ON METHÆMOGLOBIN.

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Plate IX.

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Since Hoppe-Seyler first described the derivative of blood-pigment, which he called "Methæmoglobin," this substance has received a large amount of attention and has been the subject of many elaborate researches. As a result of these researches we are in possession of a considerable number of facts bearing on the question, but as regards the nature of methæmoglobin all that can be said up to the present time is that, in the words of Lea[1], "the oxygen loosely united to hæmoglobin as oxyhæmoglobin becomes more stably combined,.....and further that the resulting substance (methæmoglobin) has the same composition and crystalline forms as oxyhæmoglobin, and may be reconverted into the latter body by suitable means." An abstract of the literature of the subject up to 1888 will be found in a *mémoire* on methæmoglobin published in that year by Bertin-Sans[2]. More recently Araki[3], Dittrich[4] and Vorkampff-Laue[5] have worked at the subject.

The following research arose from an attempt to determine definitely the spectrum of methæmoglobin, which is variously described by different authors as showing 1, 2, 3 and 4 absorption bands. I shall briefly describe my experiments and then formulate the conclusions to which they have led me.

In these experiments I have used the blood of the following animals —ox, sheep, pig, horse, guinea-pig, rat, newt. I have also used solution

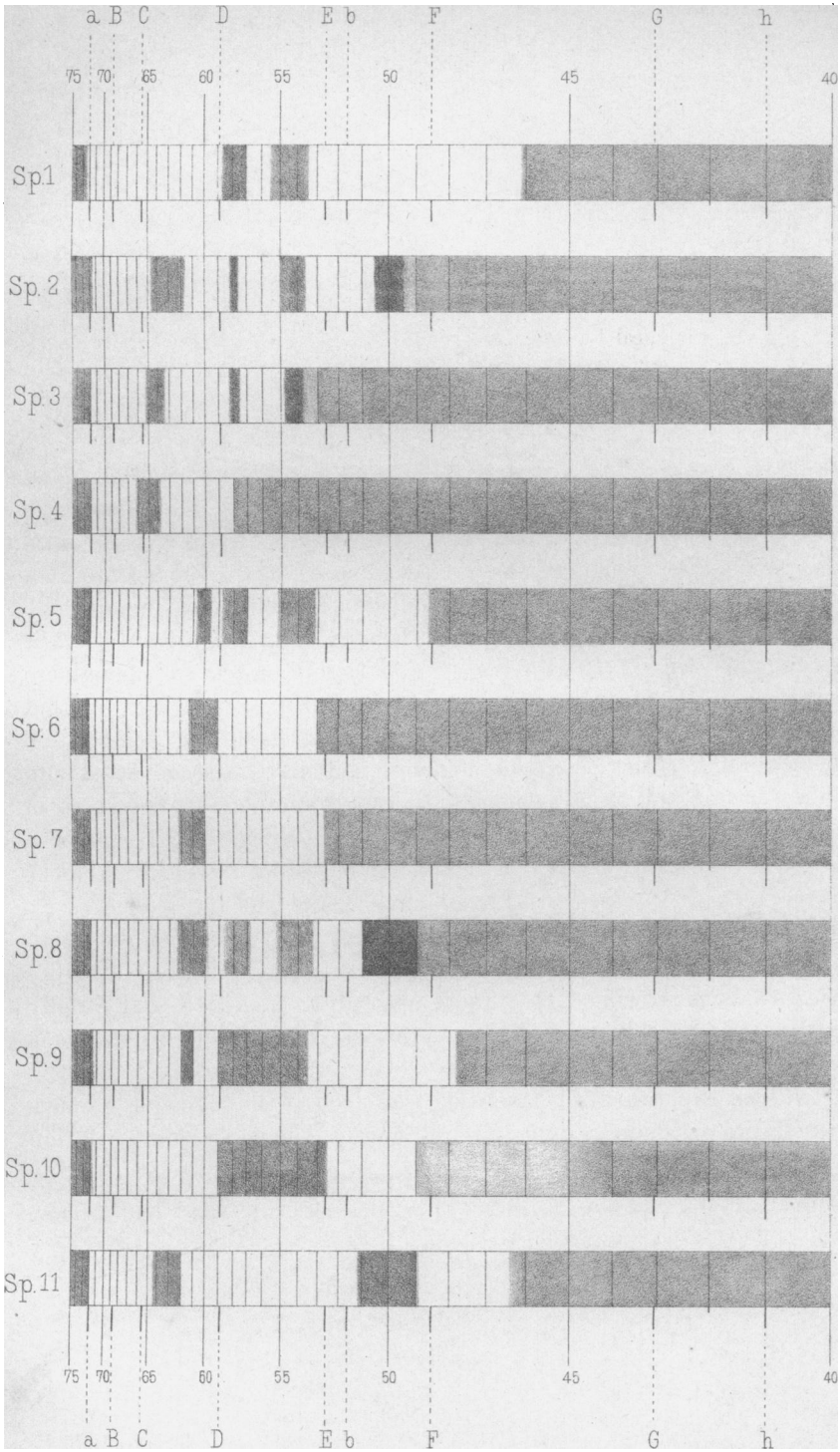
[1] Lea. *The Chemical Basis of the Animal Body*, 1892.

[2] Bertin-Sans. *Études sur la méthémoglobine*, Une broch. in 8°. Paris. J. B. Baillière, 1888.

[3] Araki. *Zeitschr. f. Physiol. Chem.* XIV. 405. 1890.

[4] Dittrich. *Schmiedeberg's Archiv*, XXIX. 280. 1892.

[5] Vorkampff-Laue. *Beiträge zur Kenntniss des Methämoglobins und seiner Derivate.* Inaug. Diss. Dorpat. 1893.



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of oxyhæmoglobin crystallised from dog's blood according to the method recommended by Halliburton[1]. The crystallisation was repeated three times. The changes observed in the pigment were the same whatever the animal from which the blood was obtained. The strength of solution used was usually such that when examined spectroscopically in a $\frac{5}{8}$ in. test-tube, the two bands of HbO_2 were almost merged into one. The experiments were conducted in flasks or test-tubes, in some cases at the temperature of the room, in others on a water-bath at 37°—40° C. The changes were produced more rapidly in the latter case. The position of the bands was measured by means of an arbitrary scale and reduced to wave-lengths by an interpolation curve.

I have observed spectroscopically the changes produced in blood-pigment by the action, more or less prolonged, of the following reagents: glycerin, potassium chlorate, permanganate and ferricyanide, iodine, amyl nitrite, potassium nitrite, chloride, bromide and iodide, and sodium fluoride. The action of acids will be dealt with in a separate paper.

Drying HbO_2 crystals.

As Hoppe-Seyler[2] and Sorby[3] have shown, on drying HbO_2 crystals at the ordinary temperature part of the mass is converted into methæmoglobin. This partial conversion also takes place in the preparation of HbO_2 crystals unless the process be carefully conducted at a very low temperature. Solutions of methæmoglobin obtained in this way have a faintly acid reaction, and, according to Hoppe-Seyler[4], contain a proteid substance and volatile fatty acids. The spectrum shows the typical HbO_2 bands well-marked, the characteristic methæmoglobin band in the red is present and there is a faintly marked fourth band (Pl. IX, sp. 2). The band in the red may be less marked or as well marked as those in the green. The centres of the bands are at λ 630, 579, 541 and (about) 500. The exact position of the last is difficult to determine, as this band appears rather as an intensification of a general shading than as a distinct band.

Such a solution of methæmoglobin neutralised with dilute Na_2CO_3 solution shows no change in its spectrum. On addition of ammonia the colour changes from brown to rich red. The band in the red slowly disappears, those in the green become more pronounced but do not

[1] Halliburton. *Text-book of Chemical Physiology and Pathology.* 1891.
[2] Hoppe-Seyler. *Virchow's Archiv*, XXIX. 233, 597. 1864.
[3] Sorby. *Monthly Micros. Journ.* VI. 9. 1871.
[4] Hoppe-Seyler. *Centralb. f. d. med. Wiss.* 1865. 65.

assume the intensity and sharp definition of typical HbO_2 bands. Band IV disappears. As band I fades, there gradually appears a faint band immediately to the red side of *D*, which, although distinct from, merges with, the adjacent band II. The centre of the new band is at λ 602 (Pl. IX, sp. 5). If a little Am_2S is now added to the solution, the band to the red side of *D* disappears, while the bands in the green rapidly assume the typical sharply defined characters of the HbO_2 bands, then slowly lose their sharp margins and gradually become changed into the one band of Hb. On shaking the solution with air the colour changes from the purple of Hb to the scarlet of HbO_2 and the spectrum again becomes that of HbO_2 (Pl. IX, sp. 1).

If Am_2S is added directly to methæmoglobin solution without the previous addition of ammonia, bands I and IV disappear, while II and III take on HbO_2 characters and then give place to the Hb band.

Stokes' fluid gives the same results as Am_2S with one difference—with this reagent I have never been able to assure myself of the intermediate appearance of HbO_2 in the course of reduction of methæmoglobin. There is an intensification of the bands in the green but it is such as might be produced by ammonia alone.

Exposure of HbO_2 solution to air.

If a solution of HbO_2 crystals or some dilute blood is left exposed to the air, especially in a water-bath at 37°—40° C., it, after 24 hours or more, developes an acid reaction, shows a brownish tinge, and shows spectroscopically, besides the HbO_2 bands, the band in the red of methæmoglobin. MetHbO_2 may be recognised by the reduction test. A day or two later the acid reaction gives place to an alkaline, putrefaction has begun, the colour becomes purple, the spectrum is that of Hb and can be converted into that of HbO_2 by shaking the solution with air.

Effect of Glycerin.

Preyer[1] found that when glycerin was added to a solution of HbO_2 and the mixture exposed to the air the spectrum showed, after 6 days, an absorption band in the red. I have found that diluted blood, treated with $\frac{1}{15}$ of its volume of glycerin, and kept at a temperature of 37°—40° C. shows after 24—48 hours a distinct band in the red. The

[1] Preyer. *Pflüger's Archiv*, I. 395. 1868.

amount of methæmoglobin then goes on increasing and that of HbO_2 diminishing for some days longer. By this means I have obtained a solution which showed bands I and IV well-marked, whereas bands II and III had almost, if not altogether, disappeared. Ammonia and Am_2S bring about the changes already described. On account of the greater density given to the solution by the glycerin, if Am_2S is carefully added to the top of the brown solution in a test-tube and the tube then set aside for 6—24 hours, there are seen 3 zones, sharply marked off from one another, and more or less equal, the upper purple and showing the spectrum of Hb, the middle scarlet with spectrum of HbO_2, and the lowest brown with spectrum of methæmoglobin. Later the whole solution becomes purple.

If the methæmoglobin solution obtained by means of glycerin is allowed to remain a few days longer (7 to 10 days altogether) in the water-bath, a brownish-red precipitate forms which is insoluble in water, but dissolves in weak acids or alkalies. The solution in dilute Na_2CO_3 shows spectroscopically two bands in the position of the bands of HbO_2 but not sharply defined. On addition of Am_2S the spectrum changes to that of hæmochromogen, so that the precipitate is probably hæmatin or a closely allied body. Thus, whereas a solution of HbO_2 exposed to air passes through a methæmoglobin stage (partial) and then becomes Hb, a solution of HbO_2 with glycerin passes into first methæmoglobin, then (?) hæmatin.

Effect of Potassium Chlorate.

If to dilute blood or a solution of HbO_2 crystals saturated solution of $KClO_3$ is added, in the proportion of 1 drop to each c.c. HbO_2 solution, and the tube placed in the water-bath, the solution gradually becomes brown in colour, and with the spectroscope the HbO_2 bands may be observed to fade and the characteristic methæmoglobin band (I) to appear. There is absorption of the violet end up to and including the βHbO_2 band. The $aHbO_2$ band becomes very faint. Ammonia and Am_2S give the usual methæmoglobin reactions.

Some hours later the solution is pale brown and spectroscopic examination shows no bands, but diffuse absorption. On addition of Am_2S the spectrum becomes that of hæmochromogen + a faint band in the red overlapping the *D* line. This latter band corresponds to that described by Bertin-Sans and Moitessier[1] as characteristic of their 'reduced hæmatin." After standing (with Am_2S) for a day this solution

[1] Bertin-Sans et Moitessier. *Comptes Rendus*, CXVI. 11, p. 591. 1893.

contains Hb, showing spectroscopically the band of that substance and becoming changed to HbO_2 on shaking with air.

When HbO_2 solution has stood with $KClO_3$ 24 hours or more in the bath, it is turbid and greenish-brown. There are no bands in its spectrum, but diffuse absorption. When Am_2S is added the fluid becomes clear and of a beautiful green colour. The spectrum then shows a distinct band in the red and two rather faint bands, apparently due to hæmochromogen, in the green. The same appearances are obtained when HbO_2, acted on for a certain time by dilute hydrochloric acid, is treated with Am_2S, so that it is possible that the green colour may be due to an Fe reaction.

In this case also we have the formation of first methæmoglobin, and second a substance which gives the reduction reaction of hæmatin, viz. the formation of hæmochromogen.

The spectrum of methæmoglobin formed in this way is the same as that of methæmoglobin formed spontaneously (Pl. IX, sp. 2), only in this case only the bands I and II are distinctly seen. Their centres are at λ 630 and 579.

Effect of Potassium permanganate.

Solution used 0·025 %. Solution of HbO_2 treated with $\frac{1}{15}$ of its volume of $KMnO_4$ solution passes through the same stages as in the case of glycerin and $KClO_3$. The spectrum of methæmoglobin formed by this reagent has the four bands in the positions already described (Pl. IX, sp. 2), viz. λ 630, 579, 541 and 500. In 24 hours a considerable brown precipitate has formed, and the supernatant fluid remains a clear pale brown solution of methæmoglobin, showing the four bands of about equal intensity. If the tube is allowed to stand 7—11 days, the pigment which remains in solution changes to Hb, proved by spectroscopic examination and shaking with air in the usual way. The precipitate now appears scarlet. It is insoluble in water, but on washing with water for 24 hours, then dissolving in 0·2 % HCl, it yields a rich dark brown solution, the spectrum of which shows 3 bands approximately in the position of I, II and III of methæmoglobin. On addition of ammonia the spectrum becomes that of alkaline methæmoglobin, but further addition of Am_2S gives hæmochromogen.

Effect of Potassium ferricyanide.

With this reagent the result is essentially the same as with those already mentioned—a passage from HbO_2 through a methæmoglobin

stage to hæmatin. To HbO_2 solution $\frac{1}{15}$ of its volume of saturated solution of K_3FeCy_6 is added. The colour immediately changes from scarlet to brown, and the band in the red of methæmoglobin appears. If the solution is diluted the four bands can be seen. Ammonia and Am_2S give the usual results. If the solution is kept two days in the water-bath or six days at the ordinary temperature, its colour changes to scarlet and a dark brown precipitate forms. The spectrum of the solution shows one band resembling that of Hb, but unchanged on shaking with air (Pl. IX, sp. 10). Am_2S added, the spectrum changes to that of hæmochromogen. This hæmochromogen solution on standing for 24 hours changes to Hb, and HbO_2 appears on shaking with air.

The scarlet solution above referred to has the characters of the "cyan-hæmatin" of Hoppe-Seyler, the "KCN-hæmatin" of Nawrocki. According to Preyer[1] the substance formed on addition of Am_2S is not hæmochromogen but "cyan-hæmatin."

The precipitate above referred to is insoluble in water, soluble in dilute alkalies or acids, the solutions giving hæmochromogen on addition of Am_2S.

If the K_3FeCy_6 action is allowed to go on in the cold, there is a hæmatin stage between methæmoglobin and "cyan-hæmatin," in which, while the spectrum is that of methæmoglobin, addition of Am_2S gives hæmochromogen.

Effect of Iodine.

I used Marchand's[2] solution, containing ·05 gram of iodine and 1 gram KI to 100 c.c. water. This reagent causes rapid conversion of HbO_2 into methæmoglobin (Pl. IX, sp. 2). At 40° the solution soon becomes turbid, and 24 hours later a dark red precipitate has fallen, while the solution is almost colourless and shows spectroscopically only two very faint bands in the green, none in the red. Am_2S, however, when added causes the appearance of first HbO_2 then Hb, so that methæmoglobin is present. Sooner or later, though the spectrum is the same as before, addition of Am_2S causes the formation of hæmochromogen, but even then shaking with air brings about the change into HbO_2.

If the dark red precipitate mentioned above is collected, washed with water and dissolved in dilute HCl, it gives a rich dark brown solution, the spectrum of which shows three bands corresponding to

[1] Preyer. *Die Blutkrystalle.* 1871. p. 202.

[2] Marchand. *Virchow's Archiv,* LXXVII. 488. 1879.

I, III and IV of methæmoglobin. Ammonia causes, when added to the solution, the change of this spectrum to that of alkaline methæmoglobin, but subsequent addition of Am_2S leads to the appearance of hæmochromogen.

Effect of Amyl nitrite.

A few drops of this reagent, pure or in alcoholic solution, added to solution of HbO_2, almost immediately produces a change of colour from scarlet to chocolate brown, and the solution gives the 4-banded spectrum of methæmoglobin, the band in the red being very distinct, those in the green indistinct. The four bands are in the usual positions (Pl. IX, sp. 2). The solution gives the usual reactions with ammonia and Am_2S.

If HbO_2 solution is allowed to stand with amyl nitrite several days, a precipitate forms, and, especially rapidly if blood which has commenced to putrefy be used, the band in the red disappears. Am_2S then brings about a change to hæmochromogen. In one experiment with pure HbO_2 (dog) the band of "reduced hæmatin" of Bertin-Sans and Moitessier[1] was also present. The precipitate exhibits the same characters as the other precipitates already described—its HCl solution shows three bands in the positions of I, III and IV of methæmoglobin. Am_2S converts the pigment into hæmochromogen + "reduced hæmatin." Solution of the precipitate in 1% Na_2CO_3 shows no bands, and Am_2S gives hæmochromogen. This solution (+ Am_2S) after one day shows a broad band in the green overlapping D, unchanged on shaking with air. I also obtained this spectrum with a solution of pure HbO_2 treated with amyl nitrite for one day, when there were no bands in its spectrum but Am_2S produced hæmochromogen + "reduced hæmatin," and, some minutes later, the bands of these substances disappeared, leaving a broad band in the green as above, unchanged on shaking with air. In this latter case, after three days the broad band was replaced by two faint bands in the position, or nearly so, of the HbO_2 bands. Am_2S now added to the solution gave hæmochromogen. The substance characterised by these two bands in the green I shall have to refer to again. It appears to be the hypothetical "oxidised form of hæmochromogen" of Bertin-Sans and Moitessier.

One more point—HbO_2 solution + amyl nitrite, after passing through the methæmoglobin and hæmatin stages, finally becomes a substance of which the spectrum shows two bands almost or altogether

[1] *loc. cit.*

in the position of the HbO_2 bands, but not so well defined. This spectrum is unchanged on treatment of the solution with Am_2S, even after 24 hours. Can this be HbNO or a compound of one of the oxides of nitrogen with hæmatin?

With regard to the action of amyl nitrite on HbO_2 a new point has been raised by D. Leech[1]. Amyl nitrite on being mixed with water is decomposed with production of nitrous acid, which in its turn is easily converted into nitric acid. In view of this fact I have made several experiments:

I examined carefully the position of the band in the red produced by the action of amyl nitrite in moderate quantity on blood, and compared it with the position of the similar bands produced by nitrous and nitric acids (1% solution used) respectively, with the result that these bands are identical in position, the centre in each case being at λ 630. On increasing the quantity of acid used, the band shifts towards *C* and its centre is found to be at λ 650. It is now the band of acid hæmatin. On adding a drop or two of pure amyl nitrite to HbO_2 solution, to test the effect of excess of the reagent, a flocculent precipitate forms and interferes with the spectroscopic examination. If the precipitate be filtered off, the spectrum shows two bands in the green, like those obtained by prolonged treatment with a smaller quantity of the reagent, and probably due to the presence of "oxidised hæmochromogen." By performing the experiment carefully and using smaller quantities of amyl nitrite I have succeeded in obtaining the spectrum of acid hæmatin, i.e. with band I at λ 650, but not so distinctly as in the case of the acids. In large quantity amyl nitrite seems to produce complex changes in the blood pigment, due in the first place to the acids produced, and secondly probably to its action as an oxidising agent. In some cases after the action of amyl nitrite I have been unable to detect any bands in the spectrum, but even then Am_2S gave hæmochromogen and this slowly passed into the condition of hæmoglobin.

Effect of Potassium nitrite.

A small quantity of a watery solution of this reagent added to HbO_2 solution causes the colour to change to reddish-brown. The spectrum shows the four bands of methæmoglobin. Am_2S converts the methæmoglobin into HbO_2 then Hb. If a sufficient amount of KNO_2 has been used the same red-brown precipitate forms in a few days as in the

[1] Leech. *Croonian Lectures on Action of Nitrites. Brit. Med Jour.* June, 1893.

case of amyl nitrite. After the precipitate has formed Am_2S added to the supernatant fluid (decanted) gives rise to a mixture of Hb and hæmochromogen. In one experiment, with horse's blood, at the temperature of the room, methæmoglobin formed first, and four days later the solution was scarlet and showed the spectrum of HbNO (?).

The action of KNO_2 is much slower than that of amyl nitrite, some hours are required for even partial conversion of the pigment, as if some decomposition of the reagent were necessary. Amyl nitrite we know is decomposed. Probably KNO_2 also is decomposed before its action is produced. We must look for an action of HbO_2 on the salt. We know that the presence of HbO_2 facilitates the splitting off of CO_2 from its compounds in the plasma. May HbO_2 not have a similar action on compounds of weak and unstable acids with K and other bases, in salts which are methæmoglobin-producing reagents?

Effect of Sodium fluoride.

Using this salt as a preservative for watery extracts of spleen, I observed that the colour of the solution changed from scarlet to a rich crimson, and on spectroscopic examination the HbO_2 bands had almost disappeared and two new bands were seen at λ 612 and 500, the former very marked (Pl. IX, sp. 8). The same change occurs if a little 1% NaFl solution is added to solution of pure HbO_2. The solution is strongly alkaline. Addition of a little acetic acid causes intensification of the band in the orange and fading of the HbO_2 bands. It is to be noted that the band in the orange is in the position of the band of alkali-hæmatin, but on addition of Am_2S there is produced in this case first HbO_2, later Hb. If the NaFl action has gone on for some days, Am_2S gives a change to Hb directly, with what seems to be the transitory appearance of the *a* hæmochromogen band superposed on the Hb band. The NaFl-HbO_2 band also differs from the band of alkali-hæmatin in being extremely pronounced. The band of alkali-hæmatin in watery solution is often shadowy, and sometimes alkali-hæmatin gives no distinct band.

If NaFl is allowed to act on HbO_2 solution for some days in the water-bath, a precipitate is produced with the same characters as similar precipitates already described. Its solution in dilute HCl shows no bands in its spectrum, but on addition of Am_2S hæmochromogen is formed.

In some experiments which I have made on the digestion of blood

pigments I have found that whereas HbO_2 or a freshly made solution of HbO_2 in NaFl solution becomes decomposed by pancreatic extracts, yielding hæmatin, on the other hand a solution of "sodium fluoride hæmoglobin" is unaltered after treatment with pancreatic extract on the water-bath for some days.

Effect of other Salts of the Halogens.

I have used KI, KBr and KCl, and find that each of these, if left for some days with HbO_2 in the water-bath brings about the conversion of the HbO_2 into a substance whose spectrum is characterised by two bands in the green, and which is converted into hæmochromogen by Am_2S. This corresponds with the description of the "oxidised form of hæmochromogen" of Bertin-Sans and Moitessier.

Conclusions.

1. HbO_2 becomes converted into methæmoglobin (*a*) as the result of decomposition of a solution of HbO_2 exposed to the air, (*b*) when a blood stain is allowed to dry exposed to the air, and (*c*) under the action of certain reagents, of which I have investigated the following—*glycerin, potassium chlorate, permanganate* and *ferricyanide, iodine, amyl nitrite, potassium nitrite, sodium fluoride* (and *certain acids*). It seems improbable that the formation of methæmoglobin from HbO_2 is either an oxidation or a reduction process. In the case of the nitrites, which are both oxidising and reducing agents, another explanation is available (p. 408).

2. When methæmoglobin is produced by decomposition the solution has an acid reaction, and, on decomposition proceeding further, whereby the reaction changes from acid to alkaline, a reconversion into Hb takes place.

3. Methæmoglobin produced by the action of the above-mentioned reagents, by further action of the same reagents, becomes changed so as to give rise to hæmatin, which is precipitated unless held in solution e.g. by acids. In the case of K_3FeCy_6 the hæmatin passes into the condition of cyan-hæmatin.

4. Methæmoglobin cannot be distinguished from hæmatin by its spectrum alone, nor by the spectrum produced on addition of ammonia to its solution, but only, in the present state of our knowledge, by the change produced by a reducing agent. Methæmoglobin yields Hb on

reduction. Hæmatin (at least in the presence of albuminoid material) yields hæmochromogen.

5. In the case of chlorate and ferricyanide of potassium, and iodine, after hæmatin has been formed, and so long as it remains in solution, addition of Am_2S gives hæmochromogen, which is gradually transformed on standing into Hb. Further light is thrown on this reconstruction of the hæmoglobin molecule by my experiments on the action of acids on blood-pigment, described in a separate paper[1].

6. Amyl nitrite acts very much more rapidly on HbO_2 than does KNO_2. The former is decomposed on mixing with water, yielding in the first instance nitrous acid. The spectrum of methæmoglobin produced in the usual way by amyl nitrite is the same as that of methæmoglobin produced by minimal quantities of nitrous or nitric, or other mineral acid. Further, excess of amyl nitrite rapidly produces acid hæmatin from HbO_2, even at the temperature of the room.

Sorby[2] observed in 1870 that the addition of a little weak acid makes KNO_2 very active, but that in the presence of a little weak alkali it has a very slight action. Is it not probable that the slow action of KNO_2 is due to the fact that acid must be set free before the action can take place, and may not the acid be set free in one of two ways, either (1) by development of the fatty acids alluded to by Hoppe-Seyler as resulting from the decomposition of hæmoglobin, or (2) by an action on the part of HbO_2 itself similar to that which it is said to exercise on the loose compounds with CO_2 in the plasma? This opens up the wider question of the possible dissociation of such unstable compounds as $KMnO_4$ and K_3FeCy_6 before the products of such dissociation act in turn on the hæmoglobin. $KMnO_4$ we should expect to be at once decomposed by an organic substance such as hæmoglobin.

I have examined the reaction of blood solutions treated with $KClO_3$, $KMnO_4$ and K_3FeCy_6, and never found it appreciably acid to litmus paper.

Vorkampff-Laue[3] found in the case of nearly all the reagents he worked with—and they were all organic compounds except $NaNO_2$—that along with the production of methæmoglobin an acid reaction appeared. Whether this was due to decomposition of the reagent or

[1] This *Journal*, xvii. 415.

[2] Sorby. *Quart. Jour. Micr. Sci.* x. N.S. 400. 1870.

[3] Vorkampff-Laue. *Beiträge zur Kenntniss des Methämoglobins und seiner Derivate.* Inaug. Diss.. Dorpat. 1893.

of hæmoglobin one does not know, but it seems to me quite possible that it may have been due to a splitting up of the reagent itself and that the products of this decomposition had to do with the formation of methæmoglobin.

7. In the spectrum of methæmoglobin the only constant feature is the band in the red, and its position is variable. Of the other bands, II and III become paler the more complete the conversion into methæmoglobin, till ultimately they are scarcely, if at all, visible. IV, speaking generally, is most marked in methæmoglobin furthest removed from hæmatin. As the methæmoglobin approaches the condition of hæmatin the absorption of the violet end spreads so as to approach and include the band IV.

I believe, with Araki[1] and Lankester[2], that the spectrum of methæmoglobin is characterised by two bands only, viz. I and IV, for two principal reasons,

(1) As Araki has pointed out, further addition of the methæmoglobin forming reagent leads to the further disappearance of bands II and III, till in some cases one can no longer see them, but the substance observed still retains the characters of methæmoglobin, i.e. it becomes HbO_2, then Hb on addition of Am_2S.

(2) As methæmoglobin is an intermediate stage (according to my experiments) between HbO_2 and hæmatin, one would not be surprised to find it mixed with both of these substances. A very minute trace of the former would account for bands II and III. Again, the spectrum of hæmatin shows absorption of the violet, blue and a large part of the green, and so, in presence of a certain amount of hæmatin, the IV band of methæmoglobin would be swamped in this absorption of the blue end.

The objection which has been brought against bands II and III being considered due to a residue of HbO_2 is that band III is darker than II and that this condition is the opposite of that which obtains in the case of the HbO_2 bands. But methæmoglobin might, without producing a definite band, lead to increased absorption in the region of βHbO_2. A trace of hæmatin certainly would do so. And it may be urged that the bands II and III in the methæmoglobin spectrum do not show the sharp definition of the HbO_2 bands but bear a marked resemblance to an intermediate stage between HbO_2 and Hb. It may be that these bands represent partially reduced HbO_2.

[1] Araki. *Zeitschr. f. phys. Chem.* XIV. 405. 1890.

[2] Lankester. *Quart. Jour. Micr. Sci.* X. N.S. 402. 1870.

8. Sodium fluoride converts HbO_2 into a form of methæmoglobin, showing spectroscopically, besides faint traces of the HbO_2 bands, two characteristic bands, one beautifully distinct in the same position as the band of alkaline hæmatin, at λ 612, the other at λ 500, i.e. in the position of band IV of methæmoglobin. The solution is strongly alkaline, and addition of Am_2S gives first HbO_2 then Hb.

PLATE IX.

Sp. 1. Oxyhæmoglobin.
Sp. 2. Methæmoglobin as usually obtained and described.
Sp. 3. Spectrum obtained by using excess of acetic, oxalic or phosphoric acid.
Sp. 4. Acid hæmatin.
Sp. 5. Alkaline methæmoglobin.
Sp. 6. Alkaline hæmatin in alcoholic solution.
Sp. 7. Alkaline hæmatin in watery solution.
Sp. 8. Sodium fluoride hæmoglobin.
Sp. 9. Hæmoglobin and sulph-hæmoglobin.
Sp. 10. ? Cyan-hæmatin.
Sp. 11. ? Pure methæmoglobin.

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