PIGMENT FORMATION IN THE LIVER DURING AUTOLYSIS AND ITS RELATION TO THE PIGMENTATION OF HEMOCHROMATOSIS.*

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While engaged in studying the problems presented by the disease known as hemochromatosis, a recent paper of Brown (1) impressed us with the possibility that the knowledge of autolytic changes might be of aid in the explanation of the pigmentation occurring in this and some other diseases.

Several authors (2) have insisted that hemosiderin occurs in the parenchymatous cells of the liver, especially in those cells which show some evidence of degeneration. Particularly is this the case in hemochromatosis in which there is an enormous production of an iron-containing pigment in the parenchymatous viscera, especially in the liver and pancreas. In the liver there may be seen all stages from the cell in a fair state of preservation with a few granules to the cell whose membrane seems stretched by the load of pigment and whose nucleus is barely perceptible. Still other masses of pigment occur about which no cell membrane can be recognized, but which in size and general appearance resemble the heavily loaded liver cells. Thus through cell degeneration and irritation of the interstitial tissues through the deposit in them of pigment, there arises a productive inflammatory process which results in a fibrosis of the organ. Similar processes, although less marked, take place in other viscera.

The understanding of the disease, the cardinal features of which are the general pigmentation, cirrhosis of the liver, and diabetes, lies in the explanation of the pigment production since most of the evidence at hand points to this condition as the primary event. Inasmuch as most of the pigment contains iron in an easily

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demonstrable form, it has been generally assumed that it had its origin in the hemoglobin of the red blood cells, and a primary disease of the blood or some alteration of the blood associated with excessive intravascular destruction of the red blood cells has been invoked to explain the unusual accumulation of hemosiderin.

One of us (3) has recently discussed this question at some length and taken the view that there is little justification for the assumption of a primary blood dyscrasia. In those cases in which records of blood studies are available, we find either normal pictures or a slight secondary anemia which might well be expected considering the condition of the patients. Examination of the bone-marrow in the cases reported from this laboratory showed no evidence of unusually active hematopoiesis. Furthermore, the distribution of the pigment in hemochromatosis greatly exceeds and varies widely from that found in pernicious anemia in which the pigmentation is universally ascribed to blood destruction. Finally, we cannot consider as due to hemolysis a striking feature in hemochromatosis, the accumulation in several organs of pigments which have no relation to hemoglobin.

The alternative suggested is that of a widespread simultaneous parenchymatous cell degeneration of a special nature affecting many organs leading to the deposition of a variety of pigments, the composition of the pigment in any one cell being determined by the chemical processes going on there. The iron of the hemosiderin we must refer to the so called masked, organic or tissue iron which we have reason to believe occurs in especially large quantities in those cells which characteristically are the seats of the hemosiderin deposits (4). The tissue iron must be replenished, doubtless, by the blood, but we believe it has been shown in the above mentioned paper (3) that this may be done by the iron derived from the physiological dissolution of the red cells. Such an explanation calls for a marked iron retention in the progressive stages of hemochromatosis, a point at present under investigation.

Brown has reported upon the pigmentary changes in the rabbit liver taking place during autolysis in moist chambers under aseptic conditions. The exuded fluid from the autolyzing tissue was studied and small pieces of tissue were removed at intervals and fixed in alcohol. He reports hemosiderin as of prac-

tically constant occurrence in the liver, and that during about 48 hours of autolysis there was a definite increase in the granular hemosiderin of the liver cells and in the formation of masses of hemosiderin in the capillaries and larger vessels. Coincident with or following close upon this increase, there was an increase in the hematoidin in the fluid and the development in the liver cells of an iron-free pigment partially soluble in chloroform and alcohol, in which solvents hematoidin and other bile pigments could then be demonstrated. Finally, the hemosiderin of the liver cells was observed to lose the iron reaction as the nuclei underwent dissolution, and large amounts of colorless and pigmented amorphous and crystalline material, containing both phosphorus and iron, appeared throughout the section, but more particularly in the portal spaces. The fact that the increase in pigmentation took place best at a temperature of 37° C. and near the exposed surfaces suggests that hemosiderin is "an oxidation product of hemoglobin due to enzyme action." This is the first instance in which hemosiderin has been shown to be formed outside the living animal body.

This method of studying pigmentary changes in organs by submitting them to autolysis offers an opportunity of testing one of the disputed features of hemochromatosis which we have mentioned; namely, the possibility of the formation of hemosiderin within parenchymatous cells in the absence of blood. Accordingly we undertook a series of experiments in which the rabbit liver was first thoroughly perfused with Locke's solution and then placed in a moist chamber under aseptic conditions and allowed to undergo autolysis.

EXPERIMENTAL PART.

Method.—The rabbit's abdomen was first opened, a clamp placed at the hilum of one lobe of the liver to prevent the perfusion of that lobe, and the abdominal incision temporarily closed. A jugular vein and carotid artery were next exposed, a cannula was inserted in the jugular vein, a nick made in the carotid artery and the perfusion begun with sterile, oxygenated Locke's solution. The operations were performed under aseptic conditions. When the effluent from the carotid became clear, the cannula was inserted into the portal vein and the hepatic vein above the liver severed. At the end of the perfusion the liver, excepting the portion previously clamped off, appeared entirely bloodless although in some cases a few red blood cells were found on microscopic examination.

The liver was then removed, a small piece taken from both perfused and non-perfused portions, and fixed at once in 90 per cent. alcohol.

The rest was placed on small glass stands in large sterile Petri dishes, 23 cm. in diameter and 8 cm. deep. A small dish of water and one containing toluol were placed on the floor of the larger vessel. Oxygen was passed into the vessels through a small tube four or five times during the day. Two vessels were used for each rabbit, usually one standing at room temperature and the

other placed in the thermostat at 37° C. In some experiments one or both of these moist chambers were placed in direct sunlight. Small pieces were removed at intervals and fixed in 90 per cent. alcohol and in formalin. The alcohol-fixed tissues were embedded in celloidin and three sets of sections prepared, one unstained, one stained in hematoxylin and eosin, and a third treated by Nishimura's method for iron. This method, which has given us the best microchemical results, consists in immersing the sections in ammonium sulphide for one hour, washing in water, treating with a mixture of equal parts of 2 per cent. potassium ferrocyanid and 1 per cent. hydrochloric acid for 20 minutes, washing in warm ½ per cent. hydrochloric acid and then thoroughly in distilled water.

Results.—The changes occurring during autolysis have been well discussed by Brown. We have been able to confirm many of the points in his report, while some few points of difference will be mentioned. We have not followed the later stages, confining our studies to the alteration occurring during the first two or three days.

The change in the color of the tissues when examined grossly is often striking and is limited, as Brown pointed out, to the exposed surfaces. When the liver rests upon a glass slide through which the light strikes it from below, the color of this surface is much paler than of parts exposed to the air. Diffuse daylight has little effect in hastening the color changes, a temperature of 37° C. in a dark thermostat being more effective.

The most rapid and striking changes, however, occur in direct sunlight. Within a very short time the darkening may be noted and especially along the edges and angles of the tissue. After several hours' exposure most of the surface of the perfused liver becomes a much darker brown while that of the blood-containing lobe is often almost black. Cross section reveals a border 2 or 3 mm. thick which is darker, dryer, and apparently much more condensed than the central part. These changes in direct sunlight occur almost as quickly when the dishes are packed in ice to prevent elevation of the temperature.

In his histological description of the alcohol-fixed tissue, Brown makes use of certain terms which it may be convenient for us to follow. He states that a section of liver from an average animal, fixed in alcohol, will show the following: "(1) A peripheral zone of pigmented material reacting for iron and containing granular, pigmented masses and patches of pigment which do not react for iron. (2) A zone of condensation with granular and diffuse pigmentation of all elements which react decidedly for iron as well as granular pigment which does not react. (3) The depths of the section show a few granules of hemosiderin and a few granules of bile pigment in the liver cells, the cells of Kupfer, and the leucocytes. (4) The tissue elements do not react for iron."

In tissues which have undergone autolytic changes he speaks further (5) of the "development of a zone beneath all exposed surfaces in which all changes are most pronounced and which might be termed the zone of reaction."

These different zones are readily recognized, but we do not find

a constant iron reaction in the peripheral and condensed zones, nor do we find in most cases hemosiderin present in the liver cells of the rabbits. In fact in only four of fifteen rabbits was hemosiderin present in appreciable amounts. In most of the others there were granules of pigment of similar appearance and distribution, which failed, however, to give an iron reaction even with prolonged treatment with ammonium sulphide, or after preliminary treatment with hydrogen peroxide. These pigments whether iron-containing or not were usually increased during autolysis both in the perfused and non-perfused liver tissue. In three cases there was very definite increase in the amount of hemosiderin in the cells of the perfused liver, especially well seen in the zone of reaction just beneath the exposed surfaces. No distinct difference was noted in these specimens at the end of twenty-four hours of autolysis at room temperature, but at 37° C. the changes during this period were indicated by the increased size and number of the hemosiderin granules throughout the section. In specimen 4, for example, the increase was very definitely shown at the end of seventy hours at 37° C., when in sections stained for iron, the zone of reaction stood out as a broad blue band beneath the surface easily visible to the naked eye. Its prominence was doubtless due in part to the shrinkage of the tissues and the consequent concentration of the increased amount of pigment into a smaller space. This zone is shown by the microscope filled with pigment granules which react for iron and also stain darkly with hematoxylin. In unstained sections they appear as bright yellow granules of various sizes. In the depths of the section, at this stage, the pigment is less conspicuous than in the control tissue fixed immediately after the animal's death.

In only one instance was hemosiderin formed during the autolysis of a liver in which it was not found in the tissue fixed immediately after the death of the animal, and this occurred in the lobe containing blood. The increase in intracellular iron-containing pigment seemed almost, if not quite as great in the perfused as in the blood-containing portion of the same liver.

In several instances the iron-free pigment was increased during autolysis and often to a greater extent in the perfused than in the blood-containing lobe. The pigment disappears under the influence

of alkalis and more slowly in hydrogen peroxide. It is not readily soluble in mineral acids. Fat stains and fat solvents have no apparent effect upon it, and we are inclined to consider it analogous to that found in the so called brown atrophy of the liver, in the reticular zone of the adrenal cortex, and in the cardiac muscle fibers, a product presumably of the proteid constituents of the cell in which it occurs.

SUMMARY.

Without claiming any close analogy between the autolytic changes and pigmentation in pathological processes, it has, we believe, been shown that iron-containing, as well as other kinds of pigments, may be formed during the autolytic degeneration of parenchymatous cells independently of the hemoglobin in the blood stream, and hence may be derived from the proteid constituents of the cell itself.

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