THE STRUCTURE AND COMPOSITION OF HEMOSIDERIN.

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INTRODUCTION.

In the spleen, liver, and kidneys of most mammals may be found varying quantities of yellow-brown granules and to these granules has been given the general name hemosiderin. Since the formation and location of hemosiderin coincides in time and place with the destruction of blood, the idea has become firmly established that hemosiderin represents some step in the disintegration of hemoglobin and the formation of bile pigment. The question has come under consideration in many connections throughout the fields of physiology and clinical medicine. The great range of literature in which there is mention of hemosiderin may be seen in the reviews of Whipple (1), Rous (2), and Rich (3). These papers summarize and discuss the present status of the problems concerning the normal and abnormal destruction of red blood corpuscles and formation of bile pigment. It is clearly brought out that hemosiderin is in some way connected with these processes but the extensive although scattered evidence does not conclusively indicate the exact nature of the reactions involved. Nor is much known of the constitution of the substance itself save that it contains iron in some form. The fact that hemosiderin has a high iron content has led many investigators to conclude that in the breakdown of hemoglobin the iron is split off from the hematin as hemosiderin and the iron-free remainder of the molecule goes to make up the bile pigment (cf. Rich (3)). This view has found its way into the text-books (such as Wells, "Chemical Pathology"). It has seemed desirable to omit entirely all con-

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sideration of the source or function of hemosiderin and to confine this investigation solely to the question of its actual composition.

The attention of investigators has been centered principally on the fact that hemosiderin contains a large amount of iron. That this is true has been often demonstrated by various means, both on the granules in situ and on various tissue preparations (cf. Abderhalden (4), Oppenheimer (5), Wells (6), Muir and Dunn (7), McMaster, Rous, and Larimore (8), Muir and McNee (9), Boycott and Douglas (10), Brown (11), Oberzimmer and Wacker (12), etc.). If it is assumed, therefore, that iron is present in the hemosiderin granules, the most important question chemically is this: in what form does the iron exist? Several opinions have been expressed, of which the more representative are these:

1. The iron is combined with an organic molecule. Brown (11, 13) thinks that hemosiderin is an iron-protein compound.

2. The iron is inorganic. The brown color of the granules and the ease with which the color is extracted with acid lend support to the idea that the granules are masses of ferric (or possibly also ferrous) oxide or hydroxide. One of the chief exponents of this view is Fischer (14). He says, "He [referring to Hueck] emphasizes that if one treats sections containing hemosiderin with acid it is possible to demonstrate the presence of iron, and with sufficiently large quantities to precipitate with soda hydrated iron oxide, 'a body which resembles hemosiderin in nearly all respects.'" Fischer says later, "regarding hemosiderin, since it cannot be extracted by water, it is most probable that it is nothing other than finely divided elementary iron, overcast with a layer of oxide which gives the pigment its color."

Aside from the fact that no evidence of any metallic iron can be discovered, the experiments reported here tend to confirm the view that in hemosiderin the iron is inorganically bound.

**EXPERIMENTAL.**

The material used was horse spleen, fresh from the slaughterhouse, which was selected because of its high hemosiderin content. Preliminary analyses showed that the hemosiderin and iron

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1 Hueck, Pigmentstudien, Habilitationsschrift, Munich, 28-29 (1912).
2 Translated by the author.
content run parallel. The cells are usually packed full of granules and in such cases the iron may be as much as 4 per cent of the fresh weight of the spleen. For work on the granules in the tissue under the microscope, sections were made with the microtome from frozen blocks of the fresh organ. They were then washed several times in distilled water to remove what blood might remain. This procedure obviates any disturbance due to fixation or preservatives. The larger granules correspond roughly in size to red blood corpuscles; the smaller are barely visible under the ordinary high power. The method used for obtaining them free from the tissue is described below.

**Chemical Examination of Granules.**

1. **Properties of Acid Extract of Granules.**—0.5 gm. of tissue was cut into thin sections and treated with 60 cc. of 30 per cent HCl. It was centrifuged and the clear liquid used for the following tests: (a) NaOH gives a brown precipitate of Fe(OH)_3. (b) K₄Fe(CN)_6 gives a heavy precipitate of ferric ferrocyanide. (c) KCNS gives a characteristic deep red color. (d) (NH₄)₂S gives a heavy black precipitate. These four qualitative tests all show the typical reactions of ferric iron. The extract with strong HCl, therefore, contains the iron in simple ionic form.

2. **Reactions of Granules in Tissue.**—Thin sections of fresh tissue were treated with the same reagents with the following results: (a) NaOH has no apparent effect whatever on the granules. (b) K₄Fe(CN)_6 + dilute HCl causes the granules to turn a blue-green which becomes true Prussian blue on warming (cf. Oberzimmer and Wacker (12)). This reaction is not typical. Free ferric iron should turn deep blue immediately. (c) KCNS + HCl causes a general, diffuse, faint coloration of the entire tissue after about 30 minutes, but there is no localization of color in the granules. The general colorization may come from free iron present either as an impurity (e.g. from the section knife) or from the slow action of the dilute HCl on the granules. The striking point in this experiment is that there is no characteristic coloration due to the iron in the granules themselves. (d) (NH₄)₂S causes intense blackening of the granules. The black may be removed by dilute acid, with the evolution of H₂S gas, indicating that the black substance is FeS which is formed from ferric salte by reduction. (e) K₃Fe(CN)₆
Hemosiderin has no effect whatever. The total absence of Turnbull’s blue suggests that there is no ferrous iron present, although the anomalous behavior of the ferric reactions makes it impossible to insist on the point.

Of the first four reagents only one, ammonium sulfide, reacted in a typical manner. The most interesting deviation from what might be expected is the failure to get a good color with thiocyanate, since this is one of the most delicate known tests for ferric iron. The presumption therefore is that the iron in the granules is not in the ionic form. But free ferric iron is obtained in the liquid when the granules are extracted with strong acid. The effect of the latter must be to remove the iron from an organic compound if there is one, or to alter some molecular to the ionic state.

3. Reactions of Granules after Extraction with Acid.—10 per cent hydrochloric or sulfuric acid causes the hemosiderin to bleach and become colorless in a few minutes. With weak acid a longer treatment in the cold, or a brief boiling, brings about the same bleaching of the tissue. After extraction the customary colors are not obtained with ferrocyanide or ammonium sulfide; in other words there is no reaction for iron at all. The latter is evidently quantitatively removed from the granules by the acid. The characteristic color of the granules is destroyed in the process.

In order to confirm the above results it seemed advisable to try to separate the granules from the mass of tissue in which they are embedded. For it is sometimes difficult to distinguish the granules from the cells around them, especially after bleaching, and furthermore no quantitative work can be done in the presence of a great quantity of organic debris. Several methods were tried, of which the following gave the best results. From a fresh spleen the pulp was scraped out and digested in the cold for several days with 3 per cent sodium or potassium hydroxide. (Previous examination had shown that digestion with alkali did not alter the characteristic reactions of the granules.) After the proteins had been hydrolyzed the somewhat syrupy fluid was centrifuged and the sediment washed (by centrifuging) several times with 3 per cent soda or potash and then with distilled water. The product was a suspension of hemosiderin almost entirely free from organic matter. The effect of digestion with concentrated alkali will be discussed in another connection.
The experiments reported above were now repeated and in every instance the results were the same; there was a blue-green color with ferrocyanide and no color with acid thiocyanate. This demonstrated that the hemosiderin had not been essentially altered during the digestion and washing.

As it was now possible to follow the behavior of the granules, unobscured by any extraneous material, the following observations were made relative to their structure.

1. It has been stated above that strong HCl completely removes the iron and decolorizes the granules. This decolorization was now carefully followed under the microscope. In the acid the granule slowly loses its brown color, becomes yellow, and ultimately entirely colorless. In the final stage it has the appearance of a ghost in that it retains its original size and shape. Although hyaline and almost transparent it remains the definite body it was in the first place.

2. At various stages of the bleaching the granules were treated with ammonium sulfide, which reacts with any iron present to give a black or gray color. In the early stages the black is merely less intense, but as the general fading continues one gets a less and less intense darkening with sulfide until finally this substance has no effect whatever. In many cases, however, the black appears as localized regions within the granule. There may be one or several of these regions in the interior, the periphery being colorless. The larger granules tend to exhibit this phenomenon more than the smaller.

From these data the conclusion may be drawn that hemosiderin granules have a definite physical structure, and are not irregular particles of a homogeneous chemical compound, such as ferric hydroxide. For were they the latter it is to be expected that removal of the iron by HCl would break up or alter the structure of the molecules and completely disintegrate the particle. At least there would be some change in the size or shape of the particle. It is more reasonable to suppose that the granule consists of a mass of inert (organic) material in some way heavily impregnated with an iron compound, and that the acid chemically or physically leaches out the iron, as it were, starting with the periphery and eventually penetrating to the deeper parts. The unevenness which characterizes the removal of iron (as indicated by ammo-
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Nitric acid may be due to variation in the physical dimensions, or the density, of the granule or possibly may be due to differences in the concentration of the iron within.

Hydrochloric acid in all concentrations removes the iron, as ferric chloride, from hemosiderin but some other acids behave very differently, especially nitric and acetic acids, as the following series of experiments shows. In all the analyses for iron the substance was evaporated to dryness, ignited, and taken up with H$_2$SO$_4$. The iron was reduced by zinc metal and titrated against standard permanganate.

**TABLE I.**

<table>
<thead>
<tr>
<th>Sample A.</th>
<th>Sample B.</th>
<th>Sample C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placed in 10 per cent HCl and allowed to stand 20 hrs.; centrifuged and fluid decanted. Fluid. Clear, yellow; 3.35 mg. Fe.</td>
<td>10 per cent HNO$_3$. Same as for Sample A. Clear, colorless; 2.5 mg. Fe.</td>
<td>10 per cent CH$_3$COOH. Same as for Sample A. Slightly opalescent semicolloidal deep red-brown; 4.35 mg. Fe.</td>
</tr>
<tr>
<td>Residue. Treated with 5 cc. distilled water and allowed to stand 1 day; centrifuged; fluid colorless and clear; 0.82 mg. Fe.</td>
<td>Treatment same. Fluid yellow-brown opalescent; 1.87 mg. Fe.</td>
<td>Treatment same. Fluid clear and colorless; 0.45 mg. Fe.</td>
</tr>
</tbody>
</table>

Three lots of hemosiderin, each containing 5 mg. of iron, were placed in three test-tubes and the procedure adopted as shown in Table I. In each case the difference between 5 mg. of iron and the total quantity shown represents that contained in the second residue which was not extracted by acid.

The HCl removes the iron in ionic form and apparently the HNO$_3$ at this concentration also does so. But the fact that when the residue from HNO$_3$ is placed in distilled water a brownish solution appears, suggests the possibility that (1) the 10 per cent nitric acid did not remove all the iron (also indicated by the iron analyses)
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or that (2) more dilute nitric acid might take out the iron in a different form. The acetic acid removed almost all the iron from the granules but not in the same manner as the other two acids. Rather the extract from 10 per cent acetic acid resembled the distilled water extract from the original 10 per cent nitric acid residue. Therefore it was necessary to investigate more closely the concentration relations of the two acids.

(a) Nitric Acid.—Suspensions of hemosiderin were made up with different concentrations of HNO₃ and the color of the extract observed. The results, which are given in Table II, show that nitric acid in great dilution has no effect on hemosiderin, in medium concentration (0.05 to 1 per cent) it extracts a red-brown pigment,

<table>
<thead>
<tr>
<th>Concentration of HNO₃</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>per cent</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Colorless, good test for ferric iron.</td>
</tr>
<tr>
<td>4</td>
<td>Same.</td>
</tr>
<tr>
<td>2.5</td>
<td>Slightly yellow.</td>
</tr>
<tr>
<td>2.0</td>
<td>Quite yellow.</td>
</tr>
<tr>
<td>1.5</td>
<td>Orange.</td>
</tr>
<tr>
<td>1.0</td>
<td>Red-brown, slightly opalescent.</td>
</tr>
<tr>
<td>0.4</td>
<td>Same.</td>
</tr>
<tr>
<td>0.05</td>
<td>&quot;</td>
</tr>
<tr>
<td>0.005</td>
<td>Colorless, very faint test for ferric iron.</td>
</tr>
</tbody>
</table>

and in higher concentration it behaves like hydrochloric acid and removes ferric iron.

(b) Acetic Acid.—A suspension of hemosiderin which by analysis was found to contain 10 mg. of iron per cc. was divided into four lots of 1 cc. each. Each sample was then made up to a definite concentration of acetic acid. From the results in the tabulation

<table>
<thead>
<tr>
<th>Acid, per cent...............</th>
<th>Sample A.</th>
<th>Sample B.</th>
<th>Sample C.</th>
<th>Sample D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color of extract.............</td>
<td>2 Red-brown.</td>
<td>0.5 Light-brown.</td>
<td>0.1 Pale-yellow-brown.</td>
<td>0.01 Colorless.</td>
</tr>
<tr>
<td>Fe in extract, mg...............</td>
<td>7.6</td>
<td>6.5</td>
<td>2.8</td>
<td>Trace.</td>
</tr>
</tbody>
</table>
it may be concluded that acetic acid extracts the pigment and also the iron in proportion to the concentration of the acid but that high concentrations do not break up the pigment into ferric iron.

The question next arises whether the iron found by analysis of the extracts is contained in the pigment or is free in solution. In other words can the iron and the pigment be separated and if so to what extent?

Use was made of the fact that the pigment which is extracted by 1 per cent HNO₃ is precipitated by 20 per cent HNO₃. This precipitate may be centrifuged off and dissolved in distilled water. If the iron is not in the pigment, it will be removed by this process and we shall eventually arrive at a point where the pigment will be free from iron. If it is in the pigment, then as long as we have the latter we shall be able to find iron on analysis.

A suspension of hemosiderin was made up in 1 per cent HNO₃ such that each 10 cc. of the suspension contained 10 mg. of iron. The acid extracted the pigment, and the following procedure was adopted. (a) A solution of 100 cc. of pigment was centrifuged. 10 cc. were taken for analysis and found to contain 7.7 mg. of Fe. The remaining 2.3 mg. of Fe were left in the granules, the extraction being incomplete. (b) 20 cc. of 20 per cent HNO₃ were added and the precipitate centrifuged. An analysis of 12.2 cc. of the fluid gave 1.5 mg. of Fe. Either it had originally existed in the ionic state in the granules, or it represented decomposed pigment. In either case there should remain 10−3.8 or 6.2 mg. of Fe in each 10 cc. of pigment. The precipitate was dissolved in 90 cc. of water, and 10 cc. of the solution contained 6.3 mg. of Fe. (c) 20 cc. of 20 per cent HNO₃ were added and the precipitate centrifuged. This time analysis of the fluid gave only a trace of iron. The precipitate was dissolved in 80 cc. of water and 10 cc. of this contained 5.7 mg. of Fe. (d) The process was repeated. The fluid again contained only a trace of iron, whereas the pigment yielded 6.3 mg.

After the first precipitation, therefore, all the iron remains with the pigment. If the latter were broken up by the acid, it would gradually lose its iron on repeated precipitation, but such is not the case. Or if it contained no iron at all, none of that element would be found after two or three analyses. Furthermore with hydrochloric acid all the iron appears as ferric chloride and there is
no sign of the pigment. The hemosiderin granules thus apparently contain an iron pigment along with a small amount of free ferric iron.

The next step was to investigate the chemical nature of the red-brown pigment. This was done in four ways.

1. **Qualitative Reactions.**—A solution of the pigment, extracted with nitric acid and freed from ionic iron by precipitation, was treated in the test-tube with the same reagents which had previously been used on the granules. (a) NaOH has no effect; i.e., it forms no precipitate of Fe(OH)₃. (b) K₄Fe(CN)₆ gives a blue-green coloration, turning to Prussian blue on warming. Ferric ferrocyanide is then precipitated. (c) KCNS gives a faint, barely perceptible, coloration, such as might be caused by the traces of free iron present. An equivalent amount of iron in the form of FeCl₃ gave an intense red color. (d) (NH₄)₂S gives a heavy black precipitate of ferric sulfide.

These reactions correspond precisely to those observed in the granules and furnish good evidence that we are dealing with one and the same substance, both in the extract and in the granules themselves. Other tests for iron were applied such as those with tannin, phosphate, pyrogallol, and H₂O₂, etc., and were checked with standard ferric salts. In every case the pigment reacted not at all, or in an abnormal manner. Tests for ferrous iron were entirely negative.

2. **Quantitative Analysis.**—The iron content of the pigment (and granules) is very high. The following analyses give an idea of its order of magnitude. The material was evaporated to dryness at 100° and ignited. The iron was determined by the permanganate method.

### Granules.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Dry weight</th>
<th>Iron (mg.)</th>
<th>Iron (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31 mg.</td>
<td>4.7 mg.</td>
<td>15.1</td>
</tr>
<tr>
<td>2</td>
<td>24 mg.</td>
<td>4.3 mg.</td>
<td>18.6</td>
</tr>
</tbody>
</table>

Average = 16.5 per cent iron.

### Pigment.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Dry weight</th>
<th>Iron (mg.)</th>
<th>Iron (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.5 mg.</td>
<td>7.7 mg.</td>
<td>49.5</td>
</tr>
<tr>
<td>2</td>
<td>9 mg.</td>
<td>4.1 mg.</td>
<td>45.5</td>
</tr>
<tr>
<td>3</td>
<td>20.4 mg.</td>
<td>7.8 mg.</td>
<td>38</td>
</tr>
<tr>
<td>7</td>
<td>11.1 mg.</td>
<td>4.7 mg.</td>
<td>42.5</td>
</tr>
</tbody>
</table>

Average = 44 per cent iron.
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In view of the peculiar reactions of this substance, it was important to know whether the iron was organically or inorganically combined. Now if a definite organic compound of iron were present, it would necessarily be of low molecular weight. For if 1 atom of iron per molecule is assumed, and the proportion of iron 45 per cent, the molecular weight would be about 125. If there were just 1 atom of carbon per molecule then the substance would have to contain at least 10 per cent carbon. Similarly there would have to be 11 to 12 per cent nitrogen, were there any at all. Accordingly a complete organic analysis was made on samples of the pigment as extracted by both nitric and acetic acids. The nitric acid extract was found to contain: Fe 55 per cent, C 1 per cent, H 12.5 per cent, O 27 per cent, and N less than 0.1 per cent. Analysis of the acetic acid extract gave approximately the same result save only that the carbon content was slightly higher due to traces of acetic acid remaining in the solution.

These analyses were twice checked with respect to nitrogen. Two analyses were made by the writer in the Cambridge laboratory, using a micro-Kjeldahl method, and only slight traces of nitrogen found. Two more were made by Mr. George Giragosintz of the Department of Biochemistry, University of California. Here different material was used but no significant amount of nitrogen could be detected.

The conclusion may reasonably be drawn from these analyses that the pigment contains neither carbon nor nitrogen. Likewise it is exceedingly difficult to assume that the iron in hemosiderin itself is organically combined. For the iron of hemosiderin has been shown to be nearly all in the pigment and the pigment does not contain an appreciable amount of carbon or nitrogen.

3. Precipitation Reactions.—The pigment when dissolved in distilled water can be precipitated by nitric acid in moderately high concentration, as has been described. This precipitate can be redissolved in water. There are also other methods of precipitation which indicate that the material is in a state of physical instability. (a) When an aqueous solution is evaporated the residue is an amorphous brown mass which is insoluble in water, acid, alkali, ether, chloroform, and all other substances tried. It is

* Analysis made by Dr. A. Schoeller, Feinchemie, Berlin-Schmargendorf.
soluble in hot dilute, or cold concentrated HCl but is thereby converted into the ferric salt of the acid. It thus behaves under these conditions as a hydrophobe colloid. (b) The pigment may be precipitated with formic acid (20 per cent). This precipitate is soluble in water. (c) It may be brought down with a mixture of alcohol and ether. This precipitate is soluble in water only with difficulty. (d) It may be brought down by a neutral solution of sodium acetate, sodium chloride, and other salts. In these cases it may or may not be redissolved by water. (e) If a precipitating reagent (e.g., formic acid, sodium acetate) be added very carefully to a solution of the pigment, the very faint opalescence of the solution becomes more marked. A definite Tyndall effect may also be observed. If it be then centrifuged 5 or 10 minutes at high speed (9000 R. P. M.), there will be a difference between the upper and lower portions of the tube. The bottom will be relatively cloudy and deeper colored, whereas the top will be clearer and lighter colored. (f) Mention should be made here of the behavior of the granules when they are separated from the tissue by concentrated alkali. The experiments hitherto reported were all made on hemosiderin which had been digested in 3 per cent alkali. The product of digestion in stronger alkali is the same as that in the weaker because all the chemical reactions are the same (iron reactions, etc.), but the physical properties appear to differ.

In this instance the red-brown pigment is extracted not by dilute acid but by distilled water itself. If the sediment is washed clean of organic matter in an alkaline medium and is then put in distilled water, the pigment is removed. This extract has the appearance of the acetic acid extract previously discussed. The pigment is, however, precipitated by dilute acid, dilute alkali, or neutral salts, and in no case can it be redissolved in distilled water. The acid precipitate is not soluble in alkali, nor the alkali in dilute acid. It cannot be dissolved in anything short of strong HCl, which of course decomposes it. The difference in coagulability between these two extracts seems to be due to the difference in concentration of the alkali used in digestion.

These six sets of observations, although purely qualitative, make it probable that we are dealing with iron in a colloidal form. The appearance of the solution, the extreme ease of coagulation, the erratic behavior in the presence of inorganic ions, all point to an existence of a colloid.
4. Some Reactions of Artificial Iron Salts.—Since hemosiderin in the granule and as an extract reacts in a peculiar manner when treated with certain reagents and since there is evidence that hemosiderin may be of a colloidal nature, it seemed worth while to try the effect of these reagents on a known colloidal solution of ferric iron. The ferric oxide sols (cf. Weiser (15)) are very numerous and differ in their properties according to their mode of preparation. One of the best known and most easily prepared is that obtained by precipitating a dilute solution of ferric chloride with a base. This method was accordingly used, and a heavy precipitate of so called ferric hydroxide obtained. The experimental procedure from this point resembled that with hemosiderin, as the following examples indicate.

Experiment 1.—An iron solution was made by running 10 per cent NH₃OH into 5 per cent saturated FeCl₃ till very slightly alkaline. The precipitate was washed several times by decantation. Acetic acid was added to a concentration of 2 per cent. A red-brown solution was obtained in 6 hours. The solution was then centrifuged to remove undissolved precipitate. The fluid was clear brown. It gave a green color with potassium ferrocyanide and only slight traces of color with potassium thiocyanate. The residue, dissolved in water, gave an opalescent, brown solution, which became green with ferrocyanide, and showed no color with thiocyanate.

Experiment 2.—A precipitate of ferric hydroxide was treated with 2 per cent lactic acid. A brown solution was obtained in 3 hours and was precipitated with 20 per cent nitric acid. The precipitate was centrifuged. The fluid became a deep red color with thiocyanate. The residue, dissolved in water, gave a light brown solution but only a very slight color with thiocyanate.

Experiment 3.—A precipitate of ferric hydroxide was treated for 15 hours with 2 per cent acetic acid. A red-brown solution was obtained, which was centrifuged to remove undissolved precipitate. (a) The fluid gave a green color with ferrocyanide, and showed slight traces of color with thiocyanate. (b) When precipitated with 20 per cent nitric acid and centrifuged, the fluid was clear and colorless. It gave some color with thiocyanate, representing iron which had been ionized by the acid. The residue from (b) dissolved in water as a clear, brown solution.
This turned green with ferrocyanide, and gave no color whatever with thiocyanate. The residue from (a) was soluble in water. It became green with ferrocyanide and gave no color with thiocyanate.

Although these experiments differ somewhat in detail from the previous ones with hemosiderin, the same general procedure was followed and the same general results were obtained. Particularly worthy of note are the facts that in both cases the original substance (hemosiderin or ferric hydroxide) may be got into a brown solution by means of dilute acids (e.g. acetic), may be precipitated therefrom by means of stronger acids (e.g. nitric), and in the sol state may both give a green color with ferrocyanide, and no color or only traces of color with thiocyanate. The analogy between the reactions of hemosiderin and those of a ferric oxide solution is thus very striking and taken together with the preceding evidence makes it very probable that hemosiderin is itself simply ferric oxide.

**DISCUSSION.**

It is clear from the foregoing that hemosiderin is not a definite chemical entity, such as hemoglobin. The granules are of no particular shape or size but are analogous to red blood corpuscles in that they form a substrate on which a chemical compound is deposited. Possibly they may carry the red-brown, iron-containing pigment in some such fashion as the corpuscles carry hemoglobin. At any rate the pigment may be removed, leaving the substrate, or stroma, intact.

This pigment is of peculiar nature. It contains no carbon or nitrogen and therefore cannot be an organic compound. Its iron content is very high, and aside from iron consists only of hydrogen and oxygen (or water). It must be an inorganic compound of iron, yet its reactions are not those characteristic of ferric iron. It gives none of the reactions of ferrous iron whatever. Hence the iron must be in the form of an oxide, or hydroxide. The behavior of the material in solution suggests that it is in the colloidal state. Furthermore the behavior of a pure ferric oxide sol toward acids, and toward ferrocyanide and thiosulfate, has been found to coincide to a reasonable degree with that of the pigment. If this is so, then we may tentatively define hemosiderin as some form of colloidal ferric oxide, physically combined with an organic sub-
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strate, the stroma of the granule. It may be adsorbed on the surface of the stroma but it is more likely that the latter is permeated by the iron compound, the molecules being held in place throughout the substance of the granule by physical forces. Were the iron held only on the surface it would be quickly removed by strong acid, but observation has shown that some of it persists in the interior and can there become blackened by sulfide after the surface is no longer affected by that substance. Another possibility is that the iron in the interior of the granule is in the form exhibited in the pigment but that the surface is covered with a thin layer of free ferric iron. This conception would coincide to a certain extent with that of Fischer, mentioned in the introduction. But there seems to be no metallic iron present. The problem of the exact method whereby the iron compound is held in the granule awaits further investigation.

In regard to the broader aspect of the question, the rôle of hemosiderin in the economy of the organism, it must be said that these experiments tend to substantiate the view that the iron of hemoglobin is split off from the organic part of the molecule, and is deposited, in an inorganic form, as hemosiderin.

SUMMARY.

1. Tests under the microscope demonstrate that hemosiderin consists of an iron compound which can be removed from the granules by treatment with acid, leaving the substrate practically intact.

2. The granules can be obtained free from tissue by a process involving digestion with alkali.

3. The iron-containing substance can be extracted and observed in vitro.

4. This material reacts with thiocyanate and other substances in a manner which is not characteristic of ionic iron.

5. This material has been found by analysis to contain only iron, hydrogen, and oxygen. It apparently exists as a colloid.

6. A pure ferric oxide solution has been found to react with thiocyanate and other substances in approximately the same manner as the extract of hemosiderin.

7. It is concluded that hemosiderin consists of organic granules impregnated with some form of ferric oxide.
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The author wishes to take this opportunity to thank Professor Joseph Barcroft both for the hospitality of his laboratory and for his kind interest and help during the course of the present investigation.

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