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HÆMATOPORPHYRINURIA AND ITS RELATIONS TO THE SOURCE OF UROBILIN.

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I would first of all like to allude to a case of abnormally pigmented urine, which I had the privilege of examining through the kindness of Professor M'Call Anderson, University of Glasgow.[1]

It was one of those very rare cases of 'burgundy-red' urine similar to two examined by M'Munn, and published by Ranking and Pardington (*Lancet*, 1890, vol. ii. p. 607).

The pigment here and in Professor M'Call Anderson's case—one of dermatitis herpetiformis bullosa—was not uro-hæmatoporphyrin, but a closely allied body, believed by M'Munn to be *less* de-oxidised than uro-hæmatoporphyrin, or than urobilin. M'Munn has not named it; and as it is awkward to have to allude to it always by a periphrase, I suggest that it be known as *Meio-de-oxy-hæmatoporphyrin.*

The urine in the case I examined was claret-coloured, did not give the guaiac reaction, did not contain biliary pigment, nor any proteid. With the spectroscope it showed very distinctly a four-banded absorption spectrum, closely resembling that of uro-hæmatoporphyrin, with one band in the red, two between D and E lines, and one band near the F line.

Such a reducing substance as ammonium sulphide had no effect whatever on the pigment, which, had it been met-hæmoglobin—a vastly commoner four-banded pigment—would have been reduced through oxy-hæmoglobin to hæmoglobin; but treatment with concentrated sulphuric acid instantly gave rise to a pigment with the characteristic double-banded absorption spectrum of acid-hæmatoporphyrin.

On careful spectroscopic examination there was no possibility of confusing this rare pigment with met-hæmoglobin, as in the

[1] The case is published in the *Scottish Medical and Surgical Journal*, February 1897. (Jack and Borland on a case of Hæmatoporphyrinuria).

former the band to the right of D is much fainter than the homologous band of the latter.

The patient was a young Highland fisherman (æt. 25), who had suffered for several years from an annual eruption (from April to October) of a vesicular type. Ten to twelve hours before the formation of the blisters there was intolerable itching, but he had no headache nor malaise. The blood showed no diminution in the number of corpuscles; the hæmoglobin, however (with Gower's instrument), appeared to be only 60 per cent. of normal; spectroscopically, it yielded no bands beyond the two of HbO_2.

Beyond all doubt, hæmoglobin, through its pigmentary element the hæmatin, is the parent of the pigments of the bile, and the chief pigment of the urine (urobilin)—(indican and other allied pigments being derived from an aromatic molecule, phenol and its substitution-compounds, originally resident in proteid material metabolised in pancreatic digestion). Hæmatin—an iron-containing substance—is the source, on the one hand, of bilirubin or biliverdin, iron-free pigments; and on the other, of urobilin, also an iron-free pigment; but in what manner, as to parentage, are these pigments of the bile and urine related to each other? Are they members of the same generation, or is the bilirubin the progenitor of urobilin? The former supposition seems to be more in accordance with facts, though certain of the text-books will be found to dismiss the subject by upholding an old view, which, stated more in detail, is, that the bile-pigment in the intestine is acted upon by nascent hydrogen there, and that a reduction-product (considered by Maly identical with hydro-bilirubin, formed by the action of sodium amalgam on bilirubin) is thereby produced, that this is absorbed into the portal system, passes through the liver without (apparently) undergoing any change or being excreted into the bile, is sent on to the right side of the heart, and so, *via* the lungs, into the arterial stream from which the kidney removes it as urobilin.

Before giving reasons for adopting the view that the bile-pigment and the urine-pigment are derivatives of hæmatin of *equal remoteness* from it, and are not derived the one from the other, we might point out the unlikely nature of the hypothetical

process just explained. In the first place, it is tantamount to saying that hydro-bilirubin exists in arterial blood—of this there is no evidence—*cf.* p. 311, Halliburton, *Physiological Chemistry*; secondly, later work has shown that urobilin is much more nearly related to choletelin—the fully oxidised biliary pigment—than to hydro-bilirubin, the least oxidised; thirdly, it seems most unlikely that so constantly-present a body as urobilin should depend upon the presence in the intestine of 'nascent hydrogen,' which must vary in amount from hour to hour, according to the amount of putrefactive decomposition; fourthly, it is purely conjectural that, whereas bilirubin gets thrown out into the biliary secretion, its next ally, hydro-bilirubin (granting that its absorption by the portal radicles is a fact, or is possible) passes on through the liver, escaping separation from the blood, and so reaches the heart and arterial circulation.

A greater objection than any of these is, that both in the lower animals and in the human being, in the case of a biliary fistula, where no bilirubin gets into the intestine at all, and where the fæces are colourless, the urine contains urobilin in undiminished amount; and in Copeman and Winston's case, at times in increased amount.[1]

In the face of these considerations, we cannot regard bilirubin as the parent of urobilin; rather, both pigments are derivatives of hæmatin, of equal degree of remoteness therefrom.

If a function is to be found for 'reduction' in the intestine, there is the obvious one of altering bilirubin or biliverdin to stercobilin, as Copeman and Winston suggest, p. 231; but if, as Garrod and Hopkins maintain,[2] the pigments of bile and of fæces are identical, all we can say is, that we have one more member of the generation—stercobilin—produced in the liver from the common ancestor, hæmatin. M'Munn, indeed, had previously contended that stercobilin was more closely allied to 'pathological urobilin' than it was to bile-pigment.

Although the bile-pigment cannot be regarded as the source of the urinary-pigment, it is highly probable that the liver is the seat of the formation from hæmatin of the antecedent of urobilin, which is not excreted externally by the hepatic cells

[1] *Journal of Physiology*, vol. x. p. 228.
[2] *Journal of Physiology*, vol. xx. Nos. 2 and 3.

into the bile-canaliculi, but internally into the hepatic blood. This is an '*internal secretion*' *for the liver*, if that of glycogen be denied it. That increased metabolism of hæmoglobin in the liver is at once followed by the secretion of an increased amount of urobilin in the urine, while the liver has additional iron deposited in it, is well known.

If hæmoglobin be by any means dissolved out of the corpuscles *in the circulating blood* we have hæmoglobin or methæmoglobin in the urine,—as, *e.g.*, after the injection of foreign blood, injections of water into the blood, in extensive burns, in Raynaud's disease, after the injection of many toxic substances, bile salt, etc.; in other words, the blood-pigment in these cases is not decomposed into hæmatin, nor is hæmatin de-oxidised in hæmoglobinæmia: the pigment circulates as oxyhæmoglobin dissolved in the plasma, and is excreted *as such*[1] by the kidneys,—another incidental proof that in the blood itself de-oxidations do not go on.

In the liver it is very different: its pre-eminence as *the* urobilin-forming body is well brought out in the case of pernicious anæmia, in which, as Mott, Delépine and Hunter have shown, there is an abnormally active destruction of red discs, with a correspondingly *augmented* secretion of urobilin, or its less fully oxidised ally 'Pathological Urobilin' (which has, besides the band at F, two between C and E lines). The amount of urobilin present in normal urine is not sufficient to yield the band at F, which appears as soon as the urine is evaporated down to about $\frac{1}{5}$ its bulk; if, therefore, one can see the band at F in a fresh specimen of urine unsophisticated chemically (and provided the urine does not give Gmelin's test for bile-pigment), one is dealing with a case of excessive excretion of the normal urinary pigment; but if, in *addition* to this band, the two between C and E are present, the pigment is 'Pathological Urobilin,' the more completely de-oxidised form of urobilin. Thus, if the hepatic metabolism be still normal in quality, but only unusually excessive in degree, we have an excessive amount of normal urobilin in the urine; if, however, the hæmatin-reduction goes beyond the limit of the

[1] The final reduction does not endure sufficiently long to form reduced hæmoglobin.

formation of urobilin, we have the more completely de-oxidised pigment (Pathological Urobilin) appearing in the urine.

So much for the *hepatic*, as opposed to the biliary, *origin* of urobilin. On this view, urobilin or its chromogen (whichever, in the absence of positive proof, be regarded as the internal secretion of the liver) would of necessity require to be found in the blood. I may say at once that I believe urinary-pigment is present in blood-plasma, and therefore in blood-serum, although bile-pigment is not.

M'Munn long ago discovered[1] that sheep's serum yielded a spectrum with a band at F, and even then (1880) he remarked it must be due to "choletelin, or a substance like it." But even at that time Maly, Neubauer, and Vogel believed it was due to urobilin; and as our knowledge of all these substances has been considerably increased in these seventeen years, we are by no means compelled to say that a pigment, choletelin, which is only known in the laboratory, is present in the blood, when we have just as much evidence for believing that another—urobilin — constantly in the body, is the pigment in the blood. The objection that we see no band at F in normal blood has no weight whatever, because (1) urobilin will be in all likelihood present in blood in much less percentage than in urine, in which the urobilin present is spectroscopically invisible; and (2) because it is quite conceivable that it might exist in the blood (partly) as the colourless chromogen of the pigment.

By a chromogen, we understand the precursor of a pigment, a colourless body, which on oxidation will yield the pigment, and which can be again obtained by de-oxidising the pigment.

But there is evidence that the katabolism of hæmatin can proceed in other organs or tissues than the liver. It is a commonplace of clinical knowledge that in febrile diseases the urine is 'highly coloured.' In recent years, largely due to M'Munn's work, it is known that this increased depth of pigmentation is the result of either an excess of normal urobilin or the presence of 'Pathological' (originally called 'Febrile') Urobilin in the urine.

Now, in fevers we have increased tissue-change, a more than

[1] "Researches into the Colouring Matters of Human Urine," *Pro. Roy. Soc.*, 1881, p. 231.

usually active blood-katabolism going on, which is evidenced not only by the elevated temperature, but by the increased amount of urinary pigment appearing. The muscles undoubtedly are the seat, in many fevers, of this excessive katabolism, part of the expression of which is the increased destruction of hæmatin owing to the exaltation of the normal tissue-avidity for oxygen, with the subsequent appearance either of an excessive amount of a hæmatin-derived pigment (urobilin), or, by reason of the very vigour of the metabolism, an unusually de-oxidised form of it,—Pathological Urobilin.

M'Munn, in his chart accompanying the paper "On the origin of Uro-hæmatoporphyrin and of Normal and Pathological Urobilin in the organism,"[1] shows that urobilin must be regarded as more fully oxidised than Pathological Urobilin, and than stercobilin if its hepatic origin and identity with Pathological Urobilin be believed in.

We have further evidence that other tissues than the hepatic katabolise hæmatin,—extravasations of blood under the skin or fasciæ usually give rise to an increase of urobilin, and we know that in these clots hæmatoidin, an iron-free though crystalline pigment, is produced. The chief *other* tissues or 'systems' which could by any possibility be sufficiently extensive seats of this metabolism are (1) the muscular, (2) the cutaneous, and (3) the connective-tissue, articular and skeletal.

The blood which reaches the kidney is entirely arterial, being a portion of the main aortic stream which has passed through the lungs, having been collected from very different sources and mixed in the right auricle. Now, from a *chemical* point of view, the blood of the right side of the heart may be viewed as having proceeded from precisely these two main sources—(I.) the liver, (II.) from the systems (1), (2), and (3), as above indicated (to which, no doubt, the venous blood of the head has to be added).

The bloods from both (I.) and (II.), which may be for physiologico-chemical purposes thought of as distinct, pass through the lungs, where oxidation is the pre-eminent process, and thereafter are distributed to all parts of the body where *de*-oxidations are equally characteristic.

[1] *Journal of Physiology*, vol. x. p. 71.

Now, M'Munn made urobilin artificially by (1) oxidising hæmatin by the action of peroxide of hydrogen, and (2) subsequently briefly de-oxidising it with sodium amalgam (*cf.* his last-mentioned paper): what have we in the body but these very two processes, for whether the hæmatin be freed of its iron in the liver, or be so in the muscles, skin, and connective-tissues, the pigment-antecedent so formed passes in both cases to the lungs, where it must be thoroughly oxidised, and then, during excretion by the renal epithelium, would suffer a "subsequent brief reduction." It may be supposed that the chromogen of urobilin is already formed when the substance reaches the lungs, and that it is there oxidised (more or less perfectly) to the pigment, which, in the kidneys, undergoes a partial de-oxidation to the chromogen. (It is a familiar fact that urine deepens in colour as it stands after being passed: this may be due to a partial re-oxidation of the chromogen.)

We are now in a position to inquire under what conditions uro-hæmatoporphyrin supplants urobilin, wholly or partially, as the urinary pigment.

Hæmatoporphyrin, as we know it in the laboratory, is either an acid or alkaline solution of iron-free hæmatin. Acid-hæmatoporphyrin can be rapidly made by adding a very little defibrinated blood to an excess of strong sulphuric acid. This gives the two-banded spectrum—one thin band to the left of D, and one much broader, darker band to the right of D, there being intermediate a narrow zone of obscured yellow light. There is no band at F.

This pigment can be precipitated by the addition of water, and then dissolved in ammonia, giving alkali-hæmatoporphyrin with a four-banded spectrum.

Uro-hæmatoporphyrin, as found in urine, has a four-banded spectrum closely resembling the last mentioned, but with this important difference, that the left of the two bands between D and E is much fainter in the former pigment; but M'Munn has managed to manufacture in the laboratory a pigment from hæmatin which yields by successive *reductions* hæmatoporphyrin (acid or alkali) and uro-hæmatoporphyrin. When his chief paper on hæmatoporphyrin was published he had not met with his cases of meio-de-oxy-hæmatoporphyrin, but he has since assigned

a place to it by regarding it as less de-oxidised than uro-hæmatoporphyrin: the series of bodies in descending scale of possession of oxygen would therefore be—hæmatin, hæmatoporphyrin, meio-de-oxy-hæmatoporphyrin, uro-hæmatoporphyrin, Pathological Urobilin.

[M'Munn actually made uro-hæmatoporphyrin by reducing hæmatin with zinc and sulphuric acid, and further reduction yielded a pigment identical with Pathological Urobilin; but he failed to produce it from bilirubin.]

If one makes a survey of the diseases in which uro-hæmatoporphyrin has been recognised, it is noticed that they all present lesions of one or other of the systems (1), (2), or (3), as given above.

Thus, those involving the muscular system or connective tissue, or most probably both at once, are acute rheumatism, pericarditis, meningitis, peritonitis, and cirrhosis of liver.

Three are the acutely febrile, croupous pneumonia, typhoid, and measles,—the last two with cutaneous involvement; also Addison's disease and Hodgkin's disease,—both disorders of pigmentary metabolism, and involving a cutaneous factor. [Hæmatoporphyrinuria has also been described by Oswald[1] in sulphonal over-dosing in the insane, and Stokvis produced it by feeding rabbits with sulphonal.[2]] Lastly, of three cases of meio-de-oxy-hæmatoporphyrin, two of them are obscure, being in neurotic women, but one was a case of profound cutaneous lesion (Professor M'Call Anderson's).

Briefly, then, we may say, that in health pigmentary metabolism in the three great systems already alluded to forms from hæmatin the urobilin-chromogen, that this on traversing the lungs is oxidised to urobilin, which on traversing the kidneys is partly de-oxidised to the chromogen, partly excreted as urobilin. It is probable that even in health a certain quantity of "Pathological Urobilin" is formed by these three systems, but is partly de-oxidised to its chromogen at renal elimination. M'Munn has, however, seen 'Pathological Urobilin' in stale *normal* urine. That on being passed the bands of Pathological Urobilin are not seen, is perfectly explicable on the supposition that it is, like urobilin, present in too small quantity to give a spectrum,

[1] *Glasgow Medical Journal*, January 1895.
[2] *Centralblatt für physiol.*, 25th July 1896.

and that much of it exists as the chromogen. It is perhaps time to re-name the pigment by a term connoting nothing pathological,—*para-urobilin* might suffice.

If, however, metabolism in these systems is excessive, as in certain febrile disorders, in which the chemical changes are carried to an abnormal extent, we have either an exaggeration of the normal formation of the urobilin-chromogen, giving rise to *an excessive quantity* of normal urobilin to be eliminated by the kidneys, or, by the initial reduction of hæmatin being carried beyond the normal limit, the chromogen of a more de-oxidised pigment than urobilin produced, viz., Pathological Urobilin, which supplants the ordinary pigment in the urine. Lastly, if the metabolic changes in these three great systems become 'depraved,' altered, or abnormal in some way as yet not fully understood, we have the initial hæmatin-reduction process arrested at some intermediate stage, so that there are formed reduction-products of hæmatin less de-oxidised than urobilin or pathological urobilin, and these appear in the urine in order of nearness to hæmatin, meio-de-oxy-hæmatoporphyrin in very rare cases, and less rarely uro-hæmatoporphyrin.

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