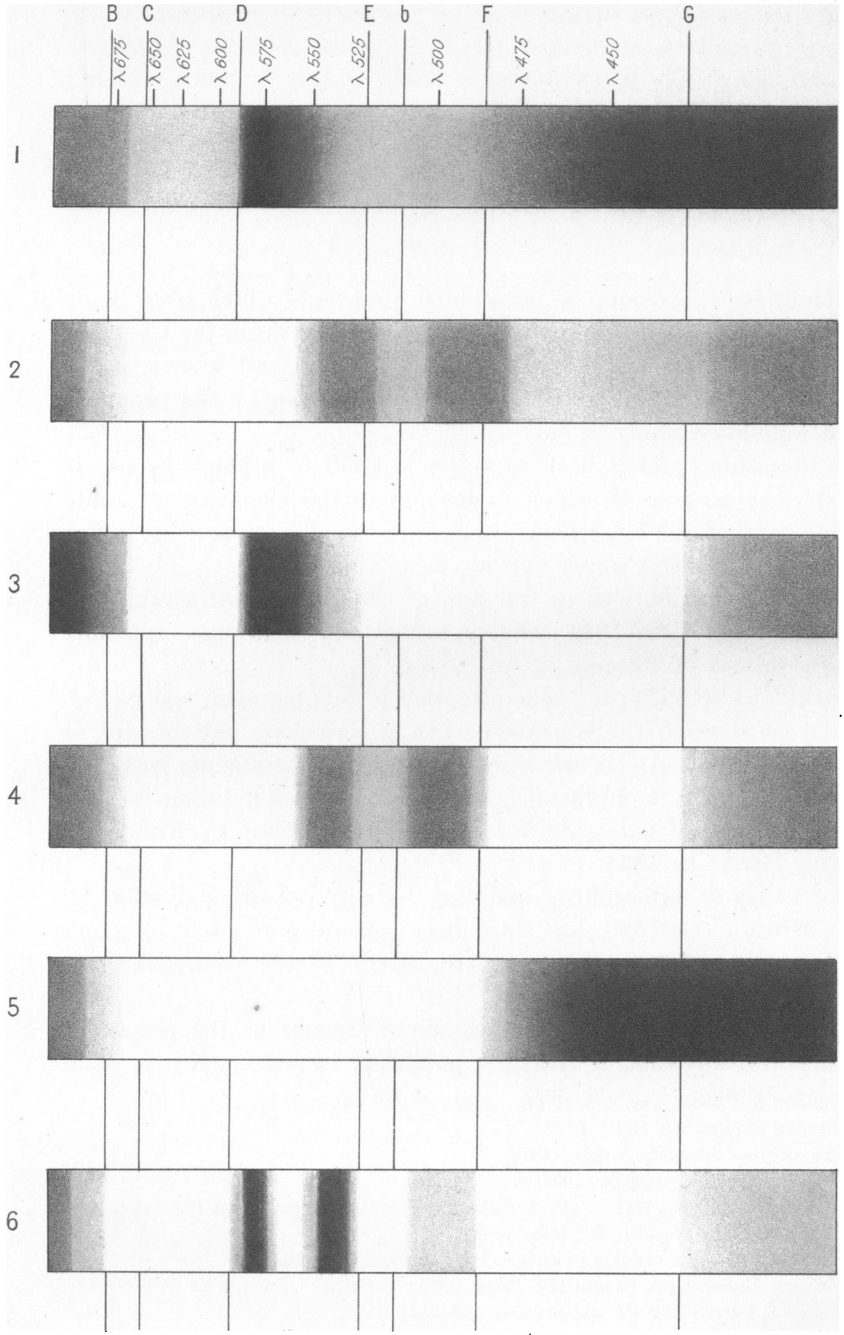

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A CONTRIBUTION TO THE STUDY OF UROERYTHRIN.

By ARCHIBALD E. GARROD, M.A., M.D., F.R.C.P. Plate X.

It is doubtless on account of its peculiar properties which place many obstacles in the path of the investigator, that uroerythrin, the colouring matter of pink urate sediments, remains one of the least known of the urinary pigments, although it was the first member of the group to receive individual study.

Its literature extends back to the year 1800 to a paper by Louis Proust[1], one section of which is devoted to the chemistry of urate sediments and of the "*substance rosacée*" or "*acide rosacique*" to which they owe the pink tint which they so often exhibit.

The next contributions to the subject came from Vauquelin[2], in 1811, and Vogel[3], in 1814, who confirmed and somewhat extended the observations of Proust.

Berzelius[4] (1833) paid some attention to this pigment, and had no difficulty in showing the incorrectness of a statement put forward in 1821 by Prout[5] that the colouring matter of the sediments was purpurate of ammonia or murexide; and the differences between its properties and those of murexide were further emphasized by Brett and Golding Bird[6] in 1834.

The name of uroerythrin, assigned to this colouring matter by Franz Simon[7] in 1840, has since been generally adopted, although that of purpurine, proposed by Golding Bird[8], is still occasionally met with.

Until quite recently the most complete account of the properties of uroerythrin was that of Heller[9], published in 1854, and it is upon

[1] *Annales de Chemie*, xxv. 265. 1800. *Scherer's Journal*, vii. 11.

[2] *Annales du Muséum*, 133. 1811.

[3] *Schweigger's Journal*, xi. 401. 1814.

[4] *Traité de Chemie*, vii. 356. 1833.

[5] *An Enquiry into the Nature and Treatment of Gravel etc.* pp. 16 and 122. 1821.

[6] *London Medical Gazette*, xiv. 600. 1834.

[7] *Handbuch der angewandten medizinischen Chemie*, i. 342. 1840.

[8] *Urinary Deposits*, pp. 57 and 107. 1844.

[9] *Archiv f. Chemie und Microscopie*, 361. 1853–4.

his observations that such brief descriptions as are to be found in works dealing with the chemistry of the urine are mainly based.

Thudichum[1] described the spectrum of uroerythrin in 1875, but his description differs materially from that which was given in 1883 by MacMunn[2], and which has been confirmed by all more recent observers.

Of quite recent years our knowledge of uroerythrin has been very materially advanced by the researches of Riva[3] and Zoja[4], the former of whom has shown conclusively that the view put forward by his countryman Reale[5] that this pigment is identical with urobilin is quite untenable.

The descriptions of uroerythrin contained in the text-books are so meagre, and the publication of Riva and Zoja's results is so recent, that it is practically necessary before speaking of the results of my own observations to sum up briefly those arrived at by the observers whose names have been mentioned in the above sketch of the literature of the subject.

The older method of extracting the pigment from urate sediments was by soaking them, after washing, in hot alcohol, but Riva describes a process of the efficacy and value of which I can speak from considerable experience, and which is briefly as follows:—The sediment is washed upon the filter-paper with ice-cold water, dried, and soaked in absolute alcohol; the sediment is next dissolved in warm water and extracted with pure amylic alcohol, which at once takes up all the uroerythrin from the aqueous solution; and after filtration a clear and concentrated solution in amylic alcohol is obtained.

Cold absolute alcohol dissolves isolated uroerythrin readily, but does not extract the pigment from urate sediments. (Riva.)

Solutions of uroerythrin have a ruddy orange tint and when concentrated absorb the entire blue end of the spectrum, but more dilute solutions show an ill-defined absorption band, consisting of two darker portions united by shading. (MacMunn.)

Strong sulphuric and hydrochloric acids impart a pink colour to the solutions (Vogel, Berzelius, Heller), but acetic acid produces no immediate change. (Brett and Golding Bird.)

[1] *Journal Chem. Soc.* XIII. 399. 1875.
[2] *Proc. Royal Soc.* XXXV. 399. 1883.
[3] *Gazetta Medica di Torino*, XLIII. pp. 1 and 923. 1892.
[4] *Archivio Italiano di Clinica Medica*, XXXII. 63. 1893.
[5] *Rivista Clinica e Terapeutica*, 1891.

Caustic alkalies destroy the pigment and change its colour to green. The green colour is most intense when the solid pigment is acted upon. (Thudichum.) Not unfrequently a play of colours is observed when an alkali is added to the solution. (Riva.) Before the spectroscope the green solutions show no absorption bands. (Zoja.)

Solutions of uroerythrin are very rapidly decolorized by actinic light, which however has little effect upon the solid pigment, and none at all upon pink urate sediments. (Riva and Zoja.)

The pigment is precipitated from its solutions by salts of lead, barium, calcium and tin, yielding pink precipitates. (Heller, Berzelius, Riva, Zoja.)

Solid uroerythrin leaves no ash on combustion, and gives off only a small quantity of ammonia when heated with caustic potash. (Berzelius, Heller.)

The colours of urate sediments. Other pigments besides uroerythrin take part in the coloration of urate sediments, as is evidenced by the variety of tints which they exhibit, from pale yellow or fawn colour to brick red or pink. Sometimes the yellow pigment of urine (urochrome) appears to be the only colouring matter present, but even the pale sediments often show some tinge of pink when filtered off. The yellow pigment is not removed by washing as urobilin is, and by its admixture with uroerythrin the various shades of red are produced. In some instances the sediments contain and may even owe the chief part of their colour to a form of hæmatoporphyrin which I have described elsewhere[1], which shows, in addition to a faint shading in the blue, two conspicuous absorption bands closely resembling those of oxy-hæmoglobin (Plate X, Fig. 6).

The bile pigments and chrysophanic acid may also be met with in the urate sediments, and these also are not removed by washing. In selecting specimens suitable for the extraction of uroerythrin, those from the urine of patients who have recently taken rhubarb or senna should be discarded, on account of the chrysophanic acid which they contain; and also such as are found to be rich in the form of hæmatoporphyrin above referred to.

The spectrum of pink urate sediments. It has been suggested by several observers that the uroerythrin contained in pink urate sediments is not free but in combination, and I am able to bring

[1] This *Journal*, xv. 116. 1893.

forward an additional piece of evidence which certainly seems to support this view, viz. that pink urate sediments constantly show a definite absorption band, which may be seen either when the deposit is examined upon the filter-paper by reflected light, or when the paper so coated is dried, oiled and examined by transmitted light. The band lies close to, and on the more refrangible side of the *D* line, extending from λ 5890 to about λ 5430 (Plate X, Fig. 1). Although the presence of this band is undoubtedly due to the uroerythrin which the sediments contain I have failed to see it either by looking through a layer of free solid uroerythrin deposited upon glass, or when the solid pigment, diluted with some inert white powder, was examined by reflected light. If however it be the case that the urate sediments contain a compound of uroerythrin it must be supposed that this compound is only formed at the moment of deposition, and ceases to exist when they are redissolved, for the spectroscope shows that both in the original urine, and in aqueous solutions of the pink deposits, the pigment exists in the free state.

A new process for the extraction of uroerythrin from urate sediments. The following process, not hitherto described, although somewhat less simple than that of Riva, affords a satisfactory means of extracting uroerythrin from pink urates, and yields solutions of the pigment in ethylic alcohol, which are almost completely free from pigmentary and other impurities.

A specimen of pink urate sediment, collected from the total urine passed during several days by a patient who has not recently taken either rhubarb or senna, is collected upon a filter, and whilst still moist is washed off the paper with a stream of cold water; more water is added according to the bulk of the sediment, and gentle warmth is applied until the urates are completely dissolved. The warm aqueous solution is next saturated with ammonium chloride, which proceeding has the effect, as Gowland Hopkins has shown, of causing the complete precipitation of the uric acid in the form of ammonium urate, and upon this precipitate the whole of the dissolved uroerythrin is once more carried down. The precipitate, which is flocculent, is easily filtered off, and the filtrate, which has a yellow colour, usually shows a distinct urobilin band. The precipitate is next washed off the filter with a saturated aqueous solution of ammonium chloride, and the filtration and washing are repeated until the washings no longer have any yellow tint. The washed precipitate has a purer pink colour than the original sediment, but shows the same absorption band near *D*.

The moist filter-paper coated with pink urate is now transferred to a wide-mouthed bottle containing warm alcohol, and is allowed to soak for several hours in a warm place, protected from light. This precipitate gives up uroerythrin to alcohol much more freely than the natural sediments do, and on filtering a more or less concentrated solution of the pigment is obtained. To the alcoholic solution there is now added at least twice its bulk of distilled water, and the liquid is shaken with chloroform, which, if enough water has been added, takes up no uroerythrin, but is rendered yellow by impurities which it removes. This preliminary washing should be repeated several times, the impure chloroform being separated off on each occasion. A fresh supply of chloroform is then added together with a single drop of acetic acid, and, on shaking, the uroerythrin will now be promptly and completely extracted, the supernatant liquid being left quite colourless. The chloroform solution of uroerythrin so obtained is separated off; shaken with distilled water; again separated, and allowed to evaporate slowly in a warm place, protected from light. The solid residue which remains is readily dissolved by absolute alcohol.

The very rapid decolorization of uroerythrin solutions by light, which is brought about even by a few hours' exposure to the full light of a winter's day in London, supplies a ready means of testing the freedom of the product from pigmentary impurities, and after such exposure the alcoholic solution obtained as above should be practically colourless, exhibiting only a faint yellowish tint (doubtless due to impurities still remaining) when examined by daylight, in depths of several centimetres.

In carrying out the above process certain precautions are necessary. If acetic acid is added to the original urine with a view to increasing the amount of sediment, the proper separation of the uroerythrin upon the ammonium urate is interfered with, the pigment tending to be deposited apart from the urate.

Again, the lowest temperature compatible with the complete solution of the original urate sediment in water should be employed, as otherwise the removal of the pigment by soaking in alcohol is apt to be seriously impaired. Even when this precaution is taken the removal by alcohol is in some instances less satisfactory than usual.

Occasionally chloroform takes up uroerythrin even before the addition of acetic acid to the aqueous-alcoholic solution, but when this is the case it is usually because not enough water has been added. Lastly, if the chloroform solution be evaporated upon the water bath, instead of

being allowed to evaporate at a lower temperature, the solid residue of uroerythrin will be found to be scarcely, if at all, soluble in alcohol.

A similar process may be employed for the direct extraction of uroerythrin from urine in which it is abundantly present, but which throws down no urate sediment. The urine itself is saturated with ammonium chloride and the precipitate which forms is treated as above described. Under these conditions the resulting product is much less pure, being contaminated with pigmentary impurities (and especially with hæmatoporphyrin thrown down from the urine by saturation with ammonium chloride) which, when urate sediments are employed, pass away in the urine which is filtered off.

Properties of solid uroerythrin. So intense is the colouring power of uroerythrin that even a considerable bulk of a concentrated solution yields but a very small amount of solid residue, and the total quantity of the pigment which can be obtained from a large quantity of urate sediment is quite minute. The solid pigment has a pink colour slightly different from that of pink urates, and its colour is only very gradually destroyed by light. As already mentioned it does not show the band near *D* of the urate sediments, but a less defined and more general absorption. Even when warmed it has little smell, and it emits no characteristic odour when burnt. It leaves no ash, and no trace of iron reaction can be obtained with hydrochloric acid and potassium sulphocyanide after the combustion of such a quantity as 0·005 gramme.

When an alcoholic solution is allowed to evaporate slowly at the temperature of the air the pigment is deposited in minute spherical granules which show no trace of crystalline structure.

The solubility of uroerythrin in various liquids is much affected by such conditions as temperature and acidity, and even those liquids which dissolve it best, do so much more readily in the presence of a trace of acid. Among the various known solvents amylic alcohol stands first as Riva states, but acetic ether is very little inferior in solvent power. Alcohol and chloroform may be placed next in order, and after these water. Ether also dissolves the pigment readily but as Riva points out the solution rapidly loses colour, even in the dark, and the uroerythrin tends to be deposited as an impalpable pink powder. When one of the above solvents is poured upon the solid pigment the change from the pink of the solid to the rich orange colour of the solution is very striking.

Properties of solutions of the pigment. Solutions of uroerythrin differ in colour according to their degree of concentration. When dilute they have a peculiar subdued pink tint, but when concentrated they assume a ruddy orange colour. In a clear glass bottle the contrast between the tints of the thinner and thicker layers of the liquid gives to the solutions a peculiar and characteristic appearance. The pink tint is only well seen in the purest solutions, and is readily masked by even a very small amount of pigmentary impurity. Spectroscopic examination fully explains this double colouring, for whereas a very concentrated solution absorbs the whole of the more refrangible rays, the absorption ceasing somewhat abruptly at about λ 5520; as dilution proceeds the more refrangible rays begin to penetrate, and some time before the pink stage is reached there appears the complex absorption band which was first described by MacMunn (Plate X. Fig. 2).

The band, which is a broad one, consists of three distinct parts, two darker portions being joined by a shading of less intensity. The edges of the several parts are ill-defined and measurements are difficult to obtain, and necessarily only approximate. Even the darker portions never appear black, for before such a degree of intensity is reached, they become merged in the general absorption of the blue end of the spectrum.

If the solution shows a narrow band close to the *D* line, some two-banded hæmatoporphyrin is present as an impurity, its other band being hidden by that of uroerythrin.

The following approximate measurements of the uroerythrin band, obtained from a pure solution in rectified spirit, agree as closely as can be expected with the figures given by Zoja (viz. 1. λ 550—λ 525, 2. λ 510—λ 484):

1. λ 546—λ 520.
Shading.
2. λ 506—λ 481.

The more refrangible of the two darker bands corresponds somewhat closely with that of urobilin, but as Riva has pointed out it cannot be due to contamination with that substance, since it disappears completely when the liquid is decolorized by light.

Pure alcoholic solutions are practically neutral in reaction, tending if anything towards acidity rather than towards alkalinity. This result is at variance with the observations of some early observers, who ascribed to the solutions highly acid properties. No fluorescence is visible even when the liquid is examined by the light from a Geissler's tube.

Reactions of uroerythrin. From a chemical point of view uroerythrin is an extremely unsatisfactory substance. Its rapid destruction by light renders it difficult to work with, and the uncertainty of its behaviour under apparently similar conditions is calculated to make one despair of getting any further with its investigation. Its most striking properties are its great affinity for uric acid and its compounds, and that of yielding a green colour with caustic alkalies. There are however certain remarkable colour reactions of this pigment, especially with various acids, which must be briefly described, but they cannot be reckoned up as constant phenomena, although they are shown to be true reactions of uroerythrin by the fact that solutions which yield them cease to do so after their colour has been discharged by exposure to daylight for a few hours. These reactions seem to be only explicable on the hypothesis that uroerythrin tends to form a series of highly unstable compounds, which are decomposed by mere dilution of their solutions, but that under certain conditions these compounds fail to be formed, the pigment being instead straightway decomposed by the reagent employed. I have known the same solution of uroerythrin to yield the characteristic colour reaction with one specimen of sulphuric acid and to fail to do so with another. It is doubtless owing to this uncertainty of the behaviour of the pigment, that whereas Vogel, Berzelius and Heller all alluded to the appearance of a pink colour on the addition of sulphuric or hydrochloric acid to a solution of uroerythrin, Riva and Zoja merely state that the pigment is destroyed by mineral acids.

Colour reactions with acids. *a. Sulphuric acid.* On the addition of strong sulphuric acid to a solution of uroerythrin a brilliant carmine tint appears, and on shaking with chloroform much of the carmine pigment is taken up by that solvent. The chloroform solution shows a dark absorption band near *D*, λ 5860—λ 5520 (Plate X. Fig. 3), similar to that of pink urate sediments, and a second fainter band in green is sometimes seen. On dilution with alcohol the original colour and spectrum of uroerythrin are again obtained. The carmine solution is rapidly decolorized by light, and there remains only a greenish yellow tint. When the reaction fails a similar greenish tint is at once produced on the addition of the acid. This reaction is no longer obtained after the uroerythrin solution has been decolorized by light.

b. Hydrochloric acid. Hydrochloric acid produces a rose-pink colour, and the liquid when concentrated shows a shading too ill-defined

for accurate measurement from about λ 6080—λ 5170. The pink product is readily taken up by chloroform, and after evaporation of the chloroform the residue dissolved in alcohol has the colour and spectrum of uroerythrin solutions. The pink colour rapidly disappears on exposure to light, and a greenish tint remains. The reaction is uncertain; it is not obtained with uroerythrin solutions which have been decolorized by light.

c. Phosphoric acid. Phosphoric acid turns the colour of the solutions to a salmon pink, and the product is readily taken up by chloroform. On dilution the original colour and spectrum are restored. The spectrum of the pink solution resembles that of uroerythrin, but the complex band is nearer to the red, and the blue end of the spectrum is much less obscured (Plate X. Fig. 4). The parts of the band read as follows:—

1. λ 557—λ 524.

Shading.

2. λ 515—λ 489.

The phosphoric product is less rapidly decolorized by light than the original uroerythrin. The reaction is not obtained with previously decolorized solutions.

Action of alkalies. Alkalies have a much greater destructive action upon uroerythrin than acids, but it is not improbable that the play of colours which is so frequently observed when caustic potash or soda is added to the solutions, and which Riva regards as affording evidence of the existence of more than one variety of the pigment, indicates the formation of compounds with the alkali which are rapidly destroyed, with the formation of the ultimate green product. This explanation receives support from the fact that in the earlier stages of the reaction immediate acidification with acetic acid restores the uroerythrin spectrum, but this is no longer the case when once the green stage is reached.

The series of changes through pink, purple and blue to grass green occupies only a portion of a minute, but I have frequently watched the process with the spectroscope, and have witnessed the appearance and rapid disappearance of well-defined absorption bands. At an early stage a band is seen from λ 672—λ 6425; when the purple stage is reached two bands are seen resembling those of the indigo pigments, and the ultimate green product shows no bands, but absorbs the violet end of the spectrum to about λ 4660 (Plate X. Fig. 5). Solutions of the

green product are decolorized by light but not so rapidly as those of the original pigment.

There is a very remarkable colour reaction of uroerythrin-green which is by no means constant, and is difficult to obtain. If a small quantity of caustic soda be added to a solution of uroerythrin in amylic alcohol much of the green product leaves the amylic alcohol, which however retains a pure green tint. If now the amylic alcohol be evaporated off, and the residue be treated with strong sulphuric or hydrochloric acid in great excess, a carmine-coloured solution is obtained. With sulphuric acid a dark band is seen from λ 5825—λ 5490, with hydrochloric acid a band from λ 6080—λ 5490. In either case the liquid turns green on dilution with alcohol, but again changes to carmine when a great excess of acid is added. From the carmine solution chloroform takes up the pigment but, being unable to take a sufficient excess of acid with it, assumes a green colour.

Effects of oxidizing and reducing agents. Solutions of uroerythrin are decolorized, even in the dark, by both oxidizing and reducing agents. With nitric acid the effect is immediate, but peroxide of hydrogen acts more slowly, its action being greatly aided by the temperature of the water bath.

Among reducing agents that which has the most rapid effect is, as might be expected, nascent hydrogen produced by the action of hydrochloric acid upon tin or zinc.

Precipitation by metallic salts. It has long been known that uroerythrin is carried down upon the precipitates formed on the addition of lead acetate or of barium chloride to urine; and as Riva says, when a few drops of a solution of lead acetate are added to a solution of uroerythrin in amylic alcohol, obtained by shaking the pure alcohol with an aqueous solution of well-washed pink urates, there is formed, in the course of a few hours, a scanty precipitate of an intense pink colour. Riva speaks of the substance of which this precipitate is composed as "*uroeritrina piombica*," and I gather that he regards it as a compound of the pigment with the metal. He adds that similar precipitates are obtained with salts of barium and of tin.

My own observations, whilst they fully bear out Riva's statement, have led me to think that we have here merely fresh examples of the tendency of uroerythrin to be carried down upon precipitates formed in its solutions, and not a true precipitation of the pigment in combination with the metal. In the instance under consideration the deposit obtained undoubtedly consists of deeply pigmented insoluble urates, for

the amylic extracts prepared in the manner above described always contain some urate, as is easily seen by substituting an aqueous solution of snake's urine for that of a pink urate sediment, when a precipitate of lead urate is obtained on adding a small quantity of lead acetate to the amylic alcohol.

When a few drops of a solution of barium chloride are added to a solution of uroerythrin in absolute alcohol, prepared by the ammonium chloride process, the barium chloride separates out in crystalline form and takes some of the pigment with it, but if enough distilled water is added to redissolve this salt the solution remains clear, showing no turbidity and depositing no sediment. If a drop of a solution of colourless urate be now added a brilliantly pink precipitate will form.

If equal quantities of an absolute alcoholic solution of uroerythrin, of the same solution completely decolorized by light, and of absolute alcohol alone be placed in three similar bottles, and if there be added to each equal quantities (three or four drops) of a saturated aqueous solution of neutral lead acetate; the three specimens will all be rendered turbid; the turbidity will gradually increase, and in time a scanty flocculent precipitate will fall. In the case of the pigmented solution this precipitate will have a deep pink colour, whereas the sediments in the other bottles will of course be colourless. The turbidity is not removed by the addition of distilled water.

It will be seen from what has gone before that the results obtained from the investigation of uroerythrin during a period extending over nearly a century, although not devoid of interest in themselves, and sufficing to show that the pigment differs conspicuously in its nature and properties from the other colouring matters which exist as such in the urine, cannot be regarded as satisfactory; and this is readily explained by the extreme instability of the pigment, its ready decomposition by light, the uncertainty of its reactions, and the very minute quantities in which it is obtained.

Clinical evidence points strongly to the liver as the probable seat of its formation, but no clue has yet been obtained indicating a relation to or derivation from the colouring matters of the blood and bile. In some respects uroerythrin resembles some of the pigments obtained by the action of acids upon urine, but there does not appear to be any sufficient ground for accepting the statement of Bruno Mester[1], for

[1] *Zeitschrift f. physiol. Chemie*, XII. 143. 1888.

which he gives no reasons, that it is a skatol pigment; seeing that alike in colour, spectrum and reactions it differs widely from the ordinary pigment which is developed on the addition of acids to urines rich in skatoxyl derivatives.

[The expenses of this investigation were amongst those covered by a grant from the Government Grants Committee of the Royal Society.]

PLATE X.

Fig. 1. The spectrum of pink urate sediments, as seen when a layer of the sediment upon oiled filter-paper is examined by transmitted light.

Fig. 2. Spectrum of uroerythrin in solution in ethylic alcohol.

Fig. 3. Spectrum of the carmine product obtained by adding sulphuric acid to a solution of uroerythrin. (In chloroform.)

Fig. 4. Spectrum of the pink product obtained by adding phosphoric acid to a solution of uroerythrin. (In alcoholic solution.)

Fig. 5. Absorption of the green product formed by the action of sodium hydrate upon uroerythrin.

Fig. 6. Spectrum of hæmatoporphyrin as it is obtained from urate sediments. On the addition of a mineral acid the ordinary spectrum of acid hæmatoporphyrin at once appears.