

The Bradshaw Lecture

ON

THE URINARY PIGMENTS IN THEIR
PATHOLOGICAL ASPECTS.*Delivered before the Royal College of Physicians of London
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MR. PRESIDENT AND GENTLEMEN,—The invitation to deliver a lecture before such an audience as this at the same time confers a great honour and imposes a heavy responsibility. For the honour I beg to offer my sincere thanks, and to the distinguished men who have preceded me in the position which I for the moment occupy I desire to apologise in advance for any failure on my part to attain to the high standard which they have set up.

THE CLASSIFICATION OF URINARY PIGMENTS.

The grouping together, under the collective name of "urinary pigments," of a number of substances which need have nothing in common beyond the facts that they are met with in the urine and are possessed of colour, can only be looked upon as a temporary expedient, although it may be justified on grounds of convenience, and is even rendered necessary by the incompleteness of our knowledge. It may confidently be predicted that in the course of time this will share the common fate of such elementary classifications and that the group will undergo a gradual disintegration, as one by one its individual members can be referred to their true places in the chemical scheme. Some of the substances usually included do not even fulfil the above two conditions, but are merely coloured products formed by the action of reagents upon constituents of urine which are themselves colourless. Among these are urochrome and the indigo pigments, although of these latter indigo blue is occasionally met with as a sediment or in impalpable suspension in urine in which it has been formed by the decomposition of indoxyl-glycuronic acid, the less stable of its parent substances.

Even of the urinary pigments more correctly so-called not a few are excreted, either wholly or in part, as colourless or faintly-coloured parent substances, which quickly become converted into their coloured derivatives when the urine is exposed to light and air. These very unstable precursors are known as "chromogens," a convenient term which makes no assumption as to their true nature. When once the chromogen has been isolated and sufficiently studied we no longer speak of it as such. Thus it is not customary to describe indoxyl-sulphuric acid as the chromogen of indigo blue and red, or homogentisic acid as the chromogen of the alkapton pigment.

Coloured substances may enter the alimentary canal in articles of food, in sweetmeats or in medicines, and may be thence absorbed and excreted either altered or unaltered by the kidneys. With these may be included pigments formed during the passage through the body of constituents of food or drugs which are not themselves coloured, such as the oxidation products of the dioxybenzenes present in carboluria, and the yellow santonin pigment. It is important that one should have some acquaintance with these substances in order that one may distinguish them from others, but in themselves they have no special pathological significance.

In yet a third sub-group may be classed certain pigments which are normal constituents of the body, but which only find their way into the urine under morbid conditions. Such are hæmoglobin, bilirubin, biliverdin, and melanin, which last may appear in abundance in the urine, mainly as a chromogen, in cases of disseminated melanotic sarcoma. The excretion of such pigments by the kidneys affords diagnostic indications of great value, but these indications are, for the most part, well recognised, and their interpretation forms part of the daily routine of clinical practice.

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Lastly, there is a group of colouring matters, far less familiar, which may claim to be urinary pigments in a more special sense, seeing that they may all be present, if only in traces, in the urine of persons who may fairly be considered to be in normal health. I refer to urochrome, urobilin, hæmatoporphyrin, and uroerythrin, and it is to the consideration of these four pigments that I propose to devote the time at my disposal this afternoon. Urochrome is the most abundant of the urinary pigments, and to it the familiar yellow colour of normal urine is probably entirely due. Urobilin, which only occurs in very small amount in normal urine and wholly in the form of chromogen, has no influence upon its colour, but in many morbid conditions it appears in greatly increased quantity and to a great extent as the formed pigment. Under such circumstances it has a distinct effect upon the colour of the liquor, and its single dark absorption band is conspicuously visible on direct spectroscopic examination without the addition of any reagent. Hæmatoporphyrin is present in mere traces in normal urine and often in increased quantity in disease, but only very seldom does it occur in sufficient abundance to have any material effect upon the colour. Uroerythrin, if not strictly a normal urinary constituent, may appear in small amounts as the result of very trifling deviations from perfect health. In morbid urines it is often abundantly present, and it is chiefly conspicuous as the colouring matter of pink urate sediments.

UROBILIN.

Since its discovery by Jaffe,¹ in 1868, urobilin has attracted far more attention than the other members of the group, and there has grown up around it a literature which far exceeds in bulk that of all the other urinary pigments put together. On this account it will be more convenient to consider it first. The interest which it has awakened has been largely due to Maly's² observation that by the action of sodium amalgam upon bilirubin a product is obtained which in its main properties resembles urobilin very closely, so closely, indeed, that the identity of the two has been widely assumed, and the name of hydrobilirubin is not infrequently applied to the urobilin of urine, as also to the stercobilin obtained from fæces by Vanlair and Masius.³ However, there have always been some who have questioned the identity, although not the similarity, of urobilin and Maly's hydrobilirubin, which differ not a little in their minor properties, and some combustion analyses of urobilin carried out by Mr. F. G. Hopkins and myself⁴ show that whereas the urobilin of urine and the stercobilin of fæces are identical in composition as also in properties they differ conspicuously from hydrobilirubin, especially in the much smaller percentage of nitrogen which they contain.

—	Hydrobilirubin (Maly).	Urobilin (Hopkins and Garrod).
C.	64.68	3.58
H.	6.93	84
N.	9.22	4.11
O.	19.17	24.47
—	100.00	100.00

It would seem, indeed, that there is a group of substances which agree in showing the absorption band of urobilin and in exhibiting a brilliant green fluorescence with zinc chloride and ammonia, but which differ in stability and other minor respects and are not chemically identical. Such urobilinoid products have been obtained from bilirubin, hæmatin, hæmatoporphyrin, and urochrome by the action of reducing agents, and from some of these by what appear to be processes of oxidation. That they have something in common seems evident, and we are surely justified in regarding them as affording important evidence of the chemical relationship of the substances from which they are obtained.

Of the chromogen of urobilin we know very little as yet,

¹ Centralblatt für die medicinischen Wissenschaften, 1868, Band vi., p. 243; Virchow's Archiv, Band xlvii., 1869, p. 405.

² Centralblatt für die medicinischen Wissenschaften, 1871, Band ix., p. 839; Annalen der Chemie und Pharmacie, 1872, Band clxiii., p. 77.

³ Centralblatt für die medicinischen Wissenschaften, 1871, Band ix., p. 369.

⁴ Journal of Physiology, 1898, vol. xxii., p. 451.

for its great instability renders its isolation and study a matter of no small difficulty. We do know, however, that it is precipitated, as urobilin is, by saturation of the urine with ammonium sulphate (Eichholz⁵) and that it is readily soluble in chloroform. As Riva has pointed out, a chloroform extract of fæces is often practically colourless, but may nevertheless show a urobilin band of great intensity on the addition of an acid. Whatever be the nature of this chromogen it is certainly not urochrome.

Urobilin and its chromogen are widely distributed in the human body. In the fæces both in health and disease they are present in far larger amounts than in the urine. They have been found in bile removed from the gall-bladder during life, as well as in that obtained post mortem and are met with in the blood and in serous effusions.

As obtained from all the above sources specimens of urobilin agree in all their properties and are clearly identical in their nature. Time forbids me to enter here into the evidence upon which this assertion is based or to discuss the views of those who have maintained the contrary thesis and who hold that even in urine more than one kind of urobilin is met with. For such discussions I must refer you to the writings of MacMunn and others upon the chemical and spectroscopic sides of the subject, with the pathological aspects of which we are now concerned. I would merely recall that as regards urinary and fæcal urobilin not merely their properties but also the results of ultimate analyses bear witness to their identity.

Various theories have been put forward to account for the origin of urobilin and for the conspicuous variations in the amounts excreted in the urine. The earliest of these, the theory of Maly, assumes that it is formed by the reduction of bilirubin in the intestine, and is thence in part absorbed, and excreted by the kidneys. In order to meet the objections which present themselves to such an intestinal theory in its simplest form more than one hepato-intestinal theory has been propounded in more recent years. According to the purely hepatic theory of Hayem⁶ which was adopted by Tessier⁷ in his thesis urobilin is formed in the diseased or disordered liver as bilirubin is by the same organ in health, and the appearance of excess of urobilin in urine affords evidence of a derangement of the hepatic functions. Others maintain that urobilin is formed in the tissues at large, either by the reduction of bilirubin or directly from the blood pigment. Kunkel,⁸ and more recently Mya,⁹ with Giarre¹⁰ and others of his pupils, have advocated the former or histogenic theory, and some, including Leube,¹¹ have ascribed to the kidneys the chief share in the process. Dietrich Gerhardt¹² is the most recent upholder of the second or hæmotogenous theory, although he considers it to be proven that some of the urobilin of urine is of intestinal origin.

It will be seen that the above theories one and all start with the assumption that urobilin is derived from hæmoglobin, either directly or through the intermediate stage of bile-pigment. Thudichum¹³ alone maintains that urobilin is nothing more or less than a product of the decomposition of urochrome, and that neither urochrome nor urobilin are related to, or derived from, the pigments of the blood or bile.

The chief seat of the formation of urobilin (for it will be convenient to employ this term as including both pigment and chromogen) is undoubtedly the intestinal canal. This can only be gainsaid by denying the identity of the urinary and fæcal pigments. The quantity normally present in the fæces is far larger than that which enters the intestine with the bile, and, as we shall presently see, there is strong evidence that the urobilin in bile is itself of intestinal origin. This being so, it is clear that all theories other than the intestinal and its modifications merely attempt to trace a second source for the urobilin of the urine. It is equally clear that the substance from which the intestinal urobilin is formed is the bile-pigment. Under ordinary conditions the bile-pigment is destroyed in its passage along the intestine

and does not appear as such in the fæces. In its place we find large quantities of urobilin which in its turn disappears when occlusion of the common duct prevents the entrance of bile into the intestine. Again, when under certain morbid conditions the bile-pigment passes along the intestine unaltered urobilin is absent from the fæces. However, the conversion of bilirubin into urobilin is no mere process of reduction but involves a much more radical change with elimination of nitrogen.

That the change is brought about by bacterial action there is much evidence to show. When bile is inoculated with fæcal material and kept in an incubator a rapid formation of urobilin takes place and at the same time the bile-pigment diminishes and ultimately disappears. This has been repeatedly observed and the fact can be verified without difficulty. More striking results have been got by Friedrich Müller,¹⁴ Adolf Schmidt,¹⁵ and Esser¹⁶ who obtained urobilin by cultivating intestinal bacteria in broth to which an alkaline solution of bilirubin had been added. The best results were obtained under anaerobic conditions. The formation of urobilin began about the second day of incubation and reached its maximum before the seventh day. At a later period the pigment formed appeared to be again destroyed. Schmidt and Esser failed to obtain this result with pure cultures of various fæcal bacteria, but it was shown by control experiments that the urobilin found was not introduced with the material used for inoculation.

Dr. J. H. Drysdale and I are engaged upon a further study of this subject and we have found that the suitable conditions are not very readily reproduced. I need only state here that we have obtained results agreeing with those of other observers. In our anaerobic cultures the band has only appeared after the broth has been allowed to stand for some time exposed to the air and after the addition of an acid. This is a point of some importance, as one might be led to conclude from a hasty examination that no urobilin has been formed when it is present in a chromogen form in the culture medium which has been kept deprived of all oxygen. The product so formed has the chief properties of urobilin, but it has still to be proved that it is identical with the natural pigment. Esser who made some experiments with the unorganised ferments of the intestine failed to find that they had any power of converting bilirubin into urobilin.

Further evidence is afforded by the examination of the excreta of new-born infants. As a rule the meconium and solid excreta of the first day or two of life are urobilin free and sterile, but about the third day traces of urobilin are usually found and cultures yield a few bacterial colonies. Some observations of Dr. Drysdale and mine agree with those of previous observers on this point, but in one instance we found a considerable amount of urobilin in the meconium expelled immediately after the infant's birth. The mother, who had been ill for some time previously, died comatose on the second day after parturition and was found to have multiple abscesses of the kidneys. Cultures from the specimen of meconium remained sterile, and this exceptional observation can only be reconciled with the bacterial theory by supposing that the urobilin present was of maternal origin. Lastly, Dr. Vaughan Harley has found that the green stools passed after the administration of calomel are rich in bile-pigment and poor in urobilin and this he attributes to the antiseptic action of the drug.

It is in the large intestine, and especially in its upper regions, that urobilin is mainly formed. This has been shown by Schmidt, by Vaughan Harley,¹⁷ and by Macfadyen, Nencki, and Sieber.¹⁸ However, the range of the pigment in the intestine varies very widely in different individuals and it may even extend to the jejunum. Of two dogs deprived of their large intestine, upon which Vaughan Harley experimented, one produced considerable quantities of the pigment and the other very little; and it is noteworthy that the estimation of the aromatic sulphates of the urine showed that in the intestines of the dog with fæces rich in urobilin the bacterial processes were much more active than in those of the other animal.

A question of great interest from the theoretical standpoint,

⁵ Ibid., 1893, vol. xiv., p. 326.

⁶ Gazette Hebdomadaire, 1887, tome xxiv., pp. 520 and 534; Gazette des Hôpitaux, 1889, tome lxii., p. 1314.

⁷ Essai sur la Pathologie de la Sécrétion Billaire, Paris, 1889.

⁸ Virchow's Archiv, 1880, Band lxxix., p. 655.

⁹ Archivio Italiano di Clinica Medica, 1891, vol. xxx., p. 101. Lo Sperimentale, 1896, vol. i., p. 71.

¹⁰ Lo Sperimentale, 1895, vol. xlix., p. 89; ibid., 1896, vol. i., p. 81.

¹¹ Verhandlungen der Würzburger Phys. Med. Gesellschaft, 1886, Band iv., p. 23.

¹² Zeitschrift für klinische Medizin, 1897, Band xxxii., p. 303.

¹³ Virchow's Archiv, 1897, Band cl., p. 536; ibid., 1898, Band cliii., p. 156.

¹⁴ Schlesische Gesellschaft für Vaterland Kultur, January, 1892.

¹⁵ Verhandlungen des XIII. Congresses für Innere Medizin, Wiesbaden, 1895, p. 320.

¹⁶ Untersuchungen über die Entstehungsweise des Hydrobilirubins, &c., Dissertation, Bonn, 1896.

¹⁷ Brit. Med. Jour., 1896, vol. ii., p. 898.

¹⁸ Archiv für Experimentelle Pathologie und Pharmacologie, 1891, Band xxviii., p. 311.

but which has received singularly little attention, is whether urobilin is the only product formed from bilirubin in the intestine or is only one of several derivatives. If the former it is clear that, under normal conditions, the bacteria of the intestine are able to change the whole of the bile-pigment supplied to them into urobilin and increased formation can only have one cause—viz., an increased supply of bile-pigment. If, on the other hand, urobilin is only one of two or more products formed, the quantity of bile-pigment and the degree of bacterial activity may have an important influence upon the yield. The question is one to which it is not easy to give a decided answer. Undoubtedly the colour of the faeces affords little indication of the quantity of urobilin which they contain, for this pigment may be, and usually is, chiefly in the form of chromogen. Repeated extractions with alcohol, chloroform, and ether diminish their colour but slightly, whereas obstruction of the bile duct renders them almost colourless. This strongly suggests that urobilin is not the only coloured product formed from the bile-pigment, but when further extraction with acids or alkalies is resorted to it is difficult to be sure how far the colour of the extracts is due to pigments formed under the influence of the reagents employed. Culture experiments *in vitro* may perhaps throw important light upon this question.

The possible influence of the amount of bacterial activity in the intestine upon the quantity of urobilin formed has received little attention except from Dr. Vaughan Harley, who attaches considerable importance to it and whose observations on the relation of the excretion of aromatic sulphates to that of urobilin are of much interest.

The reaction of the intestinal contents probably plays an important part in this connexion. Esser found that acidity of the culture medium inhibited the change which took place under the influence of bacteria in alkaline broth. Macfadyen, Nencki, and Sieber have shown that the contents of the small intestine are acid and that an alkaline reaction is only acquired below the ileo-cæcal valve. These two observations show why it is that the colon is the chief seat of urobilin formation.

The green stools occasionally passed in typhoid fever are rich in biliverdin, to which they owe their colour, but they contain no urobilin. Although bile-pigment is abundantly available the urobilin-forming process is suspended, and it is a significant fact that such stools are always distinctly acid in reaction. Acid stools of a like consistence but of a yellow colour are sometimes passed in the same disease and these also are free from urobilin, and the fact that diarrhoea may persist unchecked after the stools have resumed their ordinary character excludes the effect of mere rapid transit of the intestinal contents.

The next question which calls for consideration is whether or no the urobilin of urine is derived from the large supply of the pigment in the intestine. Riva has shown by experiments upon animals that urobilin introduced into an isolated loop of intestine undergoes absorption, and Achard and Morfaux¹⁹ found that the chromogen diffuses through membranes more readily than the formed pigment; but the most convincing observations are those of Friedrich Müller²⁰ who showed, in the first place, that complete occlusion of the common bile duct causes urobilin to disappear from the faeces, and a few days later from the urine also. This observation has been repeatedly confirmed, and of its accuracy anyone can convince himself who examines a suitable case. It is true that the faeces usually retain a mere trace of urobilin, which has been ascribed, and probably correctly, to the entry of small quantities of bilirubin into the intestine through its walls. Examination of cases of biliary fistula with occluded duct, in which the jaundice has had time to disappear, would settle this point. I may mention that in the faeces of two infants with congenital atresia of the bile ducts I have failed to detect even the usual trace of urobilin. Müller further observed the remarkable fact that when pig's bile was introduced through a tube into the stomach of his patient not only did urobilin reappear in the faeces, but the urine also, after removal of the bile-pigment showed a pronounced urobilin band which remained visible for several days, only to disappear again after the faeces were once more urobilin free. Nature performs a similar experiment whenever a temporary obstruction of the bile duct is removed, and the urobilinuria

which is associated with the entrance of the pent-up bile into the intestine is a well-recognised phenomenon.

In typhoid fever urobilin and its chromogen disappear from the urine when green stools free from this pigment have been passed for a day or two.²¹ In one such case the urine, which I was watching from day to day, showed one morning an intense urobilin band after a period of complete absence and the stools of the same day were found to have regained the ordinary consistence and colour of typhoid motions, to be alkaline in reaction, and rich in urobilin. Beck²² has shown, and Vitali has confirmed the observation, that when the common bile duct of a dog is tied and a biliary fistula is formed the disappearance of urobilin from the faeces is followed by its disappearance from the bile also, but when the fistula bile is introduced into the stomach of the animal urobilin quickly reappears in the bile, and in this connexion it is interesting to note that I have failed to detect urobilin in the fistula bile of a patient with occluded bile duct whose faeces were practically urobilin free. Hence it would appear that the urobilin present in bile is also some of that which has been absorbed from the intestine and is not produced by the liver.

Such is the evidence upon which the intestinal theory of urobilinuria rests, and it seems to be clearly established that absorption from the intestine does take place and that of the urobilin so absorbed some is excreted with the bile and some in the urine. When, however, we attempt to apply this theory to the explanation of the clinical facts difficulties are encountered which cannot as yet be wholly met, and it soon becomes evident that the theory as above stated is inadequate and that some amplification or modification of it is necessary.

Observations bearing upon the ratio of urinary to faecal urobilin are not very numerous and the results obtained are somewhat conflicting. Friedrich Müller states that a parallelism is only observed in extreme cases. Gerhardt,²³ who employs a spectro-photometric method of estimation, obtained widely different ratios in different cases, varying as greatly as from 1:3 to 1:40. Vitali,²⁴ too, has noticed similar variations in different cases. Riva²⁵ and Zoja, on the other hand, have found that in individual cases the fluctuations of urinary and faecal urobilin are in the main parallel and that an increased amount in the faeces is attended by an increased excretion by the kidneys. They attribute the observed departures from this rule in part to the slow excretion of the absorbed pigment, which renders possible continued urobilinuria after the amount in the intestines has become conspicuously diminished, and in part to the dependence of the urinary excretion of urobilin upon the integrity of the kidneys. They rightly urge that isolated observations are of little value in this connexion and that a just notion can only be obtained by continuous observations over a considerable period.

Leaving aside the question of the relative value of the methods employed, other influences than those already referred to suggest themselves as possibly disturbing the ratio. Even if it be granted that the estimation of the amount of urobilin present in the solid excreta of a given period affords a true indication of the quantity of the pigment present in the intestinal canal—and it is by no means certain that it does so—its distribution must surely materially influence absorption. The researches of Schmidt and Vaughan Harley have shown how widely the distribution of the pigment varies in the intestines of different individuals. The consistence of the intestinal contents suggests itself as another disturbing factor, and one can imagine a disturbance of the ordinary relation between the amounts excreted in the bile and urine respectively.

Last, but by no means least, it is not certain that all the urobilin absorbed from the intestine is excreted as such. The very small amount present in normal urine and its complete absence from that of certain animals, such as dogs, in whose intestines it is present in considerable quantity, rather suggest that the pigment is in part subjected to change in its passage through the body and is excreted in some altered form.

In view of the conflict of evidence further observations bearing upon the ratio between urinary and faecal urobilin are needed, but it seems clear that in many instances

¹⁹ Comptes Rendus de la Société de Biologie, 1899, tome vi., p. 50.

²⁰ Loc. cit.

²¹ St. Bartholomew's Hospital Reports, 1897, vol. xxxiii., p. 13.

²² Wiener Klinische Wochenschrift, 1895, Band viii., p. 617.

²³ Zeitschrift für klinische Medizin, 1897, Band xxxii., p. 303.

²⁴ La Clinica Medica Italiana, 1899, vol. xxxviii., p. 674.

²⁵ Archivio Italiano di Clinica Medica, 1896, vol. xxxv., p. 367.

increased excretion in the urine is connected with increased production in the intestine, as evidenced by the presence of unusual quantities in the fæces,

The effect of constipation upon the excretion of indican, the parent substance of which, indol, is formed by bacterial action in the intestine, is so marked that it is natural to expect that it will have a similar effect upon the excretion of urobilin in urine, the stagnation of the contents of the colon allowing more time for absorption. George Hoppe-Seyler states that retention of the contents of the colon does increase the urobilin of urine, but that obstruction of the small intestine has no such effect. Dietrich Gerhardt, on the other hand, attaches little importance to constipation in this connexion and quotes cases of perityphlitis treated by opium in which when the temperature fell there was an enormous decrease of the urinary urobilin, although the intestines were still kept at rest. He also gives a series of observations upon a convalescent patient whose bowels were only moved every fourth or fifth day. Here, it is true, the maximum of urobilin in the urine was reached on the day following an action of the bowels, but if we admit (as I think we must, from what is seen when occlusion of the common bile duct occurs) that the absorbed urobilin is only slowly excreted, his figures point to a distinct increase of excretion under the influence of constipation, the full effect of the evacuation being seen only on the third day after its occurrence. Bargellini,²⁶ who made an elaborate series of observations bearing upon this point, arrived at the conclusion that in simple atony of the bowel the degree of constipation is without influence upon the amount of urinary urobilin, but that in cases of typhoid fever it causes an obvious increase, whereas disinfection or emptying of the lower bowel caused a noticeable decrease in the amount. If we study Bargellini's tables we find that a similar explanation may be given of many of his results. In several typhoid fever cases a continuous series of daily observations were made, whereas in none of the other cases were observations carried on to the second and third day after the evacuation of the bowel. Further observations upon this point, bearing in mind the slowness of the excretion of the absorbed urobilin, would be of much interest.

The most serious objection which has been raised to the intestinal theory is based upon the connexion which undoubtedly exists between urobilinuria and liver diseases. It is by no means the case that marked excess of urinary urobilin is always associated with obvious hepatic derangements, but there can be no question that conspicuous urobilinuria usually accompanies such diseases as cirrhosis and carcinoma hepatis. This fact, coupled with the presence of urobilin in the bile, formed the basis of the hepatic theory of Hayem and Tessier. However, the theory that the liver itself, and especially the disordered liver, manufactures urobilin, whilst it will explain the association under discussion entirely fails to explain the disappearance of this pigment from the bile and urine alike when the bile duct is occluded and its reappearance in both when bile is introduced into the stomach.

There are two different ways in which the influence of the liver may be reconciled with the intestinal origin of the urobilin of urine. We may suppose either that the condition of the liver exerts its influence at the beginning of the cycle by affecting the quantity and quality of the bile entering the intestine, and so indirectly the amount of urobilin formed, or that the liver lays hold upon the urobilin after its absorption and excretes it in part in the bile and in part converts it into some other substance. There can be little doubt that the state of the liver exerts an influence upon the quantity of bile-pigment formed and Riva and Vitali have shown that in phosphorus poisoning and acute yellow atrophy respectively the destruction of the liver cells practically puts an end to the formation of bile-pigment and that as a consequence urobilin may disappear from the fæces and urine. We should, indeed, expect that such quantitative influences would tell in the direction of diminution rather than increase. On the other hand, the hepato-intestinal theory of Riva,²⁷ which he has worked out with the aid of L. Zoja²⁸ and other members of

the school of Parma, attributes to the condition of the liver an important influence upon the quality of the bile-pigment formed. The differences of quality are evidenced by the colour of the bile, of which the reddish-brown variety yields urobilin much more readily than the greenish. According to Zoja²⁹ the essential difference lies in the relative amounts of bilirubin and biliverdin present. These authors, together with Cavalli³⁰ and Chiodera,³¹ have clearly shown that different biles exhibit marked differences in respect to the ease with which their contained pigment is converted into urobilin and that the bile of different animals differs widely in this respect, the green bile of vegetable feeders yielding urobilin less readily than that of carnivora. Cavalli also found that the bile obtained from the bodies of human patients agreed in its characters with what might be inferred from the excretion of urobilin during life. It is evident that this theory involves the formation from bile-pigment of other products besides urobilin, for if under normal conditions it is all converted into urobilin in the intestine increased convertibility can obviously lead to no increase of the yield. It also supposes a direct dependence of the quantity of urobilin in the urine upon that in the intestine, unless, as Riva himself suggests, the absorbed pigment be not all excreted unchanged. Granting that, as is most probable, urobilin is not the only product formed from the bile-pigment it seems clear that the quality of the bile must have a material effect upon the quantity of urobilin produced in the intestine, and the question is rather whether the qualitative changes in the bile formed by disordered livers are so great as this theory demands—in a word, whether it is capable of explaining all the facts.

An alternative theory, originally suggested by Murri and independently by Beck, has been elaborated by Vitali,³² a pupil of the former. Vitali maintains that the hepatic cells have a strong affinity for urobilin, which they pick out from the blood, after it has been absorbed from the intestine, and re-convert into bilirubin. He further supposes that these cells have a still greater affinity for hæmoglobin and bilirubin, and that if they are called upon to deal with these latter pigments the urobilin brought to them may escape conversion. Thus when excessive hæmolysis is going on the liver cells may be so occupied that the urobilin passes them unchanged, or more urobilin may be brought to them than they are able to cope with, whereas disease of the liver may be supposed to lead to the same result by impairing the power of the cells to deal with the pigment. Morfaux,³³ who was evidently unacquainted with Vitali's writings, propounded a similar theory in his thesis, but offered no suggestion as to the nature of the change which the pigment undergoes in the liver. It may be pointed out that this theory includes two distinct hypotheses, and even if we doubt the re-conversion of urobilin into bilirubin we may still accept the first proposition that the liver cells are capable of laying hold upon the urobilin in the circulation. That they do so is indeed shown by the presence of urobilin in the bile whenever this pigment is being formed in the intestine. Some at least of the urobilin waylaid by the liver is excreted unchanged by that organ. If urobilin were merely a reduction product of bilirubin its re-conversion into that substance would be comparatively simple, but no one has yet obtained bilirubin by the oxidation of urobilin, and, as has been pointed out, the change involved is of a much more radical nature. To this objection Vitali replies by dismissing the urobilin analyses made by Hopkins and myself as proving nothing, because the material analysed was in his opinion obviously contaminated by an organic iron compound, since some of the products yielded a trifling ash in which iron could be detected. In so doing he ignores the close agreement of the results obtained from both fæces and urine by three different processes and also the fact that the figures obtained from the products with ash agreed with those got from wholly ash-free specimens.

He made some interesting experiments upon dogs and obtained evidence of increased formation of bile-pigment during the circulation of blood containing urobilin. The bile formed appeared more highly coloured and caused more absorption of the violet end of the spectrum, but

²⁶ *Lo Sperimentale*, 1892, vol. xlvii., p. 119.

²⁷ *Le Scuole Italiane di Clinica Medica*, Milan, 1894; *Lo Sperimentale*, 1896; *Gazzetta Medica di Torino*, 1896, vol. xlvii., No. 12.

²⁸ *Archivio Italiano di Clinica Medica*, 1893; *Conferenze Cliniche Italiane*, vol. i., p. 261; *La Clinica Medica Italiana*, 1893, vol. xxxvii., p. 535.

²⁹ *Bollettino della Società Medico-Chirurgica di Pavia*, 1897.

³⁰ *Archivio Italiano di Clinica Medica*, 1896, vol. xxxv., p. 394.

³¹ *Gazzetta Medica di Torino*, 1896, vol. xlvii., No. 39.

³² *Il Morgagni*, 1897, vol. xxxix., p. 225; *La Clinica Medica Italiana*, 1899, vol. xxxviii., p. 674.

³³ *Recherches sur l'Urobiline*, Paris, 1899.

the quantity of bile was not increased. No such results were obtained when a saline solution containing urobilin was substituted for blood. The obscuration of the violet end of the spectrum, upon which Vitali chiefly relied as evidence of an increased formation of bile-pigment, was shown not to be due to an excess of urobilin as such. Moreover, he could obtain no evidence that the presence of urobilin caused hæmolytic or had a cholagogue effect. It is, however, clear that more complete proof is required before so remarkable a phenomenon as the re-conversion of urobilin into bilirubin in the liver can be accepted as an established fact. Riva³⁴ objects to this theory that in cases of cirrhosis and carcinoma hepatis the intestinal as well as the urinary urobilin is conspicuously increased, a fact of which the theory takes no account. To this Vitali replies that the increase of intestinal urobilin in such cases is not constant, and when present may be ascribed to excessive hæmolytic and increased excretion of urobilin in the bile.

These two hepato-intestinal theories embody the only attempts yet made to explain what is a very real difficulty in connexion with the intestinal theory. It is, of course, possible that both are to some extent true, and that the influence of the liver is felt both at the beginning and the end of the cycle. As has been mentioned, Riva himself thinks it probable that some of the absorbed urobilin is changed in its passage through the body, and suggests that it may be converted into urochrome. This suggestion will be again referred to when we come to speak of that pigment.

One more objection must be mentioned. Urobilinuria is not infrequently observed when the bile duct is partly obstructed and the quantity of bile entering the intestine is diminishing. In considering such cases the possibility of antecedent urobilinuria and slow excretion must be considered. However, Vitali records a case in which this could be excluded and explains the facts by supposing that the liver cells are occupied with the absorbed bilirubin, and that consequently the urobilin which is still being formed in the intestine so escapes conversion. Vaughan Harley, on the other hand, suggests that the partial removal of the antiseptic influence of the bile encourages bacterial activity, and that in spite of a diminished supply of bile-pigment the yield of urobilin is increased. An investigation of the fæces in such cases would supply important evidence upon this point.

There remains to be considered the question whether there are sources of urobilin in the body other than the intestinal. Its disappearance from the urine when the bile duct is occluded shows that no such second process is usually at work and disposes of the theory that urobilin is formed from bilirubin in the tissues at large, for under such circumstances although the tissues remain loaded with bilirubin the excretion of urobilin is arrested. There are, however, some, and notably Gerhardt, who consider that a direct formation of urobilin from hæmoglobin cannot be excluded, although the urobilinuria which accompanies hæmolytic and the absorption of extravasated blood may be explained by the increased formation of bile-pigment which is known to take place under such circumstances. If it can be shown that in some cases of occlusion of the common bile-duct of sufficient standing to allow of the excretion of all absorbed urobilin urobilinuria is still observed, there is no alternative but to admit the existence of a second source. I have never met with such a case, and the only recorded instance of the kind with which I am acquainted is one referred to by Gerhardt of a patient the complete occlusion of whose bile-duct by carcinoma was verified post mortem, who developed hæmorrhagic ascites, and whose urine showed a pronounced urobilin band. It is not stated how long the intestinal contents had been practically urobilin-free at the time of observation. Beck injected blood under the skin of a dog with ligatured common duct and a biliary fistula and found a little urobilin in the bile, very little in the urine, and none in the fæces. Hence he concluded that the possibility of hæmatogenous urobilinuria could not be dismissed offhand, and it is evident that further observations are necessary before the question can be regarded as definitely settled.

Lastly, it is necessary to say something of the clinical aspects of urobilinuria. Increased excretion of the pigment, evidenced by a dark absorption band seen when the urine is examined with the spectroscope, is a very common phenomenon in disease, and we may distinguish between cases in which the band is seen for only a few days in

succession and those in which it is present over a long period. A distinction may also be drawn between cases of what may be called "pure urobilinuria" in which a markedly increased urobilin excretion is the only obvious pigmentary abnormality and those in which uroerythrin and hæmatoporphyrin are also present in excess. In febrile disorders of almost every kind temporary urobilinuria may be met with, the duration of which usually corresponds with that of the pyrexia. In diseases of the liver the urobilinuria is usually persistent, as is well seen in cases of cirrhosis, malignant disease, or passive congestion secondary to cardiac or pulmonary troubles. In such cases the urobilin band is apt to be masked to some extent by a general absorption of the violet end of the spectrum due to other pigments. In diseases attended by excessive hæmolytic, and during the absorption of extravasated blood, there is apt to be conspicuous urobilinuria, and unless complications are present there is no corresponding increase of uroerythrin or hæmatoporphyrin. Such urines have a warm orange colour, which is readily recognised by a trained eye, and at the apex of a conical glass a pinkish tinge is usually seen. The occurrence of persistent urobilinuria in pernicious anæmia was first described by Dr. F. W. Mott³⁵ and Dr. William Hunter,³⁶ and it supplies a diagnostic sign of real value and affords an indication of the progress of the case. In association with it I have observed a marked excess of urobilin in the fæces. When blood extravasations are being absorbed a temporary urobilinuria is apt to occur in a day or two after the occurrence of the hæmorrhage, and this again may prove of service in the diagnosis of deep-seated hæmorrhages, such as pelvic hæmatocœles. Diminished excretion or absence of urobilin from the urine may be due to diminished formation of bile-pigment, as in chlorosis, phosphorus poisoning, or acute yellow atrophy of the liver; to suspension of urobilin formation in the intestine as in typhoid fever with green stools; as well as to occlusion of the common bile duct. Viglezio³⁷ suggested that renal permeability has an important influence upon the excretion of this pigment, and it is a clinical fact that albuminuria and urobilinuria very seldom co-exist. There is, moreover, experimental evidence in support of this view. As might be expected all diseased kidneys do not hold back urobilin to the same extent, and Zoja thinks that a parallelism may be traced between its excretion and that of urea. Morfaux,³⁸ who, with Achard, has recently worked at this subject, states that a kidney which is impermeable to methylene blue is impermeable to urobilin also, and that the more diffusible chromogen may pass through a kidney which does not allow the passage of the formed pigment.

HÆMATOPORPHYRIN

When we pass to the consideration of urinary hæmatoporphyrin and of the pathological significance of its presence in excess we enter upon comparatively little-trodden ground. Hæmatoporphyrin has been found by MacMunn in the integuments of certain invertebrata, some of which have no hæmoglobin in their blood. Krukenberg and Sorby have shown that it is one of the colouring matters of the shells of birds' eggs, and Tappeiner found it in the bones of certain diseased swine. Pigments very closely resembling it have been obtained by the action of reagents upon substances other than hæmoglobin and hæmatin. Such are the turacoporphyrin obtained by Church from the copper-containing pigment turacin, and phylloporphyrin prepared by Schunck and Marchlewski from chlorophyll. In normal human urine hæmatoporphyrin is present in minimal quantity, as can readily be demonstrated by appropriate methods. In many morbid urines it is found in larger but still small amounts, and under exceptional circumstances, and especially as a result of the administration of sulphonal, it is much more abundantly present in urines which have a deep port wine tint. However, such urines owe but little of their peculiar colour to this pigment. In the fæces it is also present, as Stokvis³⁹ has recently pointed out, and its presence may be demonstrated by a method employed by Sallet⁴⁰ for its detection in urine, the basis of which is extraction with acetic ether after the addition of acetic acid. I have recently made a number of examinations of the solid excreta

³⁵ THE LANCET, 1889, vol. i., p. 520.

³⁶ Practitioner, 1889, vol. xliii., p. 161.

³⁷ Lo Sperimentale, 1891, vol. xlv., p. 225.

³⁸ Loc. cit.; Comptes Rendus de la Société de Biologie, 1899, dixième série, tome vi., p. 50.

³⁹ Nederl. Natuur-en Geneeskundig Congres, 1899, p. 378.

⁴⁰ Revue de Médecine, 1896, tome xvi., p. 542.

by this method and have always invariably found traces of hæmatoporphyrin, comparable with those met with in urine. I have also found that considerably larger amounts of this pigment may be extracted from meconium by the same method, both from that expelled during the first day or two of life and from that removed from the intestines of still-born infants. Stokvis also obtained hæmatoporphyrin from bile. Here its detection is rather less easy and the results which I have obtained with this material have been much less constant. Lastly, in a case of sulphonal poisoning A. E. Taylor and J. Sailer⁴¹ have recently recovered it from blood collected post mortem.

Hæmatoporphyrin yields to no other pigment in the distinctive characters of its spectra, and at least four characteristic groupings of absorption bands serve for its identification under different conditions. However, the amount present in urine is usually so small that the bands are quite invisible on direct spectroscopic examination or are so faint that they can only be recognised by a trained eye. The natural product so closely resembles the artificial product, and especially that obtained by Nencki and Sieber's⁴² process, both in its spectroscopic and other properties, that their identity is hardly open to doubt. Certain properties regarded by Sallet as peculiar to the urinary pigment can be shown to be shared by the artificial product, and further evidence of identity is furnished by the nitrogen determinations carried out by Nebelthau⁴³ on the urinary hæmatoporphyrin from a remarkable case which he observed, which gave results according perfectly with the requirements of Nencki and Sieber's formula. We are, therefore, justified in concluding that the hæmatoporphyrin of the body has hæmoglobin for its parent substance and is isomeric with bilirubin.

Nencki and Sieber suggested that hæmatoporphyrin might represent a stage in the building up of hæmoglobin, basing this suggestion on the fact that when the pigment was injected beneath the skin of dogs the amount excreted in the urine represented only a small part of that which was absorbed from the seat of injection. However, Otto Neubauer has recently offered another explanation of this, and has shown that much of the pigment absorbed is excreted in the bile. Assuming, then, that the hæmatoporphyrin of the body is a product of the downward metabolism of hæmoglobin, and possibly of the histohæmatins of MacMunn, the next question to be considered is whether it is derived from the human blood-pigment or from that introduced in the food. Stokvis⁴⁴ holds that part of the hæmatoporphyrin of urine is derived from the food and that even chlorophyll, from which, as has been mentioned, a very closely allied, if not identical, pigment can be obtained, may contribute to its formation. He does not doubt, however, that some is formed from the human tissues. His pupil, Keyser,⁴⁵ found that a change of diet from red meat to white meat without vegetables caused the disappearance of the normal trace from the urine, but that it returned when green vegetables were added to the food. However, he only gives a single set of observations extending over a few days, and marked fluctuations of the normal excretion are not uncommon apart from changes of diet. I have myself been unable to notice any appreciable effect of changes from a milk diet to a similar diet with spinach, and afterwards to red meat. However this may be, it is certain that the bulk of the urinary and fæcal hæmatoporphyrin has no such origin. Patients on milk diet excrete hæmatoporphyrin both in urine and fæces, and I may quote the single instance of a boy who was in good health save for a cicatricial stricture of the œsophagus of a year's standing. When on a diet of pure milk this child regularly passed traces of the pigment, and it may also be found in the fæces of suckling infants. Perhaps the most important evidence is afforded by the presence of hæmatoporphyrin in considerable quantity in the ante-natal meconium. On the other hand there is no obviously increased excretion in the urine in cases of gastric hæmorrhage with melæna, unless some other cause be present, such as cirrhosis of the liver.

Seeing, then, that at least the greater part of the hæmatoporphyrin of the body is derived from human sources, it is a

natural supposition that its excretion in excess is an indication of increased hæmolysis. There are two ways in which this proposition may be put to the test—viz., by examining the blood in cases in which unusual amounts of the pigment are being excreted and by examining the urine in cases in which active hæmolysis is known to be in progress. Both methods return the same answer—viz., that there is no necessary connexion between excess of hæmatoporphyrin in the urine and excessive hæmolysis. It is only in extreme cases, such as those due to sulphonal, that the quantity of the pigment excreted is sufficiently large to promise any cogent evidence by the first method, and although excessive hæmolysis and hæmatoporphyrinuria may naturally coexist, such evidence of undue blood-destruction as the hæmometer affords is usually wanting in these cases. In the case of a female patient observed by Dr. L. R. Oswald⁴⁶ three blood examinations during its progress gave figures varying between 3,520,000 and 3,150,000 red corpuscles. Dr. Hotchkiss has kindly sent me the following counts obtained in the course of a case of hæmatoporphyrinuria also due to sulphonal which he has recorded.⁴⁷ Red corpuscles: Jan. 5th, 3,600,000; Jan. 6th, 4,044,000; and Jan. 12th, 3,961,000. Dr. J. Purves Stewart also was good enough to make some blood examinations for me in a similar case in the National Hospital for the Paralysed and Epileptic with the following results. April 13th: red corpuscles, 4,560,000; hæmoglobin, 70 per cent. April 17th: red corpuscles, 4,360,000; hæmoglobin, 72 per cent. April 21st: red corpuscles, 4,500,000; hæmoglobin, 72 per cent. April 26th: red corpuscles, 4,560,000; hæmoglobin, 70 per cent. The force of such negative results is not impaired by the occasional coexistence of conspicuous anæmia with urinary change. Mr. F. G. Hopkins⁴⁸ has shown, and I have repeatedly confirmed the observation, that in pernicious anæmia, a disease in which hæmolysis is an essential feature, the urinary hæmatoporphyrin is not increased unless complicating conditions are present. In such cases, as we have seen, it is urobilin which appears in abnormal amounts in the urine. Thus one is driven to the conclusion that hæmatoporphyrin is either an intermediate product of metabolism, which normally to some extent, and in many morbid conditions to a larger extent, escapes further change and is excreted as such, or else that it is a bye-product of metabolism which may be formed in undue amount in the place of some other hæmoglobin derivative such as its isomer bilirubin. Whatever be the nature of the process involved it is one which is constantly active to some extent in health and even in intra-uterine life.

Although, like urobilin, hæmatoporphyrin is found alike in the urine, the fæces, and the bile, the quantitative distribution of the two pigments is altogether different. The quantity of fæcal hæmatoporphyrin is quite minute and some of it, at any rate, enters the intestine with the bile. As far as my own observations go there appears to be a parallelism between the quantities in the urine and fæces respectively, increased excretion in the urine being accompanied by an increase in the solid excreta, and *vice versa*. However, when the common bile-duct is occluded and urobilin disappears from the intestine some hæmatoporphyrin remains, which shows that the bile is not the only source of supply. That the remaining pigment is not derived from food is shown by its persistence upon a diet wholly free from meat and green vegetables. I even found a minute trace of the pigment in the collected solid excreta of several days of an infant, aged four months, with congenital atresia of the bile ducts (afterwards verified post mortem), and who had never had any other diet but milk. One is therefore driven to conclude that some of the intestinal hæmatoporphyrin makes its way into the intestine through its walls. There is clearly no reason to suppose that this pigment has its place of origin in the intestinal canal.

It seems probable, *a priori*, that the liver, which is the seat of the manufacture of bilirubin, is also the organ in which hæmatoporphyrin is formed. Riva and Zoja's⁴⁹ clinical observations led them to the opinion that hepatic disorders, functional and organic, are mainly responsible for the appearance of increased amounts of this colouring matter in the urine. Keyser, too, found it to be specially abundant in cases of hepatic disease, and my own observations, extending over a

⁴¹ Contributions from the William Pepper Laboratory, Philadelphia, 1900, p. 120.

⁴² Archiv für experimentelle Pathologie und Pharmacologie, 1888, Band xxiv., p. 430.

⁴³ Zeitschrift für physiologische Chemie, 1899, Band xxvii., p. 324.

⁴⁴ Loc. cit.; Zeitschrift für klinische Medizin, 1895, Band xxviii., p. 1.

⁴⁵ Ueber Hæmatoporphyrin im Harn, Dissertation, Freiburg, 1897.

⁴⁶ Glasgow Medical Journal, 1895, vol. xliii., p. 1.

⁴⁷ Brit. Med. Jour., 1898, vol. ii., p. 685.

⁴⁸ Guy's Hospital Reports, 1893, vol. i., p. 349.

⁴⁹ Gazzetta Medica di Torino, 1892, vol. xliii., p. 423; Archivio Italiano di Clinica Medica, 1893, vol. xxxii., p. 63.

number of years, have led me to exactly the same conclusion. In this connexion the impressions gleaned from the examination of large numbers of cases are really of more value than statistics, for statistics necessarily fail to take into account the contributory factors in individual cases. For example, in valvular heart diseases the amount of hæmatoporphyrin in the urine is often conspicuously increased, but in such cases the increase is closely connected with the amount of passive congestion of the liver, as evidenced by enlargement and tenderness of that organ. In phthisis, too, the marked increase not uncommonly met with has appeared to me to stand in direct relation to fatty degeneration of the liver, either evidenced by enlargement made out during life or seen at post-mortem examinations. Again, fatty degeneration of the liver and occasional cirrhotic change are mentioned by Dr. Thomas Oliver among the more constant effects of lead poisoning, a condition in which, as Stokvis has pointed out, increase of urinary hæmatoporphyrin is well-nigh constant. Nor can the influence of hepatic disorders be excluded in some diseases in which they play no recognised part. In gout, for example, there is usually an excess of urinary hæmatoporphyrin, and on referring to the post-mortem records of St. Bartholomew's Hospital I found that in a series of 50 cases in which uratic deposits were present in the toe-joints some naked-eye change in the liver was noted in no less than 39. In 15 cases the liver was described as fatty, in four as both fatty and cirrhotic, and in six others as merely cirrhotic.

In febrile disorders some evidence of hepatic change, such as "cloudy swelling," is sufficiently common, and in acute rheumatism, the disease in which hæmatoporphyrin in the urine was first detected by MacMunn⁵⁰ and in which an increase is very generally observed, it will, I think, be found that hepatic changes, evident on microscopic examination, are fairly constant. Dr. F. J. Poynton has found such changes in some livers which he has kindly examined for me. Once more, in a case of spleno-medullary leucocythæmia in which hæmatoporphyrin bands were visible, on direct spectroscopic examination of the urine, over a long period the liver showed general leukæmic infiltration to a conspicuous degree.

We are not yet in a position to assert that the liver is disordered or diseased in all cases in which hæmatoporphyrin is present in excess in the urine, but the evidence, as a whole, is strongly in favour of the view that its disorders play the chief part in bringing about this result.

That the spleen takes any important share in the process is excluded by the fact that removal of that organ has no obvious effect, either immediate or remote, upon the normal minimal excretion of the pigment. By the kindness of Mr. J. Bland-Sutton and Mr. D'Arcy Power I have had opportunities of examining the urine in three cases of splenectomy, both within a short time of the operation and in one case at long intervals after it. In all three cases the ordinary trace of hæmatoporphyrin was present without noteworthy increase or diminution. It may also be noted that diseases of the spleen are not accompanied by any marked disturbance of the excretion unless the liver is also implicated, as in the case of leucocythæmia referred to above.

The cases of hæmatoporphyrinuria due to sulphonal and those much rarer ones in which similar dark red urines are passed apart from the taking of this drug or its allies call for special consideration. In them we are confronted with something more than a mere exaggerated excretion of hæmatoporphyrin, and, as Hammarsten⁵¹ first pointed out, the peculiar colour of such urines is almost wholly due to other abnormal pigments as yet not studied. The removal of the hæmatoporphyrin from such urines causes little or no reduction of colour and when this pigment is added to normal urine until bands of similar intensity are seen the change of tint produced is comparatively slight. In one such case, not due to sulphonal, I was able to isolate a purple pigment which differed in its properties from any known urinary colouring matter and to which the colour of the urine in question was obviously in the main due. There is, indeed, in these cases a profound disturbance of pigment metabolism and even the more familiar pigments present in such urines are apt to exhibit peculiarities of behaviour. It is worthy of note that in sulphonal cases fatty degeneration of the liver has been repeatedly observed, and Taylor and Sailer state that in their case the most conspicuous anatomical changes were found in the liver, which showed

widespread degeneration of the hepatic cells, the nuclei of which exhibited advanced degenerative changes and their protoplasm refused acid stains. Glycogen was reduced to a trace and small foci of necrosis were scattered through the tissue. Stokvis was able to produce a degree of hæmatoporphyrinuria in rabbits by the administration of sulphonal, and this observation has recently been confirmed by Otto Neubauer⁵² who also found the pigment in the bile of the animals. He was unable to detect it in the blood or any organ except the liver, in which it was constantly present, but he suggests that its presence there may be due to an excretory action of the gland and not necessarily to its formation *in situ*. On the other hand, it is difficult to ascribe all the phenomena of such hæmatoporphyrinuria to hepatic changes, seeing that changes of like degree may occur without any conspicuous urinary abnormality, and there is still much that is mysterious in this condition. Stokvis was inclined to ascribe the increased excretion in lead poisoning and the far greater increase in the sulphonal cases to hæmorrhages into the mucous membrane of the alimentary canal, the conversion of the blood-pigment into hæmatoporphyrin being favoured by sulphonal. In rabbits poisoned with the drug he found hæmorrhagic areas in the stomach walls which showed the spectrum of acid hæmatoporphyrin by transmitted light. He also found that when fresh blood was digested with pepsin, hydrochloric acid, and sulphonal at from 38° to 40° C. hæmatoporphyrin was formed in small amount, but Kast and Weiss⁵³ and Otto Neubauer have failed to obtain this result. Intestinal hæmorrhages have certainly been described in some sulphonal cases, but in others they have been looked for in vain, and very extensive extravasations would be necessary to produce the observed excretion.

In the cases in which dark red urine is passed by patients who have not taken sulphonal the significance of the symptom is apparently not so grave and pathological data are wanting. In no two of the few recorded instances have the morbid conditions present been similar, the patients having suffered from such diverse maladies as phthisis, exophthalmic goitre, typhoid fever, and hydroa æstivalis. Perhaps the most remarkable case is that of Nebelthau, in which a female patient the subject of congenital syphilis had passed dark red urine as long as she could remember and continued to do so whilst under observation. No similar instance of such persistence of hæmatoporphyrinuria has hitherto been placed on record.

UROCHROME.

Of the remaining members of the group, urochrome and uroerythrin, our knowledge is as yet too imperfect to allow of the framing of definite theories of their modes of origin or of the conditions which control their excretion. As has already been mentioned, Dr. Thudichum,⁵⁴ who for a long time stood almost alone in maintaining the existence of urochrome as a definite chemical individual, holds that this, the most abundant of the urinary pigments, has no chemical relationship with either hæmoglobin or bilirubin. Urobilin, too, he regards as merely a decomposition product of urochrome, identifying it with omicholic acid, with his analyses of which substance our analyses of urobilin certainly show a very close agreement. The relationship of urobilin to the pigments of the blood and bile appears to me to admit of no doubt, nor can I accept the view that the urobilin of urine is formed by the decomposition of urochrome. Granting that it is possible to obtain from urochrome a product having the main properties of urobilin, this fact appears to me rather to afford evidence that to urochrome itself a place must also be assigned among the derivatives of hæmoglobin.

By acting upon urochrome with acids, in strict accordance with Dr. Thudichum's directions, I have not succeeded in obtaining any product showing the urobilin band or yielding the well-known fluorescence with zinc chloride and ammonia; but a substance having both these properties is readily obtained by the action of aldehyde upon an alcoholic solution of this pigment.⁵⁵ In a short time—shorter still when the liquid is warmed—an absorption band appears like that of urobilin, and the tint of the solution deepens to a

⁵² Archiv für experimentelle Pathologie und Pharmacologie, 1900, Band xliii., p. 455.

⁵³ Berliner klinische Wochenschrift, 1896, Band xxxiii., p. 621.

⁵⁴ Brit. Med. Jour., 1864, vol. ii., p. 509; Virchow's Archiv, 1897 Band cl., p. 586, and 1898, Band cliv., p. 154.

⁵⁵ Journal of Physiology, 1897, vol. xxi., p. 190.

⁵⁰ Proceedings of the Royal Society, 1880, vol. xxxi., p. 211; Journal of Physiology, 1889, vol. x., p. 71.

⁵¹ Skandinavisches Archiv für Physiologie, 1891, Band iii., p. 319.

rich orange-yellow. With zinc chloride and ammonia a brilliant green fluorescence appears, and the band is shifted towards red, as that of urobilin is under like conditions. The process can be stopped at this point by the simple addition of water, for aldehyde has no such action upon aqueous solutions of urochrome. If, however, the action be allowed to continue a further change ensues; the liquid reddens and a second band appears in the violet. The fluorescence can still be obtained with zinc chloride and ammonia, and both bands are shifted towards red and are closer together than before. This reaction with aldehyde affords a very delicate test for the presence of urochrome in alcoholic solutions. The product of the earlier stage although it is not identical with urobilin, resembles that pigment quite as closely as the products obtained from bilirubin and hæmatin by the action of reducing agents; but no second band is developed when aldehyde is added to an alcoholic solution of urobilin. On the other hand, by the action of potassium permanganate upon urobilin Riva and Chiodera⁵⁶ obtained a substance closely resembling urochrome, and a similar product is formed when an aqueous solution of urobilin containing ether is evaporated upon a water bath. Neither product shows any absorption band and both behave as urochrome does when it is acted upon by aldehyde. Riva and Chiodera also detected the presence of urochrome in fæces and in serous effusions, and its presence in both may readily be demonstrated. I have found that the most satisfactory method for its detection in fæces is to extract with a saturated solution of ammonium sulphate, which takes up neither urobilin nor its chromogen. From the yellow extract so obtained the urochrome can be separated with alcohol in the ordinary way,⁵⁷ and precipitated from alcoholic solution by ether. Its identity can be established by means of the aldehyde reaction. As judged by the colour the quantity obtained is small and there is no relative excess in the intestine, as in the case of urobilin.

When ascitic fluid is saturated with ammonium sulphate the serum pigment is all thrown down with the last of the proteid precipitate, and from the almost colourless filtrate alcohol extracts a minute amount of a yellow pigment, which yields the aldehyde reaction of urochrome. Riva's⁵⁸ suggestion that some of the urobilin absorbed from the intestine may be converted into urochrome in the tissues is in part based upon the scanty pigmentation of the urine of young infants and in part upon the almost complete decolourisation of the urine of a patient with obstructed bile duct, when the bile-pigment was removed by means of baryta mixture. The case or cases which he observed were certainly exceptional in this respect, for in the urine of several patients with occlusion of the bile duct which I have examined I have never failed to find abundance of urochrome present after similar treatment. Vitali has had the same experience, and it is a well-known fact that in cases of biliary fistula, with occluded duct, the urine is not decolourised even after it ceases to contain bile-pigment. Moreover, urochrome is present in the urobilin-free fæces and even in those of infants with congenital atresia of the ducts. One is, therefore, driven to conclude that the formation of urochrome is quite independent of that of urobilin, and although it is probably a derivative of hæmoglobin there is as yet no evidence to show how or where it is formed. It may be mentioned as a negative point that the excretion of urochrome is not obviously affected by removal of the spleen.

Clinical evidence bearing upon this question is at present wholly wanting, and although there can be little doubt that the excretion of urochrome is subject to wide fluctuations apart from the apparent variations due to concentration or dilution of the urine, we know nothing of the conditions under which they occur. The pigment has no characteristic spectrum and the want of a satisfactory method for its estimation makes it difficult to form even a rough notion of the quantity present in morbid urines, in which its colour is so often masked by that due to other pigments.

UROERYTHRIN.

Of the mode of origin and the relationships of uroerythrin we know even less notwithstanding that its power of imparting a pink colour to urate sediments renders its detection extremely easy and caused it to be the first of all the urinary pigments to attract individual attention. Uroerythrin is a potent colouring matter, far excelling urochrome and urobilin

in this respect, so that the presence of even a small amount has a conspicuous effect upon the tint of the urine in which it is contained. The peculiar reddish-orange colour of the pigment in solution contrasts strongly with that of the pink urate sediments, in which it probably exists in combination, for they show a characteristic spectrum of their own.⁵⁹ In its properties uroerythrin differs widely from the pigments which we have hitherto been considering. The most conspicuous of these is its extreme instability, and compared with it urobilin and hæmatoporphyrin are quite stable compounds. Its solutions in alcohol or chloroform are very rapidly decolourised by light, and even when kept in the dark quickly undergo change. Alkalies destroy the pigment readily, with the production of a green tint. Neutralisation of the alkali does not restore the original colour or bring back the absorption spectrum, which is characteristic though ill-defined, consisting of two feeble bands in green and blue united by a feebler shading. One of these bands has the position of the urobilin band, but both alike disappear when the solutions are decolourised by light. These properties suggest that uroerythrin is a pigment of a different order from those which we have hitherto considered and that it is probably not a member of the family of hæmoglobin derivatives. There is, however, little to guide us in this matter and C. E. Simon⁶⁰ quotes an estimation of the carbon and hydrogen in uroerythrin which is suggestive of a relationship to bilirubin, but he gives no particulars. Hitherto this pigment has only been found in urine and there is no evidence that it is excreted in the form of a chromogen. On the other hand, it may fairly be doubted whether a substance so readily destroyed by alkalies can exist as such in the alkaline body fluids. I have sought for it in the fæces of patients in whose urine it was abundantly present by several methods, such as extraction with amylic alcohol and acetic ether, both excellent solvents of uroerythrin; by shaking water extracts, acidified with acetic acid, with amylic alcohol, and by precipitation of crystalline uric acid in watery extracts. All of these methods have failed to reveal its presence and although some method as yet untried may reveal traces it can hardly be present in any considerable amount in the solid excreta.

Although uroerythrin is probably not a strictly normal constituent of urine it may appear in connexion with most trifling deviations from the standard of health. Heller⁶¹ regarded it as the commonest of all abnormal ingredients of urine, and uratic sediments are not often entirely free from a pink tint which may, however, only be visible after their removal by filtration. Such knowledge as we possess of its pathological significance is entirely derived from clinical observation and points to disturbances of the hepatic functions as the prime causes of the increased excretion of uroerythrin. Urines which throw down intensely pink uratic sediments or have the fiery orange colour indicative of much uroerythrin in solution are most frequently passed by patients with definite hepatic diseases such as cirrhosis, carcinoma, or the passive congestion resulting from cardiac lesions. It is true that this pigment is often excreted in large amount in certain febrile disorders, such as acute rheumatism and pneumonia and also in gout, and when associated with an undue excretion of hæmatoporphyrin it affords additional evidence of the implication of the liver. In typhoid fever a large excretion of uroerythrin is exceptional and its occurrence has been connected with pulmonary complications. Riva has made the interesting observation that in such diseases as cirrhosis hepatitis the excretion of uroerythrin, as also of urobilin, is much diminished when the patient is put upon a milk diet. In nephritis uroerythrin is seldom found in the urine, but I have seen in a case of pneumonia an abundant excretion of this substance accompanying conspicuous albuminuria.

If uroerythrin in urine is always indicative of hepatic disorder it is clear that not only actual disease of the liver, but even functional disturbances of the slightest description, such as may be supposed to result from trifling errors of diet or may accompany a dyspeptic attack, suffice to bring about its appearance.

Although, as we have seen, the liver plays so important a part in connexion with the pigmentation of the urine, the various morbid changes to which that organ is liable have somewhat different effects upon the excretion of the several

⁵⁶ Archivio Italiano di Clinica Medica, 1896, vol. xxxv, p. 505.

⁵⁷ Proceedings of the Royal Society, 1894, vol. lv, p. 394.

⁵⁸ Gazzetta Medica di Torino, 1896 vol. xlvii., No. 12

⁵⁹ Journal of Physiology, 1895, xvii., p. 439.

⁶⁰ Clinical Diagnosis, third edition, 1900, p. 426.

⁶¹ Archiv für Chemie und Microscopie, 1853-54, p. 361.

pigments, and it is probable that a more detailed study of these effects would supply further diagnostic indications. However, in this review of a somewhat obscure branch of chemical pathology nothing has been further from my desire than to claim for my subject an exaggerated practical importance or to suggest that the investigation of the urinary pigments should form part of the routine of clinical examination. Such importance as the subject possesses is derived from the light which it throws upon processes which are at work in the body both in health and in disease, and especially upon those which are concerned with the disposal of effete blood pigment. Nevertheless, in certain cases the examination of the colouring matters of the urine may afford information of real diagnostic value and which is not readily obtained in other ways. It may therefore be fairly claimed that their study is one which is not simply and solely of academic interest.

A Clinical Lecture
ON
EMPYEMA FOLLOWING LOBAR PNEUMONIA
Delivered at Guy's Hospital on June 27th 1900,
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GENTLEMEN,—Most patients suffering from lobar pneumonia either recover or die without any complications, but among those few in whom complications follow there are none for whom you can do more than for those who suffer from empyema. A few years ago Dr. Chaning Pearce helped me to abstract several cases from our medical reports; lately my clinical assistants, Mr. F. W. Goble and Mr. B. W. Moss, kindly abstracted some more, and I have abstracted some myself. Altogether there are 45. 15 of these were under my care and for permission to refer to the others I am indebted to my colleagues. The results are shown in the following table:—

—	1898	1897	1896	1895	1894	1893	1892	1891
Total number of cases of lobar pneumonia in Guy's Hospital ...	122	115	114	100	67	182	88	108
Number of cases of pneumonia complicated by empyema ...	3	7	3	6	2	9	2	6
Percentage complicated by empyema ...	2.46	6.08	2.63	6.0	3.0	4.94	2.27	5.5

—	1890	1889	1888	1887	1886	1885	1884	1883
Total number of cases of lobar pneumonia in Guy's Hospital ...	88	60	43	72	48	39	26	69
Number of cases of pneumonia complicated by empyema ...	1	1	1	2	—	—	1	1
Percentage complicated by empyema ...	1.13	1.6	2.3	2.9	—	—	3.8	1.4

If we divide this period of 16 years into two equal periods we find that in the eight years 1891–98 there were 896 cases of lobar pneumonia, of which 38, or 4.24 per cent., had empyema, while in the eight years 1883 to 1890 the total number of cases of pneumonia was 445 among which there were seven cases, or 1.57 per cent., of empyema. Thus we see that both relatively and actually the number of cases of empyema due to pneumonia has increased of late years, and the rarity of this complication a few years ago explains the fact that in many text-books published then it was not mentioned that empyema was a complication of pneumonia, and it also goes to confirm what I think experience tells us—namely, that pneumonia is a more severe disease than it was; indeed, pneumonia has since influenza appeared, become much more formidable than formerly, for not only do these figures prove that the number of admissions for pneumonia have been very great during the last eight years, but the frequency of empyema has increased. There is very little

doubt that these figures understate the number of cases of empyema following lobar pneumonia, for it is almost certain that some of the patients who are admitted for pus in the chest have had pneumonia shortly before they came into the hospital. Only recently a man was under my care for empyema which was opened. He died very shortly after the operation and pneumonia was found at the post-mortem examination. Had he recovered we should not have known that his empyema was consecutive to pneumonia. Another reason which makes it impossible to give precisely the proportion of cases of pneumonia which are followed by empyema is that the pneumococcus may be the means of setting up an empyema, even although it does not induce pneumonia, and in such a case the general symptoms and physical signs may be such that it is very difficult to deny certainly the presence of pneumonia. I have seen three or four cases illustrating this point; perhaps the best is that of a man who was under my care but whose case is recorded by Dr. J. W. Washbourn in the Transactions of the Royal Medical and Chirurgical Society, vol. lxxvii. The patient was aged 36 years; his illness was ushered in by a rigor, he had persistent high temperature, rapid breathing, cough, herpes, and delirium. His general aspect was so like that of a patient suffering from pneumonia that both the medical registrar and I pointed him out to the students as having the exact appearances of a patient suffering from pneumonia. The physical signs were until the day before death those of solid lung—viz., loud bronchial breathing, bronchophony, dullness, and consonating râles. Not until the day before death did signs of fluid appear at the base of the lung. At the necropsy the lung was normal both to the naked eye and the microscope, 54 ounces of pus were found in the chest, and this was proved by microscopical examination, by cultivations, and by inoculations to contain no micro-organisms but pneumococci. It is of great interest to notice that such cases as these remind us that although the pneumonia due to the pneumococcus does lead to a pulmonary abscess, yet the same micro-organism may do so when it invades other parts of the body.

We see, therefore, that it is impossible to state with accuracy the percentage of cases in which pneumonia is followed by empyema, but it is interesting to note that out of 325 consecutive cases of empyema in the medical wards of Guy's Hospital there were 41, or 12.6 per cent., in which it appeared that the empyema followed a lobar pneumonia. But although this is so, it is easy by systematic examination of the pus to find out the proportion of cases of empyema due to the pneumococcus. I have not searched through all our records to find the exact proportion, for several authors have published results from which it appears that the pneumococcus is a very common cause of empyema, probably the commonest, that it is especially common as a cause in children, some considering that 75 per cent. of all empyemata in children are due to it.

Perhaps the best way to consider the subject is to see what we can learn from the 45 cases of empyema following upon lobar pneumonia which occurred in Guy's Hospital from 1883 to 1898 and to check the knowledge thus gained by the patients now in the hospital and one who has recently died.

By far the most important aid to diagnosing that empyema has followed pneumonia is the temperature. The usual thing, if empyema follow, is for the temperature to fall when the crisis takes place, for it to remain down two or three days, for it then to rise again, so that it soon becomes from 2° to 4° or 5° above normal in the evening and about 1° or 2° in the morning; this continues until the pus is evacuated. Chart 1, taken from a patient under the care of Dr. Frederick Taylor, shows this admirably. Sometimes the apyrexial interval is only one day, sometimes it is four or five days, and sometimes there is not strictly an apyrexial interval, for the temperature does not fall at the crisis to normal, but only to nearly normal, and then soon begins to rise again, so that instead of an apyrexial interval we have an interval of lower temperature. There is a fall of temperature at the pneumonic crisis with a subsequent rise in about a third of all the cases in which empyema followed pneumonia. This fall of temperature is a very curious phenomenon, for we believe the pneumonia to be due to the pneumococcus and the empyema to be due to the same micro-organism, and that it is in some way responsible for the pyrexia; that being so it is difficult to understand why, although these micro-organisms are constantly present, there should be