lesions; while the blood poisons, repeatedly administered, injure other tissues besides the blood, with complex results. In view of these circumstances the unsatisfactory outcome of experiments is easily understood.

Method.

It has seemed to us that better results than those described might be obtained through a utilization of the animal's own ability to break down blood under circumstances such that more pigment is liberated than spleen and marrow can deal with. A means for the work has come to hand in the method of repeated transfusion employed by one of us with Robertson for the study of normal blood destruction.[6] Rabbits rendered plethoric by the day to day injection of 10 cc. of the citrated whole blood of other rabbits soon acquire the ability to dispose

[4] Schurig, *Arch. exp. Path. u. Pharmakol.*, 1898, xli, 29.
[5] Muir, R., and Dunn, J. S., *J. Path. and Bacteriol.*, 1914–15, xix, 417.
[6] Robertson, O. H., and Rous, P., *J. Exp. Med.*, 1916, xxv, 665.

rapidly of the foreign blood, and this in many instances without the development of demonstrable immune bodies. We have accordingly transfused eleven animals for months, injecting them 6 days out of every 7, and towards the end increasing the daily amount of blood to 15 cc. For each recipient a number of donors were used in rotation. In the early weeks some of the recipients developed weak agglutinins for the foreign blood. Later, as the injections were continued, such antibodies disappeared.

Findings in Relation to Pernicious Anemia.

In the present paper a general description of the results of the work will be given. A study of certain of its special features is to be published later.

The rabbits killed after a few weeks of plethora induced by the repeated injection of blood showed merely the lesions already described by many observers[7] as following one or two massive transfusions; namely, an enlarged spleen, turgid with cellular debris and pigment, and phagocytes full of red cells; a considerable siderosis of the marrow; and a slight one of the abdominal lymph nodes—in other words, the findings usual after blood destruction in normal animals. When the injections had been kept up for 2 months there was in addition a pigmentation of the parenchyma of the liver and kidneys with fine yellow-brown granules which on test proved iron-containing. The pigment was abundant in the peripheral cells of the hepatic lobules and in the convoluted tubules of the kidneys, as is the case in pernicious anemia. Save for the siderosis, a general plethora, and a consequent slight, central atrophy of the liver cells, the organs appeared normal. In human pernicious anemia the spleen is relatively free from pigment, whereas in our animals this organ was always most heavily loaded with it. The difference is an important one, indicating as it does either that the spleen is altered in pernicious anemia, and unable to take up the products of blood destruction, or that its activity is somehow circumvented, which is the supposition in Hunter's theory of a portal blood destruction.

[7] For instance, Boycott, A. E., and Douglas, C. G., *J. Path. and Bacteriol.*, 1910, xiv, 294.

Findings in Relation to Hemochromatosis.

Seven rabbits were transfused over a period of from 3 to $6\frac{1}{2}$ months. The findings in these have great interest, for they are such as are associated, in human beings, with hemochromatosis and with this disease alone. Most of the animals were healthy when killed and had gained weight despite the long standing plethora which involved a day to day hemoglobin of 120 to 160 per cent Sahli, and, as the autopsies showed, a pronounced general congestion. Jaundice was not noted at any time, nor was hemoglobin or sugar found in the urine. The absence of the latter is accounted for by the state of the pancreas which showed lesions of a mild grade in contrast to the serious changes which accompany the *diabète bronzé* of man.

In comparing the rabbit material with that of human hemochromatosis, we have made use of the recent excellent descriptions of Sprunt,[8] Gaskell and his associates,[9] and also of specimens from an outspoken case of hemochromatosis studied at the Hospital of The Rockefeller Institute, and autopsied by one of us.[10] The older studies of hemochromatosis are in general marred by the use of unsatisfactory methods to demonstrate iron-containing pigment.

The changes in the rabbits were, in general, much less marked than those observed post mortem in human hemochromatosis, as would naturally follow from the fact that the pigmentary process had not gone so far in the animals as even to menace health. Nevertheless, many of their organs, notably the liver, kidney, heart, visceral lymph glands, pancreas, spleen, and marrow, had, to the naked eye, a distinct brown tint. The liver was in several instances of a light chocolate color and showed a slight, diffuse cirrhosis, indistinguishable in the gross from that so frequently intercurrent in rabbits. The spleen was never larger than after several weeks of transfusion and often smaller. The skin of a long transfused white rabbit had a brownish tone. In every case the vessels were engorged, the liver was swollen, owing to congestion, and the heart was enlarged and dilated. The histological findings were essentially similar in all the animals, differing only in degree.

[8] Sprunt, T. P., *Arch. Int. Med.*, 1911, viii, 75.

[9] Gaskell, J. F., Sladden, A. F., Wallis, R. L. M., Vaile, P. T., and Garrod, A. E., *Quart. J. Med.*, 1913–14, vii, 129.

[10] Rous, P., *J. Exp. Med.*, 1918, xxviii, 645.

Pigments.

Most of the pigment in the organs differed not at all in appearance or reactions from the hemosiderin observed in human beings. It had the form of orange-brown, moderately refractile granules, usually minute, but sometimes coarse and irregular, as in the spleen. When exposed to ammonium sulfide the granules turned black, and when tested by Nishimura's method[11] they took on a fine, deep blue color. In determining their distribution to the various organs, we have regularly used the latter method, which Sprunt and others, working with human material, have found satisfactory.

Hemofuscin, a yellow or brown pigment which fails to react for iron, occurs in quantity in some cases of human hemochromatosis, whereas in others it is practically absent. As Sprunt has pointed out, its amount varies greatly with the method used to demonstrate it. With the Nishimura test there is always far less than with Perl's reaction which the older authors generally employed for the demonstration of iron. The hemofuscin is to some extent characterized by its situation, being found especially in the capsules and trabeculæ of lymph nodes and in the smooth muscle of the walls of blood vessels. Very little was present in the numerous preparations from our recent case of human hemosiderosis. In the transfused rabbits a yellow pigment which failed to respond positively to the Nishimura test was observed in scattered connective tissue cells of the lungs and choroid plexus.

Comparison of the Human and Rabbit Organs.

Spleen.—The condition of the spleens of rabbits long transfused justifies the conclusion already expressed that this organ serves as a buffer depot for hemosiderin, and that its activity in this direction must be overcome if the pigment is to reach the other viscera in quantity. In animals killed after only 3 to 4 weeks of plethora the spleen was much enlarged, turgid with cellular debris and with phagocytes crowded with red cells. The liver and other viscera were at this period practically non-pigmented. But when the plethora had been maintained for months an entirely different state of affairs was found. The spleen, no larger than before, and often rather small, was a mere

[11] Nishimura, J., *Centr. allg. Path. u. path. Anat.*, 1910, xxi, 10.

congeries of sinuses, containing large, irregular masses of hemosiderin, and distended with blood, but almost empty of other cells (Fig. 1). The Malpighian corpuscles were atrophic, and many had nearly disappeared. The organ was, in fact, a mere shell, scarcely to be thought of as functioning longer for the retention of blood pigment. And, as would naturally follow from this fact, the liver and many other organs now showed profuse deposits of hemosiderin.

In the hemochromatosis of man there is chronic passive congestion of the spleen consequent on the liver cirrhosis characteristic of the disease. Phagocytes distended with hemosiderin are present in moderate number, but the greater part of the pigment, which at most is not abundant as compared with the amount in other organs, lies in the reticular cells. The Malpighian bodies are not atrophied. A diffuse increase in the connective tissue may usually be noted. There is no sign that a great activity of the organ to store up pigment was once present and has been overcome.

Bone Marrow.—The marrow of the rabbit, after months of plethora from transfusion, showed little more pigment than after a few weeks (Fig. 2). Evidently the limit of its capacity for the reception of hemosiderin had soon been reached. The greater part of the pigment lay in distended mononuclear cells. The entire quantity was only moderate. In man, too, this is the case. No distinct changes in the blood-forming tissues are to be noted in either animal.

Liver.—In the rabbits this was, after the spleen and certain of the abdominal lymph nodes, the most heavily pigmented organ. In one instance the degree of siderosis approached that in some patients dying of hemochromatosis (Fig. 3). The parenchyma contained everywhere numerous pigment granules but notably at the periphery of the lobules, where often the cells were filled with coarse yellow lumps that turned deep blue when tested by Nishimura's method. Similar iron-containing pigment lay in the capillaries, much of it in great rounded masses enclosed more or less completely in multinucleate giant cells. Many Kupffer cells were filled with pigment granules. Towards the center of the lobules the capillaries were widely distended, without doubt as a result of the plethora, and there was some pressure atrophy of the otherwise normal looking parenchymal elements. In two of the most pigmented livers there was some intralobular cirrhosis, but in a

third this element was quite lacking, and careful study has convinced us that such cirrhosis as was found was intercurrent in character of a sort frequently seen in supposedly normal rabbits. The question of its relationship to the pigmentation will be discussed in detail further on. The cirrhotic tissue contained some new-formed bile ducts, but neither these nor the normal ones were pigmented, though here and there a connective tissue cell was laden with the characteristic brown granules.

In man the siderosis of the liver parenchyma has the same general appearance and an identical distribution with that in the rabbit, being more abundant towards the periphery of the lobules. Here many of the parenchymal cells may become so filled with pigment as to break down, resulting in connective tissue proliferation and the appearance of islands of new-formed liver cells. There is regularly present a pronounced hypertrophic cirrhosis with many new-formed and heavily pigmented bile ducts. This characteristic feature of the human disease was absolutely lacking in our rabbits.

Kidneys.—There was in the rabbits a marked siderosis of the kidney parenchyma (Fig. 4) somewhat more general in its distribution than is the case in human hemochromatosis. In this latter disease the pigment is, as a rule, sharply localized to the ascending limb of Henle's loop and to the distal convoluted tubules, where it is abundant; while the rest of the parenchyma, save in some cases the glomeruli, is free from it. Gaskell and his associates[9] point out that in pernicious anemia, by contrast, the pigment is found almost wholly in the proximal convoluted tubules. In our rabbits the glomeruli never showed pigment, but the cells of nearly all the cortical tubules contained it. Very fine granules were present in moderate number in the proximal convoluted tubules; more were to be seen in the descending limb of Henle's loop; and the ascending limb and distal convoluted tubules were very heavily pigmented with coarse, granular agglomerates, just as in the hemochromatosis of man. And, as in this disease, the collecting tubules and medulla were free from pigment.

Pancreas.—Only in the animals transfused during a long period was this organ pigmented. Fine hemosiderin granules were then demonstrable in the alveolar cells, often in considerable quantity (Fig. 5). The islands of Langerhans were always normal and there was an en-

tire absence of the cirrhosis which in human beings may be marked. But there was an absence too of the destruction of gland tissue through excessive pigmentation which many authors hold to be the cause of the cirrhosis.

Adrenals, Stomach, and Intestines.—In two rabbits the adrenal glands showed a slight siderosis and in precisely the region where pigmentation is found in man; namely, in the zona glomerulosa of the cortex (Fig. 6). The intestines, as in the human instance, were free from hemosiderin save for the granules in a few connective tissue cells scattered throughout the coats. But the stomach also was unpigmented whereas in the human stomach the glands of the mucosa are usually the subject of an outspoken siderosis, especially at their base.

Heart.—This organ showed pigment only in the rabbits that were transfused longest. The hemosiderin was deposited in fine granules throughout the muscle fibers, being especially abundant close to either end of the nucleus (Fig. 7). An identical localization of the pigment is found in human beings, but the amount is, as a rule, far greater, sometimes leading to death of the muscle fibers with connective tissue overgrowth as a further result.

Lungs.—Neither in man nor in the rabbit is there a noteworthy pigmentation of the lungs. A few iron-containing cells may be present here and there in the reticulum and sometimes in the capillaries.

Lymph Nodes.—In the rabbits with most advanced siderosis large masses of free iron-containing pigment were present in the sinuses of the lymph nodes draining the liver (Fig. 8) and granules were present in many cells, as well. The other abdominal lymph nodes also contained iron in some quantity, but so sometimes do those of the normal rabbit. The mediastinal lymph glands of the transfused rabbits and those from the groins and axillæ showed at most only a few granules. This localization of hemosiderin to the nodes draining heavily pigmented organs is the rule in human hemochromatosis.

Skin.—The bronzed portions of the human skin usually contain slaty brown pigment granules in the basal layers of the epithelium, which do not react positively to the tests for iron. Ordinary hemosiderin is present as well in connective tissue cells of the corium, especially about the sweat glands. In our rabbits the epidermal pigment was lacking, but connective tissue cells containing hemosiderin were often fairly numerous in the corium (Fig. 9).

Other Organs.—In one of Sprunt's cases the tracheal cartilage was pigmented and in another the submaxillary gland. Both these lesions have been found in our rabbits. Siderosis of the submaxillary gland was well marked in one animal, the granules lying grouped in the alveolar cells (Fig. 10). The rabbit thyroid, on the other hand, was never affected, whereas in man it may be loaded with pigment. The rabbits transfused longest happened to be old females. In them a pronounced siderosis of the mammary gland was regularly present, certain cells of the epithelium being distended with pigment, while still others containing it had desquamated and could be found free in the lumen of the ducts (Fig. 11). There is no parallel in man for this lesion, since human hemochromatosis is a disease of the male. Abbott's description of the single case on record in a female does not include a report on the mammary gland.

Relation of Cirrhosis and Pigmentation.

Hypertrophic cirrhosis of the liver is a constant feature of human hemochromatosis, but whether it is a primary or secondary factor in the disease has not been determined. There is no doubt that a fibrous overgrowth is often present that has been induced by parenchymal destruction. The evidence of such a secondary connective tissue proliferation is so clearly visible in autopsy material that many authors have been led to consider the entire cirrhosis as secondary. But actually the recognition of areas of induced cirrhosis proves nothing either way, since they would be present in any event were the pigmentation sufficient to cause parenchymal destruction.

The transfused rabbits were killed at a relatively early stage of hepatic siderosis, and the ability of this latter to induce cirrhosis in the absence of cell destruction can be well judged from the findings. At the periphery of the lobules the parenchymal cells were heavily pigmented, but as yet only here and there had one broken down. Cirrhosis was practically absent, save for that of an intercurrent nature, which at most was slight, and obviously it had not followed the pigmentation, being often well marked where the latter was negligible. Here and there in the lobular capillaries were great masses of iron pigment, some of them as much as 80 μ across, more or less completely enclosed in multinucleate giant cells. But though these pigment

masses had induced pressure atrophy of the surrounding parenchymal cells, no connective tissue reaction had taken place about them. It may be urged that proliferation might have ensued in time, and this is not impossible. Yet in view of the lack of an immediate reaction the conclusion seems justified that hemosiderin when enclosed in living cells has no noteworthy stimulative effect on connective tissue.

Hepatic changes similar in degree to those in the rabbits cannot be expected at the average human autopsy, since patients with hemochromatosis do not, as a rule, succumb to the disease until the pigmentation has destroyed large numbers of parenchymal cells with a resulting connective tissue overgrowth. Only by some chance may one expect to obtain the liver at an earlier stage. Such a chance has recently come to us in the case of hemochromatosis already mentioned. The patient died, not of this disease, which apparently had lasted about $1\frac{1}{2}$ years, but of an old chronic myocarditis.[10] The cells at the periphery of the liver lobules were pigmented only to the degree found in rabbits and showed no evidence of injury. But here the parallel ceases. For whereas in the rabbits an hepatic cirrhosis was absent, in the human liver a strikingly abundant one was found, interlobular, and of long standing, as shown by its character. Save in the cells of the many new-formed bile ducts, the cirrhotic tissue contained almost no pigment. That the connective tissue overgrowth was in this instance the result of parenchymal destruction from excessive pigmentation is out of the question. One is led inevitably to conclude that it must have been a primary feature of the patient's disease.

If the fact is granted, as the evidence warrants, that cirrhosis is a primary occurrence in some cases at least of hemochromatosis, this does not mean that it is without a relation to the characteristic pigmentation. Kretz[12] has shown that cirrhosis of any sort is frequently accompanied by a more or less marked siderosis of the liver. He found such a pigmentation in fourteen out of twenty-six cirrhotic livers, whereas in only one of every fifteen or twenty that were non-cirrhotic was it present. The rabbit material has furnished striking proof that when a tendency to siderosis exists an intercurrent cirrhosis will

[12] Kretz, R., *Centr. allg. Path. u. path. Anat.*, 1897, viii, 620.

greatly increase the deposition of pigment. In one transfused animal with a very moderate general siderosis of the liver an adhesion had occurred connecting the anterior edge of a lobe with the abdominal wall and thus inducing a well marked local cirrhosis. Here the liver cells were so heavily pigmented as in many instances to be scarcely recognizable and in others to have broken down (Fig. 12). The contrast to the parenchyma elsewhere in the organ was extreme. In a second animal, as well, a local cirrhosis of unknown origin was accompanied by great local pigmentation. It is perhaps not a matter of accident that the rabbit with the most pigmented liver had not been transfused longest but was the subject of an intercurrent cirrhosis. In the course of 167 days it had received 139 transfusions—52 of 10 cc. each and the last 87 of 15 cc. each. A companion animal of the same weight and sex, receiving in 194 days 156 transfusions, the last 99 of 15 cc. each, had no cirrhosis of the liver and exhibited by contrast little pigmentation.

All this gives good reason for the supposition that the pigmentation of the liver cells in human hemochromatosis is in large part, if not entirely, secondary to the cirrhosis. Whether the connective tissue overgrowth of the pancreas that is so often observed is an essential phenomenon of the human disease or is secondary to the abundant parenchymal destruction, our work does not enable us to say. But a similar fibrous overgrowth, which is obviously of secondary character, may often be observed in the heavily pigmented heart of hemochromatosis cases, and sometimes in the thyroid gland.

Injury and Pigmentation.

Signs of an old perirenal inflammation existed in one of the transfused rabbits, and in the scar tissue here numerous connective tissue cells were noted containing hemosiderin. The observation led us to inject agar into the other animals of the series some days before killing them, with a view to determining whether iron would be laid down in the reactive tissue. Such was the case. The cells in the interior of the agar mass were unpigmented, which was to have been expected, since they were in process of rapid proliferation, but in the older reactive tissue hemosiderin was fairly abundant as small granules in the fibro-

blasts (Fig. 13). Its constant presence and general distribution rules out the possibility that it was derived from local hemorrhages.

A similar relation of injury to pigmentation was observed in the human instance of hemochromatosis already several times cited. The arteries showed scleroses here and there. In the sclerotic patches iron was sometimes encountered in quantity (Fig. 14), whereas in the healthy arterial wall it was entirely lacking. The ability of hepatic cirrhosis to induce pigmentation is, of course, only another example of the effect of injury, but of injury this time to an organ essentially concerned in the degradation of blood pigment.

A simple explanation now suggests itself for the curious distribution of the cutaneous pigmentation in human hemochromatosis. The face and neck to the collar line, and the backs of the hands are the surfaces which become most bronzed and are often the only ones affected. These are precisely the surfaces most subjected to light, wind, and other injurious influences, such as may be supposed from the evidence just given to conduce to a deposition of pigment. There is, however, no gainsaying the fact that not every sort of injury can bring about cutaneous siderosis, else one would expect it on the soles of the feet, for example. But the data given by Kretz show that even in the liver the injury must be of a special sort if iron is to be laid down. He has pointed out that neither the liver degenerations which result from phosphorus and arsenic poisoning, nor chronic passive congestion, nor cholelithiasis with jaundice suffices to cause the least hepatic siderosis.

An Aid to Diagnosis.

Hemosiderin granules were frequently found lying free in the lumen of the kidney tubules of the heavily pigmented rabbits, and they were also noted embedded in casts (Fig. 4). The fact suggested that in human beings a diagnosis might be made through the demonstration of hemosiderin granules in the urine. This would be far preferable to the excision of a piece of skin, the procedure usual at present. A diagnosis has been accomplished by the method, as is shown in another paper.[10]

On the Sequence of Events in Human Hemochromatosis.

The hemochromatosis of transfused rabbits, as thus far studied, has points of difference from the human disease, but also important points in common with it. The points of difference are in large part those of degree and of time. For instance, none of the animals developed diabetes; but for that matter in none was the pancreas greatly damaged as it is in *diabète bronzé*. The pigmentation of the pancreas, as far as it went, was like that occurring in man. This example well illustrates the general variances and likenesses found. The pigmentation of the rabbits was strikingly similar to that in human beings, not merely in kind but in distribution to the organs and in them. The sole difference in the pathological findings which must be considered as essential was the total absence in rabbits of the hepatic cirrhosis which in man is a constant and pronounced feature of hemochromatosis. There are good reasons, already stated, for the belief that this cirrhosis is a primary factor in the disease.

Most of the recent evidence in the literature is against the older view of excessive blood destruction in an otherwise healthy organism as the cause of hemochromatosis. So too are our experiments with rabbits. The transfused animals had disposed of immense quantities of blood before any noteworthy pigmentation appeared. Some of them received in the course of each week during a long period an amount of blood almost as great as their own original total quantity (5.5 per cent of the body weight).[13] The organism was required to dispose of this and of its own worn out corpuscles as well, yet at most the pigmentation was moderate compared with that found in man. Needless to say this excessive and continued blood destruction could not be supported by the human body without notable changes in the circulating red cells and the blood-forming organs, and these are completely absent.

While an increased destruction of red cells cannot be the primary cause of hemochromatosis, yet certainly one need look no further than these elements for the source of the hemosiderin. The presence of the pigment in such peculiar situations as the heart muscle and the parenchyma of many glands has led some authors to suppose that there must

[13] Boycott, A. E., and Douglas, C. G., *J. Path. and Bacteriol.*, 1909, xiii, 256.

be a derangement of these tissues, causing them to elaborate hemosiderin out of their own proper substance. But in our rabbits the pigment laid down was manifestly attributable to erythrocytes undergoing destruction, and it was found in precisely the same situations as in human hemochromatosis.

The activity of the liver is known to be profoundly important to the working over of blood pigment. The sequence of events in human hemochromatosis would seem to be the following: A liver cirrhosis of unknown origin leads to a failure of the organ to deal adequately with the iron-containing products of normal blood destruction, and the latter accumulate in the organism. Widespread pigmentation results automatically. It at length becomes so great as to cause the death of parenchymal cells, especially in the liver and pancreas, thus leading to secondary connective tissue overgrowth. This, in turn, through injury to the remaining parenchyma, hastens the accumulation of pigmentation. By a repetition of the vicious circle what is essentially a chronic disease becomes at length a rapid progress down-hill. Diabetes appears in many cases as a result of the pancreatic injury, and soon death results. There is no link in this chain of events for which the facts do not afford strong evidence.

SUMMARY.

In rabbits destroying transfused blood constantly during a period of many months a pronounced and widespread siderosis ensues, practically identical with that characterizing human hemochromatosis. The findings do not indicate the ultimate cause of this disease, but they throw light on its various features and its course, and suggest a means for its diagnosis.

EXPLANATION OF PLATES.

The sections from which Figs. 1 and 8 are taken were stained with methylene blue and eosin. Nishimura's method was used with all the other material to demonstrate iron-containing pigment, and lithium carmine was employed as a counterstain.

PLATE 64.

FIG. 1. Spleen of a rabbit (A) transfused 139 times during a period of 167 days. The sinuses are distended with blood. An atrophic Malpighian corpuscle may be

seen near the center of the photograph. The black masses consist of hemosiderin for the most part extracellular.

FIG. 2. Bone marrow of the same animal. The hemosiderin appears black, as is the case also in the photographs which follow.

PLATE 65.

FIG. 3. Liver of the same animal as in Figs. 1 and 2. The cirrhosis is intercurrent. The iron is especially abundant towards the periphery of the lobules, but the parenchyma shows granules of it everywhere.

FIG. 4. Kidney of a rabbit (B) transfused 120 times in the course of 180 days. The glomeruli are free from pigment, but it is present in all the tubules except the collecting ones, being especially abundant in the ascending limb of Henle's loop and in the distal convolutions. A cast is to be seen containing many fine hemosiderin granules.

PLATE 66.

FIG. 5. Pancreas of a rabbit (C) transfused 156 times in the course of 194 days. The alveolar cells towards the center of the photograph contain hemosiderin in especially large amount.

FIG. 6. Iron-containing pigment in the peripheral cells of the zona glomerulosa of the adrenal gland. Rabbit C. The connective tissue capsule of the organ occupies the upper part of the photograph.

PLATE 67.

FIG. 7. Heart muscle of Rabbit A. The hemosiderin is especially abundant at either end of the nuclei of the muscle fibers.

FIG. 8. Section of a lymph gland draining the liver of Rabbit A. Large aggregates of extracellular hemosiderin are to be seen.

PLATE 68.

FIG. 9. Skin from the abdomen of Rabbit B. Iron-containing pigment is present in scattered cells of the corium.

FIG. 10. Hemosiderin granules in the alveolar cells of a submaxillary gland. Rabbit C. The patchy localization here seen was present throughout the gland.

PLATE 69.

FIG. 11. Mammary gland of Rabbit A, an old female. Many of the heavily pigmented cells have desquamated. The animal had not been pregnant during the period of transfusion.

FIG. 12. Heavy pigmentation secondary to a local cirrhosis of the liver. Rabbit B. Non-cirrhotic tissue may be seen at the lower left hand corner of the photograph. Here the pigmentation of the parenchyma is by contrast very slight.

PLATE 70.

FIG. 13. Hemosiderin in cells of the reactive tissue surrounding a mass of agar-agar. Rabbit B. The clear material is the agar, which is in process of organization. Iron-containing pigment is present only in the older scar tissue, not where the cells are actively proliferating.

FIG. 14. Sagittal section of a small artery from the patient with hemochromatosis. Iron-containing pigment has been deposited where the media is degenerated. The rest of the vessel wall is free from it.

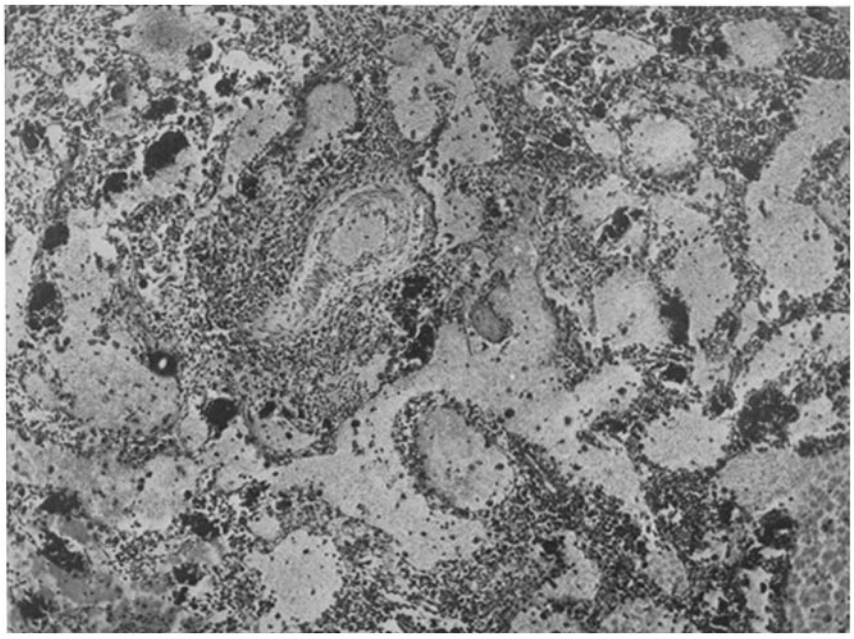

FIG. 1.

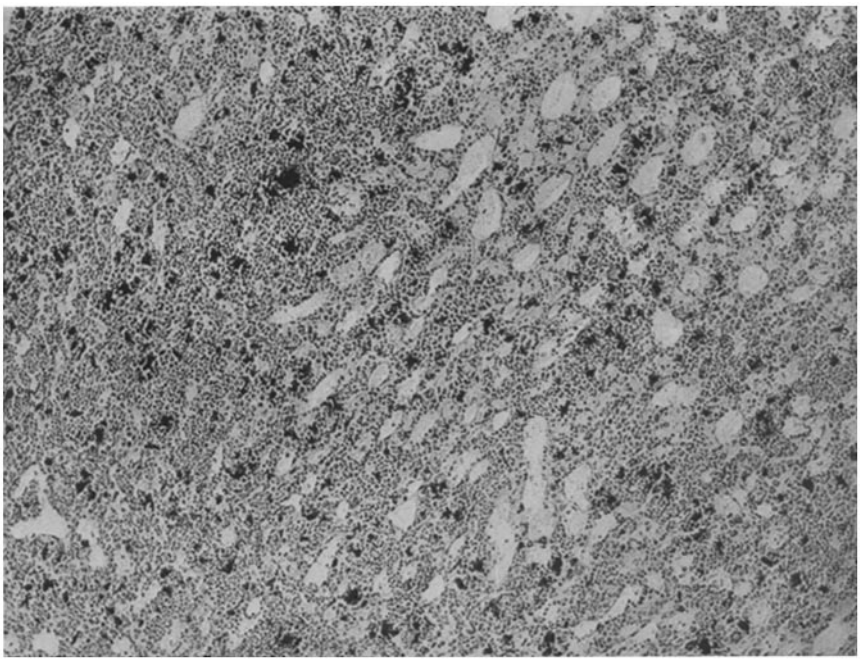

FIG. 2.

(Rous and Oliver: Experimental hemochromatosis.)

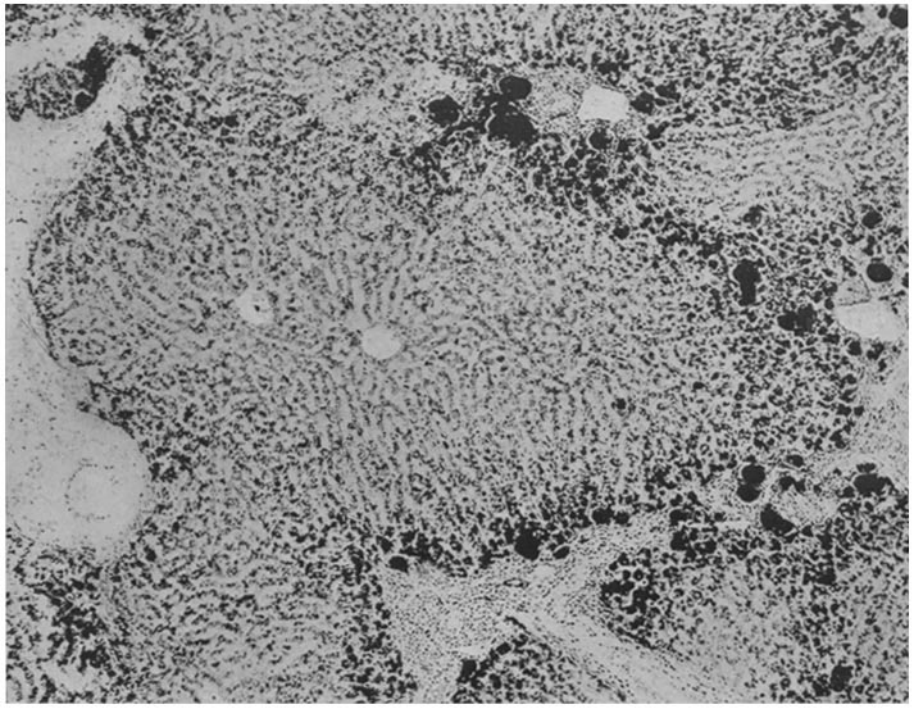

FIG. 3.

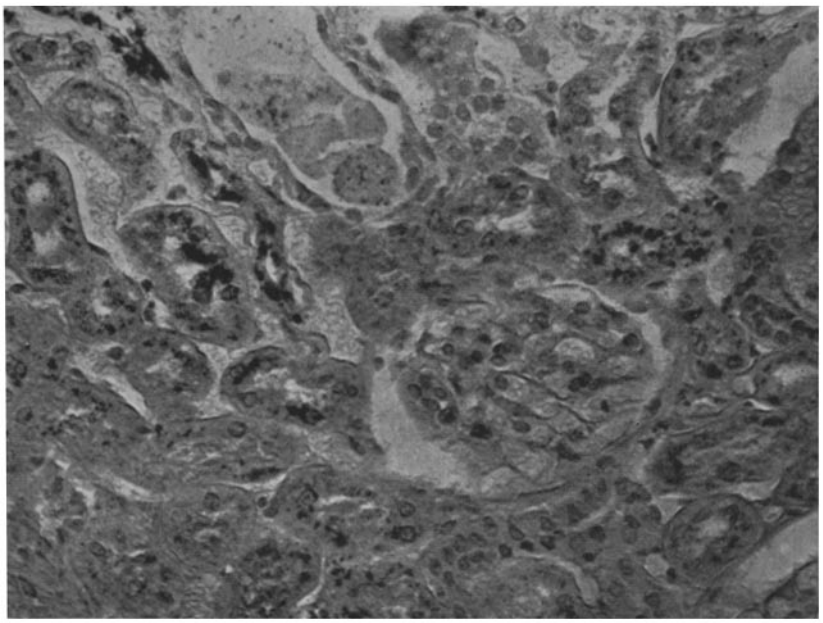

FIG. 4.

(Rous and Oliver: Experimental hemochromatosis.)

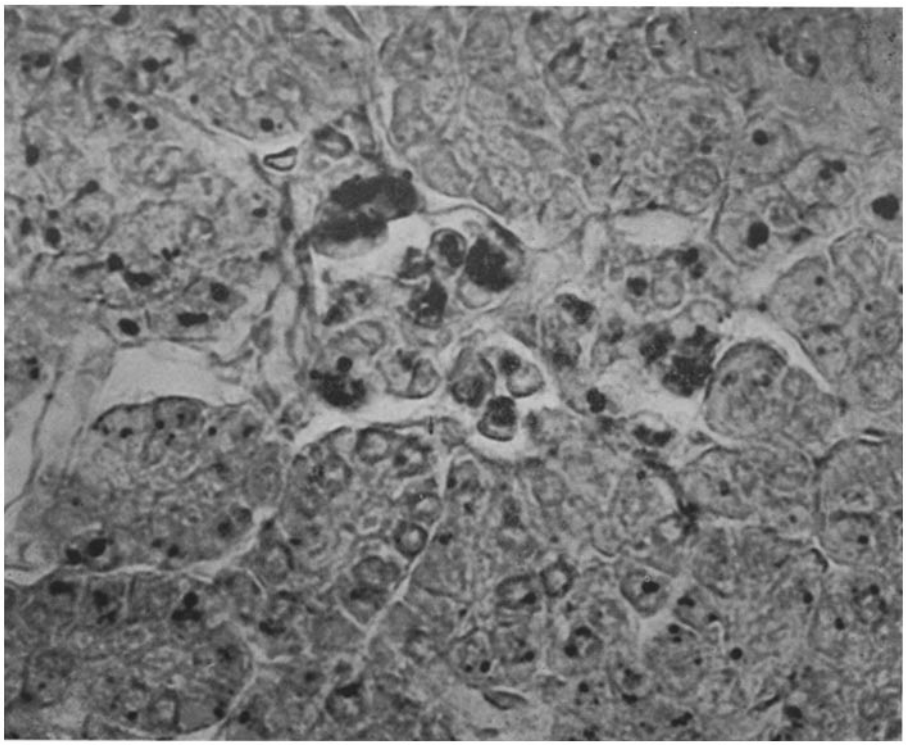

FIG. 5.

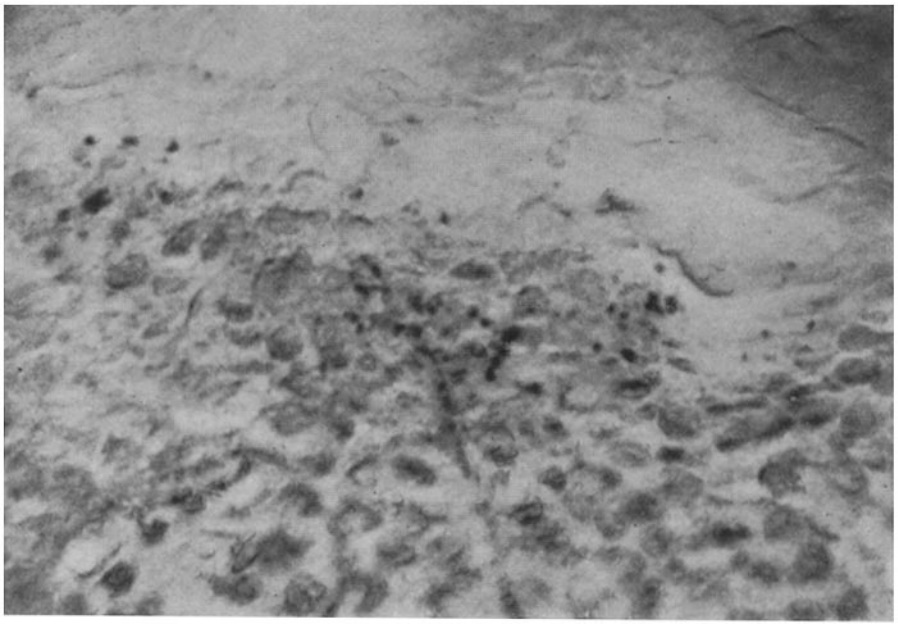

FIG. 6.

(Rous and Oliver: Experimental hemochromatosis.)

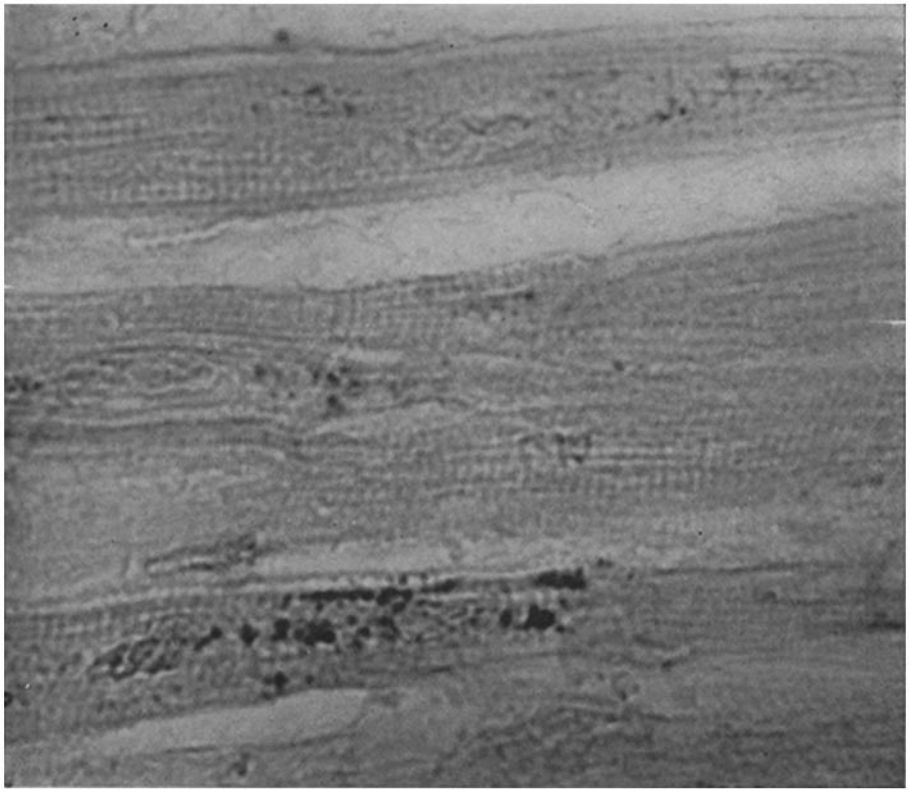

FIG. 7.

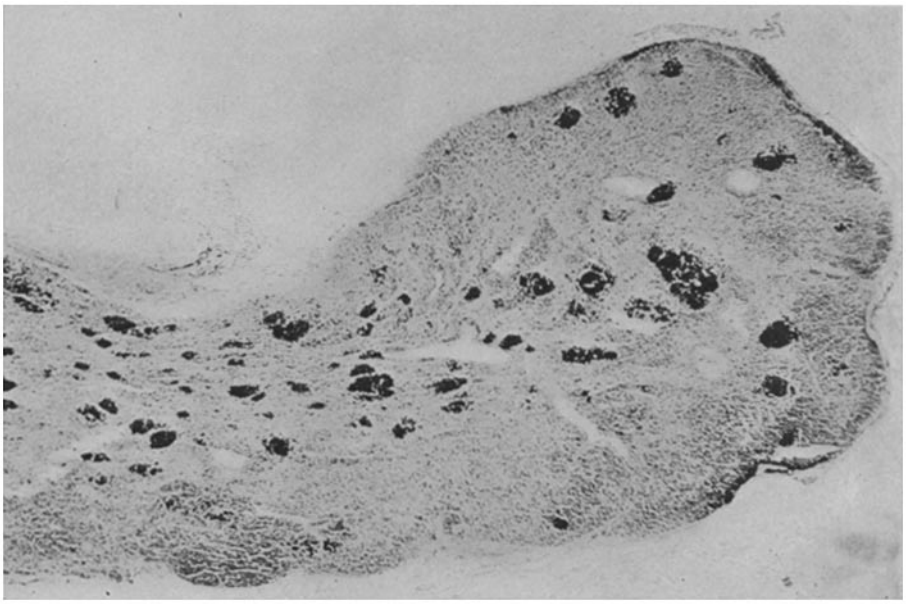

FIG. 8.

(Rous and Oliver: Experimental hemochromatosis.)

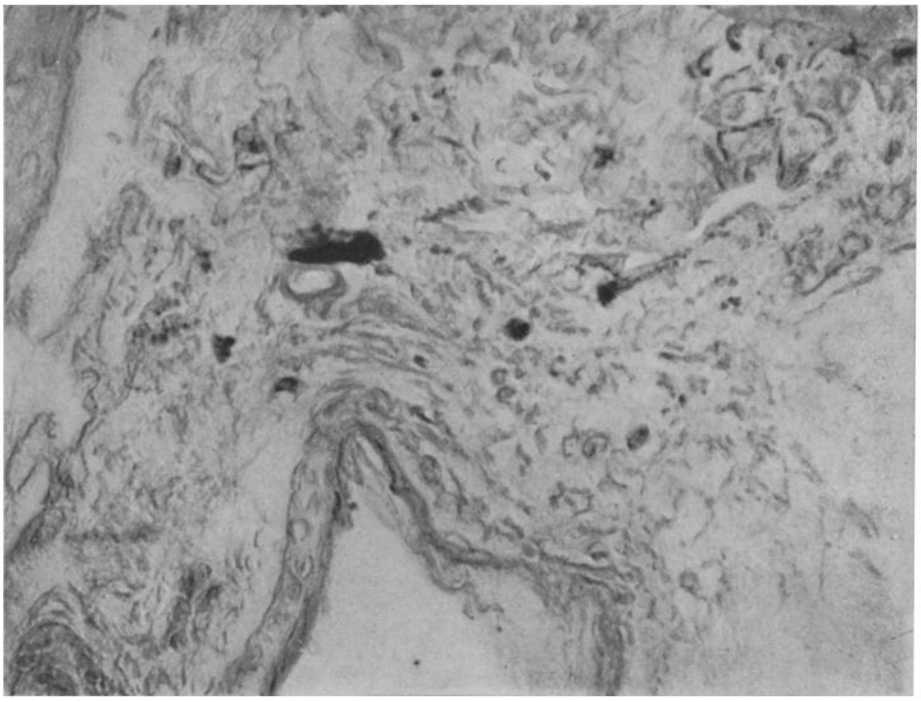

FIG. 9.

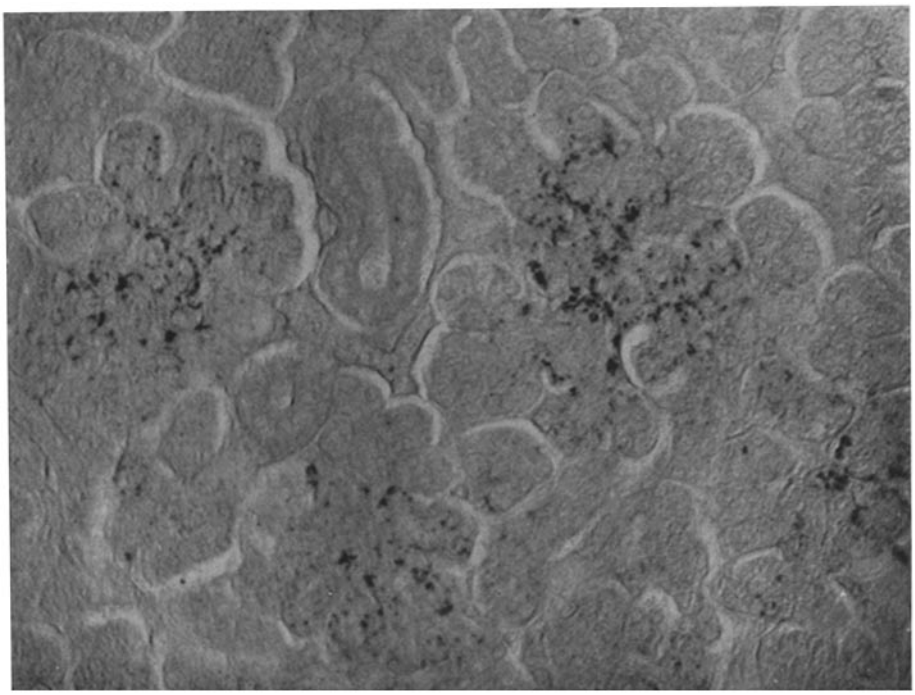

FIG. 10.

(Rous and Oliver: Experimental hemochromatosis.)

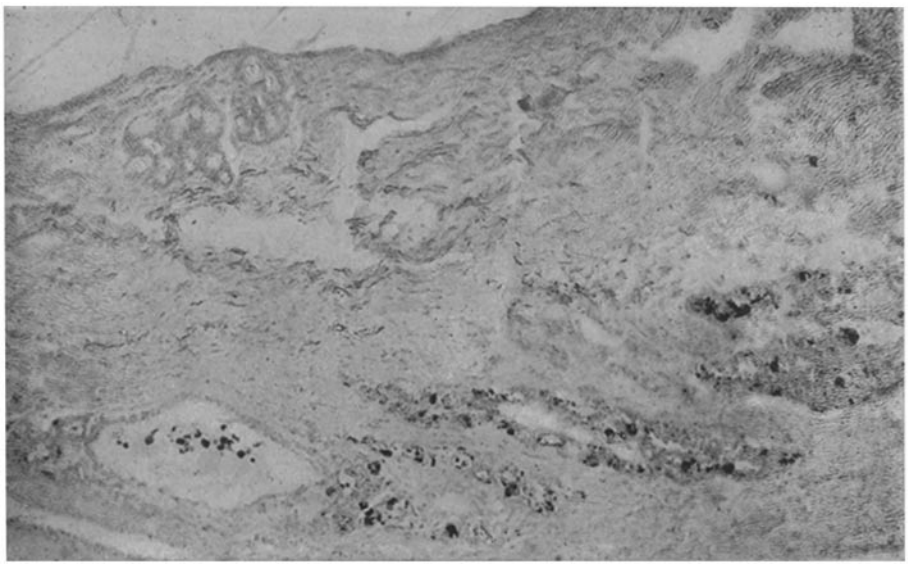

FIG. 11.

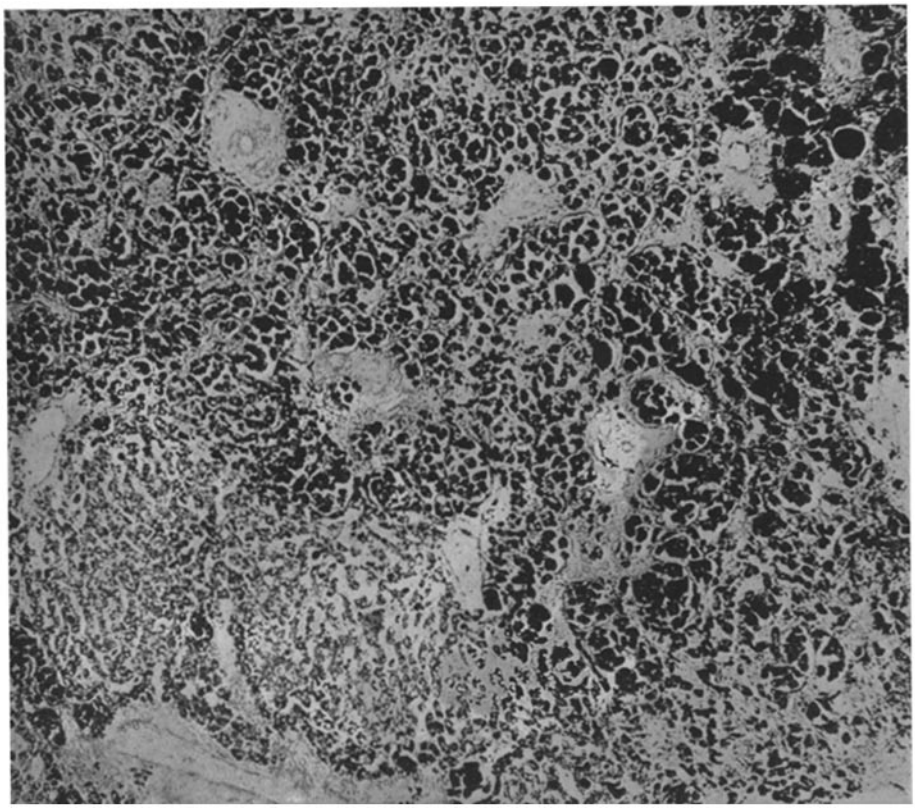

FIG. 12.

(Rous and Oliver: Experimental hemochromatosis.)

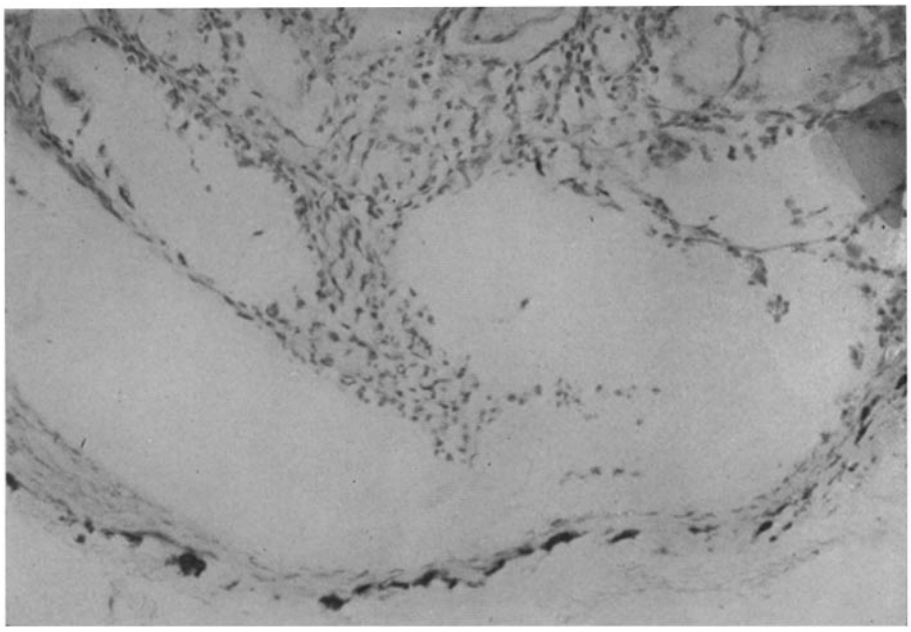

FIG. 13.

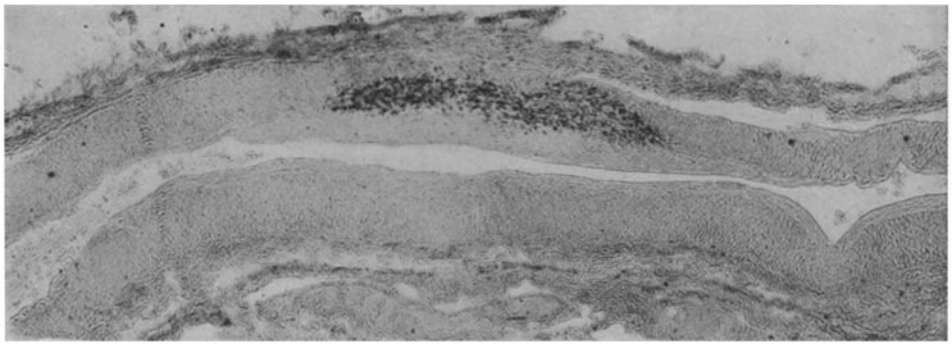

FIG. 14.

(Rous and Oliver: Experimental hemochromatosis.)