Observations on some of the colouring matters of the bile and urine, with especial reference to their origin : and on an easy method of procuring haematin from blood / by C.A. Mac Munn.

Orville Rice with Walter's Compliments

(3)

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OBSERVATIONS ON SOME OF THE COLOURING MATTERS OF BILE AND URINE, WITH ESPECIAL REFERENCE TO THEIR ORIGIN; AND ON AN EASY METHOD OF PROCURING HAEMATIN FROM BLOOD.

By C. A. MAC MUNN, M.A., M.D. Pl. I.

(1) Easy and rapid method of procuring Haematin. An easy method by means of which the decomposition products of haemoglobin can be procured in a short time has been long wanted, and the method I am about to describe is both easy and rapid.

I may remark that the methods which until very recently have been used for the preparation of haematin are[1] (1) the decomposition of haemoglobin by means of oxalic or acetic acid in the presence of ether which takes up the haematin formed, and treating with water, alcohol, and ether; (2) the very elaborate method of Hoppe-Seyler which requires the previous formation of haemin or hydrochlorate of haematin; (3) coagulation of blood by an excess of cold alcohol, and boiling the precipitate with alcohol and sulphuric acid and further treatment; besides these three there are other methods which I need not mention, as in most of them the thorough separation of proteid from coloured constituent is not accomplished. Nencki and Sieber[2] have lately published another method which depends on the formation of haemin and its solution by amyl alcohol; from the haemin thus formed and separated by amyl alcohol they readily obtained haematin and haematoporphyrin.

In 1880[3] when endeavouring to work out the connection between the colouring matters of blood, bile and urine, I found that chloroform was an excellent means of separating the acid haematin formed by the action of alcohol acidulated with sulphuric acid on blood, but owing to pressure of other work I had to postpone the further study of this subject until lately. Even now the subject is not thoroughly worked

[1] Gamgee, *Physiol. Chem.* Vol. I. p. 114.

[2] *Berliner Berichte*, Vol. XVII. pp. 2267–2276.

[3] *Proc. Roy. Soc.* No. 208. 1880.

out, but as the method may lead to good results in the hands of others, I have thought it perhaps advisable to publish an account of it in its present form.

The defibrinated blood of the sheep or other animal, or preferably the blood clot (as the pigments of serum are got rid of by using the clot), is extracted with rectified spirit containing pure sulphuric acid of a strength of 1 in 17 or thereabouts, the solution is filtered, and on examination with the spectroscope shews the spectrum of acid haematin. The solution after dilution with an equal bulk of water is agitated in a separating funnel with chloroform, which at once assumes a reddish brown colour, while at the same time precipitation of proteids and other matters takes place. After standing a little time the chloroform is separated off and filtered. The separating funnel is then well washed and the chloroform again returned into it and washed with fresh water to remove most of the acid clinging to it. It is then separated off again and filtered, and may be allowed to evaporate spontaneously or distilled on the water bath. The residue is a dark brown pigment, soluble in alcohol, ether, chloroform, aqueous alkaline solutions and bisulphide of carbon, and insoluble in petroleum and water. If allowed to remain for some time on the evaporating dish it dries up into a bluish-black powder.

Now the solubility of this pigment differs from that of haematin as it is usually described, and I at first concluded that this must be due to the fact that it does not contain a proteid, but further study of it shews that this is not the reason. The reason probably is that it carries with it, clinging to it in that tenacious manner familiar to anyone who has tried similar experiments, a small quantity of acid which increases its solubility in the above media.

This residue can now be used for preparing solutions shewing the spectra of acid haematin, alkaline haematin, haemochromogen and haematoporphyrin. Dissolved in the above-mentioned solvents it shews the spectrum of acid haematin, in rectified spirit and caustic soda that of alkaline haematin, and on adding sulphide of ammonium to the latter solution that of haemochromogen. To procure from it haematoporphyrin it is dissolved in sulphuric acid and filtered through asbestos, when the deep red filtrate shews a well marked acid haematoporphyrin spectrum. If this is poured into water and ammonia added, in a short time reddish-brown flocks form in the solution which are separated by filtration and which dissolve in alcohol and ammonia, shewing the spectrum of alkaline haematoporphyrin.

But this haematin is not pure; the readiness with which it allows itself to be reduced with sulphide of ammonium shews that some proteid is probably clinging to it, and its solubility in the media mentioned shews that it is mixed with something increasing its solubility.

I found that if instead of evaporating the chloroform after separation and filtering it was allowed to stand for a few hours, the solution became cloudy and the cloudiness was then found to be due to separated pigment. This occurs in brownish granules, mostly made up of groups of needles and rhombic prisms, and in star-shaped crystals which have a strong resemblance to some varieties of haemin crystals. The very small spicules, prisms and needles, are however arranged in crosses or rosettes and one does not find the isolated rhombic tablets of haemin. Still the resemblance to haemin is very remarkable, especially when one considers that no hydrochloric acid can be present.

If these crystals are separated by filtration, well washed with water, alcohol and ether, the mass left on the filter paper is a bluish-black colour, and now is insoluble in alcohol, ether, chloroform, water or weak acids. It is however soluble in weak solutions of caustic alkalies, and in alcohol and caustic soda (out of which it can be precipitated with hydrochloric acid, and then gives the usual reactions of haematin). By this treatment its solubility is completely changed, and it answers to all the reactions of pure haematin. From it can be got acid and alkaline haematin and haematoporphyrin, but its alkaline alcoholic solutions are hardly changed with ammonium sulphide. This shews that Hoppe-Seyler is correct in saying that the presence of a proteid is necessary for reduction into haemochromogen.

The occurrence of the crystals and their apparent dependence on the presence of acid is most remarkable, and shews that hydrochloric acid is not necessary for the formation of crystals bearing a strong resemblance to those of haemin.

Amyl alcohol as well as chloroform is a very good solvent for the impure haematin, but I have not yet examined this solution thoroughly.

(2) Cholohaematin. As is well known the bile of carnivorous animals is generally free from bands, except when treated with acids, when the band of urobilin comes into view, provided the presence of other bodies possessing an absorptive power for the violet end of the spectrum does not obscure this band. Bilirubin itself, although it has a most characteristic absorptive power for the violet end of the spectrum, gives no bands until treated with nitric acid, when the changes of colour

and spectra which it undergoes are most characteristic[1]. Biliverdin also in alcoholic solution is free from bands, whether it be prepared from bilirubin or obtained from herbivorous bile; it absorbs however a little of the red and violet ends of the spectrum and sends through green intensified, and when treated with nitric acid the same changes of spectra are seen as in the case of bilirubin. All general statements affirming the presence of absorption bands in bile are incorrect. In Dalton's *Physiology* for instance, the statement occurs that biliverdin possesses three bands, but in this instance Dr Dalton has concluded that the green colour of sheep's bile furnishes the three bands which, as I shall shew, are generally present in sheep and ox bile, but in reality it owes its green colour for the most part to a biliverdin-like pigment[2], and this when separated from the bile possesses no band whatever: the bands being due to a changed haematin as I have proved[3], and not to biliverdin at all. I have already called attention to the fact that the bile of some animals yields bands, but when present they are doubtless due to the presence of a colouring matter which is a derivative of haematin.

I wish now to call attention to the body in the bile of the sheep and ox which gives the three- or four-banded spectrum. This spectrum is not constant in the bile of the sheep and ox: it is sometimes only present in traces, and then replaced by a green pigment, which differs from the biliverdin prepared in the usual way from bilirubin in the peculiarity that it goes into chloroform when the bile—after precipitation of mucus, &c. with absolute alcohol and acetic acid, and filtering—is shaken in a separating funnel with chloroform; still it possesses the same absorptive property as biliverdin.

When the band-giving pigment is present in ox bile, its bands give approximately the following readings:

1st band centre at λ 649,
2nd band from λ 613 to 585,
3rd band from λ 577·5 to 561·5,
4th band from λ 537 to 521·5 (?).

On adding absolute alcohol, a few drops of acetic acid, filtering, agitating with chloroform, separating, and filtering, a bronze-coloured solution is obtained which gives the same four-banded spectrum:

1st band from λ 654 to 636,
2nd band from λ 607 to 580·5,

[1] Cf. *The Spectroscope in Medicine*, 1880. Also *Proc. Roy. Soc., loc. cit.*

[2] Which however goes into chloroform and amyl alcohol.

[3] *Proc. Roy. Soc., loc. cit.*

3rd band from λ 572 to 560,
4th band from λ 536 to 516.

On dissolving the residue from a chloroform solution in ether a greenish solution is obtained shewing four bands:

1st band from λ 654 to 636,
2nd band from λ 600 to 579,
3rd band from λ 567·5 to 556,
4th band from λ 531 to 513.

The pigment also went into bisulphide of carbon, forming a greenish solution giving the following bands:

1st band from λ 660 to 640,
2nd band from λ 607 to 583·5,
3rd band from λ 574 to 562·5,
4th band from λ 537 to 519.

The above solutions do not of course contain the pure pigment, and in order to obtain it purer I evaporated down an ether solution, dissolved the residue in chloroform and washed it again in a separating funnel with water. On separating off the chloroform, filtering and evaporating the solution, an amorphous resinous looking residue of a dark sap-green colour was obtained which still had a peculiar musky smell. On dissolving some of this residue in alcohol and adding ether, no precipitate formed, shewing that bile salts could not have been present.

I have shewn in another place that this banded spectrum of ox and sheep bile is not due to the presence of the same oxidation product obtained by the action of nitric acid on bile pigment, as Hoppe-Seyler[1] concluded. This can easily be seen by a comparison of the spectra. But an additional proof is afforded by the action of nitric acid on an alcoholic solution. On adding nitric acid the liquid which was olive-brown (daylight, and reddish by gaslight) became dark green, and the spectrum changed as shewn in Plate I. The dark band at D read from λ 596·5 to 583·5, the next from λ 572 to 556, and the next from λ 501 to 481 (?). On adding more nitric acid the fluid became more greenish and the spectrum changed as shewn in the plate. In the large spectroscope the band before D was seen to be composed of two, all of the bands reading as follows:

1st part of band λ 623 to 613,
2nd part of band λ 607 to 600,

[1] *Handbuch Physiol-Chem. Analyse.* 4th Ed. 1875. Cf. *Proc. Roy. Soc.* No. 226. 1883.

next band λ 577·5 to 553,
next band λ 503 to 482·5.

Sulphuric and hydrochloric acid acted in the same way, but acetic acid only caused darkening of the band between green and blue.

But the most interesting result was obtained from this colouring matter by the action of sodium amalgam. I had already concluded that a similar spectrum to that yielded by the present colouring matter could be obtained by the short action of sodium amalgam[1] in the cold upon alkaline haematin, and the truth of that supposition was confirmed by reducing an alcoholic solution of the pigment itself in rectified spirit with sodium amalgam. An alcoholic solution was sufficiently diluted with water to enable a reaction to be developed with the sodium amalgam and it was then heated on the water-bath. At first but little change was produced; after three hours the colour had become paler, but after some time it again got darker. It then shewed three bands, two faint between D and E, and a darker at the blue end of the green. It was then treated with sulphuric acid in excess and filtered; the filtrate was reddish and shewed a spectrum which is that of acid haematoporphyrin, (mixed with urobilin?) The bands read:—

1st λ 609—600,
2nd λ 582—548·5,
3rd λ 505—484·5.

The colouring matter was then isolated by means of chloroform in the separating funnel, the chloroform evaporated down leaving a brown residue which had a smell of butyric acid. It was then dissolved in absolute alcohol, forming a red solution, and this shewed the spectrum of acid haematoporphyrin, of which the bands read:—1st from λ 611 to 600, 2nd from λ 582 to 548·5, the darkest part of the last being from λ 569 to 551, the third band from λ 505 to 484·5. And on adding ammonia until an alkaline reaction was obtained and filtering the solution, it was found to be yellowish red and gave the following bands:—

1st from λ 649 to 642·5,
2nd λ 628 to 619,
3rd λ 582 to 564·5,
4th λ 551 to 531,
5th λ 517·5 to 494·5.

If one compares these measurements with those of a similar solution of haematoporphyrin they are found to agree closely.

[1] *Proc. Roy. Soc.* No. 208. 1880.

Now I formerly found haemochromogen in mammalian bile; and a similar colouring matter in the bile of invertebrates[1] e. g. among pulmonate mollusca, in the crayfish, and latterly in *Patella*. In invertebrates it is undoubtedly connected with the pigments found in their tissues which I have named histohaematins, and it is quite possible that in the sheep and ox it may be a metabolite of the same pigments, and of myohaematin.

If the spectrum of ox and sheep bile when it contains the above pigment be compared with that of the histohaematins, there is a strong likeness (cf. also spectrum of medulla of adrenals). These observations so far prove that in the bile of the sheep and ox a colouring matter is found which must be a haematin derivative, (and a mere inspection of its spectrum would suggest that it may be a mixture of alkaline haematin and haemochromogen).

Does this furnish biliverdin? or is biliverdin formed from it? Physiologists are fond of appealing to the simple structure and functions of the lower animals for a reply to complex questions of human physiology, and in the present case some observations recently made by me on Sea-anemones may help to answer the question. In the common red *Actinia mesembryanthemum* a colouring matter occurs which is changeable into haemochromogen and haematoporphyrin. Now in the mesoderm and elsewhere in the same animal, I have found biliverdin beyond doubt. There at least it no doubt is the metabolite of the useless pigment which is no longer fit for respiration, and I believe it is the same with the biliverdin of bile. I have already shewn that biliverdin was present in a hydrocele fluid into which blood had been extravasated, and I have seen in the lobule of the liver of a pigeon squeezed out in the compressorium and examined in the manner described before the Physiological Society, in one part haemochromogen, in another biliverdin, which filled the ultimate radicles of the biliary ducts in the lobule. Hence it appears to me that biliverdin is broken down and changed haematin.

With regard to this pigment of the bile of the sheep and ox, in order to avoid confusion, I think it ought to be called by a name which will distinguish it from other pigments: perhaps cholohaematin is as appropriate as any other.

The idea that the pigment described above is chlorophyll can be disproved by a comparison of its spectra with those of chlorophyll.

[1] *Proc. Roy. Soc.* No. 226. 1883.

(3) Peculiarities of the colouring matters in bile from a biliary fistula[1]. As the bile acids are occasionally absent from bile obtained from biliary fistulae it becomes of interest to see whether the pigments may not be absent, or in what they differ from the normal ones. The specimens examined were obtained from a biliary fistula in a woman upon whom Mr Lawson Tait had performed cholecystotomy for impacted gall stones, and the bile was obtained some time after the operation. The first specimen was obtained after dinner. It had a bronze-green colour and transmitted red and a little green in a deep layer, but shewed no bands. After treatment with acetic acid it became very much greener, shewing that a chromogen existed in it which became oxidized by the acid; this is interesting as in sea-anemones a similar chromogen is found. On agitating with chloroform no bilirubin could be got into solution, for the bile contained none, but on standing a little while in contact with the chloroform the latter shewed a band at F, and as the original bile gave no band, it is quite evident that a chromogen belonging to the pigment giving the band at F was also present. On evaporating the chloroform and dissolving the residue in absolute alcohol a yellow solution was obtained which gave a band from λ 519 to 503.

On treatment with zinc chloride a band appeared in the red and a feeble one[2] at D, also a band nearer the violet end of the spectrum which also appears when febrile urobilin or stercobilin is treated with zinc chloride; on adding a few drops of ammonia and filtering, a green fluorescence was noticed. The band of the alcohol solution was much nearer red than that of urobilin, yet the other characters of the pigment leave no doubt that it is closely related to urobilin.

The next specimen was obtained from the woman while fasting. This was also a bronze-green colour and was free from bands, and gave a play of colours with nitric acid and the usual spectrum changes of Gmelin's reaction, as also did the former specimen. It also became greener with acetic acid. It contained no bilirubin and no urobilin, and water extracted the green colouring matter from the residue left after the evaporation of an alcohol extract better than any other solvent. From the same residue ether and chloroform failed to take up anything.

The third specimen came from the same source and was obtained before dinner: it had a brownish-yellow colour but on adding a little acetic acid and alcohol and filtering the filtrate was green. On agitating

[1] I have to thank my friend Prof. Windle for these specimens.

[2] Similar bands can often be seen when the bile of some animals is treated with caustic soda and zinc chloride, e.g. that of toad, frog, cat &c.

with chloroform the latter became greenish-yellow, and gave a band between green and blue from λ 506 to 489, and this band was narrow and distinct.

On evaporation of the chloroform and on dissolving the yellow and green residue in absolute alcohol a greenish yellow solution was obtained which also gave a band like the chloroform solution: on treatment with caustic soda and zinc chloride the same bands appeared that are seen when urobilin is treated in the same way: moreover a faint green fluorescence was now seen. This specimen therefore contained the chromogen of biliary urobilin and very little of the usual bile colouring matters.

The fourth specimen from the same source was obtained after dinner and had a golden-yellow colour and a feebly acid reaction, sp. g. 1007. On treatment with acetic acid and absolute alcohol and filtering, the filtrate was green. On shaking this with chloroform a greenish-yellow solution was obtained which gave the same band as that noticed in the last specimen from λ 506 to 489. The yellow and green residue left after evaporating the chloroform formed a green solution in absolute alcohol and absorbed a little of the red and violet ends of the spectrum, and shewed also a band in the blue end of green: on treatment with caustic soda alone, and with caustic soda and zinc chloride, the solution showed similar appearances to those seen by similar treatment of an alcoholic solution of stercobilin. The narrow band nearer violet extended from λ 513 to 503 with caustic soda alone. In this specimen the principal colouring matter present was urobilin; there was no bilirubin and not much biliverdin.

Mr Tait very kindly ordered the bile of 24 hours to be collected for me from the same case, a portion being collected every hour. The specimen had a bronze-green colour, a sp. g. of 1006·5, and a feebly acid reaction. I found that in 100 parts of this bile there were 1·4915 parts solids, and 98·5085 water. On treating with acetic acid and alcohol and filtering, the filtrate had a brownish-green colour, and on agitating with chloroform the latter took up the same pigment as in the other cases, giving a band from λ 507 to 490·5. The yellow and green residue left on evaporation of the chloroform was dissolved in absolute alcohol, and on treatment with zinc chloride and caustic soda this yellow solution shewed a distinct green fluorescence and the same spectrum appearances noticed before.

Hence four of these specimens contained the chromogen of a colouring matter which appears to be very similar to febrile urobilin

and stercobilin, no bilirubin, but some biliverdin, and the chromogen of biliverdin. The occurrence of urobilin in bile is very constant, so much so that it has as much, if not more, right to be enumerated among the biliary constituents as bilirubin or biliverdin.

I regret that I am not able to find out whether the urobilin in this bile had found its way from the intestine to the liver, but I think it highly probable, as Mr Tait was going to close the fistula at this time[1].

(4) The colouring matters of Fæces. I have already shewn that there is a difference between stercobilin obtained from the fæces of the cat and febrile urobilin, but on examining human fæces I find that the difference is hardly perceptible; when one says that the fluorescence of a zinc-chloride-treated alkaline solution of stercobilin is much greener than a similarly treated solution of febrile urobilin, he has given the only difference worth mentioning. The differences between stercobilin and normal urobilin are greater, and I may state at once that these pigments are not identical as can easily be shewn.

Vaulair and Masius[2] so far as I know are the only investigators of stercobilin so called by them, but the proof of the identity or non-identity of this colouring matter with the urinary pigments or one of them is of great importance to the physician. If stercobilin is the source of febrile urobilin or its chromogen it is quite evident that the amount of this pigment in urine is the measure of the amount of absorption from the intestine. That this is a matter of immense importance in medicine no one can deny who has read Dr Lauder Brunton's recent lectures on Digestion, for if a large quantity of unchanged stercobilin can be taken up from the intestine, it is reasonable to suppose that some ptomaine-like bodies may accompany it, and may escape the destructive action of the liver.

The best, and at the same time the least unpleasant, method of proceeding in examining fæces is to divide the mass into several portions and cover them up at once with different solvents. I have examined the colouring matters obtained from these solutions:—(1) absolute alcohol, (2) chloroform, (3) ether, (4) rectified spirit and sulphuric acid—1 in 15, (5) water.

[1] I have noticed the appearance of the band of urobilin in chloroform solutions—got by shaking bile with this solvent—repeatedly, so that the presence of this chromogen in bile is tolerably constant.

[2] *Centralb. f. d. med. Wissensch.*, 1871. Nr. 24.

The mass being stirred up in each solution by means of a glass rod. The following results were obtained.

Alcohol solution. The alcohol solution was a deep orange red colour. In a deep layer it transmitted red, in thinner red and a little green, and, on diminishing the depth still more, an abrupt shading was seen at the blue end of green due to the presence of a band, which on adding an acid (acetic 1 drop) stood out quite black and extended from about λ 506 to 482·5. On adding caustic soda a narrow band less shaded and nearer the red appeared from about λ 521·5 to 505. On again adding acid the band appeared as before.

With zinc chloride (alone) a precipitate was produced, and after filtering, the solution gave a well-marked green fluorescence, and this solution gave a band from λ 517·5 to 501. This green fluorescence is much more intense than that obtained with either normal or febrile urobilin.

Ammonia also produced a similar band to that produced by caustic soda, also a very feeble band in red. The former read from λ 517·5 to 503. On treatment first with ammonia and then zinc chloride, the green fluorescence was very remarkable and the band produced had the peculiarity of shading noticed in a similar solution of febrile urobilin, its redward edge being abruptly shaded while the other less shaded edge extended far over towards violet. In one solution the band extended as far as from λ 520 to 467 (!). If a deep layer of alcoholic solution be treated with caustic soda a band is seen in red and one at D besides that nearer violet referred to. The first extended from λ 649 to 629. Zinc chloride caused exactly the same appearance, and the bands gave the same reading.

Ether solution. The ether solution was yellow and gave a band in red, belonging to chlorophyll from the food. a double band far over in the green, and a third in violet belonging to lutein. The last three gave the following measurements: 2nd band λ 519 to 501, 3rd λ 492·5 to 470, 4th λ 458·5 to 445 (approximate). It seems to me that the second band like the fourth may belong to lutein, and its apparent darkening with zinc chloride is only due to the production of the usual zinc chloride band over it; the whole of the black band does not shift because zinc chloride does not act well in an ether solution. On treatment with acetic acid the darkest part of the dominant band reads from λ 504 to 482·5. Possibly the second band may be due to a pigment arising from the action of trypsin on a proteid, as Krukenberg figures a band almost in

this place which was obtained by the action of trypsin on fibrin and treatment with oxidising reagents[1].

Chloroform solution. The chloroform solution was orange in colour and gave also a chlorophyll band in red and one between green and blue, but no band before this latter as in the case of the ether solution. On treatment with an acid the band between green and blue became quite black, on suitable dilution it was found to extend from λ 507 to 486·5, but in a deeper layer it had a great breadth: in one experiment from λ 507 to 462. The absence of unchanged bilirubin was proved by appropriate tests.

Aqueous solution. The solution obtained by means of water was yellow in colour and gave only a double band at F, reminding one of the ether solution. The feeble part of this double band begins at λ 519 and the dark part extends from λ 503 to 484·5 with one drop of acetic acid. On treatment with zinc chloride a faint green fluorescence was seen and the zinc chloride band appeared from λ 521·5 to 503.

Rectified spirit and sulphuric acid. The solution obtained by means of rectified spirit acidulated with sulphuric acid was a dirty brown colour, and in a deep layer allowed only the red of the spectrum to pass; but in a thinner layer a band became detached at D, while in a still thinner layer a dark broad band became detached at the blue end of green. The first one read from λ 613 to 582 and is that of indigo blue, while the dark band extended from about λ 507 to 462, its darkest part being included between λ 501 to 475.

On diluting this solution with water and shaking in a separating funnel with chloroform and separating this off and filtering, a red solution was obtained which gave the same two bands, the first from λ 613 to 585 and the second from λ 509 to 482·5. On pouring on a white dish the fluid at its edge had a dirty violet colour due to the presence of indigo-blue.

On evaporating the chloroform solution on the water-bath a dark-brown, amorphous residue was obtained, which gave practically almost the characters of febrile urobilin both as to solubility and spectra of its solutions; these it is unnecessary to repeat. In none of the solutions could I detect unchanged bile-pigments. The position of the bands in an alcoholic solution with zinc chloride and with caustic soda, both of that close to C and at D, differs very slightly from that of those

[1] *Verhandlungen der Physikalisch-med. Gesell. zu Würzburg.* N. F. B. XVIII., Nr. 9. 1884.

of a similar solution of febrile urobilin and resembles closely that of the bands of a similarly treated solution of the urobilin of bile. Indeed I think that my former conclusion "that the urobilin of bile is formed in the intestine" is justified from the appearances seen[1].

(5) Colouring matters of Urine. The colouring matters of urine have been the subject of more controversy among physiological chemists than most other things in physiology. The mistake which most people are guilty of is that of supposing that urine, either normal, or pathological, owes its colour to one colouring matter. There are in pathological urines—excluding the influence of bile and blood—a great number of pigments, and chromogens, of which some are derivatives of bile and of haematin, others oxidised derivatives of indol, skatol and other bodies of the aromatic series whose radicals are produced by the action of trypsin and other ferments on the proteids in the intestine. Then there is, even in normal urine after boiling with acids, a body soluble in amyl alcohol which becomes quite black on evaporation as Plosz has shewn[2], and also other bodies reddened by boiling with mineral acids. Most of these substances occur in the form of chromogens which are with difficulty separated from each other. The idea that normal urine only contains one yellow colouring matter which becomes oxidised into uroerythrin is to anyone who has made a special study of urinary chromatology perfectly absurd; but I hope at some future time to be able to publish an account of my experiments on those chromogens of urine which are remotely derived from indol and skatol and other digestion-formed radicals, but for the present I must confine myself to those pigments which are derived from haematin, bilirubin, and biliverdin.

I have already shewn that there are two distinct kinds of urobilin, (1) an incompletely metabolised body which is that known as febrile urobilin[3] and which is found in a great number of pathological urines, and whose *chromogen* occurs in most normal urines. Now I have already said that this kind of urobilin is almost the same as stercobilin; it differs from it principally in the fact that on treating its alcoholic solution with ammonia and zinc chloride the green fluorescence is not so well marked, and there are also some trifling differences in the spectra with regard to the feeble bands towards the red end of the

[1] *Proc. Roy. Soc.*, No. 208. 1880.

[2] P. Plosz: *Zeitsch. f. Physiol. Chemie*, Band VI., Heft 6, p. 504, and Band VIII., Heft 1 and 2, p. 85.

[3] The band at F of febrile urobilin in absolute alcohol extends (in one depth) from λ 505 to 475, and (in a less depth) from λ 503 to 482·5.

spectrum. It is this febrile urobilin which is called urobilin by continental chemists and their English copyists. I often regret that this pigment has been named *febrile* urobilin, as it occurs in many cases where there is no elevation of temperature: for example, in cases of functional derangement of the liver, dyspepsia, chronic bronchitis, valvular diseases of the heart and other conditions. It seems to me that anything which alters the blood-pressure in the liver may cause pathological urobilin to appear in the urine in large quantity; and under normal conditions all that part of it which escapes conversion into a chromogen is further oxidised in the blood into normal urobilin and appears as such in the urine. The band at F of febrile urobilin is much darker than that of normal urobilin and much broader, and its replacement by a narrower band nearer the red by the use of caustic soda and zinc chloride is much better marked than in the case of normal urobilin, whose band at F often disappears with caustic soda.

(2) The other kind of urobilin is not—when in the solid state—the deep brown or reddish brown of the former, it is a dull brown. The dominant band of its acid solution is much feebler than the last, and it certainly is very like, if not identical with, choletelin, as I have shewn[1]. I also produced a similar colouring matter by acting on acid haematin with peroxide of hydrogen, but it is only lately that I found that this peroxidised pigment when dissolved in alcohol and treated with ammonia and zinc chloride gives a green fluorescence. Hence I believe there can no longer be a doubt that we may produce from haematin, by oxidation, a pigment very like, if not identical with, normal urobilin. This pigment is not the same as stercobilin and I believe it occurs in blood serum. I have made a great number of experiments on pathological serum-colouring-matters. The albumen was coagulated by heat, the coagulum dried and pulverized, and extracted with different solvents, and I frequently found traces of a colouring matter which has a close resemblance to the above oxidation product. But in a considerable proportion one also meets with biliverdin or a closely related pigment when the powder is extracted with alcohol acidulated with sulphuric acid[2]. It would therefore appear that we may have normal urobilin formed entirely from haematin, and that, under normal conditions, only a chromogen belonging to changed stercobilin occurs in the urine, and that it is not always present.

[1] *Proc. Roy. Soc.* No. 208. 1880.

[2] I do not wish it to be understood that I deny the presence of "serolutein" in blood serum; I think it is frequently present.

But there is another pigment present occasionally in urine which cannot be prepared from bilirubin or biliverdin, and this is a pigment of great interest from a pathological standpoint. It is that which I named urohaematin but which might with greater propriety have been called urohaematoporphyrin, since it bears a very striking resemblance to haematoporphyrin, as can be seen by comparing the spectra of its alkaline and acid alcoholic solutions, with similar solutions of haematoporphyrin. It is now five years since I found this colouring matter in the urine of a case of rheumatic fever, and shewed that I could produce it artificially from haematin either by means of reduction with zinc and sulphuric acid or sodium amalgam[1]. I notice that Nencki and Sieber[2] have produced a body from haematin by the action of tin and hydrochloric acid which they call hexahydrohaematoporphyrin, which doubtless is the same body. I may recall to mind that, according to Hoppe-Seyler, urobilin is produced by this treatment; but I was the first to shew that the body obtained by zinc and sulphuric acid and haematin is the same as the pigment I obtained from urine and named urohaematin. An inspection of its spectra shews that it differs remarkably from urobilin, and resembles closely—except for the exact position of its bands—haematoporphyrin. (See Pl. I.)

I may here state that it is obtained from urine[3] by precipitating the urine with neutral and basic lead acetate as long as a precipitate falls, separating the precipitate by filtration, decomposing it with rectified spirit and sulphuric acid, filtering and agitating the filtrate with chloroform in a separating funnel after dilution with water. The chloroform is then distilled off and the residue examined. It is a brown pigment and is soluble in alcohol, chloroform and the other solvents already mentioned in former papers But the presence of this pigment can be easily detected in the urine by merely adding a little mineral acid and examining in a deep layer, when the characteristic bands of acid urohaematin are seen.

In the acidulated alcohol solution[4] its bands read: 1st, λ 595 to 587; (2nd, λ 576 to 566? a band which I had previously missed); 3rd, λ 557 to 541·5; 4th, λ 503 to 482·5. I believe the darkness of the band at F is due not only to this pigment but also to the presence of urobilin; still

[1] *Proc. R. S.* No. 208. 1880.

[2] *Loc. cit.*

[3] Like febrile and normal urobilin.

[4] From urine of Addison's disease. It shews, with zinc chloride and ammonia, only a faint green fluorescence.

its alcoholic solution on treatment with ammonia and zinc chloride shews only a feeble green fluorescence.

The pigment also goes into amyl alcohol, and this solution shews the same bands as a chloroform solution, but it also takes up a chromogen which becomes oxidised to a black brown pigment[1] on evaporating the amyl alcohol.

On treating an absolute alcohol, solution of the isolated pigment with caustic soda a five-banded spectrum is obtained, the bands of which read as follows: 1st, λ654 to 640; 2nd, λ627 to 618; 3rd, λ582 to 563; 4th, λ 540 to 527; and 5th, λ 509 to 488. An absolute alcohol solution with sulphuric acid gave: 1st band, λ 596·5 to 587; 3rd, λ 558·5 to 544; and 4th, λ 503 to 481. This pigment possesses a peculiar interest, as I find it in the urine of rheumatic fever with great constancy, and I also found it, as communicated to the *British Medical Journal*[2], in the urine of a case of "idiopathic" pericarditis which doubtless owed its origin to the *materies morbi* of rheumatism. In very slight cases I find it present in mere traces, and in cases which were apparently going to end in rheumatic fever, but in which the pain ceased after a few doses of salicylate of soda, I could as a rule find only pathological urobilin.

Latterly, I have also found urohaematin in the urine of Addison's disease, which appears to me to be of importance, as it supports the view as to the function of the adrenals which I published in abstract last December[3]. If the function of the adrenals is to remove effete blood-pigment from the body or to convert it into something which can easily be removed, disease of them may prevent this taking place and the effete pigment or its imperfect metabolite may appear in the urine as urohaematin. Hence then urohaematin may appear in the urine under two totally different conditions: firstly, from over-production of effete blood-colouring matter or effete histohaematin, in greater quantity than the blood-metamorphosing glands can deal with; or secondly in normal quantity but too much for diseased blood-metamorphosing glands to deal with, as in Addison's disease.

I may here remark that the occurrence of Addison's disease without

[1] The "uromelanin" of Plosz, *loc. cit.*

[2] *Brit. Med. Journal*, Dec. 1883.

[3] *Proc. Physiol. Soc.*, No. IV. 1884. The urohaematin of Addison's disease may shew some trifling differences in the position of its bands from those of the urohaematin formerly described by me and referred to *supra*, but this can easily be explained, as the difference depends on the amount of reduction.

disease of the adrenals is no argument against my theory, because disease of any other organ in which metabolism of effete pigment takes place might bring about the same result, nor is the occurrence of diseased adrenals without bronzing of the skin an argument against it, because the other pigment-metabolising glands (among which is the liver) may take on increased activity, and be able to deal with the excess of effete pigment. I have thought it necessary to introduce these remarks, as physicians are apt to draw conclusions from imperfect data and often fail to take a broad grasp of all the bearings of the case.

It is not necessary to suppose that the mother pigment of urohaematin is worn-out haemoglobin, as a very curious fact found out by me teaches. If the integument of brown specimens of Uraster rubens or of the slugs Limax and Arion[1] be extracted with alcohol and sulphuric acid a solution is obtained which shews all the characters of haematoporphyrin; and this haematoporphyrin exists as such in the integument, as can easily be proved. Now in the bodies of these animals there is not a trace of haemoglobin, all their tissues and organs being free from it: but they contain histohaematins. Hence the histohaematins may furnish the mother pigment of the integumental haematoporphyrin. If this is the case in invertebrates it may also occur in vertebrates; and may not the myohaematin of the muscles furnish it? Can some chemical change take place in the muscles of rheumatism accompanied by the setting free of an acid such as lactic acid and of a metabolite of myohaematin? Whether this be the case or not we must acknowledge that under certain diseased conditions a pigment is found in the urine which has its origin not from bile pigments, but from haematin.

Putting all the facts obtained together, it would appear that the stercobilin resulting from the putrefactive processes in the intestine, and accompanied by imperfectly changed biliary pigments, is taken up by the branches of the portal vein and carried into the liver, where it is probably again changed by the action of a ferment into a chromogen: a portion of this chromogen gets into the blood and is excreted in the urine as a chromogen. A portion may escape in the condition of biliary urobilin as such and appear in the urine in a further oxidised condition, or owing to disturbance of circulation in the liver a large portion of unchanged biliary urobilin may appear in the urine. Besides this, the urine under normal conditions may contain a pigment which has no biliary origin and may be derived entirely from haematin; while in certain diseased states a

[1] *Proc. Birm. Philos. Soc.* Vol. III. (1883), p. 378 and seq. I have repeatedly verified the truth of this statement since 1883.

reduction product of haematin, having no connection with bilirubin or biliverdin, and closely related to haematoporphyrin, may appear in the urine and to a great extent, if not altogether, may replace urobilin.

It is only by the careful use of the spectroscope that these colouring matters can be distinguished from each other.

I wish to say in conclusion that the above paper only represents the present state of my knowledge with regard to these colouring matters, and may be considered merely a preliminary account of a difficult subject.

EXPLANATION OF PLATE I.

Sp. 1. Alcohol solution of cholohaematin isolated by means of chloroform and dissolved in absolute alcohol. (Ox-bile.)

Sp. 2. Action of nitric acid on this solution, first stage.

Sp. 3. The same, later stage.

Sp. 4. Alcohol solution of the above cholohaematin after the action of sodium amalgam and isolation, solution in alcohol and treatment with sulphuric acid ; this is acid haematoporphyrin mixed with urobilin.

Sp. 5. The absolute alcohol solution of the same colouring matter after isolation treated with ammonia ; this is alkaline haematoporphyrin mixed with urobilin.

Sp. 6. Absolute alcohol solution of febrile urobilin isolated from urine : a combination spectrum of a deep and a shallow layer.

Sp. 7. Normal urobilin isolated and dissolved in absolute alcohol.

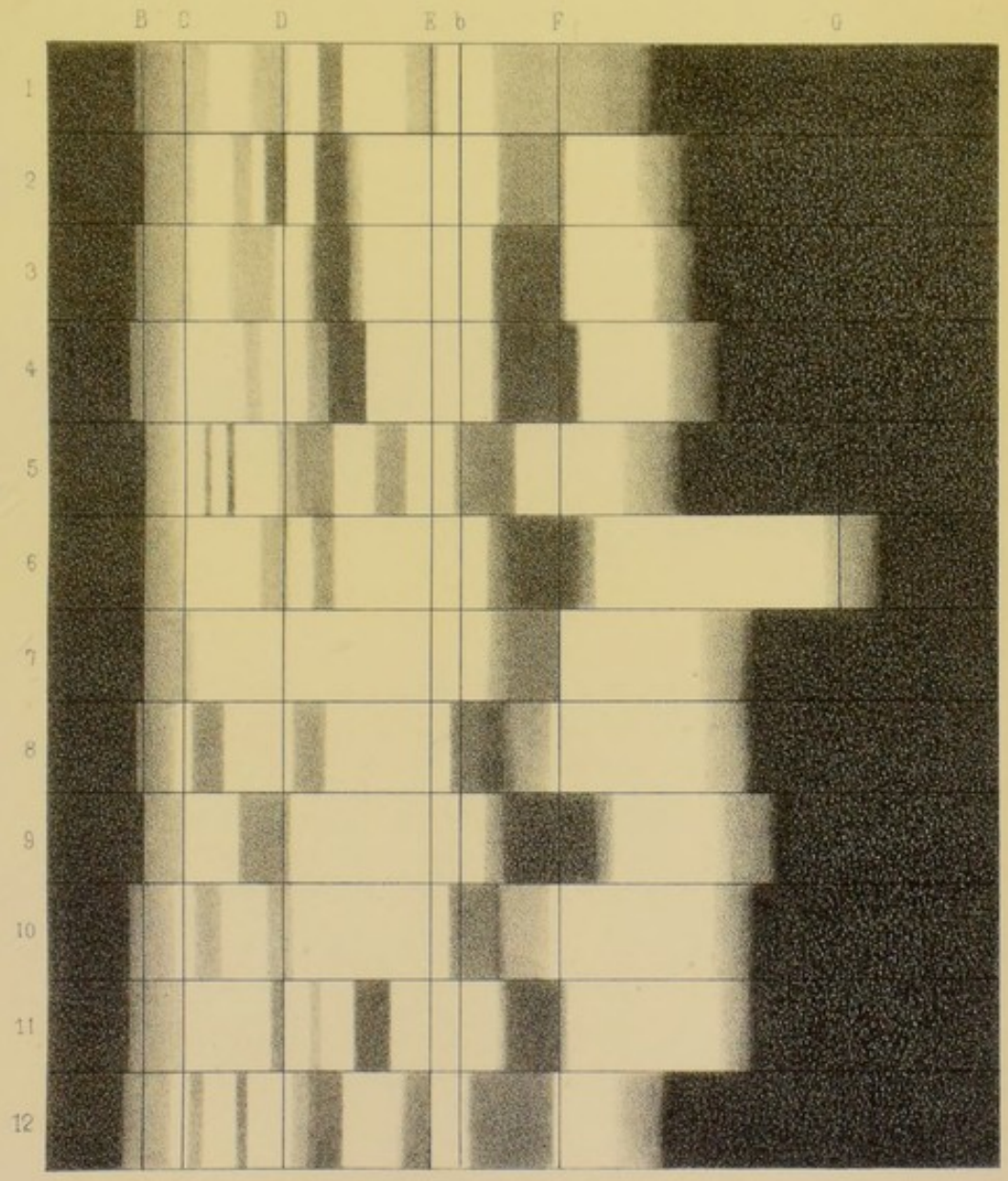
Sp. 8. Absolute alcohol solution of febrile urobilin treated with ammonia and zinc chloride (combination spectrum).

Sp. 9. Acidulated alcohol (rect. spirit and H_2SO_4) extract of fæces (stercobilin and indigo-blue).

Sp. 10. Absolute alcohol extract of the isolated stercobilin treated with zinc chloride.

Sp. 11. Urohaematin from urine of Addison's disease as it is seen in the acidulated rectified spirit extract of the lead precipitate from urine. This is the spectrum of acid urohaematin.

Sp. 12. Alkaline urohaematin in an absolute alcohol solution of the isolated pigment after treatment with caustic soda. On comparing this spectrum with Sp. 5, they are seen to have some resemblance to each other, but the differences are well marked.



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