FURTHER OBSERVATIONS ON MYOHAEMATIN AND THE HISTOHAEMATINS. By C. A. MACMUNN, M.A., M.D. Pl. II.

In a former paper[1] I have shown how by the examination of the fresh organs and tissues of Vertebrates and Invertebrates by means of the microspectroscope I was led to discover the presence of the histohaematins and of myohaematin; and I stated that I could not isolate these colouring matters unchanged. I was able to say that they are joined to proteids or that they are coloured proteids, and when isolated in the changed condition soluble only in water.

In the present paper I can go further and show how they can be got into solution, and although as yet the pigmented portion cannot as in the case of haemoglobin be separated from the proteid constituent, yet one can definitely prove: (1) that a peculiar colouring matter is present in solution, (2) that it yields decomposition products which prove its near relationship to haemoglobin, while at the same time it fails to yield all such decomposition products, proving that it is distinct from haemoglobin or any of its decomposition products; and (3) one can form a fairly accurate idea as to the physiological rôle which it may play and as to its reactions.

While I have not yet succeeded in getting relatively large quantities of pigments so as to be able to deal with them analytically, yet the amount is sufficient for enabling me to prove their great importance from a physiological point of view.

The proof of the existence of these colouring matters then rests no longer on the observation of the spectra of the fresh organs and tissues of animals, as I can now easily obtain coloured juices from muscle, and from certain organs, in such quantity as to enable them to be dealt with in solution, and their reactions followed, by means of the spectroscope.

[1] *Phil. Trans.* Part I. 1886.

Myohaematin as such, although obtainable from the rapidly frozen muscle of birds and mammals in sufficient quantity to enable one to say definitely that it occurs in plasma, yet is only obtainable in mere traces owing to the rapidity of muscle coagulation in warm-blooded animals. In cold-blooded animals, such as the frog from whose muscles plasma can be obtained in greater quantity, myohaematin is present also in mere traces, so that the difficulty is not overcome by using such plasma.

Hence attempts at its extraction are limited to experiments on dead muscle in which coagulation has occurred, and I thought that I might get myohaematin into solution by extracting the myosin in the usual manner.

Experiments on Salted Muscle.—In most of the voluntary muscles of mammals and birds myohaematin and haemoglobin are present together, as I have proved. In the pectorals of the pigeon, however, the former appears to be the sole pigment present.

If the pigeon be bled to death by severing the large blood-vessels in the neck and allowing all the blood to drain away, very little haemoglobin is left in the pectoral muscles, especially if the muscle be well washed and subsequently pressed. The muscle is then finely divided, and rubbed up with sodium chloride which is added in sufficient quantity to form a 10 per cent. solution with the water subsequently added. After standing 24 hours the magma is pressed in linen, and filtered under pressure. I sometimes washed out the blood-vessels previously with salt solution, but the results obtained were the same as in those cases where the animal was merely bled to death. The filtered extract of muscle thus obtained is reddish-yellow and shows the dominant, dark, narrow band of myohaematin well marked, sp. 1. In this spectrum other bands are seen due to traces of haemoglobin, as is proved by adding ammonium sulphide, as we then find the band of reduced haemoglobin, with another, but those of reduced myohaematin are very distinct, sp. 2. In this latter solution the bands were found to read:—

1st, λ 625 to λ 610,
2nd, λ 553·5 to λ 547,
3rd, λ 526 to λ 514.

If such a solution be treated with acetic acid the myohaematin bands disappear, and two very faint shadows in green may be seen, the first of which is nearer the violet than the first oxy-haemoglobin band. Portions of the filtrate from salted muscle which came through later than the solu-

tions mentioned above showed only myohaematin bands, no haemoglobin, as shown in Pl. II. sp. 3. Upon boiling such a solution the coagulum which forms no longer shows any distinct bands; this is in accordance with a former result, as I found that sufficient heat destroys the bands in muscle itself. Coagulation by means of absolute alcohol on the contrary allows the bands to be recognised in the yellowish coagulum, especially if it be treated with a reducing agent.

In some filtrates obtained from salted muscle, the band before D was missing, sp. 4 being seen instead.

The faint band after D did not disappear with ammonium sulphide, showing that it did not belong to haemoglobin.

In some cases the coagulum, produced by adding alcohol to the filtrate from salted muscle, when extracted with rectified spirit and caustic potash showed a band like that of alkaline haematin at D, sp. 5; but it is worthy of notice, although I do not attach much importance to this result for reasons to be mentioned again, that this band did not go with ammonium sulphide, while another faint band between D and E appeared, this latter however is not the dominant reduced myohaematin band as it is nearer the red than the latter. Such coagula were extracted by various solvents including glycerine and water with a negative result, the colouring matter clinging most tenaciously to the coagulated proteid.

Several proteids appear to be present in the filtrate from salted muscle, and all the myohaematin is not carried down completely until the solution has been heated sufficiently to carry down the last proteid.

When the filtrate from salted muscle is concentrated *in vacuo* over sulphuric acid, it becomes redder and the bands can be seen very distinctly, they read after concentration as follows:—

1st, λ 589 to λ 571,
2nd, λ 553·5 to λ 545,
3rd, uncertain (see sp. 7).

But it is quite evident that the myohaematin here is not the same as it exists in muscle, the bands being those of modified myohaematin. The first band of sp. 7 does not belong to oxyhaemoglobin as it persists after the use of sulphide of ammonium. Probably during the extraction of the myosin and during the subsequent concentration *in vacuo*, changes take place which account for the change of spectrum. I succeeded in procuring haematoporphyrin by acting with sulphuric acid on the coagulum produced by absolute alcohol on the filtrate of salted muscle, and filtering through asbestos, but owing to the doubt which I felt

as to whether traces of haemoglobin might not be present, I do not attach much importance to these observations; although as I shall subsequently show, haematoporphyrin may be procured from myohaematin alone, when the influence of haemoglobin may be entirely excluded. The influence of acids and alkalies, of oxygen and carbonic acid, &c. was also studied, but the same remark applies to them.

On a Method of obtaining Myohaematin in solution by means of Ether. —By the method now to be described myohaematin may be obtained perfectly free from haemoglobin, and it is so simple that it is surprising no one has discovered myohaematin before. I am indebted to some observations of H. Struve[1] for this method of extraction. Struve says that he has discovered a body giving the spectrum of oxyhaemoglobin but which is not that body for certain reasons which he mentions. He obtained it by extracting "flesh" with ether; that from the ox and calf gave the best results, but it was not present in the ether extract of all flesh; he also found it in the liver of the calf and ox and elsewhere. Struve goes on to say that when flesh is treated with ether one obtains on the one hand a more or less strongly coloured muscle-juice as a result of osmotic phenomena, and on the other hand an ether solution, which according to the kind of flesh used is coloured more or less strongly yellow. On evaporation of the first ether extract one obtains a residue containing different kinds of fat, which separate out on cooling the extract. The flesh is then treated with ether again, when the latter becomes less deeply coloured than before, and on concentration traces of fat separate from it, while it remains yellow and now gives two bands like those of oxyhaemoglobin[2]. Struve therefore turned all his attention to the ether, not to the "juice" which had exuded "as a result of the osmotic phenomena;" had he examined the juice probably he might have found myohaematin.

Struve's body giving the oxyhaemoglobin spectrum resembled myohaematin in being yellow, but differed completely in spectrum. It is however worthy of notice that myohaematin may also at times be found in the ether, as will be shown, though this is an exceptional occurrence.

The most interesting point in Struve's experiments was the

[1] *Berichte der deutschen chem. Ges.* Band IX. S. 623—627.

[2] He found the bands to correspond with those of oxyhaemoglobin as figured by Preyer in *Die Blutkrystalle*, 1871.

separation of a coloured "juice" under the influence of the ether, and it is in this "juice" that myohaematin is found.

I repeated Struve's experiment, in the first place on the heart of an ox, which was finely divided, and freed from fat as much as possible, also washed free from blood and then subjected to pressure in a linen cloth. It was then put into a large bottle provided with a glass stop-cock and covered with ether, the bottle inverted in a suitable stand, so as to allow any juice which exuded to cover the neck of the bottle and to be removed by the glass stop-cock. After two days' extraction the reddish juice gave the spectrum of methaemoglobin mixed with that of myohaematin, as shewn in sp. 8. Here the dominant myohaematin band read λ 552 to λ 545. On adding sulphide of ammonium, the two oxyhaemoglobin bands momentarily appeared, then that of reduced haemoglobin accompanied by those of myohaematin, sp. 9. As this juice exuded under the influence of the ether it was removed from time to time for a fortnight until at the end of that time a pale yellow juice exuded which no longer contained haemoglobin, and which on treatment with sulphuretted hydrogen gave sp. 10. This spectrum evidently is that of altered myohaematin, and the juice which showed it was treated with a stream of oxygen and with a stream of carbon dioxide for a considerable time without producing any change whatever. The ether extract itself after the lapse of a month showed the myohaematin bands (modified) mixed with those of a lipochrome. The former read:—

1st, λ 552 to λ 545,
2nd, λ 521·5 to λ 518·5 = narrow part,

the broader shading over which the latter was placed could not be measured. See sp. 11. This ethereal solution became redder on concentration and deposited a good deal of fatty matter; on extracting the residue with petroleum ether an orange coloured solution was obtained showing similar bands. Here then in all probability the coloured constituent had separated from the proteid, the former uniting with the fat from which it could not be separated. It is possible that some constituent such as lactic acid may have united with the myohaematin and increased its solubility, presenting in this a parallel to haematin which when pure is much less soluble than when an acid clings to it. It is worthy of remark that no myohaematin can be got into ether by extracting coagula from muscle-juice with it; and another point noticed was this: that while myohaematin is soluble in water, none could be got

from the residue mentioned above—which was left by evaporation of the ethereal solution—by the use of either cold or hot water.

But in this and in other experiments I experienced great difficulty in separating the accompanying haemoglobin from the myohaematin, so that I again returned to the source where myohaematin is the sole colouring matter: namely the pectoral muscles of pigeons. I found that if the pigeon be bled to death, there is no need to wash out the blood-vessels with salt solution. The method of doing this has been referred to before. On the other hand if death take place from pithing, chloroform narcosis, drowning, or other method whereby the animal is not bled to death, then the results are vitiated by the presence of haemoglobin. It is necessary too in removing the muscles to dissect them away from the blood-vessels, and to squeeze them well in a linen cloth. They are then cut up small and covered with ether in a bottle as described above. After the lapse of some hours the ether becomes yellow, and contains a lipochrome as Halliburton has shown,[1] but he has seen only one band while I can see two:—

1st, λ 490 to λ 469.
2nd, λ 458 to λ 441 (approximate).

A yellowish-red juice also falls to the bottom of the vessel, which on spectroscopic examination is seen to contain no haemoglobin, but does contain myohaematin in abundance, present generally as modified myohaematin, sp. 12; the bands of which read:—

1st, λ 552 to λ 545,
2nd, λ 532 to λ 506 over which is placed a narrower, darker part from λ 521·5 to λ 517.

This juice is acid in reaction, it is precipitated by heat in brownish flocks, and by absolute alcohol in fine granules. The bands are unchanged with sodium hydroxide in slight excess, and are diminished and then made to disappear with acetic acid[2].

The juice does not always show this spectrum, but another figured in sp. 13, which can however be changed into the last by ammonium sulphide. The bands of sp. 13 read:—

1st, about λ 569 to λ 562,
2nd, „ λ 553·5 to λ 547,
3rd, „ λ 542 to λ 509.

[1] *Journal of Physiol.* Vol. VII. No. 4.

[2] In some solutions two faint bands may be seen in the green between D and E, in a deep layer.

We notice that the band at D of the normal spectrum of muscle is wanting, doubtless due to changes produced during extraction and the development of the acid reaction. Two of these bands will be found to be coincident with those of myohaematin in the muscle itself.

If this solution be treated with a stream of carbon dioxide for an hour, or if the former solution be treated with a stream of oxygen for the same time, as I have repeatedly done, no change of spectrum takes place. If however ammonium sulphide be added to the latter the spectrum changes. Two well-marked bands appear like those of sp. 12 now reading:—

1st, λ 553·5 to λ 545,

2nd, λ 529 to λ 509, with a darker part within it from λ 523 to λ 517.

This reduced solution cannot be however changed by oxygen as regards its spectrum, showing clearly that this myohaematin is not capable of uniting with oxygen after it has become reduced; and as I said before the oxidized modification of sp. 13 cannot be changed into the reduced by CO_2. Therefore myohaematin does not enter into a loose combination with oxygen like haemoglobin, so far as one can judge in dealing with the slightly altered pigment.

The bands of the oxidized variety of sp. 13 may vary very slightly in different cases; sometimes they read:—

1st, λ 569 to λ 562,
2nd, λ 552 to λ 545,
3rd, λ 542 to λ 514.

These measurements are those of a solution concentrated *in vacuo*, and after ammonium sulphide they read:—

1st, λ 553·5 to λ 545,
2nd, λ 530·5 to λ 506, over which was placed a narrow, darker part from λ 523 to λ 517.

Sometimes too a band or bands at D may be visible in concentrated solutions as shown in sp. 14, where the great darkness and breadth of the other bands are remarkable.

Although solutions of myohaematin are reddish-yellow yet when concentrated they are quite red, so that a red muscle may owe all its colour to myohaematin. The muscle-juice thus obtained yields a precipitate with absolute alcohol, and by heat, by heat and acetic acid, and other proteid precipitants. The precipitate becomes a fine red when boiled with Millon's reagent and shows the xanthoproteic reaction with nitric acid. The precipitate with absolute alcohol shows the

bands of the original solution, and changed in the same manner with sulphide of ammonium, but when heated to a certain point the bands disappear, as they do in muscle itself. Incinerated the precipitate showed the presence of iron, and it also contained sulphur, besides carbon, oxygen, and nitrogen. Whatever precipitates the proteid out of solution also precipitates the colouring matter; and from the coagulum no solvent that I have used will extract the colouring matter; hence pigment and proteid are closely united. In fact, myohaematin appears to be a coloured proteid.

On certain Crystals observed in Solutions obtained as above.—When muscle-juice obtained as described is concentrated *in vacuo* over sulphuric acid crystals separate out, which belong to the rhombic system[1] and are coloured yellow or yellowish-red; each crystal when large enough showing the bands of the mother solution. They readily dissolve in water, the solution being precipitated by heat and by absolute alcohol; these crystals were incinerated and also yielded traces of iron. Hence I was inclined to believe that they must have been crystals of myohaematin; but subsequent experiments showed that this is not the case. In colourless muscle-juice from the rabbit I observed similar crystals and these showed no absorption bands whatever. Subsequently I found that I could deprive coloured crystals of their colour, while at the same time they lost their absorption bands, by washing them with water containing enough rectified spirit to keep them from dissolving; and when thus prepared their aqueous solution was no longer precipitated by heat or alcohol, and boiling with Millon's reagent gave a negative result. Why did crystals from coloured juice give a spectrum and proteid reactions, and why did their ash contain iron? Simply because each crystal as it formed became coated with a film of coloured proteid to which these reactions were due.

When the colourless crystals were dried *in vacuo* over H_2SO_4 they effloresced, each crystal breaking down into a white powder. Finally I proved them to be crystals of kreatin, and doubtless the muscle-juice obtained as I have described may be found useful for the demonstration of kreatin. By following Hoppe-Seyler's directions[2] I changed them into kreatinin and obtained the very characteristic crystals of the combination of zinc chloride and kreatinin by treatment with the latter reagent and allowing the solution to stand some hours.

[1] "Oblique rhombic columns,"... "belonging to the monoclinic system."

[2] *Handbuch der Phys. und Path. Chem. Anal.* vierte Auflage, S. 178.

Although ether does not usually take up myohaematin from pigeon's muscle, yet in one instance the ether extract did contain some, as shown in sp. 15. The band at D did not go with sulphide of ammonium.

Ammonia added to pigeon's muscle forms a glutinous solution of an orange colour, in which no distinct bands may be detected, yet on adding ammonium sulphide two modified myohaematin bands may be seen, like those of sp. 12; but if this solution is boiled they can no longer be brought back with sulphide of ammonium.

From the *red* muscles of the rabbit myohaematin may be procured, and the juice obtained as above gives the same spectrum, as in the case of pigeon muscle, the pale muscles yielded only traces.

On the decomposition products of Myohaematin.—In endeavouring to procure these, the coagula[1] from the muscle-juice perfectly free from any trace of haemoglobin were used.

Treated with rectified spirit and caustic soda or caustic potash—in order to find out whether alkaline haematin can be procured—a gelatinous mass which filters with difficulty is obtained. In this two very faint bands in green may be just visible, but on adding sulphide of ammonium the bands of modified myohaematin are reproduced, there is no band at D, therefore no alkaline haematin can be procured.

If rectified spirit and sulphuric acid be used, no result follows until the solution has been heated, then a faintly reddish solution is obtained which after filtering gives some feeble bands (sp. 16), which read (approximately):—

1st, λ 636 to λ 601,
2nd, λ 585 to λ 569,
3rd, λ 545 to λ 526,
4th, λ 511 to λ 485.

These resemble the bands of acid haematin, but do not exactly correspond; at the same time I believe this to be a kind of acid haematin.

When the coagula were treated with strong sulphuric acid and filtered through asbestos, I did obtain acid haematoporphyrin undoubtedly. In the dark red filtrate a well-marked narrow band was visible before D and another between D and E, also a third simply due to the presence

[1] By the word "coagulum" here and elsewhere I mean that produced by adding absolute alcohol to the muscle-juice.

of proteid, the two former vary slightly according to concentration of the solution. In one experiment they read:—

1st, λ 601 to λ 589,
2nd, λ 559 to λ 545. (sp. 17.)

If we compare with these measurements those of a solution of acid haematoporphyrin from the integument of a slug[1] we get:

1st, λ 600 to λ 591,
2nd, λ 561·5 to λ 547.

Hence the bands are practically coincident.

On pouring the filtrate into water and adding ammonia precipitation takes place; on dissolving the precipitate in rectified spirit and ammonia by the aid of heat a four-banded spectrum is obtained, sp. 18, whose bands are fainter and narrower—owing to dilution—than they ought to be for comparison; they read:—

1st, λ 625 to λ 612·5,
2nd, λ 587 to λ 560·5,
3rd, λ 540 to λ 526,
4th, λ 509 to λ 488.

If we now take a similar solution prepared from haematin we get the following readings:—

1st, λ 633 to λ 612·5,
2nd, λ 587 to λ 564,
3rd, λ 549 to λ 529,
4th, λ 518·5 to λ 488.

Allowing for great dilution the agreement is close enough. Hence then we can procure haematoporphyrin from myohaematin. Now I stated formerly[2] that the histohaematins may be the mother substances from which the integumental haematoporphyrin of certain invertebrate animals may be derived; a statement which is now proved to be quite correct. And since myohaematin and haemoglobin yield some decomposition products which are respectively identical, one may safely conclude that these colouring matters are nearly related to each other. Probably myohaematin is of simpler constitution than haemoglobin, since the intermediate products of decomposition are not all obtainable, while the ultimate ones agree.

[1] *Journ. Physiol.* Vol. VII. No. 3. (Cf. also Vol. VI., Nos. 1 and 2.)
[2] *Journ. Physiol., loc. cit.*

Separation of a Histohaematin.—Owing to the difficulty of excluding the influence of haemoglobin, the separation of a histohaematin from a vertebrate organ is not as easy as is that of myohaematin. I have already shown that the spectrum of a histohaematin in the blood-free organs of a cat[1] agrees remarkably in the case of each organ, and with that of myohaematin; so that if one can succeed in getting a histohaematin from any organ, it may be considered identical with that from any other organ; and if we can show that the body in solution agrees in spectrum with that of myohaematin in solution, we may conclude that all that has been said about myohaematin will apply to it.

A histohaematin may be obtained from the kidney by the same method as that required for procuring myohaematin, with this exception, that the blood-vessels have to be washed out with salt solution. The kidney of the sheep has been used for this purpose. A longer time is required for the extraction of a histohaematin than for that of myohaematin: three weeks or a month being generally necessary.

The organ, after its arteries have been injected with salt solution, is cut up small, washed, the portions pressed in a linen cloth, and then covered up with ether in a bottle. After the lapse of some days a yellow juice is found to be exuding which at first may contain a little haemoglobin, but later on this juice is of a paler tint and then is free from it. Even if haemoglobin be present the detection of a histohaematin is not interfered with, as ammonium sulphide will bring out the dominant histohaematin band, which may read from λ 553·5 to λ 547, and is therefore coincident with the first band of changed myohaematin. In some cases I could just perceive the second band from λ 526 to λ 514, which is also identical with the similar band of modified myohaematin. In sp. 19 I have given this spectrum, and in that case the bands read:—

1st, λ 551 or 2 to λ 547,
2nd, λ 523 to λ 517 = darker part.

On looking back, we now see that this darker part also corresponds to the darker part of the same band in the case of myohaematin. Sometimes a band may appear momentarily in red on adding ammonium sulphide, and the other bands are always intensified. Moreover these bands are affected in a precisely similar manner as those of myohaematin, by the action of different reagents: in fact there is no longer the shadow of a doubt that no difference exists between this modified histohaematin and modified myohaematin. The juice in which this pigment is held in

[1] *Phil. Trans.* Part I. 1886.

solution is also like the corresponding muscle-juice rendered cloudy by heat and acetic acid, and by absolute alcohol, and the precipitate is coloured red by boiling with Millon's reagent. Hence in this organ a plasma occurs which holds in solution a respiratory coloured proteid, and from the liver of the rabbit I have obtained a similar one.

Remarks.—The proof of the existence of these respiratory proteids, detected in the solid organs and tissues of animals by means of the microspectroscope, no longer rests on the observation of their spectra in those organs and tissues, as one can easily now obtain them in solution and study their changes with reagents. They are as important as haemoglobin, if not more so in some animals, and they have the right of priority in time, as they were developed at an earlier period than haemoglobin, speaking from a phylogenetic point of view. Even in the lowest of metazoa—the Sponges—I have met with histohaematins[1], where they are also capable of oxidation and reduction and are therefore respiratory. It is not improbable, but indeed likely, that by a process of physiological selection these respiratory proteids may have become more complex, and their molecular instability therefore greater as the animal body became more elaborated, and a necessity arose for the setting apart of respiratory proteids for abstraction of oxygen from the air. In this way haemoglobin may have arisen, and although it is usually said that the mere colour of haemoglobin is of no use, yet the fact cannot be denied that most respiratory proteids are coloured bodies. The molecular complexity of the histohaematins is certainly not so great as that of haemoglobin, and their respiratory capacity is apparently far inferior to that of haemoglobin, for I am convinced that the histohaematins do not take up the oxygen in a loose combination, although they certainly do unite with it in a more stable combination. At the same time, one must remember that the myohaematin or the histohaematin procurable from dead tissues differs in spectrum and therefore probably in chemical composition from that of the living tissues. It is well known that no free oxygen can be obtained from muscle, and if myohaematin be the body with which it unites, then myohaematin must certainly have something to do with the storing of oxygen in muscle; and if this be the case in muscle it must be the case in other tissues and in the organs in which the histohaematins are found.

In studying the chromatology of many invertebrates I have been struck by the fact that while some of their colouring matters can be reduced

[1] *Proc. Physiol. Soc.*, March, 1886.

by such reducing agents as sulphide of ammonium, yet by shaking with air, or by passing a stream of oxygen into them, they cannot be reoxidized; in this point they afford a parallel to the histohaematins. Krukenberg has noticed the same thing and he has justly concluded that the respiratory processes of many of these animals is not as simple a matter as it is supposed to be. There can be no doubt that the union of these respiratory colouring matters with oxygen is a much more stable one than is the case with haemoglobin. It is in the observation of such facts as these that the spectroscope comes to be of value, for if these bodies did not show absorption bands one could not determine whether they were in the oxidized or reduced state.

The question may now be considered as settled which I was not able to answer before: namely, In what condition is myohaematin present in muscular fibre? as it is certainly present in the fluid condition. After muscle-coagulation sets in it is found in the muscle serum, and in the kidney also it would appear that the "structural elements" of the organ are bathed with a "juice" which contains the respiratory proteid histohaematin. Anything which causes coagulation of the proteid or proteids in the "juice" carries down the pigment and prevents its extraction by solvents.

The presence of iron in the muscle-juice in which myohaematin occurs is interesting, and it probably belongs to the myohaematin, because there is no other constituent present in the muscle-juice which is known to contain iron. Until myohaematin shall have been definitely isolated this point must however remain undecided. If iron is necessary for the formation of myohaematin and the histohaematins, an interesting medical point is raised: and another equally interesting question is this: May not oxygen starvation of the tissues occur from a deficiency of the respiratory proteids? And if these are not present in quantity or quality sufficient for the discharge of tissue- or internal respiration, then metabolism cannot be properly performed in the various organs and tissues, and these may become laden with products of incomplete metamorphosis leading to the production of disease.

These are not mere hypotheses, for I have definitely proved that myohaematin and the histohaematins are not themselves products of the metabolism of haemoglobin, but are mother substances of great importance from a physiological point of view. And the fact that they can be converted into decomposition products, some of which are identical with those of haemoglobin, such as acid haematin and haematoporphyrin, teaches that the view hitherto held that most of the biliary and urinary

pigments are derivatives of haemoglobin must be considerably modified.

The likeness of modified myohaematin to haemochromogen is striking, but I find that the bands of the former are much nearer the violet end of the spectrum, and the dominant myohaematin band is very much narrower than the first band of haemochromogen.

EXPLANATION OF CHART OF SPECTRA. PLATE II.

Sp. 1. Filtrate from pigeon muscle which had been treated with chloride of sodium, after addition of water.

2. The same with ammonium sulphide.

3. Filtrate obtained later and filtered under pressure.

4. A similar filtrate.

5. This filtrate was precipitated by absolute alcohol, the precipitate extracted with rectified spirit and caustic potash and the solution filtered.

6. The same with sulphide of ammonium showing band at D still present. Note however that the 2nd band is not in the same place as that of myohaematin[1].

7. The muscle extract from salted muscle somewhat concentrated *in vacuo*.

8. The reddish juice from heart of ox obtained by the ether process, showing spectrum of myohaematin mixed with that of oxyhaemoglobin or methaemoglobin.

9. The same with ammonium sulphide.

10. Purer "juice" from same source treated with sulphuretted hydrogen in aqueous solution.

11. Ether extract of heart muscle of ox, showing how as an exception myohaematin may be taken up by the ether.

12. The red "juice" from blood-free pigeon muscle which exudes under the influence of ether shows generally this spectrum.

13. Sometimes this spectrum is seen instead of the last and appears to belong to oxy-myohaematin.

14. "Juice" from pigeon muscle obtained by the ether process, containing a good deal of myohaematin and showing some bands at D.

15. An ether extract of pigeon muscle gave this spectrum on one occasion; this is however the exception to the rule, as one generally only finds lipochrome bands in the ether extract.

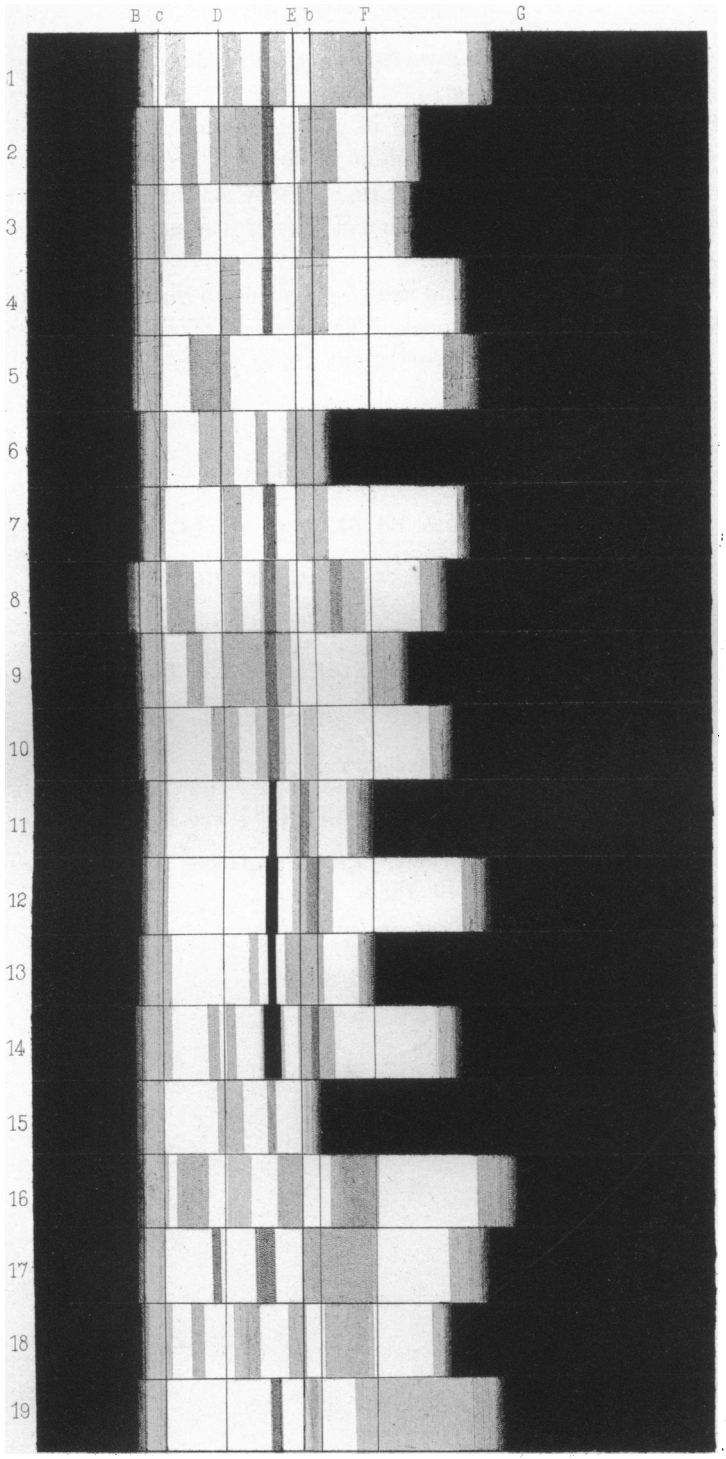
[1] As stated above I attach no importance to this spectrum, it may be only that of haemochromogen mixed with alkaline haematin.

16. A spectrum somewhat like acid haematin, got by acting on myohaematin with rectified spirit and sulphuric acid.

17. Acid haematoporphyrin from myohaematin.

18. Alkaline haematoporphyrin from myohaematin.

19. Spectrum of a histohaematin from the kidney of the sheep. This can be seen after removing the juice, which exudes from the organ—previously injected with salt solution and finely divided—under the influence of ether, twice or three times, and again covering with ether. Finally a pale yellow juice exudes which shows these bands.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