alkali albumen, and the bacilli themselves afterwards separated by filtration through porcelain, the filtrate contains a mixture of proto- and deutero-albumose, and a trace of peptone formed by the action of the bacilli on the albumen. The albumoses produce, like those of snake poison, much local œdema, sluggishness, coma, and death. Like the venom-albumose, its activity is lessened, but not destroyed, by boiling. But, in addition to these substances, he has obtained an alkaloid which has the same action as the albumose, but much more powerful, and this alkaloid, he thinks, is present in a nascent condition in the albumose, and is separated from it by the tissues of the living animal into which it has been introduced. Whether something of the same sort exists in serpent venom or not one cannot say, but the analogies between it and the products of disease germs are becoming every day more apparent, and it seems highly probable that while studies of serpent venom may throw light on the products of disease, a study of the latter may lead to the discovery of an efficient antidote to the former.

AN ADDRESS

ON SOME OF THE APPLICATIONS OF THE SPECTROSCOPE TO MEDICINE.

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I MUST ask your forgiveness for the incomplete manner in which I have to deal with my subject; and if what I have to say may appear somewhat dry, I hope that I, and not my subject, may be blamed. It is difficult to compress an account of the applications of the spectroscope to medicine into the small space at my disposal. In fact, it is impossible to give even an epitome of all the work that has been done, so that I shall merely refer to what appear to me to be some of the most important points.

It is hardly necessary to insist upon the importance of the spectroscopic study of animal pigments to-day as one felt called upon to do some years ago, because already very important facts have been discovered by means of this mode of investigation.

By means of the spectroscope we can detect substances which by no other chemical or physical method can be discovered, and just as in chemistry and in astronomy by its means we are able to detect substances present in mere traces, and without isolating them, so in morbid fluids we can do the same. The merest trace of a blood stain, long after all traces of the blood corpuscles have disappeared, and after attempts to procure hæmin crystals must fail, can still be made by appropriate treatment to yield characteristic absorption spectra. So in the case of urine containing blood which within the body has been changed into the decomposition products of hæmoglobin, when the microscopic and other tests fail, the spectroscope applied properly comes to our aid, and also tells us exactly in what condition the pigment is present. Besides, by carefully following in the laboratory the downward metabolism of hæmoglobin, we can tell exactly when we meet with products of its downward metabolism in the urine, in the bile, and elsewhere, how much change the pigments have undergone in the body, provided we have produced each stage of decomposition by artificial means in the laboratory, isolated the pigments corresponding to these stages, and mapped their spectra carefully.

The reactions required for proof of chemical identity are few and simple, and lead to absolute certainty. Of course great accuracy is required, and mere identity of spectrum does not prove chemical identity in every case, but the application of certain reagents settles the matter. The spectroscope, therefore, not only enables us to detect certain colouring matters, but it also gives us a clue as to how and where they are produced in the organism, and it enables us to form an opinion as to the energy of metabolism, not only as regards the pigments themselves, but as regards the substances with which they are associated.

Instruments.—As regards the instrument itself, it is hardly necessary to say much about its construction. It will suffice to mention that every spectroscope consists essentially of a slit to admit a pencil of light-rays and a prism in which these rays undergo refraction. Of course, this statement does not hold good for a diffraction spectroscope in which the rays are broken up by a grating, but from a medical point of view a grating is of little use—at least, at present. Besides the slit and the prism, other optical adjuncts are necessary according to the kind of spectroscope. For instance, in the chemical spectroscope the slit is set in a long tube in the focus of a lens, which makes the rays parallel before entering the prism, and after their emergence from the prism the now coloured but still diminutive image of the slit is magnified before reaching the eye, by having to pass through a small astronomical telescope. It would not be easy, however, to apply such a large and such a peculiarly shaped apparatus as the chemical spectroscope is to the microscope, so that another arrangement is necessary for this purpose. The problem is to have slit, and prism, and lenses so placed that the light-rays can pass through them in a more or less straight line, while the spectrum is sufficiently long to enable parts of it to be measured. By using a compound prism composed of flint and crown glass by which enough dispersion (or the spreading out of the spectrum) is retained, while the deviation is got rid of, this is accomplished, the necessary enlargement of the spectrum being provided for by the use of a lens similar to that used in a microscopic eye piece, placed under the compound prism.

For comparing spectra this spectrum eye piece has a right-angled prism covering half the slit, to which the rays from a second light source are admitted through a window in the stage fastened to the side of the instrument.

Besides these instruments—the microspectroscope and the chemical spectroscope, small, handy, direct vision spectroscopes, amply sufficient for the rapid detection of blood, bile, and so on, are supplied at a trifling cost by most of the opticians.

Kinds of Spectra.—The appearances seen on analysing spectroscopically the rays proceeding from all sources of light may be grouped under three heads: (1) Bright line spectra. (2) Continuous spectra. (3) Continuous spectra interrupted by dark lines or bands, or shadings.

(1) Bright Line Spectra consist of bright narrow lines occurring singly or in groups, either placed in the same or in different parts of the spectrum. They are yielded by glowing gases, or by the glowing vapours of the metals, and as each element gives its own lines it is easily detected by its spectrum. In using a spectroscope for such spectra it is obvious that the larger the spectrum is the more accurately one can measure the position of, and the distance between, such lines. But with the exception of the detection of the lines of sodium, potassium, and calcium, in the ash of calculi, the study of bright line spectra is of little use in medicine, except one wishes to study the "chemical circulation," as the late Dr. Bence Jones[1] did when he determined the time that salts of lithium occupied in reaching different parts of the body. The use of the purest reagents and great accuracy is required in such work, which perhaps is best left to the pure chemist.

(2) Continuous Spectra are yielded by almost all solids and fluids at a white heat. The continuous spectrum is a band of coloured light beginning at one end as red and passing through orange, yellow, green, blue, and violet. Such a spectrum is seen when the slit of the spectroscope is illuminated by gas or candle light, by the electric, magnesium, or lime light, by white hot platinum, and so on.

(3) Absorption Spectra are those in which spaces of darkness in the form of fine lines, or broader spaces of darkness, known as absorption bands, interrupt the continuity of this rainbow band—the continuous spectrum. If we illuminate the slit of the spectroscope by means of sunlight, and if the instrument

[1] Bence Jones: Lectures on some of the Applications of Chemistry and Mechanics to Pathology and Therapeutics. London, 1867. *Proc. Roy. Soc.*, vol. xiv., p. 400; *Proc. Roy. Inst.*, May 26th, 1865.

is sufficient for the purpose, thousands of fine lines are seen to cross the spectrum at right angles to its length. These are the lines of Frauenhofer, a German optician who first mapped them. He called the principal ones A, a, B, C, D, E, b, F, G, and H, and the first thing anyone should do when he comes to possess a spectroscope is to find out these more prominent lines and determine their position on the scale of his spectroscope. I may merely state here that these lines are the lines of elements present in the sun. The reason why they are black and not bright is because the light emitted by the elements burning in the sun has to traverse the vapours of the same elements in the sun's atmosphere. The vapour of each has the property of arresting the rays which that element emits while in the incandescent state. Accordingly these lines in the spectrum are caused by the absence of certain rays belonging to various elements. Many stars possess similar spectra, but anyone who wants to follow this subject in its applications to astronomy can do so by consulting such books as Roscoe's[2] or Schellen's[3] on spectrum analysis.

But the absorption spectra with which the physician or the physiologist has to deal are different. In these the spaces of darkness in the spectrum, known as absorption bands, are produced by causing the light from an Argand burner or other source furnishing a continuous spectrum, to traverse a fluid which has the property of arresting certain of the rays passing through it. Corresponding to the parts of the spectrum where these rays are deficient are spaces of darkness, more or less completely dark, and these spaces of darkness are the absorption bands. Coloured fluids, more especially, possess this property, although lately it has been shown that colourless fluids give absorption bands in the invisible parts of the spectrum—parts, however, which can be printed on the photographic plate. The latter method of investigation, although useful to the pure chemist in showing the intimate connection between the physical and chemical properties of organic bodies, has yet done very little for physiology.

Many coloured fluids, such as solutions of the aniline dyes and many vegetable colouring matters, block out one end or the other of, or it may be a large space in, the middle of the spectrum, thus giving what is known as "general" absorption, but, in the case of many of the animal-colouring matters, both normal and pathological, these solutions give a special or particulate absorption, showing in some cases a very complicated spectrum, by means of which their presence is readily recognised. Among plant colouring matters, chlorophyll, or leaf-green, gives a most complicated spectrum, by means of which it can be readily recognised, and others belonging to the red groups of algæ give equally characteristic spectra, but it is the animal pigments which, luckily for physiology, give the most characteristic absorption spectra.

Apparatus Required for the Study of Physiological and Pathological Spectra.—In studying these absorption spectra the source of illumination should be either an Argand gas-burner or an incandescent light, the fluid under examination being placed in a test tube, a small beaker, or in a Preyer's hæmatinometer,[4] a vessel which has parallel plane glass sides, one centimètre apart from each other (that is, when working with a chemical spectroscope). A bull's-eye condenser between the light source and the slit is a useful help, as by its means the light can be condensed on the fluid and the illumination thus increased. Sunlight is not suitable as an illuminant, owing to the presence of Frauenhofer's lines. Sometimes we may require "spectroscope bottles," which enable a considerable depth of fluid to be examined.

In working with the microspectroscope the fluid should be placed in a small glass tube let into a piece of wood, or, if a very deep layer is required, a test tube filled with the fluid may be let into the tube of the microscope above the objective, and over this the microspectroscope placed. For examining undiluted blood Hénocque's hæmatoscope[5] is useful. It consists of two glass plates, one placed above the other; they touch at one end, the space between them at that end being nothing, while at the other they are separated by an interval of 0.3 millimètre. A graduated millimètre scale engraved on the lower plate, reading from 0 to 60, and reading from left to right, enables the depth of fluid to be calculated at each division of the scale, for by multiplying the figure on the scale at any point by 5 the depth is given in thousandths of a millimètre. By means of this simple piece of apparatus one can calculate the amount of hæmoglobin in a specimen of blood, and judge of its spectrum without the addition of a reagent and undiluted.

Watch glasses, small beakers, short bottles, and so on, can also be used with the microspectroscope. For examining fresh slices of organs or bits of fresh tissue a "compressorium" may be used, the cover glass being screwed down until the spectrum is seen distinctly. Other vessels may be used, but each individual worker will soon learn for himself what he requires. Anyone who has once learnt how to use the spectroscope will find it an indispensable help in examining blood, bile, urine, and other fluids.

The first thing he should do is to map the spectrum of sunlight; after illuminating the slit of the apparatus by means of good diffused daylight, he should observe and map the principal Frauenhofer lines, carefully comparing his results with such maps of the solar spectrum as he can find in the textbooks. The mapping is most easily done by having a photographed scale adapted both to the microspectroscope and the chemical spectroscope. This scale should be divided into at least 100 parts for the microspectroscope and 120 for the chemical spectroscope. The adjustment of the scale can be learnt from the optician, who, as a rule, sets it for distinct vision. Sometimes the buyer of a chemical spectroscope is assured by the seller that it is much more accurate to measure and map by means of a graduated circle and vernier; but such is not true. Such angular measurements are required for the calculation of refraction indices, or if one happens to possess a diffraction grating, he can amuse himself by endeavouring to work out the wave-lengths of the bright lines of the elements, and so on; but for ordinary work I advise the student to use a photographed scale.

Before mapping an absorption spectrum, the scale must be set. By making a certain number on the scale exactly to correspond with the D line when mapping the solar spectrum, the observer always has his zero point to start from; and to find out at night where his D line should be, it is only necessary to illuminate the slit with the flame of a spirit lamp or Bunsen's burner into which a salt of sodium has been introduced, when the sodium line will be seen, which is coincident with the D line. In order to set the scale, suppose one has set the D line at 50 of the scale when he mapped the solar spectrum, then before beginning an observation on the spectrum of a fluid the scale should be set until the yellow sodium line again stands at 50 on the scale.

Both Hilger and Browning supply chemical and microspectroscopes provided with such photographed scales, and Zeiss, of Jena, supplies a microspectroscope provided with a photographed scale giving the readings in wave-lengths. With regard to wave-lengths: Although a busy practitioner will be perhaps satisfied in detecting blood or bile or other pigment in a fluid without troubling to map its spectrum, yet he will perhaps sooner or later find that he is coming upon new facts which he may wish to record for publication. If so, it will not do to say that he found a band in the red from 40 to 42 of his scale, because the band on another scale might read from 42 to 46, as it is impossible to make the scales—that is, the arbitrary scales—of two different spectroscopes to agree. Hence he must make use of numbers which are universally true for that part of the spectrum. Now the wave-lengths of the different parts of the spectrum never vary, so that all readings should be expressed in wave-lengths. It is quite easy to reduce readings to wave-lengths. All that is required is to draw a curve on logarithm paper supplied by Letts and Co., of London; and I have shown elsewhere how this can be made.[6] On running the eye down from the number representing that on the scale of the spectroscope, along the perpendicular line until it comes upon the point when the curve cuts this line, and then along the horizontal line from that point to the side of the paper where the wave-lengths are given, the required wave-length is found.

[2] Roscoe: *Lectures on Spectrum Analysis*, 4th ed., 1885.

[3] *Die Spectralanalyse, etc.*, 3rd ed., 1883, and English translation of the same, edited by Abney.

[4] Hermann's hæmatoscope is also a useful adjunct.

[5] Hénocque, "Notice sur l'Hématoscope," Paris, 1886; *Comptes Rendus de la Société de Biologie*, 8e. Série, t. ii, p. 12, No. 1, 11 Janvier, 1885.

[6] MacMunn: *Spectroscope in Medicine*, 1880.

The reagents required for the detection of blood, bile pigments, and so on are few in number: sulphide of ammonium, acids and caustic alkalies, solvents, such as ether, alcohol, chloroform, benzol, and a few others complete the list.

The beginner should first of all study the different kinds of spectra, and compare them together; and perhaps one of the most useful examples of an absorption spectrum, to begin work with, is that of a solution of permanganate of potassium. By diluting this gradually, he sees the whole series of bands become visible, and then grow feebler until the most deeply shaded is the last to disappear.

Spectra of Hæmoglobin and its Compounds.—The spectrum of oxy-hæmoglobin is so familiar to readers of physiological text-books, that it is mere waste of time describing it again. It is merely necessary to say that before concluding that it is present from its spectrum alone, a little sulphide of ammonium ought to be added, when the single broad band of reduced hæmoglobin appears, especially after applying a gentle heat, and again on shaking with air the bands of oxyhæmoglobin return, to again disappear on standing. If this test were not applied, then possibly some other pigment might be mistaken for that of blood, such as carminate of ammonia, or purpuric sulphuric acid, which later shows a band like that of reduced hæmoglobin.

Suppose the blood was obtained from a case where death had taken place from coal gas poisoning, or from inhaling the fumes from smouldering charcoal, or other source in which carbonic oxide[7] is the lethal agent, then reduction would not take place; the two bands—which are, however, nearer the violet than those of oxyhæmoglobin—would remain. In that case other tests might be tried, such as adding to a solution of the blood in water a 10 per cent. solution of caustic soda; on warming a cinnabar-red colour appears, whereas, in the case of normal blood, the colour would be brownish green (Hoppe-Seyler,[8] Otto[9]); or Salkowski's modification,[10] in which the blood is diluted to twenty times its bulk, and an equal quantity of caustic soda solution (specific gravity 1.34) added. If CO is present the fluid first turns white and cloudy, then bright red, and on standing red flakes form. In the case of normal blood the colour is dirty brown. Or Kuniyosi-Katayama's[11] test may be tried; a little yellow sulphide of ammonium and dilute acetic acid are added to the blood, which then turns a beautiful red colour, whereas normal blood becomes grey or greenish grey.

In poisoning with sulphuretted hydrogen a spectrum, belonging, according to Hoppe-Seyler, to sulphide o methæmoglobin, may be seen.[12] It is only necessary to pass a stream of this gas through a solution of blood to see the spectrum. The blood becomes dark, sometimes a dull green, and the distinction between venous and arterial blood disappears (Lewin).[13]

In poisoning with chlorate of potassium, Marchand[14] has shown that the blood is much changed; a sepia-coloured decomposition product being formed, which Hoppe-Seyler found to be methæmoglobin. In children, especially, poisonous doses change the hæmoglobin into methæmoglobin.

Methæmoglobin is distinguished by a spectrum, which differs totally from that of oxyhæmoglobin; thus in acid or neutral solutions it shows four bands, these bear some resemblance to those of acid hæmatin, but sulphide of ammonium distinguishes between them, as on its addition the two bands of oxyhæmoglobin appear first, then that of reduced hæmoglobin, whereas if hæmatin in solution is thus treated we get the spectrum of reduced hæmatin. Moreover, methæmoglobin has been crystallised by Halliburton,[15] Hüfner and Otto,[16] and Copeman, proving that the reagents required to produce it are not sufficient to split up the molecule of hæmoglobin into a coloured and a proteid constituent. Again, as Jäderholm[17] has shown, the spectrum of alkaline methæmoglobin is peculiar; a narrow band occurring between C and D, close to the latter, and two broader ones between D and E. Sometimes mixtures of oxyhæmoglobin and methæmoglobin are mistaken for the latter, but as H. Bertin-Sans[18] has shown—and I have repeatedly confirmed this statement—the bands of methæmoglobin placed between D and E are distinguished from those of oxyhæmoglobin in this, that the one nearest E is far more intense and about twice as broad as that nearest D. Methæmoglobin was at one time supposed to be an oxidation product of oxyhæmoglobin, but since it can be produced by the action on hæmoglobin of sodium hypochlorite, ferrous sulphate, and ferricyanide of potassium, which exert rather a reducing than an oxidising action, such cannot be the case. Other reagents produce methæmoglobin, such as dilute acids, nitrite of amyl, and other nitrites, also kairin, thallin, hydrochinon, pyrocatechin, iodin, bromine, terebenthine, ether, perosmic acid, permanganate of potassium (Hayem)[19], and antifebrin (Müller).[20] According to J. G. Otto,[21] the change of oxy- into methæmoglobin is a change from a loose molecular into a chemical combination.

Methæmoglobin is very frequently present in the urine in cases of intermittent hæmoglobinuria, although sometimes the change to methæmoglobin, as Copeman[22] has shown, may take place in the urine itself. However, even if this be so, it still proves that in that condition the hæmoglobin is more unstable than it ought to be. It is very often also present in different pathological fluids, and sometimes pigments intermediate between this and hæmatin are present in them.

In hæmoglobinæmia the hæmoglobin is found dissolved in the plasma, and this is followed by hæmoglobinuria, when the spleen and liver are unable to convert the free pigment into others. Von Jaksch[23] directs the experiment for its detection to be performed thus: The blood is drawn from the patient by means of a cupping glass, placed immediately in a refrigerator, and allowed to remain there twenty-four hours. A ruby-red stratum is seen over the blood, whereas in normal blood it should be yellow; on examination with the spectroscope the bands of oxy-hæmoglobin are seen.

In poisoning—in the case of dogs at least—with nitro-benzol it is said that hæmatin is produced (Filehne).[24]

As is now well known, Hénocque[25]—who has done so much to make the spectroscope useful in medicine—has applied this instrument in a novel manner. He observed that the oxy-hæmoglobin bands could be observed in transparent parts such as the lobe of the ear, and the ungual phalanges, if they were illuminated by diffuse sunlight. In the case of the ungual phalanx, when the finger was ligatured he found that these bands sooner or later disappeared, to be replaced by that of reduced hæmoglobin. He then found that the rapidity of reduction varied according to the amount of hæmoglobin in the blood, thus with a normal proportion of hæmoglobin reduction took place in 70 seconds, while with anæmic blood the time might be only from 30 to 40 seconds. Finally he has given a formula by means of which the energy of reduction may easily be calculated. It is as follows:

$$E = \frac{M}{D} \times 5$$

In which E = energy of reduction; M = mean proportion of hæmoglobin, obtained by the hæmatinometer of Hénocque; and D = the time in seconds in which reduction is accomplished. Hénocque calculates that the amount of oxyhæmoglobin reduced in one second under quite normal conditions is 0.2 per cent.

Hæmatin and Hæmatoporphyrin.—Before going any further I must make some remarks on the decomposition products of hæmoglobin as briefly as possible. These are hæmatin and

[7] Hoppe-Seyler: *Virchow's Archiv.*, xi, 288, 1857; Lewin: *Lehrbuch der Toxikologie*, p. 23, Wien, 1885; Böhm: *Ziemssen's Handbuch*, xv, 158, 2nd edition, 1880.

[8] Hoppe-Seyler: *Virchow's Archiv*, xiii, 1858.

[9] Otto: *Anleitung zur Ausmittlung der Gifte*, p. 246, 6th edition, 1884.

[10] Salkowski: *Zeitschrift f. physiol. Chem.*, xii, 227, 1888.

[11] Kuniyosi-Katayma: *Virchow's Archiv*, cxiv, 53, 1888.

[12] Hoppe-Seyler: *Physiol. Chemie*, p. 386.

[13] Lewin: *Virchow's Archiv*, lxxiv, 220, 1878; and *Lehrbuch der Toxikologie*, p. 48.

[14] Marchand: *Virchow's Archiv*, lxxvii, 488, 1879.

[15] Halliburton: *Quart. Journ. Micros. Sc.*, vol. xxviii.

[16] Hüfner and Otto: *Zeits. f. physiol. Chemie*, Bd. vii, 1883, p. 65, and Bd. viii, p. 366.

[17] Jäderholm: *Zeits. f. Biologie*, xiii. 193, 1877.

[18] Bertin-Sans: *Compt. Rend.*, cvi, pp. 1243-1245.

[19] Hayem: *Compt. Rend.*, cii, pp. 698, 700.

[20] Müller: *Deutsche m.d. Wochenschr.*, xiii, 27, 1887.

[21] Otto: *Biol. Centralbl.*, 1884, 212.

[22] Bristowe and Copeman: *Lancet*, July, 1889.

[23] Von Jaksch: *Clinical Diagnosis*, by Stirling and Cagney, p. 43.

[24] Filehne: *Archiv f. experiment Pa'hol.*, ix, 329, 1878.

[25] Hénocque: Etude Spectroscopique du Sang à la Surface Sous-unguéale du ponce, *Compt. Rend. de la Société de Biologie*, 8th serie, t. i, p. 671, No. 41, 6 Décembre, 1884; also, *Ibid.*, p. 700, 13 Décembre; and t. ii, pp. 760 and 762, No. 44, 1884.

hæmatoporphyrin, speaking broadly. When hæmoglobin is treated with certain reagents, such as acids and caustic alkalies in the presence of oxygen, it is split up into one or more proteids, and a coloured body, hæmatin, which contains all the iron of the hæmoglobin. If, however, as Hoppe-Seyler[26] has shown, these reagents are made to act upon the hæmoglobin in the absence of oxygen, another substance which that observer named hæmochromogen, is formed. On admitting air to the apparatus in which this is produced the bands of hæmochromogen disappear, and, according as an alkali or an acid has been used to act on the hæmoglobin, alkaline or acid hæmatin, respectively, is produced.

To study the spectra all one has to do is to act upon defibrinated blood with alcohol containing caustic soda or potash and acids, when the band of alkaline hæmatin in the first and then of acid hæmatin in the second case are produced. As is well known, the band of alkaline hæmatin occurs at the D line, while those of acid hæmatin closely resemble those of methæmoglobin, being, like them, four in number, but differing in the second band from the red, which is very difficult to see as a rule. The beginner can generally make out the first and third bands easily, but the second and fourth require more careful observation.

Hæmatin may be obtained in various ways. Thus, if blood is agitated with ether, to which some acetic acid has been added, the hæmatin produced is taken up by the ether and can be obtained by evaporating it and washing the residue with ether, alcohol, and water, as a reddish-brown amorphous powder, having a metallic lustre, and insoluble in water, alcohol, and chloroform, but soluble in acidulated alcohol and alkalin. Or it may be obtained by Hoppe-Seyler's method[27] from hydrochlorate of hæmatin or hæmin ($C_{68}H_{70}N_8Fe_2O_{10}$, 2 HCl—Hoppe-Seyler). This is the crystalline substance which one gets in treating a dried blood spot with a trace of chloride of sodium and acetic acid, and when crystals are of so much importance in the medico-legal detection of blood. This method of procuring hæmatin is fully described by Hoppe-Seyler, and need not detain us now. It will suffice to say that the substance so obtained is an amorphous powder, insoluble in water, alcohol, and ether, soluble in dilute solution of caustic alkalin, insoluble in dilute acids, soluble with difficulty in hot alcohol containing sulphuric acid, in acetic acid, and in fuming hydrochloric acid.

Lately Nencki and Sieber[28] have been reinvestigating these decomposition products of hæmoglobin. At first they prepared hydrochlorate of hæmatin by a special method, which need not be mentioned here. They isolated it by means of amyl alcohol, but Hoppe-Seyler has since shown that the amyl alcohol is decomposed by the process adopted, and the product must have been contaminated by the presence of its decomposition products. While Hoppe-Seyler assigns to hæmatin the formula $C_{68}H_{70}N_8Fe_2O_{10}$, these observers make it $C_{32}H_{32}N_4FeO_4$, and they found that it agreed with the hæmatin of Hoppe-Seyler in its character.

The method which I found out some years ago[29] enables one to procure hæmatin very easily: The defibrinated blood of the sheep is treated with rectified spirit acidulated with sulphuric acid (1 in 17), the solution filtered, diluted with water, and agitated in a separating funnel with chloroform, the latter separated, filtered, returned into the funnel, and again washed with water until free from acid. If evaporated after this treatment it leaves an amorphous impure hæmatin, but by allowing the chloroform to stand in a corked bottle in a dark place, very soon minute rhombic crystals form, making the chloroform turbid, which can be separated by filtering, and purified by washing with water, alcohol, and ether. The hæmatin thus prepared agrees in its characters, except for the occurrence of crystals, with that described by other observers.

To see the spectrum of hæmochromogen (the reduced hæmatin of Stokes) and its spectrum is one of the most important of all physiological spectra; it is not necessary to prepare it by Hoppe-Seyler's method. All that is required is to act upon some defibrinated blood with alcohol containing caustic soda or, better, ammonia, filter, and add to the filtrate some yellow ammonium sulphide; when this is done the bands of hæmochromogen are seen. They bear a resemblance to those of oxyhæmoglobin, but are much nearer the violet end of the spectrum and differ in the remarkable sharpness and darkness of the first band, and other particulars which need no mention.

It may appear superfluous to go into this detail, but otherwise I could not make the subject clear to those who have not studied it.

I stated that the bands of hæmochromogen are of great importance, and I will now give my reasons for saying so. It has happened to me more than once to have to give an opinion upon blood-stains on the blade of a penknife and on cloth. Now in some of these cases I could neither obtain hæmin crystals nor blood corpuscles; the stain was also insoluble in water. On putting the knife-blade to stand in a narrow tube just wide enough to admit the blade, and covering the latter with rectified spirit containing ammonia, the stain was dissolved out; although the fluid was barely coloured to the naked eye, and failed to show the absorption band of alkaline hæmatin, yet on adding one drop of ammonium sulphide the two bands of hæmochromogen stood out with extraordinary distinctness. Again, in the case of a blood-stain on cloth, the same very simple method was found to apply, the piece of cloth being cut up into small pieces and digested in the same solution, and then treated with ammonium sulphide. So in the case of urine containing blood;[30] sometimes the blood clings to the sediment and then fails to reveal its presence by the ordinary tests. I find, however, that by filtering off the sediment, and treating it and the filtering paper through which it has been filtered, with alcohol and ammonia, and then adding ammonium sulphur, the presence of blood is put beyond all doubt. Further on I shall have to return to hæmochromogen.

There is another very interesting decomposition product of hæmoglobin, namely, hæmatoporphyrin; this was described by Dr. Thudichum[31] under the name of "cruentine." It is produced by the action of strong sulphuric acid on hæmoglobin or hæmatin, and to the substance obtained by his own method Hoppe-Seyler[32] assigns the formula $C_{68}H_{74}N_8O_{12}$. It contains, therefore, noiron, which is said to be separated and to be present in the solution as a ferrous salt. Nencki and Sieber[33] have lately obtained it by other methods—in the first by the action of concentrated sulphuric acid on hæmin, and in the second and (according to them) easier method by the action of acetic acid saturated with bromin water on hæmatin. So obtained it is soluble in alkaline and alkaline carbonates, dilute mineral acids, and alcohol. It agrees, when thus prepared, with the hæmatoporphyrin of Hoppe-Seyler, but Nencki and Sieber assign to it a different formula, namely, $C_{16}H_{18}N_2O_3$, and they find this agrees with the formula given by Maly for bilirubin, the red colouring matter of bile, to which Maly assigned the formula $C_{32}H_{36}N_4O_6$. I would call special attention to this fact here, because it gains additional significance from what I shall have to say further on about the occurrence of hæmatoporphyrin in urine. These observers also prepared its hydrochloride $C_{16}H_{18}N_2O_3$ Hcc, and its sodium salt $C_{16}H_{17}NaN_2O_3+H_2O$, and other salts.

Further—and this is of great importance—they found that when the sodium compound was given to animals the greater part was retained in the body, possibly being there again utilised in the formation of hæmoglobin. It can also be produced by the action of energetic reducing agents on hæmoglobin or hæmatin, such, for instance, as that of sulphuric acid and zinc, hydrochloric acid and tin, and sodium amalgam; but in these cases, as Hoppe-Seyler[34] has shown, the pigment is apt to pass on to a lower stage of transformation. The spectrum of hæmatoporphyrin in a solution treated by a mineral acid, or acid hæmatoporphyrin, consists of two remarkable bands, one at D, and another between D and E; while when present in an alkaline solution it shows four or five bands. In fact, these two spectra serve well for the recognition of hæmatoporphyrin.

[26] Hoppe-Seyler: *Med. chem. Untersuch.*, Heft iv, 1871, p. 523; *Ibid.*, pp. 377-335; *Zeit. f. physiol. Chem.*, Bd. 1, p. 138.

[27] Hoppe-Seyler, *loc. cit.*

[28] Nencki u. Sieber: *Ber. d. deutsch. chem. Gesell.*, xvii, pp. 2267-2276; *Ibid*, xviii, pp. 392-399; *Monats. f. Chem.*, ix, pp. 115-132; *Archiv. f. exper. Path. u. Pharm.*, xviii, xx, xxiv.

[29] MacMunn: *Journ. Physiol.*, vol. vi., Nos. 1 and 2.

[30] MacMunn: BRITISH MEDICAL JOURNAL, July 19th, 1879.

[31] Thudichum: Tenth Rep. Med. Off. Privy Council, 1867.

[32] Hoppe-Seyler: *Physiol. Chemie.*, p. 397.

[33] Nencki and Sieber: *loc. cit.* (see Note 28).

[34] Hoppe-Seyler: *loc. cit.*

It is a very strange fact, and one upon which I came in working at the pigments of the lower animals, that hæmatoporphyrin may be present in the integument of an animal, and yet no hæmoglobin can be found in that animal.[35] At first this fact staggered me completely. I found the pigment present in star fishes, slugs, and in a mollusk allied to the razor fish, namely, *Solecurtus strigillatus;* and a comparison of my maps with those of Professor Moseley, and an examination of the pigment named by him polyperythin,[36] have led me to conclude that this pigment occurs in many invertebrate animals. I found, however, that in many, probably in all, these instances it is derived from pigments closely allied to hæmoglobin and hæmatin, and which I have named histohæmatin. It would appear, therefore, that in such animals hæmatoporphyrin is a waste product; and here permit me to give a quotation from Geddes and Thomson.[37] In their interesting book on the *Evolution of Sex*, they say: "In spite of the researches of Krukenberg, Sorby, MacMunn and others, our knowledge of the physiology of many of the pigments is still very scanty. Yet in many cases, alike among plants and animals, pigments are expressions of disruption processes, and are of the nature of waste products."

But anyone may well ask what has that to do with pathology? Allow me to reply that it has everything to do with it. In the urine of Addison's disease[38] a kind of hæmatoporphyrin occurs; and how do we explain its presence? Here it is a waste product too; and to explain why it occurs there, let me for a moment say what the spectroscope tells us about that disease.

In the adrenals of several mammals I found that hæmochromogen[39] is present in the quite fresh organ. Now previous observations had taught me to conclude that wherever that pigment occurs in the human body it is an excretory product; for instance, it was found in the liver, and in the bile, and elsewhere. Putting this fact together with the results of the analysis of the adrenals made by many competent observers, I could not help concluding that the adrenals are concerned in the downward metabolism of hæmoglobin and of its decomposition products. If they are diseased, then these decomposition products circulate in the blood, producing pigmentation of skin and mucous membrane, and appearing in the urine as urohæmatoporphyrin.

I feel bound to add, however, that I found this latter pigment—urohæmatoporphyrin—in the urine of other diseases, such as acute rheumatism, cirrhosis of the liver, croupous pneumonia, Hodgkin's disease attended by pigmentation of skin, so-called idiopathic pericarditis, peritonitis, meningitis, measles, and typhoid fever; and lately I have found a kind of hæmatophorphyrin present, to an extraordinary degree, in the urine of a case of Graves's disease, to which I shall have to refer again. However, their observations do not weaken my theory, but really support it.

Thinking over the matter, I conclude that this pigment may appear in the urine under two conditions: (1) when an excess of effete blood-colouring matter, or perhaps effete histohæmatin, is present in the blood, the glands whose function it is to change this to a simpler substance being incapable of dealing with the excess of the pigment, although they may be quite healthy; or (2) when these glands are diseased, and therefore incapable of metabolising the normal amount of effete pigment. In either of these conditions we should have urohæmatoporphyrin present in the urine.

Lately, Mr. Cant, of Lincoln, sent me a most extraordinary specimen of urine. It had a deep Burgundy-red colour,[40] which at first sight might lead one to suppose that it contained blood; but on testing for blood chemically, microscopically, and spectroscopically, none could be found, nor could any proteid be detected; it contained, in fact, no serum-albumen, globulin, or peptone, no excess of indican; but it did contain, and owed all its colour to, a kind of hæmatoporphyrin, easily demonstrated by the spectroscope. Now I need hardly say that, if the spectroscope were not available, I could have made nothing out of this urine. Without any treatment whatever, it showed a splendid hæmatoporphyrin spectrum, which, with a little sulphuric acid and a little ammonia, changed into that of acid and of alkaline hæmatoporphyrin respectively.

What was the meaning of this? Of course, in the first place, a large amount of blood pigment was being destroyed somewhere in the body; and in the second, some poison of a hæmolytic nature must have been present. Mr. Cant has sent me this urine several times, and each time the same pigment was found. It has been excreted for some years—at least three—by a woman now aged 40, who has exophthalmic goître, this disease having begun three years ago. Mr. Cant tells me that the red blood corpuscles, counted with the hæmocytometer, amount to 2,250,000 per cubic millimètre—about half the normal—and the hæmoglobin is only 40 per cent of the normal, pointing, of course, to destruction of blood, and confirming the spectroscopic appearances.

The idea suggests itself, is the blood broken up in the thyroid? If so, does that gland in health pick out effete blood pigment from the blood circulating in it, and metabolise it into something else? That events of a catabolic nature take place, then, seems likely, from the results arrived at by Horsley and others, that its extirpation is followed by myxœdema.

Another curious fact noticed by Mr. Cant was that, on drawing a little blood from the patient's finger, and putting it under the microscope, many of the red corpuscles extruded their hæmoglobin, just as they are known to do under the influence of certain weak acids. My observations teach me that in all probability blood pigment is broken up in the tissues, and by an energetic reduction process. I may recall the beautiful experiments of Ehrlich[41] in this connection; he found that certain blue colouring matters, such as alizarin blue and indophenol blue, lose their colour in the tissues of living animals, and regain it on contact with the air, proving the existence of energetic reductive processes in the tissues; but, as most people know now, it is not the nascent hydrogen, but the nascent oxygen, which brings about the final change; just as it does in the case of palladium-hydrogen.[42] The product of this is hæmatoporphyrin, which is readily changed into bile pigment when brought to the liver, or perhaps it is changed into other metabolites in other organs, such as the adrenals. I mentioned above that Nencki and Sieber[43] find that bilirubin and hæmatoporphyrin have the same formula; therefore the change from one into the other is easily accomplished. But I shall have a few more remarks to make bearing on this interesting subject directly.

The Histohæmatins.—In 1883 I found a peculiar pigment in the muscle of insects, and from a shred of its bands I came to the conclusion that it was nearly allied to hæmatin. These bands are different from any known decomposition product of hæmoglobin; they are very sharp and narrow, and the pigment is easily changeable into another which gives bands like those of hæmochromogen, only narrower and much nearer the violet end of the spectrum. I named this pigment "myohæmatin,"[44] and traced it among different groups of invertebrate, and then among vertebrate, animals up to man. I found that other organs and tissues of invertebrates and vertebrates contain a similar class of pigments, which I named "histohæmatins." Now, these pigments occur among animals which contain no hæmoglobin whatever, therefore they cannot be derived from it in such animals. Levy,[45] a pupil of Hoppe-Seyler, has tried to prove that the myohæmatin of pigeon muscle is nothing but hæmochromogen, and Hoppe-Seyler has supported him.[46] Of course I feel like David before Goliath in daring to indulge in polemics with such a great investigator; but David in this case feels that he is right. I have found evidence of the presence of a histohæmatin in sponges;[47] indeed, it would seem to have a greater right to priority in

[35] MacMunn, *Journ. Physiol.*, vol. vii, No. 3; vol. viii, No. 6.
[36] Moseley: *Quart. Jl. Micros. Sc.*, vol. xvii. 1877, pp. 1-23.
[37] Geddes and Thomson, *The Evolution of Sex*, 1889, p. 23.
[38] MacMunn, BRITISH MEDICAL JOURNAL, February 4th, 1888.
[39] MacMunn, *Philos. Trans.*, Pt. I, 1886.
[40] Dr. S. Monckton Copeman brought me two specimens of urine about a year ago, which had the same colour as the above, and both owed their colour to the above pigment. This was the first time I had met with it. Dr. Noel Paton recently sent over a specimen of urine containing an allied substance.
[41] Ehrlich: *Das Sauerstoffbedürfniss des Organismen*, 1885.
[42] Hoppe-Seyler: *Zeits. f. physiol. Chem.*, vol. ii, p. 22, 1878; vol. x, p. 35, 1886; *Ber. d. deutsch. chem. Ger.*, xii, p. 1551; xvi, pp. 117, 1917, 1883.
[43] Nencki u. Sieber: *loc. cit.* see (27).
[44] MacMunn: *Philos. Trans.*, Pt. I, 1886.
[45] Levy: *Zeits. f. physiol. Chemie.*, xiii, Heft 4.
[46] Hoppe-Seyler: *Zeits. f. physiol. Chemie.*, xiv, Heft 1. My reply is given in the same Bd. xiv. Heft 4, and xiii, Heft 6.
[47] MacMunn: *Journ. Physiol.*, vol. ix, No. 1.

time than has hæmoglobin. Like the hæmatin which Sorby[48] found in the bile of snails and slugs, and which I found in the limpet, the crayfish, and elsewhere,[49] it seems to be a pigment on its way, as it were, to become hæmoglobin. Neither Hoppe-Seyler nor anyone else can for one moment maintain that in a butterfly, a beetle, a snail, or a crayfish the histohæmatin is derived from hæmoglobin, because they possess none. It may be that if, as is likely, it has become in time replaced by hæmoglobin, owing to greater respiratory needs—that is, it may have furnished, as it were, the nucleus around which the large hæmoglobin molecule has been built in time—then possibly in the downward metabolism of hæmoglobin in the vertebrate body it may reappear as one of the intermediate products of such downward metabolism. Or how do we know that it may not precede the formation of hæmoglobin in the vertebrate body, just as it has preceded it in time?

If anyone will take the trouble to catch a bluebottle fly (*Musca vomitoria*), open its thorax, place the large alar muscle in a compressorium, and examine it under his microspectroscope, he will see the spectrum of myohæmatin. A faint band will be seen just before D; then two narrow bands, of which the second is much darker than the first, between D and E; and a third, broader and fainter, covering E and *b*. I succeeded in getting oxyhæmatin into solution in the case of pigeon muscle, and I found that I could convert it into hæmatoporphyrin, thus proving, as I said before, that the latter pigment is derived from the histohæmatin when it is found in animals possessing no hæmoglobin.

These pigments are without doubt respiratory, as I have shown, and, although attempts have been made to minimise their importance, yet I believe by-and-by someone whose eye has been properly trained to the use of the spectroscope by long and careful study will come forward and confirm my results. Doubtless, if he is not an Englishman, he will saddle the pigment with a new name, and express regret that I had missed some points which he alone is able to point out. However, I shall be happy to show anyone who cares to see it the spectrum of myohæmatin.

Piyments of the Bile.—As all readers of textbooks of physiology know, there are two colouring matters in vertebrate bile: the red one, bilirubin $C_{16}H_{18}N_2O_3$ (or double that formula), and the green one, biliverdin $C_{16}H_{18}N_2O_4$ (Maly). Neither gives absorption bands. Bilirubin cuts off, in even weak solutions, the whole violet end of the spectrum, and that abruptly; biliverdin both ends: transmitting green. Both of them, when treated in solution with nitric acid, give the colour reaction of Gruelin and Heintz, and each stage of the reaction is marked by a characteristic spectrum, which may be made use of in their detection. It is only necessary to treat a chloroform solution of bilirubin, or a weak alcoholic solution of biliverdin, in a test tube with nitric acid, and observe the solution with the spectroscope, to enable one to follow this change. At first a band appears before D, then another after D, and one at F; the second fades away first, then the first one, leaving that at F, which also in time fades away. The pigments corresponding to these changes have some of them received names; for instance, that corresponding to the blue and violet stage is the bilicyanine of Heynsius and Campbell, and that indicated by the band at F, when it is becoming feebler, is known as cholitilin. It is hardly necessary to say that bilirubin is identical with hæmatoidin,[50] a pigment found in old hæmorrhages in the brain, thyroid, and elsewhere; also occasionally in the lungs, and its crystals have been found also in the fæces. Hence there is no doubt that hæmoglobin is readily connected with bile pigment. But I must remark that, although under pathological conditions hæmoglobin is converted into bilirubin, yet it seems to me that under normal conditions the change is probably from hæmatin or hæmatoporphyrin directly into bilirubin. My reasons for saying so are as follows:[51] (1) In bile obtained from a biliary fistula in a case where cholecystotomy had been performed by Mr. Lawson Tait, I found only biliverdin in fresh specimens.[52] Copeman and Winston[53] had a similar experience. (2) A bilirubin-like pigment has been found in the placenta of the bitch.[54] (3) In the eggshells of some birds hæmatoporphyrin and biliverdin have been found side by side by Sorby,[55] Krukenberg,[56] and others. Here the transformation probably takes place in the glands of the oviduct. (4) I have found biliverdin in a hydrocele fluid, into which blood had been effused.[57] (5) In the liver of the pigeon I have seen in one part hæmochromogen and in another biliverdin, which latter was seen filling the ultimate radicles of the biliary ducts in the lobule.[58] (6) In the red sea anemone (*Actinia mesembryanthemum*) I have found biliverdin in the mesoderm, while in the ectoderm a kind of hæmatin occurs: here there is no doubt that the green pigment is formed from the hæmatin.[59] I may also refer to the interesting experiment of Haycraft and Scofield.[60] These observers found that they could change bilirubin into biliverdin by contact with the mucus of the gall badder, and by different reducing means. Hence it is probable that this change takes place in the gall bladder, but the change in the liver is probably from hæmatin or hæmatoporphyrin into biliverdin.

I have described another pigment as occurring in the bile of the sheep and ox, namely, chlorohæmatin.[61] I proved that it is a kind of hæmatin, and changeable by means of energetic reducing agents into hæmatoporphyrin. It gives a curious series of bands which have been taken by MM. Wertheimer and Meyer[62] for methæmoglobin, but they do not belong to that substance. These observers found that in dogs poisoned with aniline and toluidine, oxyhæmoglobin passed over into the bile. But in some cases, though rarely, they found a substance which gave the bands of reduced hæmatin when treated with ammonium sulphides, and in other cases a substance which they call chloro-methæmoglobin, which they say is identical with my chlorohæmatin. In any case, this pigment is evidently on its way to be changed into biliverdin.

The spectroscope may also serve as a help in the detection of bile acids by Pettenkofer's test, as this is denoted by a peculiar spectrum, which I have described elsewhere.

There is no doubt that if the bile were systematically examined in many morbid conditions, most interesting results would be obtained. Indeed, I hope when I get sufficient leisure to do this.

Pigments of Urine.—Most urines, provided they do not contain bile or blood, show an absorptive band at the F line. Now in febrile cases, and in certain pathological conditions, the urine will be found redder than normal, and the band at F will be found broader and darker. Normal urine may not show the band at F owing to its containing enough reducing substance to convert the pigment giving that band into a chromogen; but if it be treated with a little solution of potassium permanganate, the chromogen becomes oxidised into the pigment, and the band at F appears. The pigment which gives this band is urobilin, and when isolated from normal urine it possesses characters which distinguish it from the corresponding pigment present in certain pathological urines. In either case the pigment may be isolated by precipitating the urine with neutral and basic acetate of lead, filtering off the precipitate, decomposing it with rectified spirit and sulphuric acid, filtering, diluting with water and agitating with chloroform in a separating funnel. On evaporating the chloroform the pigment is obtained.[63] Just as in the urine so in solutions of the isolated pigments, certain differences are

[48] **Sorby**: *Quart. Journ. Micros. Soc.*, vol. xvi. p. 77, *et seq.*

[49] **MacMunn**: *Proc. Roy. Soc.*, No. 226, 1883.

[50] For the literature on the identity of bilirubin and hæmatoidin, *cf.* Halliburton in the new edition of Watts's *Dictionary of Chemistry*.

[51] Copeman and Winston (*Journ. Physiol.*, vol. x, No. 4) have come to the same conclusion, and believe that the bile when freshly secreted is green, and that the change to yellow or reddish is due to the reduction of the biliverdin in the gall bladder and in the intestines.

[52] **MacMunn**: *Journ. Physiol.*, vol. vi.

[53] **Copeman and Winston**: *Journ. Physiol.*, vol. x, pp. 213-231.

[54] **Hoppe-Seyler**: *Handb. d. phys. u. path. chem. Analyse*, 3rd ed., p. 180; **Btti**: *Oesterr. vieteljahr. f. wiss. Veterinärkunde*, xxxvi.

[55] **Sorby**: *Proc. Zool. Soc. Lond.*, 1875, pp. 351-365.

[56] **Krukenberg**: *Verhandl. d. phys-med. Gesell. zu. Wurzburg*, N. F., Bd. xvii; **Thudichum**: *Annals. Chem. Med.*, i, 1879, pp. 93-103; **Dickie**: *Ann. and Mag. Nat. Hist.*, xii, 1848, p. 169; **Liebermann**: *Bericht der deutsch. chem. Ges.*, xi, 1878, etc.

[57] **MacMunn**: *Proc. Roy. Soc.*, No. 226, 1883.

[58] **MacMunn**: *Journ. Physiol., loc. cit.*

[59] **MacMunn**: *Philos. Trans.*, Pt. II, 1885, pp. 644, 645.

[60] **Haycraft and Scofield**: *Zeits. f. physiol. Chem.*, xiv, pp. 173-182.

[61] **MacMunn**: *Proc. Roy. Soc.*, No. 208, 1880, No. 226, 1883; *Journ. Physiol.*, vol. vi, Nos. 1 and 2.

[62] **Wertheimer and Meyer**: *Archives de Physiologie*, No. 3, July, 1889, pp. 438-448 and 600-605.

[63] **MacMunn**, *Proc. Roy. Soc.*, No. 208, 1880; *Journ. Physiol.*, vol. x, Nos. 1 and 1; *Clinical Chemistry of Urine*, pp. 104-110.

found in the spectra which prove beyond doubt that normal and pathological urobilin differ in certain particulars. The band of pathological urobilin at F is dark, broad, and shades off towards violet, while that of normal is narrow, is not deeply shaded, and does not shade off towards violet.

In acid solutions of pathological urobilin other bands are seen, which are missing in the corresponding solutions of the normal pigments.[64] The idea occurred to me in studying the spectrum produced by the action of nitric acid on solution of bile-pigment, that the stage in which the band at F is dark corresponds to the production of pathological urobilin, while that in which the band at F is faint corresponds to the production of the normal pigment. So it has turned out, but we are not to conclude that either of these pigments as it occurs in urine is necessarily derived from bile pigments. By the long continued action of sodium amalgam and of zinc and sulphuric acid, aided by heat on hæmatin, a substance is eventually formed which possesses many of the characters of (pathological) urobilin.[65] And by the action of hydrogen peroxide on hæmatin in acid solutions, another substance is found which possesses the character of normal urobilin, as I have proved, and by reducing this with sodium amalgam it changes into a substance resembling pathological urobilin.[66]

For this and other reasons we may conclude that normal urobolin is a more completely oxidised substance than the pathological pigment, but it is possible—and, indeed, probable—that both are produced under different conditions of metabolism. In this they afford a parallel to urea and uric acid; we know that uric acid is a less completely oxidised substance than urea, yet uric acid is not produced by the same metabolism that produces urea. I believe that normal urobilin may be produced elsewhere than in the liver by the action of nascent oxygen on worn-out hæmoglobin and its decomposition products, and in many cases it does not originate from bile pigments. Pathological urobilin may result from the incomplete oxidation of worn-out hæmoglobin and its decomposition products, and may not in many cases have its origin from bile pigment. It would appear to be excreted, as is now well known, after extravasations of blood into the tissues, into the peritoneum, and elsewhere; in such cases, there is too much material for complete oxidation to take place. On the other hand, there is a pigment present in fæces—stercobilin—and this presents nearly all the characters of pathological urobilin,[67] so that in some cases the presence of the latter in the urine may indicate the absorption of fæcal matters from the intestine. I am convinced that some so-called bilious attacks are due to this cause, for if the pigment is absorbed it must be accompanied by ptomaines, which produce the headache, aching of the limbs, vomiting, and so on. Whether this absorption takes place owing to the liver not performing its guardian function owing to vasomotor disturbance, or whether absorption takes place directly from the intestine, is not clear at present. At all events, everyone knows the beneficial effects of a saline purge in such cases.

In spite of the assertion that the urobilin found in urine is always due to the absorption of hydrobilirubin from the intestine, this hydrobilirubin being supposed to originate from the action of nascent hydrogen, generated by putrefactive processes in the intestine, on the pigments of the bile (Maly and Hoppe-Seyler), I say, without fear of contradiction, that the former may arise in other ways, and that the urobilin of normal urine is a product of oxidation, and that pathological urobilin is an incompletely oxidised substance. By the action of zinc chloride, of that and ammonia, and of other reagents on those pigments, it is easy to prove, as I have elsewhere shown,[68] that hydrobilirubin is not identical with either normal or pathological urobilin. The presence in the urine of urohæmatoporphyrin and the replacement of this by pathological urobilin, and *vice versâ*, teach, when taken in connection with other facts, that the latter may, as I said before, be derived in many cases from hæmatin, and may have no biliary origin. In some cases, of course, we may have urobilin derived from bile pigment as well as directly from hæmatin, but in such cases there is generally evidence of the passage of bile pigment into the blood. It is a well-known fact that in recovery from jaundice the bilirubin may appear in the urine as urobilin.

The spectroscope is helpful, also, in enabling us to detect indican. If the urine, after applying Jaffe's test, is shaken with chloroform, the latter, if indican is present, will show a band before D due to indigo blue, and another after D, said to be due to indigo red. Or even when Jaffe's test fails, it may be detected by boiling the urine with common hydrochloric acid, cooling and agitating with chloroform; the latter shows the same bands as in Jaffe's test.[69]

There are different pigments which become red on treating the urine with nitric acid and other acids, either at the ordinary temperature or after heating, but at present their origin is uncertain, although at least one is derived from a skatol compound.

Other Pigments.—I have now described some of the most important absorption spectra, speaking from a purely medical point of view, but the subject is far from being exhausted. I have not mentioned the lipoderms or fat pigments, which term includes under it the pigments formerly known as luteins, nor the black pigments known as melanines, some of which latter do not owe their origin to hæmoglobin, as is commonly supposed; nor have I been able to furnish more than a partial and unsatisfactory account of the other pigments. Still what I have said will serve to show that the spectroscope has taught us a good deal, and, I believe, will teach us more when it comes to be more generally used. In biology generally it has recently brought many new facts to light; these in turn must help to advance comparative physiology, which at present is not advanced enough to enable anyone to write even an elementary book upon it.

[64] MacMunn, *loc. cit.*
[65] Hoppe-Seyler, *Physiol. Chemie. loc. cit.*
[66] MacMunn, *loc. cit*
[67] MacMunn: *loc. cit.*
[68] Hoppe-Seyler: *Physiol. Chemie.*, p. 297; Maly: *Centralbl. f. d. med. Wiss.*, 1871, No. 54.

ESTIMATION OF URIC ACID BY HAYCRAFT'S PROCESS.

Read in the Section of Pathology at the Annual Meeting of the British Medical Association, held at Birmingham, 1890.

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The process consists in (1) combining all the uric acid in a given amount (25 cubic centimètres) of urine with silver contained in a solution of nitrate of silver with addition of ammonia. The result is a gelatinous precipitate; (2) the gelatinous precipitate is collected and well washed on an asbestos filter; (3) the precipitate, after washing, is dissolved in nitric acid; (4) the silver thus got into solution is estimated by means of a colour test (Volhard's method). From the silver found the amount of uric acid combined with it is calculated.

Apparatus. — Flasks, pipettes, measuring-glasses, etc., a flask fitted with a rubber bung perforated in two places. Into one of the perforations fits the stem of a funnel containing broken glass and asbestos to form a filter. Into the other perforation fits a glass tube connected with a Sprengel's pump or other means of producing a vacuum.

[The working of the process was then shown on a specimen of urine.]

Addition of bicarbonate of soda—Haycraft says about 15 grains, Hermann considers that it is better to add about 30 grains; the object of the addition of the bicarbonate is to prevent the reduction of the silver combined with the uric acid.

It is always well to add an excess of the silver nitrate solution, as it is probable that when this is done the resulting urate of silver has a tolerably constant composition. When the patient is taking an iodide a precipitate of iodide of silver falls on adding the silver solution to the urine. This does not necessarily interfere with the process of estimating the uric acid; but it should always be an indication for adding more silver, so that the iodine may not deprive the uric acid of its share.

In collecting the urate of silver precipitate on the filter, if the filtrate is not quite clear it may be necessary to pass it a

[69] MacMunn: *Clinical Chemistry of Urine*, pp. 96-98.