CHANGES IN THE HEMOSIDERIN CONTENT OF THE RABBIT'S LIVER DURING AUTOLYSIS.[1]

By W. H. BROWN, M.D.

(From the Pathological Laboratory of the University of Wisconsin.)

The investigation of the changes in the hemosiderin content of the rabbit's liver during autolysis was undertaken with the hope that such an investigation might shed some light on the vexed problems of the mode of formation of the hematogenous pigments, their relation to one another and to hemoglobin, and their ultimate disposition. The work has yielded some very definite results which will be described in the present paper. The author feels that the study of hemosiderin and related pigments under autolytic conditions presents so many technical difficulties and so many sources of possible error as to render a report of the more minute details inadvisable at the present time. For this reason, the scope of this investigation, and of this report, has been confined to those changes which are most evident, and where comparative estimates are based on changes so manifest as to preclude all possibility of error.

It is well to note that the term hemosiderin is here used, as originally applied by Neumann,[2] to indicate the yellow or yellowish brown, hematogenous pigment which reacts for iron with potassium-ferrocyanide and hydrochloric acid or with ammonium sulphide.

The rabbits used for this work may be grouped in four classes: (1) normal (for laboratory animals); (2) infected animals; (3) animals injected with saponin; (4) miscellaneous animals used in the laboratory for other purposes.

TECHNIQUE.

Both control and autolysed tissues have been fixed in 95 per cent. alcohol and in 10 per cent. formalin, as a routine. These have been supplemented by other fixatives, in some instances.

[1] Received for publication May 31, 1910.

[2] Neumann, *Virchow's Arch.*, 1888, cxi, 25.

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Autolysis.—Both toluol and chloroform have been employed as preservatives in separate series. Autolysis has been conducted on two general plans.

1. Autolysis in moist chamber with toluol or chloroform as preservative. The liver has been cut into strips 6 to 8 mm. in thickness (the larger the piece that can be kept sterile the better), and placed in large air-tight Stender dishes. The atmosphere of the dish is renewed by frequently removing the cover, and moisture is maintained by moist cotton or water in evaporating dishes.

2. Autolysis in liquids. Salt solution (0.85 per cent.) and distilled water have been employed. Autolysis has been attempted by placing tissues directly into chloroform and toluol. This was done solely as a check upon the effects of these agents upon the tissues.

Autolysis has been carried out at room temperature (20° to 22° C.) and at 37° C. for periods of time up to 17 days.

Sections.—Paraffin and celloidin have been used for sections. In all cases sections stained with hematoxylin and eosin have been compared with unstained mounts and with sections tested for iron. Other procedures have been employed occasionally in attempts to clear up the nature of certain substances.

Determination of Iron.—(1) Ferrocyanide or ferricyanide of potassium (2 per cent. aqueous solution) and 1 per cent. aqueous hydrochloric acid, employed together in equal volumes or used separately. 1 per cent. acid alcohol has been used in a few instances as a check. (2) Ammonium sulphide, diluted one-half.

So much has been said of micro-chemical methods for demonstrating iron that I feel that any attempt to explain my choice of these methods would only add to the existing confusion. The one point I wish to emphasize is the necessity of performing the reaction on the slide and observing the development of the reaction under the microscope. If this precaution is not observed, many important features of the reaction may be entirely overlooked. The time allowed for the end reaction has in no case exceeded one hour, where comparative estimates were concerned.

HEMOSIDERIN CONTENT AND IRON REACTIONS OF NORMAL RABBIT'S LIVER.

The liver of the average laboratory animal varies so widely that it is difficult to establish an absolute normal for either cellular picture or pigment content. From the study of a large number of livers, I find that many livers show some degree of fatty degeneration. The cells are prone to be coarsely granular or "foamy" in character, and practically all livers show some granules of yellowish, or greenish brown hemosiderin as well as similar granules which give no iron reaction, presumably bile pigments. It is not uncommon to find the hemosiderin in the form of clear, refractile granules with a pigmented periphery. The hemosiderin frequently stains slightly with hematoxylin or with eosin, producing almost

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black granules in the one case, and copper colored granules in the other. Hemosiderin that stains with hematoxylin usually reacts more quickly and more intensely for iron than that staining with eosin. The type of iron reaction, as here indicated, is quite variable. Using the ferrocyanide method, one may obtain all shades of blue from an exceedingly dark blue to a very light or pale blue. Kupfer's cells and the cells in the liver capillaries occasionally show hemosiderin granules. The amount of hemosiderin varies within narrow limits in different portions of the same liver. No other elements regularly react for iron with the technique here employed. Exceptionally, however, some liver cells show a few colorless or faintly pigmented granules in the nucleus which stain with hematoxylin or, more rarely, with eosin, and which give a decided iron reaction. Occasionally, the entire nucleus reacts for iron, and the cell protoplasm may give a faint, diffuse reaction. Likewise, the connective tissue, particularly about portal vessels, and the vessel walls may give the most intense iron reaction. All of these reactions are more marked and more frequent after alcohol than after formalin fixation, and when tissues are not fixed immediately on the death of the animal. None of these features can be considered strictly normal.

One other feature deserves special mention and this is not peculiar to the rabbit. All tissues fixed in alcohol show a peripheral zone of condensation. This entire zone, in the rabbit's liver, both tissue elements and vascular contents, takes a copper-colored stain with eosin and gives a definite reaction for iron. Outside of the tissue proper is a layer of coagulum showing the above character and containing a number of irregular black or greenish brown, refractile, pigmented masses. In the rabbit, patches of greenish yellow are not uncommon and may extend into the zone of condensation. Unstained sections show these zones to be truly pigmented, containing both granular pigment and a diffuse pigmentation of tissue elements and vascular contents. These are features which have undoubtedly been noted by many but no one seems to have attached any importance to them.

In brief then, a section of liver from an average animal, fixed in alcohol, will show:

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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.

DELAYED FIXATION.

As a preliminary to the study of the effects of true autolysis, some experiments were undertaken to determine the changes in the hemosiderin content of the liver after the fixation of large pieces of liver or whole livers in alcohol of various strengths (50 per cent. to 95 per cent.). Under such conditions fixation is delayed, in some cases for from three to four days where whole livers and weak alcohols are used. The center of such tissues shows changes comparable, in some respects, to autolysis. The changes in this series of experiments are described as the effects of delayed fixation.

The change from the normal picture in delayed fixation is quite striking in many cases and may best be illustrated by a typical experiment.

H. R. E.—Normal rabbit, male, albino, weight 2,500 grms. Killed by blow on head. Small pieces of liver fixed in 95 per cent. alcohol and 10 per cent. formalin for control. One of the largest lobes placed in a small amount of 95 per cent. alcohol at 37° C.

Control.—The control (alcohol fixed) corresponds closely with the above description of the normal liver though slightly more fatty.

Twenty-one hours at 37° C.—Section taken which extends through entire thickness of the lobe. The central portion is not yet fixed. The liver cells stain more decidedly with eosin and the protoplasm is more granular. Most of the granules show a slight affinity for hematoxylin. The pigment granules of the liver cells average twice that of the control. The peripheral and condensation zones appear on the two capsular surfaces and correspond closely with the control. Central from the zone of condensation, about 2 mm., there is an irregular band, parallel to the surface, several millimeters in breadth, in which the cells show a slight, copper colored staining. The granular pigment in this area is strikingly increased, averaging 4 to 6 times that of the control.

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Unstained sections show peripheral and condensation zones comparable to the control. There is a band of faint, yellowish, diffuse pigmentation just central from the zone of condensation. This also shows marked increase in the granular pigment of the cellular elements. The granular pigment is increased throughout the section to an average of double the control.

Iron Reactions.—Grossly, there is a broad band of marked reaction on the two capsular surfaces. Central from this, there is a second band of slightly less decided reaction, while the entire section shows a heavier reaction than the control. Microscopically, the peripheral and condensation zones show a reaction about parallel with the control. The second band, above mentioned, shows a marked reaction in the cell protoplasm, though more pronounced in the nuclei. There is also a marked increase in the granular pigment reacting for iron. Cell nuclei in the depths of the section show a faint iron reaction with intranuclear granules which react sharply. The cell protoplasm reacts very slightly. Pigment granules (hemosiderin) are increased throughout. The connective tissue throughout the section also reacts faintly for iron.

Forty-eight hours at 37° C.—The section taken as above. Fixation is complete. The features of the 21-hour stage are but very slightly altered. The iron reaction in the central portion is slightly more pronounced and the hemosiderin slightly increased.

This experiment shows the lesser changes which have prevailed with normal animals and those showing minor degrees of fatty degeneration in the liver. Animals with infections, such as staphylococcus, and animals injected with saponin to produce destruction of red blood corpuscles and increased pigment production, present the same general changes, though to a much more marked degree. This is illustrated by the following case.

H. R. I. Gray and white, weight 1,900 grms. Between Jan. 7 and 24, the animal received seven injections of saponin in the ear vein, amounting to 19 mg. The animal died soon after the last injection. Autopsy was performed one hour after death. Tissues were treated as in H. R. E.

Control.—Liver shows foci of necrosis and hemorrhage with marked congestion. The liver cells contain numerous dark brown granules of hemosiderin, grouped particularly about the nuclei. The nuclei are large and vesicular with many slightly pigmented basophilic granules which react for iron. The foci of necrosis and hemorrhage show a faint diffuse iron reaction in all structures and slightly more granular pigment than other portions of the section. The connective tissue throughout reacts faintly for iron. The zones of condensation and peripheral coagulum react strongly for iron.

Twenty-one hours at 37° C., in 95 per cent. alcohol.—Section taken as with H. R. E.; center is unfixed. Cell nuclei are large and vesicular. The granular bodies of the nucleus have largely disappeared. The nuclei and remaining granules react strongly for iron. The hemosiderin of the liver cells is increased, on an average, 4 fold. The cell protoplasm and connective tissue throughout react strongly for iron. While there is a marked increase in iron

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reacting material of the capillaries and deeper vessels, those nearer the capsular surfaces (exposed surfaces) show the most marked changes. In these areas the entire red blood corpuscle content is fused into large and small globular and angular masses, many of which are grouped into clusters with clear refractile bodies at the center. Most of these bodies show a darkly pigmented periphery and are identical with granules of hemosiderin in the section. When unstained, all such masses are yellowish brown in color. They stain a coppery red with eosin. The entire mass reacts strongly for iron, perhaps the central crystalline body most intensely. The capsular surfaces show the two zones of the control section and a third zone which is identical in character with that described for H. R. E. This zone shows intense pigmentation, diffuse and granular, and the iron reaction is so intense as almost to obscure the structural elements.

Ninety-six hours at 37° C., in 95 cent. alcohol.—This stage shows but little change from the 21-hour stage. The granular hemosiderin is slightly increased and Zone "3" is broader. The iron reaction is intensified in all particulars.

While these two examples, taken from eighteen such experiments, represent, in type, the usual findings, such changes have not always been so marked, nor have I found them constantly. In a few instances, I have obtained a reduction in the hemosiderin content, but this has been accompanied by the appearance of a marked nuclear reaction for iron and, occasionally, by a diffuse reaction of liver cells and connective tissue similar to that just described.

The changes which should be particularly noted in delayed fixation are as follows: (1) increase in the hemosiderin content of the liver cells; (2) formation of hemosiderin in large amounts directly from the red blood corpuscle contents of vessels; (3) development of a diffuse pigmentation and iron reaction in the cellular elements; (4) development or intensification of a nuclear reaction for iron; (5) development of a zone beneath all exposed surfaces in which all changes are most pronounced and which might be termed the "zone of reaction."

AUTOLYSIS.

For maintaining antiseptic conditions during autolysis, toluol is to be preferred to chloroform. Though the latter is more efficient in preventing bacterial growth, it is very apt to fix the tissues and prevent autolysis. Pieces of tissue placed directly in chloroform are fixed slowly with marked shrinkage and distortion of cellular elements. All red blood corpuscles are laked and the hemoglobin

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coagulated on the surface in irregular globular masses which are yellow or brownish in color. A large part of such masses manifests a faint though definite iron reaction. Other changes, comparable to those noted under delayed fixation, are observed, though all such changes are on the surface or in superficial parts of the tissue. The pigment content in the depths of the section is usually reduced.

Of the methods of autolysis which I have employed, the moist chamber with toluol as preservative has given the most constant and trustworthy results. Submersion in liquids, except perhaps toluol, is not permissible, as some products formed during autolysis are soluble in such liquids and are lost from the section. In order not to burden this report with too many details of the different stages of autolysis, I will give the essential facts deduced from this entire series of experiments.

A definite increase in the hemosiderin content of the liver has been noted as early as the fifth hour, but, as a rule, the change is not marked before the twelfth or even the twenty-fourth hour. After such an interval, the exposed surfaces of the liver have become brown in color, while the side upon which the strip rests is of its original color or paler. Cross section shows this pigmentation extending two to four millimeters into the substance of the tissue. If the strip is turned frequently during autolysis all surfaces will show this change. Examination of the material oozing out of the liver, shows large and small masses of both colorless, refractile material, and pigmented material, reacting for iron, as well as numbers of hematoidin crystals. Sections, at this stage, show on the surface, variable amounts of angular and globular pigmented masses reacting for iron. There is a narrow zone of condensation (alcohol fixed tissue) which usually reacts only faintly for iron, and a third "zone of reaction" similar to that described with delayed fixation. This zone is much more pronounced in autolysis and, in sections tested for iron, it stands out grossly, as a band two to four millimeters in breadth about all surfaces exposed to the air during autolysis, while the unexposed surface shows little or no trace of such a zone.

In the depths, the granular hemosiderin in the liver cells is in-

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creased, on an average, two fold, and there is a corresponding increase in iron-containing pigment in the capillaries and larger vessels. Stained sections show that as the nuclei undergo autolysis the hemosiderin and granular material of tissues and vessels take a more or less decided hematoxylin stain, as might be expected. The granules in cell nuclei, and occasionally entire nuclei, react for iron. All other tissue elements react with varying intensity. These changes in iron reaction are nearly all referable to varying degrees of pigmentation shown in unstained sections. The maximum change of this character has usually been reached before forty-eight hours at 37° C. Occasionally, the maximum was shown at seventy-two hours, 37° C.

After the maximum of pigmentation and iron reaction has been reached, a second series of changes gradually becomes predominant. These changes are first noted in the depths of the tissues and are coincident with the dissolution of the cell nuclei, succeeding the stage noted above when the hemosiderin and other granules show definite hematoxylin staining. The hemosiderin granules gradually become brighter in color and more decidedly yellow, both in stained and unstained sections. The iron reaction in pigment granules gradually decreases and ultimately fails entirely. There is no apparent reduction in the amount of such granular pigment, and in some cases there seems to be an increase. Irregular, pigmented masses become more numerous in the capillaries between the columns of liver cells. Large amorphous and crystalline masses of both colorless and dark greenish brown material collect irregularly through the section and particularly in and about the vessels and bile ducts of the portal spaces. The material in the portal spaces is practically always pigmented. The crystalline masses occur in many forms, irregular plates, rosettes or clusters of needles or rhombic plates, and large feathery crystals. The latter are usually colorless, exceedingly hard and glass-like, and it is almost impossible to obtain good sections where such are abundant. The colorless masses and crystals stain a pale slaty blue with hematoxylin, while the pigmented forms appear unaffected by stains.

Such pigmented material is slightly soluble in water, more so

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in alcohol and is quickly dissolved or destroyed by dilute mineral acids or by alkalies. The solution in any case, unless it be with alkalies, is not complete as there always appears a dark or yellowish brown residue when the process is observed under the microscope. In a few instances, where one per cent. aqueous hydrochloric acid was employed, fine crystals of hematoidin appeared about the site of the original mass. The solubility of the colorless forms has been more difficult to follow, though it is, apparently, similar to that of the pigmented forms.

A large part, and perhaps all, of these masses react for iron and for phosphorus by the method of Lilienfeld and Monti.[3] If the Prussian blue method is employed in testing for iron, by following the reaction under the microscope, the amorphous or crystalline material will be observed to disappear with the production of a diffuse flocculent precipitate of Prussian blue about its former site. Portions of such masses, or an occasional crystal, may react without dissolution, in which case the Prussian blue is formed *in situ*. Material, apparently identical with that described above, occurs in abundance as a gritty, crystalline deposit in the *brei* in the dish used for autolysis. When tissues are autolyzed in liquids, no such crystals and amorphous masses are found in the sections, nor to any extent in the liquids.

The series of changes initiated by the disappearance of the iron reaction from the hemosiderin of the liver cells in the depths of the tissue, and culminating in the formation of amorphous and crystalline masses containing both iron and phosphorus, is in progress for several days. During this period, such changes approach the more superficial parts where pigment production and iron reaction are most pronounced. This zone resists such transformation for a variable length of time, and, while the change ultimately becomes marked, it is never so complete as in the other parts of the section, as far as my experiments have extended (seventeen days at 37° C.).

Returning to the yellowish granular pigment in the liver cells, which no longer reacts for iron, I may say that it has been impossible for me to determine with certainty what this pigment is. The color is slowly and incompletely extractable with alcohol and with

[3] Tracy, *Jour. of Med. Research*, 1906, xiv, 447.

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chloroform, apparently more readily soluble in alcohol than chloroform, but with difficulty in either. Alkalies and mineral acids either extract or destroy the color. After the extraction of the color of these granules I am convinced that the body of the granules still persists as an insoluble residue. From the above facts I believe that the pigment in question belongs to the class of bile pigments.

I have found it impossible to exercise a check on the changes in the bile pigment content of the liver cells with any degree of accuracy. The use of mercuric chloride as a fixative has been recommended, but I have found it of little value. The occurrence of hematoidin crystals in the *brei* and the solution of bile pigments in the alcohol used as a fixative have served as rough guides. In the *brei,* hematoidin crystals usually appear within ten to twelve hours and gradually increase in number for two to three days, after which they decrease, and are perhaps replaced by other oxidation products. In stages as late as fifteen days, I have found them practically absent.

In studying the *brei* for hematoidin crystals, I became impressed by the fact that they were almost always attached to or imbedded in slightly pigmented or, more often, perfectly colorless, refractile masses along with a few small masses of hematin. Such a matrix always reacts most intensely for iron and is incomparably greater in amount than either, or both, the hematoidin and the hematin. The hematin apparently develops after the addition of dilute acid to clear the preparation or in applying the test for iron. Hematin has never been found in any considerable amount in any of my preparations except in sections and tissues kept for some time in alcohol.

This raises a point in technique which is of utmost importance, namely, that the tissues should be sectioned and studied at once, without being allowed to stand in alcohol. Tissues or sections kept in alcohol change very much in a short time. The three changes of most importance which I have noted are: (1) solution of certain products; (2) decrease and marked alteration in the type of the iron reaction coincident with; (3) the appearance of enormous amounts of hematin throughout the section.

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In one instance a pronounced iron reaction was present in the nuclei and diffusely through other elements when tested immediately after fixation. One month later, section left in 80 per cent. alcohol showed practically no iron reaction, but, instead, granules of hematin were found in practically every nucleus and diffusely throughout the sections. Similar changes, though less marked, have been noted in a number of instances.

In recasting the results obtained in this investigation, certain features stand out prominently. First, in both the experiments with delayed fixation and with autolysis, there is 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 as well as in the diffuse type of pigmentation. Second, coincident with this increase in hemosiderin during autolysis, or, more likely, following closely upon such increase, there is an increase in the hematoidin in the *brei,* and the development in the liver cells of granules of pigment which do not react for iron, but are partially soluble in alcohol and in chloroform; hematoidin and other bile pigments are demonstrable in such solutions. Finally, the hemosiderin of the liver cells loses its iron reaction as the nuclei undergo dissolution, and large amounts of colorless and pigmented, amorphous and crystalline material, containing both phosphorus and iron, appear throughout the section, but more particularly in the portal spaces.

These findings show, most conclusively, that hemosiderin may be formed in organs outside the animal body, such formation continuing, under the conditions herein employed, for twenty-four to forty-eight hours after the death of the animal. The conversion of large amounts of hemoglobin *en masse* in the vessels shows that hemosiderin can be formed directly from hemoglobin.

The evidence as to the character of the process and the factors concerned is not entirely conclusive. The fact that such changes take place best under conditions favoring enzyme action suggests the possibility of such an agent. The action of the agent in question is not confined to the tissue cells but is equally active on the contents of relatively large vessels and on hemoglobin on the surfaces of tissues as well as in the *brei* in the moist chamber. The invariable predominance of pigment production on and in surfaces

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exposed to the air deserves especial emphasis, as it suggests most strongly that oxygen is a potent factor in hemosiderin production.

The idea that hemosiderin can be produced *post mortem,* or can be formed except by the activity of a living cell, is opposed to present teachings.[4] The activity of an oxidizing enzyme has been suggested by Neumann.[5] So far as I am aware, all theories that have been advanced to explain hemosiderin production have considered hemosiderin as a derivative of hematin. If it is intended to convey the idea that hemosiderin is derived from the hematin moiety of hemoglobin, without the necessity of hematin being formed as an essential step in hemosiderin production I can offer no objection to such theories on this ground. On the other hand, the idea that hematin is a necessary antecedent of hemosiderin is untenable. I have carefully traced the formation of hemosiderin from hemoglobin through all stages of autolysis and have observed nothing that would lead me to suspect that the production of hematin was in any way essential. Further than this, hemosiderin is a proteid substance while hematin is the non-proteid or pigment radicle of hemoglobin, a cleavage product. Hemosiderin can also be shown to be capable of cleavage into hematin and a proteid by the action of acids as dilute as 5 per cent. sulphuric. This can be applied to sections and watched under the microscope.

The relation of hematoidin to hemosiderin is not entirely clear. It seems to be formed in greatest abundance subsequent to the formation of hemosiderin. The strongest evidence of the relationship of these two pigments has been derived from the phenomena described in the *brei* of autolyzing tissues. It will be recalled that I mentioned the occurrence of large, faintly pigmented, or colorless, hyaline masses, reacting strongly for iron, attached to or imbedded in which were numerous hematoidin crystals. Such pictures may have been chance findings but they have been exceedingly frequent. Virchow,[6] in his classical monograph, both described and pictured this relationship essentially as I have observed it, except for the iron reaction. While he refers to hematin as the mother substance, it must be borne in mind that the terms "hemoglobin" and "hemo-

[4] Neumann, *Virchow's Arch.,* 1904, clxxvii, 401.

[5] Neumann, *loc. cit.*

[6] Virchow, *Virchow's Arch.,* 1847, i, 379.

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siderin" had not been introduced at that time, and he must have referred to the coloring matter of the red blood corpuscles. This picture represents my views at the present time of the relationship of hemosiderin and hematoidin, namely, that hematoidin is the pigment radicle of hemosiderin. When the hematoidin is removed, the residue is a colorless, refractile substance, reacting for iron; or, stated differently, hemosiderin is a colorless iron-containing proteid plus hematoidin.

Before passing to the final series of events, I wish to consider briefly those anomalous iron reactions occurring in nuclei, cell protoplasm, and connective tissue, which as I have mentioned are occasional findings at death, and develop during delayed fixation, and in the early stages of autolysis. Such phenomena occurring under the last two conditions are capable of at least three interpretations: (1) changes in the cell constituents capable of rendering masked iron demonstrable; (2) the formation from hemoglobin of a substance capable of manifesting an iron reaction; (3) the decomposition or diffusion of some iron-containing substance similar to hemosiderin.

The first of these explanations seems the least likely of the three. It is wholly inadequate to explain those cases which occur at death. Such reactions occur in animals in which blood destruction and hemosiderin formation are above normal. Granular, pigmented bodies are occasionally demonstrable in such nuclei. These granules stain with greater or less intensity with hematoxylin, but frequently show a refractile, unstained center. The iron reaction is usually confined to these granules, though the entire chromatin network may react. Further, the hemosiderin is closely packed about the nucleus and can be found penetrating the nuclear membrane. The explanation of the nuclear reaction, in these cases, is undoubtedly referable to the hemosiderin and, as I believe, to its decomposition rather than to its production, though both are possible factors. The reaction of other elements is more likely due to excessive production of hemosiderin.

The development of an iron reaction in nuclei during autolysis is undoubtedly due in part to hemosiderin production, as the reaction is shared by all structures. Further, the formation of hematin

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in such nuclei, and its diffuse distribution through the tissues after keeping sections in alcohol, coincident with the disappearance of the iron reaction, indicate that hemosiderin is an important factor in such iron reactions. It must be admitted, however, that it is impossible to prove that autolysis has not rendered demonstrable the masked iron of such tissues, though I hardly believe the autolytic changes, occurring in the alcohol experiments, can be sufficient to account for the iron reactions obtained in this series.

From the explanation of these peculiar iron reactions, and from changes noted in the exposed portions of tissues during autolysis, we find an explanation for the iron reacting coagulum and zone of condensation of normal liver fixed in alcohol. While it is impossible to explain either of these solely on the theory of solution or diffusion of hemosiderin, or contraction *per se,* and while both of these factors may be accessory, there is certainly an actual conversion of hemoglobin into hemosiderin in these areas.

The final series of changes concerned with the disappearance of the iron reaction from the hemosiderin and the formation of amorphous and crystalline material containing both phosphorus and iron, is of especial interest. While it is impossible to exclude other factors absolutely, the circumstances under which these compounds are formed indicate strongly some interaction between the nuclear phosphorus and the iron of the hemosiderin. It is quite certain that the iron of the hemosiderin is split off coincidently with nuclear dissolution which probably supplies the phosphorus. Further than this we can not go. The exact nature of the compound in question has not been investigated. Bile pigments are an admixture in some cases. The residue of the hemosiderin, after the cleavage of the iron, also contains extractable bile pigments.

This peculiar series of phenomena, taken in conjunction with the nuclear picture in animals where hemosiderin production and metabolism are excessive, furnishes an interesting clue to the vital metabolism of hemosiderin in the liver and the fixation of its iron content. Hemosiderin seems to be formed as an oxidation product of hemoglobin, and in its further metabolism an acid radicle of the nucleoproteid binds the iron, while the coloring matter, hematoidin, is excreted as the bile pigment.

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Changes analogous to those described for the liver have been observed in the kidney, spleen and mesenteric lymph nodes of the rabbit, though they have not been studied to any considerable extent.

Further experiments upon the relation of oxygen to hemosiderin production and the effect of organ extracts upon hemoglobin are in progress. Results, thus far obtained, seem to confirm the results of the present investigation.

CONCLUSIONS.

1. Hemosiderin may be produced outside of the animal body and is increased in the liver of rabbits, during autolysis, for a period of from twenty-four to forty-eight hours.

2. Post-mortem hemosiderin formation is most marked in parts of livers exposed to air, and hemosiderin is, apparently, an oxidation product of hemoglobin due to enzyme action.

3. Hemosiderin is derived from hemoglobin directly and not from hematin as an intermediate product.

4. The later stages of autolytic changes show that the acid products of proteid autolysis, especially the phosphorus acids, are capable of producing a cleavage of hemosiderin and uniting with the iron to form a new series of products which react microchemically for both phosphorus and iron. In such cleavage, pigments analogous to the bile pigments are formed.

5. The relationship observed between hemosiderin and hematoidin is such as would indicate that hematoidin is the pigment matter of hemosiderin.

6. Further, it seems probable that the vital cycle of hemoglobin metabolism in the liver is largely intranuclear; the hemoglobin is converted into hemosiderin either within the nucleus or cell protoplasm; the iron of the hemosiderin is bound by an acid radicle of the nucleo-proteid, and the hematoidin is excreted as bile pigment.

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