THE
Hastings Prize Essay.

UROCHROME;
THE COLOURING MATTER OF URINE.

BY
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PREFACE.

THE author has selected a chemico-physiological subject for competition for the Hastings Medal, because it admitted of the combination of literary research with original investigation, and of the direct application of the results of this investigation to the practice of medicine. The greatest care was bestowed upon the original research, so that every datum there given may be considered as finally proved. But the application of the results to pathology and the practice of medicine could not (owing to the limitation of the space) be pointed out, excepting in a few propositions. These propositions the author pledges himself to prove at length hereafter.

The author has made numerous experiments, and always upon large quantities of material; so that upwards of one hundred gallons of urine were evaporated, and a similar quantity worked up in the natural state. His expenses to carry out his investigations have been very considerable. He has devoted a great part of his time to this inquiry during the past twelve months. He will consider himself highly honoured and amply rewarded should he be so fortunate as to become the first recipient of the Hastings Medal of the British Medical Association.

HISTORY OF INVESTIGATIONS ON THE COLOURING
MATTER OF URINE.

Colouring matter is the most obvious of all ingredients of the urine. It has not to be discovered like urea or creatinine, but it strikes the eye at the first

* Extract from the Report of Council, presented to the annual meeting of the British Medical Association, held at Cambridge on August 3rd, 1864.

"The Hastings Prize Medal. The adjudicators of this prize have forwarded the following report.

"To Sir Charles Hastings, M.D., D.C.L., President of the Council of the British Medical Association.

"Dear Sir Charles,—We, the undersigned, being the Committee appointed by the Committee of Council to adjudicate the Hastings Gold Medal for 1864 for an Essay on Physiology, beg leave to state that, of six essays sent in in competition, an Essay on Urochrome, bearing the motto 'Original Research', is the one most worthy of the prize.

"We therefore inclose to you the letter accompanying the MS., containing the name of the author, with the seal unbroken; and we will ask you to be kind enough to break the seal, and to communicate the award to the President, Dr. Symonds. We will further ask you—in order that he may be present at Cambridge on Thursday next to receive the medal—to inform the author himself of his success, and to express to him our congratulations on the result of his learned and successful effort."

"We remain, dear Sir Charles, yours faithfully,

W. SHARPEY, M.D.

B. W. RICHARDSON, M.D.

A. T. H. WATERS, M.D.

"London, 29th July, 1864."

"The President of the Council having broken the seal, and examined the inclosure, in accordance with the wish of the adjudicators, your Council has the pleasure to announce that Dr. Thudichum is the successful candidate."

glance, touches the nose by its odour, and tastes bitter and nauseous when put upon the tongue. It is so prominent, and essentially characterising an ingredient of the renal secretion, that its study and consideration should precede that of any other. It is the alpha and omega not only of urinary analysis, but of urinary pathology.

Nevertheless, it has hitherto eluded the scrutiny of the inquirers who have endeavoured to extract it from the complex mixture to which it is peculiar. Each analyst, it is true, obtained a product more or less defined, and referable to or derived from the colouring matter. Each, too, named his product, without inquiring whether it had not been observed and named before. It was thus that urology became enriched with names apparently denoting different substances. Urophæine, uroxanthine, urerythrine, urrhodine, uroglaucline, urohematine, purpurine, indican, indigo-red, indigo-blue, oxide of omichmyl—these and other names indicate the stages of the inquiry, which had for its object the chemical knowledge of urinary colouring matter. But the results of these labours were so little satisfactory to critical minds, that Vogel, one of the latest authors on urology, declared it impossible that anybody should ever analyse the colouring matter of urine in accordance with the requirements of chemical science. If Vogel had been acquainted with the researches of Proust, he would not have ventured to express such an opinion.

Sixty-three years ago, a French chemist, then resident at Madrid, had more correct notions and better knowledge of the colouring matters of the urine than the authors of the newest treatise on urology. Sixty-three years ago, Louis Proust gave to science the account of researches, which for importance and variety of results, and correctness and beauty of description, stand unrivalled in the literature of animal chemistry; or, indeed, chemistry at large. He published them in the Spanish *Annales de Historia Natural*, March 1800, No. III, p. 275; and in the old *Annales de Chimie*, vol. xxxvi, p. 258. But the beginning of the century was not favourable to the spreading of information regarding purely scientific results. The invasion of Spain by the French destroyed Proust's existence at Madrid. He was robbed of the "silver vessels in which he had certainly evaporated two thousand pints of urine" by the soldiers of his own nation, and his laboratory was destroyed. Scientific men took no notice of his researches then as now. Of this neglect, Proust gently but justly complained; and republished the researches, with few additions or alterations, in 1820, in vol. xiv, p. 260 *et seq.*, of the new series of the *Annales de Chimie*. But it did not avail. Proust was before his time by more than half a century; and he was forgotten by his contemporaries as completely as he is ignored by our own. In 1844, Liebig (*Ann. Chem.*, vol. I, p. 161) drew attention to Proust's papers, remarking that they appeared to have escaped the attention of all authors who had hitherto written upon the subject of urine. But by misapprehending the nature of the bodies discovered by Proust, as derivatives of the colouring matter of urine, and by declaring that they could only be obtained from putrid and not from fresh urine, he neutralised the effect of his own literary discovery, so far as the colouring matter was concerned, and Proust's researches were anew assigned to oblivion.

I will here quote what Proust has said about the matters which he discovered, and which he declared to derive from the colouring matter of the urine.

"A fallow, odorous, and resinous substance, appears to me to be the seat" (I read *siège*, instead of *signe*, as probably misprinted) "of the odour, the colour, and the bitter taste of urine. If this resin were not dissolved in urine by the concurrence of so many salts, very little ammonia would suffice to keep it in solution, as it is very soluble in alkalies. If sulphuric or muriatic acid be mixed with an extract which has already been separated from its principal salts, there are immediately set free vinegar, benzoic acid, and resin, which deposits in the form of a black thick oil. On washing it with warm water, and collecting it in a beaker, it assumes the consistence of black pitch."

This description certainly only applies to extracts obtained from previously putrid urine; and from which, consequently, all the urea has been removed in the form of carbonate of ammonia. In so far, Liebig was quite justified in ignoring the word "*recent*", by which Proust describes the urine which served him for the extract from which he obtained the vinegar. If a similar extract is made from fresh urine, such as we define it now, neither vinegar, nor benzoic acid, nor resin, are obtained immediately on addition of an acid. It requires considerable management to obtain the resin from fresh urine; but in the manner to be described hereafter, it can be done with certainty. Liebig then erred only in this, that he asserted that these resinous matters could not be obtained from fresh urine. I continue the extract from Proust.

"It is possible, however, that this product does not exist in the urine as such, but in the form of an oily or greasy substance of the same colour as the urine; perhaps even its resinous character is only produced by the combined action of the air and the heat of the evaporating process. Nevertheless, as it is found with the same properties in various calculi, I do not hesitate in considering it as decidedly resinous. However that may be, its perfect solubility in alcohol, its pitch-like tenacity, and an aromatic odour which approaches that of castoreum, make me inclined to consider it as belonging to the inflammable or over-hydrogenised products.

"Its solution in alcohol does scarcely become pale when mixed with water; it becomes subdivided, and seems at first to dissolve; but afterwards it separates from the fluid, and then gives a black insoluble deposit.

"A very weak solution of potash dissolves it without evolution of ammonia; acids precipitate it from this solution, and with the assistance of warm water the particles reunite, and assume again the consistence of soft resin.

"It dissolves in the mouth and causes a very remarkable impression: it is an acrid bitter taste, which obliges one to spit in a disagreeable manner, analogous to the taste which is experienced when tasting the bulbs of arum. It is consequently its bitterness which is found in the extracts of urine, and in the urea which has not been sufficiently purified from it. Its brownish-yellow and dark colour is exactly the same as that which urine assumes on the surface which is in contact with the air. All these attributes do not permit me to doubt that it is the essential principle which colours and flavours the urine, and the linen moistened with urine when it is heated.

"Its resinous qualities are also supported by the results of its distillation. It fuses, puffs up, yields

little or no water, a slight ammoniacal odour, a large quantity of thick oil, and above all a considerable quantity of charcoal, amounting to 46 per cent. I forgot to incinerate it and to examine its ash; I might have done it; I have had as much as eight ounces of this resin.

"It has also a singular disposition to become reduced to a powder when kept under water. It shows itself in this form even when it is precipitated from too clear an extract. In order to get it coherent and consistent, it is advantageous to take extracts of the thickness of a syrup of melasses. The resin contains benzoic acid, as, when kept in paper during drying, the acid becomes attached to the paper, having left the resin."

The singular disposition to become reduced to a powder when kept under water, can only be explained by the mechanical separation of the brown matter to be mentioned hereafter.

The account which Scharling (*Ann. Chem.*, vol. xlii, p. 265) has given of a body which he extracted from urine, and termed "*omichmyl-oxyd*", tallies to a great extent with the description which Proust gives of his resin. To guard against the changes which he supposed urine to undergo during evaporation, Scharling concentrated urine by freezing. The particles of ice were removed as long as they were colourless. When they became coloured yellow, the concentration was discontinued. Artificial freezing Scharling found unserviceable, as he could not succeed in making the urine freeze from above downwards, which seems necessary for allowing the solution of increased specific gravity to sink. Urine so concentrated, was mixed with an equal volume of ether, and allowed to stand for twenty-four hours or more, being shaken at frequent intervals. The ether dissolved a part of the oxide of omichmyl, a little urea, and several other matters, which were not determined. The extraction with ether was repeated several times, the ether was distilled off, and the residue was washed with cold, afterwards with hot, water. By this treatment, the urea and the other matters soluble in water were removed, while oxide of omichmyl was left.

Unless the urine upon which Scharling operated had become decomposed during the freezing, he could not have extracted the oxide of omichmyl. In case, however, the urine was not decomposed, then I incline to the belief, from many experiments which I have made upon this point, that what Scharling extracted in the ether was the yellow colouring matter of the urine in its undecomposed state, which, by decomposition under the influence of heat and acids, in particular, the distilling off of the ether, and the action of the hippuric and other acids probably contained in the ether, yields the resin of Proust, or oxide of omichmyl of Scharling. It was, therefore, a mere accident that Scharling obtained any of his oxide; the greater part of the substance furnishing it, the yellow colouring matter, he certainly lost in the washings.

For the purification of the omichmyl oxide, Scharling proceeded as follows. To decompose ammoniacal salts, which he alleges to have adhered to it, it was dissolved in caustic potash, the solution heated to ebullition, and afterwards precipitated with dilute sulphuric acid. The oxide was thereby precipitated in brown flocks. They were collected on a filter, dried, and dissolved in ether; the solution was filtered, and, after addition of a little water, was

evaporated, when the pure oxide of omichmyle remained. The following are the chemical characters of this substance.

It fuses in boiling water, forming a brownish-yellow oil, which, on cooling, solidifies to a resin. It is soluble in ether, spirit of wine, ammonia-water, dilute caustic and carbonated potash and soda. The solution in spirit has an acid reaction. In its dry state, it has a strong odour of castoreum; but on boiling with water, a faint urinous odour is perceived. When the ethereal solution is mixed with a very small quantity of spirit of turpentine, the mixture, after evaporation of the ether, assumes a sweetish aromatic odour, resembling the smell of urine after taking spirit of turpentine or juniper-oil internally. Heated strongly, after moistening with water, until decomposition ensues, it gives out a strong penetrating odour of old urine. Heated more strongly, it takes fire, and burns with a white strongly lighting flame. Red-heat leaves only a faint trace of ash. Boiled with aqua regia, the oxide leaves a yellow semifluid resin. This boiling, performed in a retort, yields a greenish-yellow oil in the receiver, together with some nitric acid and water. This oil, boiled with water, leaves a little yellow resin. The watery solution, on cooling, deposits scaly crystals, which are easily soluble in spirit, volatile, and, when saturated with ammonia, produce a red precipitate in a neutral solution of chloride of iron. This makes it probable that the acid is benzoic, which Scharling believes to have been produced by the action of nitric acid upon the oxide of omichmyle.

The benzoic acid was, of course, derived from the hippuric, with the existence of which in human urine Scharling was not acquainted when he wrote. The oil was partly derived from the hippuric acid, partly from alcohols of the phenyl series, or phenylic alcohol itself. The oxide of omichmyle was, therefore, very impure, as is also evident from all other reactions. But it was nothing else than one of the ingredients of Proust's resin. (See Omicholic Acid.)

Had Scharling been acquainted with the proceedings of Proust, he might have obtained his oxide in quantity, and employed for an useful purpose the labours which he wasted upon the chlorinated and nitrated bodies extracted from the mother-liquor of nitrate of urea. But, finding that his chloro- and nitro-omichmylic acid turned out to be substitution-products of benzoic acid, which he had also obtained from his oxide directly, he, after the discovery of hippuric acid as a natural ingredient of the urine, became disheartened, and abandoned his researches. Omichmyle-oxide was either forgotten entirely, or dragged on a pitiful existence in the small print notices of handbooks.

I repeated the experiment of Scharling upon urine concentrated by evaporation on the water-bath. The ethereal extract yielded the oxide, and yielded the more of it the longer it was heated by itself after the evaporation of the ether. No oxide was obtained, if all superfluous heating was carefully omitted. From this I learned that the oxide was not contained as such in evaporated urine, and that it was formed by heating the yellow ethereal extract with hippuric acid. The yellow matter contained in the ethereal extract, after purification, fitted the description, in some respects, which Heller has given of uroxanthine, and Schunck of indican. By boiling with dilute acids, it yielded the fallow resin of Proust, or the

omichmyle-oxide of Scharling. It never yielded indigo-blue, although a matter was always obtained which had some features analogous to indigo.

Heller precipitated urine by acetate of lead, and evaporated the filtrate to dryness at a low temperature. He then extracted it with ether, which, after evaporation, left uroxanthine, a yellow matter, soluble also in water and alcohol. This, by treatment with acids at a higher temperature, decomposed, yielding a blue matter, uroglaucine, declared subsequently to be indigo-blue; and a red matter, termed urrhodine, declared to be identical with indigo-red.

Heller's urrhodine is "an uncrystallisable brownish red resin, of acid reaction (pigmento-resinous acid), whose solutions in alcohol and ether present so beautiful a red colour," that the name was hence derived. It contains nitrogen, and burns on platinum foil without leaving any residue. (See Omicholic Acid.)

Schunck treated urine with lead salts, and decomposed the precipitates by sulphuric acid. He obtained yellow solutions, which yielded him on standing, or on boiling, indigo-blue and indigo-red. He claims the yellow matter to be indican. This indigo-red, or indirubine, from urine, is not described any further than that it is extracted by alcohol from the mixture of substances which acids produce on boiling in a solution of the yellow matter termed indican. It has a fine purple colour in solution.

This process of precipitating urine with lead-salts, and decomposing the precipitates with acid, had been before adopted by Scherer. He employed alcohol with the hydrochloric acid, and evaporated the solution. The residue, after washing with water, he assumed to be the colouring matter of urine. It was a blackish brown matter, which could be powdered when dry. On burning, it left a mere trace of inorganic residue. It was insoluble in water, a little soluble in hot water, easily soluble in alkalis and alcohol. Its odour on combustion was peculiar, but differed from that of burning urine. A repetition of Scherer's experiments shows that he had obtained principally the red resin of Proust, or oxide of omichmyle of Scharling; mixed, however, with a considerable amount of impurities.

Scherer was succeeded by Harley, who adopted a new process, in so far as he evaporated urine, extracted with alcohol, treated the extract with caustic lime, and dissolved the lime compound so obtained in a mixture of alcohol and hydrochloric acid. From this acid solution the colouring matter was extracted by ether. This ethereal solution left a residue which was dark red, amorphous, resinous, and, dissolved in alcohol or ether, imparted to it a beautiful red colour. It was insoluble in acids, easily soluble in alkalis. On combustion, it left an ash, consisting exclusively of oxide of iron. There is, therefore, no doubt that what Harley isolated, and termed urohematine, in token of its supposed derivation from the colouring matter of blood, was one of the ingredients of Proust's resin and Scharling's omichmyl-oxide. (See Omicholic Acid.)

Marcet evaporated the urine to dryness, extracted with boiling absolute alcohol, and precipitated the urea by absolute ether. The mixture of ether and alcohol was decanted, concentrated on the sand-bath, and its acidity neutralised by some baryta water. After renewed concentration, a few drops of sulphuric acid were added, and the whole treated with a mixture of ordinary alcohol and ether. The fluid

separated into two layers, of which the uppermost was washed with water, filtered and evaporated at a low temperature. There resulted a colourless watery liquid, filled with crystals, which were not hippuric acid; and at the sides of the evaporating dish, red drops deposited. These united to form a resinous matter; had a strongly acid reaction; and, on standing twenty-four hours, crystallised in groups of needles, the centre of the group being in the centre of the drops. It was soluble in alcohol and ether only. The same product could be obtained from the urine without the addition of sulphuric acid, and is, therefore, claimed to have been present in the urine in the free state, or as an immediate principle. I believe this, again, to have been one of the ingredients of the red resin of Proust (and Scharling's omichmyl-oxide), encrusting crystals of urea or benzoic acid. (See Omicholic Acid.)

To my mind there is no longer any doubt that Proust's fallow resin, Scharling's omichmyl-oxide, Heller's urrhodine or indigo-red, Schunck's indirubine or indigo-red from urine, Scherer's colouring matter of urine as subjected by him to elementary analysis, Harley's urohematine, and Marcet's immediate principle, are *different expressions for one and the same mixture of substances—namely, of some of the products of decomposition by acids or ferments, under the influence of air, time, or heat, of a yellow colouring matter contained in the urine.*

Equal in importance to this fallow resin, in the appreciation of the whole subject of the colouring matter of urine, is another chemical compound, already described with great accuracy by Proust just sixty-three years ago, in the article already quoted, under the heading of "Particular Black Substance", in the following sentences.

"I have certainly caused two thousand pints of urine to evaporate, and all in silver dishes; this has put me in a position to seize certain products, which no doubt would have escaped me, had I worked upon smaller quantities. Such, for example, is the resin.

"I must now speak of a black matter, which acids separate at the same time with the resin. For a long time I have suspected that it might be a portion of the resin, which was altered in the relations of its elements, degenerated or hardened by a kind of particular over-oxidation; but I see on the other hand, that it differs from it by properties, which are too much pronounced for it not to be a substance of its own kind.

"It is a black powder, which separates from the resin when it is repeatedly washed with water.

"It is as insoluble in water as it is in alcohol, which latter purifies it from the last traces of resin.

"It dissolves with great facility in potash; but not after the manner of animal substances, which are all destroyed in it with evolution of ammonia and sulphuretted hydrogen.

"From this solution acids precipitate it in the form of a cheesy clot, which is black and voluminous; when dry it shines, and resembles asphalt.

"The resin of the urine is quite different; dissolved in potash, then precipitated by an acid, it collects as a pitch-like matter, like a kind of turpentine; its residues make a dirty colour, and infect with their odour the liquids from which it is precipitated; but nothing of the kind occurs with the black matter; its separation is complete, and it leaves the liquids white. One hundred parts of this matter so purified give by distillation water, very little ammonia, a trace of oil,

and 65 of charcoal residue, which is as much as is ordinarily yielded by common coal.

"This coal after incineration leaves much silica, but I have not sufficiently examined how much, and what the other constituents of the ash are.

"As it is soluble in the most feeble solution of potash, I have no doubt that it is so in ammonia also, which is always so abundant from the destruction of urea. But what is it? whence does it come? It is found in the urine! Is it in its primitive state? Is it a quantity of carbon of which organic assimilation has disembarrassed itself? I know nothing about it; but I was obliged to make it known, because some day one may discover relations for it—a filiation which we do not expect at the present moment."

Here, then, is a substance obtainable from every urine, fresh or stale, sweet or putrid, which, having been made known to the scientific world, has nevertheless for sixty-three years eluded the scrutiny of the most caustic analysts, and escaped the notice of the most learned authors. Every person who has read the account which Heller gives of uroxanthine, and Schunck of indian, and of their splitting up into indigo-red and indigo-blue, will of course conceive the probability of this matter being indigo. But it is not indigo. However carefully treated, it yields no crystalline sublimate of indigo-blue; it yields no aniline by dry distillation; and no volatile alkali is obtained amongst the products of its destruction by heat.

As it dissolves with a violet colour in fuming sulphuric acid, forming a sulpho-acid, the baryta-salt of which is insoluble, the substance has an analogy to indigo; but as I have never once, in many reactions, obtained any blue matter, and always have obtained the brown, I have become incredulous as regards the occurrence of indigo among the products of decomposition of colouring matter. I can certainly not explain how the chemists who found indigo failed to find the particular black matter of Proust. I would certainly be less inclined to doubt the occurrence of indigo-blue as a product of decomposition of the yellow colouring matter, had I not the perfect certainty that the red matter, or red resin, the product of the decomposition of colouring matter already discussed, and claimed by Heller, Kletzensky, Schunck, and others, as being identical with indigo-red or indirubine, has nothing in common with this substance. Indigo-red, as described by Berzelius, is insoluble in dilute acids and alkalies, and thereby differs from the resin obtained from urochrome, which is soluble in both, in particular easily soluble in alkalies. Moreover, I have never found sugar* as one of the products of the decomposition of urochrome, even when I decomposed it under circumstances where sugar, when once formed, could not have been destroyed again. In short, I was thoroughly unsuccessful in finding indian, or any of its products of decomposition, indigo-blue, indigo-red, and sugar. I was unsuccessful in finding uroxanthine, which had the property of splitting up into uroglaucine and

* Schunck's reaction for sugar in urine which had been boiled with acids, is described as follows: "If the liquid filtered from these flocks be mixed with a salt of oxide of copper and an excess of caustic soda, it becomes greenish; and if, after being filtered, it be heated for some time, it gradually deposits a tolerably large quantity of suboxide of copper." The reduction of alkaline copper solution by sugar, appears at once, and is rapidly completed. As the boiled urine required heating for some time, in order gradually to deposit suboxide, this is of itself evidence against the presence of sugar. We shall see subsequently that urine contains always a powerful reducing agent, which is not yet known any closer.

urrrhodine. But I was always successful in finding a yellow colouring matter peculiar to the urine, and, as products of its decomposition, the fallow resin of Proust, the particular black matter, and several volatile substances to be discussed hereafter.

Having thus, as I believe, put the question of the colouring matter of the urine upon its proper basis, I shall next describe the experiments, which led to the above and the following conclusions. I consider that there is one colouring matter in the urine, to which I appropriate the name of *UROCHROME*. By oxidation under the influence of air, this yellow matter passes into a red modification, corresponding to the matter hitherto described as *urerythrine*. By decomposition under the influence of acids and time, or of acids and heat, this yellow soluble matter, as well as its red modification, yield three insoluble ones: the fallow resin of Proust, or "*Uropittine*"; a resinous acid, corresponding with *omichmyl-oxide*, "*Omicholic acid*"; the particular black matter of Proust, or "*Uromelanine*"; and several volatile bodies; amongst them, perhaps, a neutral essential oil of peculiar properties, and several acids. Perhaps a fixed soluble product of decomposition remains in the mother liquor. Under any circumstances, the process of decomposition is very complicated, and will yet require considerable study before it can be satisfactorily formularised.

While I shared the impression, that evaporation of the urine destroyed the colouring matter, I employed metallic salts for its precipitation from variously prepared urine. Salts of iron, tin, copper, lead, and mercury, were thus employed upon large quantities of material, and the precipitates further treated as far as appeared expedient. Each of the experiments afforded important information. Some yielded curious reactions; the most remarkable being those of chloride of iron and nitrate of oxide of mercury. When I had found that unchanged *urochrome* could be extracted from evaporated urine by means of ether, I combined the methods of precipitation, in which lead and mercury salts had been found to be most useful, with those of extraction. But ultimately, I found it practical to prepare a concentrated extract of urine, and to precipitate the *urochrome* by lead and mercury salts, and to disregard entirely the slight change which takes place in a small portion of the *urochrome* during evaporation. For I had found that an extract of urine boiled for six hours with ten per cent. of concentrated sulphuric acid, did yet contain a large amount of unchanged *urochrome*; the colouring matter was protected from the effect of the acid by the urea in the same manner as I have shown* the *hippuric acid* to have been under the same circumstances.

MODE OF OBTAINING UROCHROME.

Prepared Urine.

The urine is made alkaline by dissolving in it fused or unslaked caustic baryta (5 grammes to 1 litre) contained in a little sieve suspended in the fluid. When the fluid, after brisk stirring, remains strongly alkaline, it is treated with a saturated solution of acetate of baryta, as long as a precipitate is thereby produced. The deposit is allowed to settle during twelve hours, after which the fluid is filtered.

Urine thus prepared is free from phosphoric, sulphuric, and carbonic acids, and has lost a portion of its uric acid. It contains free alkali, combined acetic acid, and a little baryta, besides the other ingredients of urine. Nitrate or chloride of barium should not be employed in this preparation, as the presence of nitric acid and an augmentation of chlorides, interfere with subsequent operations for the isolation of *urochrome* by means of mercury-salts.

Limed Urine.

The urine is treated with unslaked lime in the form of powder, or slaked lime in the form of dry powder, until it presents a strongly alkaline reaction; after twelve hours standing, it is filtered.

Urine thus treated contains no phosphoric acid; if it do give a precipitate with chloride of calcium, enough lime has not been added. It contains free alkali, particularly lime, and is in a fair state of preparation for the application of lead-salts.

Prepared Extract of Urine.

The urine is evaporated over the free fire to one-tenth, and filtered from the phosphates, urates, and gypsum. It is then slowly evaporated on the sand-bath, until a pellicle of urates forms. After cooling and filtering, it is evaporated on the water-bath to a syrup, and allowed to cool slowly with the water-bath, to effect a good crystallisation of chloride of sodium, urea, and other salts. The decanted syrup, if necessary, mixed with a little water, is treated with best calcined magnesia, until alkaline, and until a filtered sample, treated with acetic acid and chloride of iron, does not give any immediate precipitate. The extract is then filtered. An extract thus prepared, contains neither phosphoric nor uric acid, a diminished amount of sulphates, and a small amount only of chlorides. It contains free alkali, particularly ammonia and creatinine, but no magnesia. It is, therefore, particularly suitable for the application of lead-salts.

Mode of Obtaining Urochrome from Inspissated Urine by Ether.

Fresh urine is evaporated on the water-bath, treated with a little hydrochloric acid in the cold, and extracted with ether. The ethereal solution, on distillation of the ether, leaves a residue which deposits *hippuric acid* on mixing with water and standing. The acid is separated by the filter, and the washings are united with the filtrate, whereby a deposit of resin is produced. This is allowed to subside, and the fluid is separated again by filtration or simple decantation, the resin adhering mostly to the walls of the glass vessel. The fluid now represents a beautiful bright saturated golden-yellow solution, having a strong odour of phenylic acid, mixed with a reminiscence of the ether. It contains *hippuric acid*, *phenylic acid*, and a trace of hydrochloric acid, besides *urochrome*, which constitutes the greater part of its ingredients. A solution of basic acetate of lead is now added to this fluid; at first, this causes only a turbidity, and no permanent precipitate is produced. It is mostly only after large quantities of basic acetate solution have been added, that a permanent bay-coloured, or Naples yellow, flaky precipitate of considerable bulk is produced. When the fluid is almost colourless, and the acetate produces no further precipitate, all the *urochrome* is in insoluble combination with lead. This precipitate is now collected on a

* In my "Researches on the Physiological Variations of the Quantity of Hippuric Acid in Human Urine."—*Journal of the Chemical Society*, February 1864, ser. 2, vol. ii, p. 55, *et seq.*

filter, and washed. It is well disposed towards this operation, which must, therefore, be performed thoroughly. It may even be removed from the filter and boiled with water, and filtered while hot; for too much care cannot be taken to remove chlorides, which adhere with great pertinacity to all compounds of urochrome.

This compound of lead and urochrome may be suspended in water, and decomposed by sulphuretted hydrogen, when a yellow watery solution of urochrome is obtained, after boiling with the sulphide, cooling and filtering free from any gross impurities, but still mixed with a trace of phenylic acid.

To free it from these slight impurities, the lead compound of urochrome, instead of being decomposed by sulphuretted hydrogen, which requires subsequent boiling to remove the excess of this agent, is decomposed by the cautious addition of dilute sulphuric acid in the cold, while the salt is triturated in a mortar. A slight excess of the acid is useful to decompose the salt entirely, but is immediately neutralised by the addition of some carbonate of baryta. The yellow solution is now separated by filtration, treated with baryta-water until alkaline, and then treated with carbonic acid until neutral, and allowed to stand. By this treatment all resin is removed from the solution, which is now purely yellow.

To this solution, pure neutral acetate of mercury is now added, as long as a precipitate is thereby produced; the limit has to be ascertained by repeated filtration of small samples. When this point is attained, the bay-coloured precipitate is separated by the filter, washed with cold water, removed from the filter, triturated in a mortar with water, and again placed upon the filter, and washed until the filtrates are neutral and free from impurities. In case the precipitate change its colour to dark or grey, it must be decomposed by sulphuretted hydrogen in water, and again treated with baryta and acetate of mercury. When the mercury-compound on washing retains a bay-colour, it is free from resin, calomel, and phenylic acid; and, on decomposition in water in a flask, in a current of sulphuretted hydrogen, will yield a yellow solution of pure urochrome, which, after the removal of the sulphuretted hydrogen by boiling in a current of hydrogen, cooling, and filtering, is clear and bright.

Mode of obtaining Urochrome by means of Lead-salts directly from Urine.

Prepared or limed urine is treated with neutral acetate of lead until an excess of it ceases to react. The precipitate is collected on a filter, and washed. The filtrate, without the washing water, is treated with basic acetate of lead until it ceases to react. The precipitate is filtered off. To the filtrate some ammonia is added, and the precipitate filtered off. Each of these precipitates is now separately treated as follows.

It is placed in a mortar, and triturated with dilute cold sulphuric acid, cautiously added, until a sample filtrate shows an excess of sulphuric acid when treated with baryta-solution and nitric acid. To the filtrate from the lead-sulphate, carbonate of baryta is added, to remove excess of sulphuric acid; and, after careful mixing without employment of heat, and some standing, during which the baryta salts settle well in the yellow fluid, the latter is separated by filtration. The filtrate is made alkaline

with baryta-water, treated with carbonic acid, and filtered again. It is now precipitated with acetate of mercury; and the urochrome-mercury is washed completely with cold and boiling water, until clean and neutral. It must remain of a bay-colour, and not become grey or dark. If it do so, it must again be decomposed by hydrothion, and the urochrome retransformed into lead-salt by precipitation with neutral acetate and ammonia; the precipitate, after washing, decomposing with sulphuric acid, treatment with baryta as described, and filtration, yields a fluid from which acetate of mercury precipitates pure urochrome-mercury.

The three lead-precipitates from urine by this treatment yield one and the same colouring matter. They need not, therefore, be treated separately, unless to do so offers a convenience or a satisfaction to the inquirer. The urine may be precipitated in one operation by neutral acetate of lead and ammonia, until colourless. The employment of hot urine and hot reagents condenses the bulky precipitate, and makes it more manageable. The employment of heat after precipitation has the same effect, but is less convenient. Large quantities of materials and large sized vessels and utensils are requisite to obtain a notable quantity of product.

Prepared Urine and Corrosive Sublimate.

To prepared urine a saturated solution of corrosive sublimate was added, until it ceased to produce any precipitate. The white deposit was isolated, washed on the filter, transferred into boiling water, again filtered, and then decomposed in spirit of wine by hydrothion. The yellow filtrate was allowed to stand for six days exposed to the air, to deposit sulphur; and, after a second filtration, tested.

Boiling did not change its yellow colour. The addition of hydrochloric acid made the colour dark. Nitrate of mercury produced a pale flesh-coloured precipitate, which became a little redder on boiling. The fluid, shortly after boiling, became turbid, and deposited a precipitate. (Reaction for urochrome.) Acetate of mercury caused a copious precipitate, entirely soluble in nitric acid, leaving a yellow solution. (Reaction for urochrome.)

Nitrate of silver caused a white copious deposit, which was not entirely soluble in nitric acid, but left some chloride of silver.

Acetate of lead caused a copious white precipitate, shrinking on boiling, and yielding chloride to the water. Acetate of lead and ammonia precipitated the whole of the colouring matter, leaving a colourless fluid.

Evaporation of the filtrate left a brown residue (decomposed urochrome, insoluble in water), and some soluble matters, amongst them chloride of ammonium.

The precipitate by sublimate in prepared urine, therefore, contained urochrome, hydrochloric acid, and ammonia, which passed into the alcoholic solution. With the sulphide of mercury, there remained uric acid and xanthine, and probably much urochrome.

The alcoholic solution was now treated with acetate of lead, and a little ammonia to alkalinity. The copious yellow precipitate was removed and washed; the filtrate was colourless. The precipitate was transferred from the filter to a mortar, and carefully mixed with dilute sulphuric acid, added in slight ex-

cess. When the sulphate of lead was settled, the yellow solution was filtered. This solution was now treated with carbonate of baryta until neutral and free from sulphuric acid, and, after standing, filtered. To the solution, acetate of mercury was added as long as a precipitate was produced. The mixture was heated, the precipitate well washed with water, boiled in water, and again washed. There remained a yellow or fawn-coloured compound of urochrome-mercury, which, after decomposition by hydrothion in little water, with a little spirit added, yielded a yellow solution of urochrome. Hydrothion was driven off by boiling. The solution then presented all the characters and tests of a solution of pure urochrome.

The urine filtered from the sublimate precipitate was of a paler colour, and rather greenish; on standing, it deposited grey flakes of a compound containing reduced mercury. When this reducing action was completed (probably due to phenylic and cresylic alcohol), the fluid was filtered again. It was now treated with acetate of lead and ammonia, which produced a yellow precipitate, leaving a colourless fluid. The washed lead-compound decomposed by sulphuric acid, treated with carbonate of baryta, as above described, and ultimately with acetate of mercury, yielded, after decomposition of the urochrome-mercury, a solution of pure urochrome.

The urine, before the application of the lead, had yielded the pink reaction with nitrate of mercury. The urochrome did not yield the pink reaction. Consequently, the pink reaction was due to a body remaining in the filtrate from the lead-compound. This filtrate was evaporated to a small bulk, and remained colourless almost to the last, when it assumed a slight amber tint. It did not yield the pink reaction with nitrate of mercury any longer. The body of which it was a sign had been volatilised.

Urochrome from Extract of Urine boiled during many hours with Sulphuric Acid.

Prepared extract of urine was treated with concentrated sulphuric acid, added drop by drop under agitation. The slight precipitate which formed (gypsum) was removed, and the fluid put into a retort on a sand-bath connected with a condenser. Distillation was now proceeded with, and carried on for six hours, the water being occasionally replaced, and more acid added, in the form of dilute sulphuric acid.

There remained in the retort a dark red fluid, mixed with flakes of resin and brown granular matter. The fluid was decanted and filtered, and, on standing, deposited crystals of hippuric acid. This circumstance showed that the action of the acid had been directed mostly against the urea and a portion of the urochrome, and that some urochrome might be undecomposed. The fluid was therefore evaporated on the water-bath until it filled with crystals of sulphate of ammonia, soda, and other matters. The mixture was extracted with spirits of 56 over proof, and the dark brown solution again evaporated, until it formed a second crystallisation. This was again extracted with spirit, and the spirit was driven off. A syrup remained, crystallising but sparingly. A third extraction left an uncrystallisable syrup.

This syrup, diluted with water, was treated with carbonate of baryta, and boiled until all sulphuric

acid was precipitated. It was next precipitated with acetate of lead in slight excess, a few drops of ammonia being added. A copious precipitate of urochrome-lead, with some carbonate and oxide of lead, ensued. This was washed and boiled in water, decomposed by sulphuric acid, the excess of acid removed by carbonate of baryta, and the solution of urochrome precipitated with acetate of mercury. The urochrome-mercury, purified by boiling in water, was suspended in water with a little spirit, and decomposed by hydrothion. After standing and filtering, a solution of urochrome was obtained, having all the properties of urochrome obtained from fresh urine and fresh extracts. The syrup, after boiling with baryta-carbonate, had a neutral reaction, contained baryta in solution, yielded an immediate copious precipitate of nitrate of urea with nitric acid, a slight precipitate with chloride of zinc and spirit, and a copious one with corrosive sublimate. This latter probably contains a product of decomposition of urochrome, and deserves further investigation. The distillate from the retort was acid, and had a fearful odour. It was neutralised with carbonate of soda, and evaporated to a smaller bulk. When it was perceived to evolve a strong urinous smell, the evaporation was interrupted. It was now extracted with repeated quantities of ether; the ether was evaporated or distilled off at a gentle heat in the water-bath, when an *essential oil* was obtained as a residue, which had a powerful odour, a yellowish colour, became milky on mixing with water; on being heated with nitrate of mercury, gave a splendid purple reaction, and did not change the solution of silver even on boiling. It consequently contained no phenylic or cresylic alcohol, and was evidently an oil of peculiar properties—the *smelling principle of urine*. In the sequel, it will be termed THE VOLATILE OIL OF URINE.

Purification of Urochrome.

However great the care may have been with which the mercury-compound (by acetate) of urochrome has been washed, the solution of the colouring matter obtained by decomposing the salt with sulphuretted hydrogen will always be found to contain some hydrochloric and acetic acids. The former can be entirely removed by shaking the solution with freshly prepared oxide of silver. But the silver combines with a great part of the urochrome, forming a bulky precipitate; while the urochrome which remains in solution also contains a considerable amount of acetate of silver in solution.

This solution is yellow, but much brighter than before treatment with silver oxide. It has an alkaline reaction, yields no precipitate with nitrate of silver, but a precipitate with nitrate of mercury (which becomes red on boiling, as does also the fluid in which it is suspended), and a precipitate with acetate of mercury. It is freed from silver by hydrothion, and the filtrate evaporated to dryness on the water-bath. Pure urochrome now remains, in the form of an uncrystallisable yellow solid matter.

Physical and Chemical Properties.

On evaporation of a pure and neutral solution of urochrome, it remains in the form of yellow crusts. They are, however, not entirely re-soluble in water.

It is easily soluble with a purely yellow colour in

water, least in alcohol,* more in ether, very dilute mineral acids, and alkalis.

Its watery solution, on standing, even when precluded from contact with air, assumes a darker colour verging towards red, and becomes red at last. It next becomes turbid, and deposits flakes of resinous matter. This decomposition is effected more quickly by the agency of heat.

When a yellow, somewhat acid, watery solution of urochrome, such as can be obtained without the employment of oxide of silver, is evaporated in the open air on the water-bath, it becomes covered with a red film of resinous matter. The fluid, on cooling, becomes turbid; but ultimately is cleared up by the deposition of more resin, which was dissolved in the hot fluid.

The same acid watery solution, evaporated in a retort in a current of hydrogen, is decomposed. Resin is formed, which is dissolved in the acid liquid with a red colour; but falls down on cooling in the form of flakes. These under the microscope are seen to be composed of red non-crystalline granules. A highly acid, clear, colourless, stinking distillate, passes into the receiver. The ultimate residue in the retort is syrupy, and remains so when repeatedly treated with water to remove resin, and re-distilled in the hydrogen current.

Acids effect a similar transformation by mere contact, immediately by boiling. Hydrochloric acid immediately precipitates resin by boiling; but retains in solution much resin, of which the greater part is precipitated by the addition of water. Dilute nitric acid also effects this decomposition; but the solution cannot be concentrated, as can the hydrochloric acid solution. The hydrochloric acid solution, after evaporation of most acid and neutralisation of residue, does not yield the reaction for sugar with an alkaline solution of copper. Boiled with caustic potash and a little oxide of lead in solution, urochrome apparently undergoes no change; no sulphide of lead is deposited.

From its watery solution, urochrome is precipitated by nitrate of silver as a gelatinous mass, entirely soluble in nitric acid; neutral acetate of lead throws down a white and flaky precipitate; basic acetate of lead, a bay or yellow coloured flaky precipitate. Acetate of mercury produces a yellow fawn precipitate. Precipitation by this reagent is complete from neutral solutions. Nitrate of mercury produces a white precipitate; which, after boiling, becomes pale flesh-coloured; it is entirely soluble in nitric acid. The supernatant fluid assumes a pink colour.

Decompositions. (Red Resin, Uropittine, Omicholic Acid.)

When an acid watery solution of urochrome, or a mixture of urochrome and of a quantity of mineral acid, is boiled for a sufficient length of time, the fluid assumes a dark red or brown colour, and drops

of resin are seen in it. On the addition of water, or on cooling and standing, it deposits red or brown flocks, which can be worked into a lump by kneading with a glass rod, and removed. By repeating the boiling of the mother liquor, some more resin can be obtained; but the subsequent portions show already, by their black colour, that they are not so pure as the first portions.

When this resin is allowed to stand under water or kneaded with a glass rod, a brown powder separates from it, which is evidently mechanically mixed with it. (Uromelanine.) This powder also remains undissolved, when the resin is extracted with alcohol. Boiling alcohol dissolves a little of this brown powder, and deposits it again on cooling. If, therefore, the resin is dissolved in boiling alcohol and filtered hot, the solution has to be filtered a second time after standing for twenty-four hours.

The alcoholic solution of the resin thus obtained is of a magnificent ruby red colour. The addition of water causes at first an opacity in it; soon, however, the greater part of the resin is deposited in the form of reddish-brown flocks. If the alcoholic solution is evaporated to a high state of concentration and poured into cold water, the resin is deposited in a granular form. If the coloured liquid is now filtered off, and the granules are agitated in hot water, they again coalesce into the original soft red resin. On drying and standing, the resin becomes hard and brittle. In hot water, it becomes adhesive, like tar, and dissolves a little. It has a peculiar powerful smell, which is evidently the basis of the smell of castoreum. Heated on platinum, it fuses; and under boiling emits a powerful and disagreeable odour. It next burns with a strongly lighting flame, and is ultimately entirely consumed.

Purification of Red Resin.

The red resin was purified by fusing it repeatedly in hot water, until, on the addition of cold water, it became immediately hard and brittle. It was next dissolved in a small quantity of hot alcohol; and this solution, after filtration, thrown into a large quantity of cold water. The red powder which was deposited was washed in the filter, and dried *in vacuo* over sulphuric acid. [It was found to be a mixture of substances, which were separated as follows.

Separation of Uropittine from Omicholic Acid.

The dry resin was extracted with ether, as long as this agent dissolved anything. The ethereal solution contained all the omicholic acid; the residue, constituting the main bulk of the red resin originally employed, contained all the uropittine.

Uropittine.

When the residue, which was quite insoluble in ether, was boiled with absolute alcohol, it mostly dissolved, and, on cooling, deposited a brownish-yellow matter in crystalline granules. (The crystals are probably minute microscopic rhombic octohedra.) These granules, after repeated re-crystallisation from absolute alcohol, in which they were ultimately sparingly but entirely soluble, represented a pure well defined chemical body, to which, as being apparently of neutral properties, but having the main peculiarity of the red resin—namely, that of fusing by heat—the name of uropittine was given. It was subjected

* After I had ascertained this peculiarity of urochrome, I ceased, of course, to employ any alcohol in its preparation. Wherever, therefore, in the course of the above experiments precipitates of urochrome with metallic salts have been decomposed in alcohol or spirit of wine, I should now under the same circumstances employ water; the disadvantages of the higher boiling point of water, to which the solution must be exposed for the expulsion of sulphuretted hydrogen, notwithstanding. This slight solubility in alcohol of urochrome also explains in a measure the scanty success of former researches for its isolation; in almost all of them, alcohol was employed at one or other of the stages of the proceeding, and, instead of being extracted, the urochrome was left behind.

to elementary analysis, and yielded the following results.

First Analysis.

The substance was dried under the air-pump.
 Taken for analysis 0.2884 grammes.
 Carbonic acid found 0.5786 "
 Equal to 55.13 per cent. of carbon.
 Water found 0.1503 "
 Equal to 5.87 per cent. of hydrogen.

Second Analysis.

Substance taken for analysis... 0.3520 "
 Carbonic acid found..... 0.7170 "
 Equal to 55.46 per cent. of carbon.
 Water found 0.1677 "
 Corresponding to 5.28 per cent. of hydrogen.

Third Analysis: Determination of the Nitrogen.

Substance taken 0.3157 "
 Combustion-tube, before and after combustion,
 cleared by means of carbonic acid gas current.
 Volume of the nitrogen gas ob-
 tained 32.5 cub. centim.
 Temperature 15° centigrade
 Barometer, 30.08 inches = 764.03 millimètres.
 Quantity of nitrogen, by weight 0.0382 grammes.
 Equal to 12.10 per cent. of the substance taken.

At the beginning of the combustion, the glass-tube broke, and the mixture of substance and oxide of copper, traces of the uropittine being perhaps already decomposed, had to be transferred into another glass tube, in which the analysis was completed. A portion of the nitrogen may, therefore, have been lost.

The above elementary analyses lead to the formula $C^{18}H^{10}N^2O^6$ as representing the composition of uropittine.

	Found.			$C^{18}H^{10}N^2O^6$	Calculated.
C	55.13		55.46		55.67
H	5.87		5.28		5.15
N	—		—		12.10
O	—		—		14.43
					24.74
					99.99

The above formula for uropittine may be considered as hippuric acid, in which one equivalent of hydrogen is replaced by amide (NH^2).

Hippuric acid, $C^{18}H^9NO^6$

Uropittine, $C^{18}H^9(NH^2)NO^6 = C^{18}H^{10}N^2O^6$

The true hippur-amide being known, and not corresponding in its properties with uropittine, we have to consider the latter body only as isomeric with hippur-amide.

Omicolic Acid.

The ethereal solution of this body, as obtained by extraction from the mixture of substances termed red resin, is of a bright port-wine red colour, and a peculiar, independently of the ether, penetrating odour. On spontaneous evaporation of the ether, the acid remains as a syrup; ultimately, as a resinous hard residue, in the body of which no crystallisation is to be perceived. It is easily soluble in absolute alcohol; and this solution, when poured in water, deposits nearly the whole of the acid as a flaky red precipitate.

When its ethereal solution is allowed to evaporate spontaneously, a few needle-shaped crystals are deposited at the highest points reached by the ether. They are probably another body, and not omicolic

acid (possibly benzoic acid; the resin from putrid urine always contains some benzoic acid.)

The quantity of this acid was so small, that no analysis or other experiments could be advantageously undertaken with it.*]

Black or Brown Matter—Uromelanine.

The matter which remains undissolved when the red resin is dissolved in alcohol, is washed repeatedly with alcohol, dissolved in caustic potash, precipitated by acetic acid, and again washed with alcohol. A powder is thus obtained; which may be black, or brown, or even have a violet tinge, according to its state of subdivision.

It is soluble in acetic acid, more in hot than in cold; and from this solution, nitrate of mercury precipitates a red matter.

On dry distillation, it gives out white fumes, which condense to an oil; but no sublimate of any kind is obtained. The fumes, or oil, are neutral, bleaching, rather than changing like acid or alkali, the colour of litmus. They give no aniline reaction. But, with nitrate of mercury, they give an exquisite red reaction and precipitate. A very bulky, dense charcoal of the bulk of the original particles remains.

Nitric acid dissolves the matter without effervescence. After evaporation, two matters are left—one soluble in alcohol, the other insoluble. The solution gives the red reaction with nitrate of mercury.

Concentrated fuming sulphuric acid easily dissolves the black matter, forming a red or purple solution. Immediate addition of water precipitates all coloured matter, and the liquid is white and clear. But when the concentrated mixture is allowed to stand overnight, water precipitates a coloured portion, another coloured portion remaining dissolved. Carbonate of baryta precipitates all coloured matter on boiling, and the filtrate is again white. Consequently, the baryta-salt of the sulpho-acid is insoluble in water.

[The recently precipitated body, well washed, suspended in water, and subjected to the action of chlorine, yields a brownish substance, soluble in boiling alcohol. While the spirit is being heated, some of the substance melts to a brown resin, which dissolves by continued heating with new alcohol. The solutions, added together, yield, on cooling, yellowish-red amorphous flakes. These flakes are not again entirely soluble in alcohol, probably because the chloro-compound undergoes a change during the heating with the alcohol. This supposition was further confirmed by the results of an elementary analysis of the chloro-compound.

Elementary Composition of Uromelanine.

First Analysis.

Substance taken for combustion 0.2862 grammes
 Carbonic acid obtained..... 0.5985 "
 Representing 0.1632 carbon, or 57.02 per cent.
 Water obtained 0.1452 "
 Corresponding to 0.0160 hydrogen, or 5.59 per cent.

Second Analysis: Nitrogen Determination.

Substance taken 0.2765 "
 Volume of nitrogen obtained... 30.0 cub. centim.
 Temperature 175° centigrade.
 Barometer, 30.16 inches = 766.06 millimètres.
 Weight of nitrogen obtained... 0.03501 grammes
 Equal to 12.66 per cent.

* The brackets denote passages which have been added by the author since the prize was awarded.

These analyses yield the formula $C^{12}H^7NO^4$.

	Found.	Calculated.
C	57.02	57.6
H	5.59	5.5
N	12.60	11.2

The endeavour to control these analyses by repetition, and to determine the equivalent of uromelanine, had to be deferred on account of the necessity of printing the Essay. It is probable that the formula above given may have to be multiplied. Uromelanine presents a certain analogy to the green colouring matter of bile, cholochloine, the composition of which, according to my latest analysis, is $C^{36}H^{18}N^2O^{10}$. If the formula of uromelanine be trebled thus: $3(C^{12}H^7NO^4) = C^{36}H^{18}N^2O^{12}$ —a formula is obtained, which may be considered as cholochloine, in which one atom of hydrogen is replaced by amide (NH^2), and to which two atoms of water have been added.

The formula and properties of uromelanine differ so widely from those of indigo-blue ($C^{16}H^6NO^2$), as to require no further comment.]

Mode of Obtaining Uropittine and Uromelanine from Urine directly.

The mixture of resin and black matter can be obtained from urine in the following manner.

(a) *From Fresh Urine.* A quantity of prepared extract of urine is put into a capacious beaker, and mixed with concentrated sulphuric acid, added, drop by drop, while the fluid is being agitated. A trace of xanthine [a little uromelanine, and much gypsum, are] precipitated in flocks. After filtration, the fluid is diluted with water, and distilled in a capacious retort. When the fluid has been reduced to one-half, the black resin will be seen adhering to the sides of the glass, and to the platinum which it is well to put into the retort to prevent bumping. The boiling is now interrupted, and the fluid allowed to cool. Fluid and resin are separated by decantation or filtration. The particles of resin are united by fusion in hot water. They are then washed, dried, and the resin is extracted from the black matter by solution in alcohol.

(b) *From Putrid Urine.* Putrid urine is treated with a little lime in powder, or saw-dust, and filtered. The dark brown filtrate is evaporated in an open dish over the free fire. Although it soon assumes a strongly acid reaction, nothing but ammonia passes away. All froth which rises during the evaporation is carefully skimmed off. When black particles begin to appear on the surface, it is allowed to cool, filtered, put into a retort, mixed with dilute sulphuric acid, and distilled. A mixture of hydrochloric, benzoic, acetic, and another acid, passes over, together with a stinking matter, which deposits in flakes when the distillate is left to stand. The residue in the retort soon deposits the resin as a soft tar on the surface of the fluid, the sides of the vessel, and the platinum, put into the fluid for safe boiling. The resin is separated mechanically and by the filter, washed, and separated into its constituents by alcohol. The uropittine and uromelanine thus obtained present the same essential properties as those obtained from fresh urine.

The mixed resin obtained from putrid urine has some peculiarities by which it is distinguished from the resin obtained from fresh urine.

It has the smell of asphaltum when fresh, mixed with that of castoreum. Its taste is slightly bitter, and highly nauseous. It does not dissolve in the saliva to any great extent.

The black matter, after separation from the resin by alcohol, and purification by precipitation from potash, falls down as a bulky deposit, but shrinks on drying. It ultimately becomes hard, black, and shining, and breaks like asphalt. It differs, therefore, in its physical appearance, from the uromelanine obtained from fresh urine.

The resin, or uropittine, from putrid urine, is also peculiar in this, that it is much darker than the resin from fresh urine, and contains some benzoic acid, which is never present in the resin from fresh urine. From this it can be separated by precipitating its alkaline solution by acid in hot water; collecting the resin, by kneading; and decanting the liquor, which contains the benzoic acid and some resin in solution.

RESULTS AND THESES.

1. The colouring matter of the urine, to which the name of urochrome is given, is one of the most interesting compounds in the list of organic and physiological substances.

2. It can be isolated in a pure state; and then is yellow, easily soluble in water, less in ether, and least in alcohol.

3. When its quantity [in equal bulks of solution] is increased, its colour still remains purely yellow. Consequently, the hypothesis of Vogel, that the urine of healthy and sick persons becomes darker in proportion to the increase of colouring matter, is fallacious.

4. Under various processes of decomposition, urochrome yields a red resin, consisting mainly of uropittine [having the elementary composition $C^{18}H^{10}N^2O^6$, and of omicholic acid, mixed with small quantities of undetermined matters], black matter, uromelanine [having the elementary composition $C^{12}H^7NO^4$], and other products.

5. By a simple process, probably of oxidation, urochrome passes into a red colouring matter, uerythrine, which [sometimes] colours red the urine of disease, and any deposits of urates contained in it. Frequently, this oxidation is only effected after emission. The red colour may also be due to omicholic acid, which is a little soluble in ammoniacal salts.

6. The foetor of decomposed acid or alkaline urine is due to the uropittine and omicholic acid, and substances derived from them. It may be increased by, but is not primarily due to, carbonate of ammonia.

7. The urine of man contains an essential oil, which is volatile; has a strong peculiar odour; a curious reaction with iron chloride; and yields a diagnostic pink reaction with nitrate of mercury on boiling.*

8. Human urine also contains cresylic alcohol, which is obtained, along with urochrome, by one of the processes for its isolation.

9. One of the principal features of uræmia is the retention in the blood of urochrome. It is there de-

* This reaction is always obtained at the end of Liebig's quantitative analysis for urea, and has excited the curiosity of almost every inquirer who used that method. I have successfully used the appearance of the first trace of pink after the disappearance of all greenish yellow colour, as the sign that all urea was precipitated and excess of mercury solution present. The soda-test always confirmed the conclusion derived from the pink colour.

composed, and yields uropittine and omicholic acid, which, circulating in the blood, vitiate all tissues, can be found in the crusts on the teeth, and their smell can be perceived in the breath and the perspiration.

10. When colouring matter is retained, the typhoid symptoms of uræmia are prominent. The treatment with acids, under those circumstances, has to be set aside, as promoting the retention of the poisonous uropittine and omicholic acid, and a treatment with alkalis has to be substituted. The skin must be purified by washing, and repeated perspiration induced, until the effluvia do not any longer smell of uropittine.

[11. From healthy human urine, neither indican, nor uroxanthine, nor any other substance yielding by decomposition with acids indigo-red and indigo-blue, can be extracted; neither does it yield indigo-red or indigo-blue by boiling with acids.]

12. Urochrome has no immediately apparent relation to the colouring matters of the blood or the bile. It is a derivate of albuminous matter, and the most essentially characterising ingredient of urine.

Illustrations OF HOSPITAL PRACTICE: METROPOLITAN AND PROVINCIAL.

LIVERPOOL NORTHERN HOSPITAL.

TWO CASES OF AMPUTATION AT THE SHOULDER-JOINT.

By HENRY LOWNDES, Esq., Junior Surgeon to the Hospital.

CASE I. William Myers, aged 36, a carter, was admitted into the Northern Hospital on June 16th, 1864, at 8 P.M., with the following injuries, inflicted by a horse. The right humerus was fractured in the upper third; there was a deep lacerated wound in the front, and another still more extensive in the back of the arm; and the hand could be passed freely through them both down to the fracture and up towards the axilla, where the end of the lower fragment lay. The pulse could be felt at the wrist; the elbow was much contused; the bone was not much comminuted, but one or two small loose pieces could be felt. There was a large contusion over the right scapula and the right side of the chest. He was a good deal collapsed. He said he had been attacked in the stable by an entire horse of which he had charge. It appears to have first seized him by the arm with its teeth, and then to have got him down and kicked him. The soft parts of the arm were so much injured, that my colleague, Mr. Hakes, agreed with me that amputation was necessary; and at 10 P.M., I removed the limb at the shoulder-joint. Mr. Hakes was able to compress the axillary artery very securely, by passing a finger into one of the lacerations. I was barely able to save sound skin enough to cover the wound. The brachial and two other vessels were tied with ligatures, and a vein that was troublesome was secured with an acupuncture needle and wire. He was directed to have brandy freely given through the night. He had thirty drops of laudanum on admission.

June 17th, 11 A.M. He was heavy and had slept a good deal; his breathing was much oppressed, and mucous riles were heard in the trachea and bronchi. The pupils were contracted. Emphysema had appeared on the right side of the chest, and extended upwards on the neck. Pulse 120; tongue dry and furred. He was ordered to have a strengthening plaster applied over the right side of the chest from the sternum to the spine.

R Ammonia carbon. ʒj; spiritus ætheris sulph. comp. ʒij; aqua ad ʒviij. M. Sumat 3ss alternis horis.

He was ordered to have an ounce of brandy every two hours; also, ice and lemonade. He had passed urine; had been rather delirious.

9 P.M. He was much the same, but not delirious.

June 18th, noon. The emphysema had extended to the left side. His breathing was still much oppressed; tongue moist; pulse 120.

June 19th, 12.30 P.M. I dressed the stump, which looked well; there was a free discharge of serum. Wet lint was applied. He was ordered to have an enema of castor oil. At 5 P.M., he was seized with a violent pain on the right side, with great distress in breathing; pulse 134; respiration 56. Hot fomentations were ordered, and brandy every hour. At 9 P.M., the pain was a good deal less severe; pulse 120, sharp and hard; the face was flushed; the skin was hot; the bowels had been moved. He was directed to take a grain of powdered opium every four hours; to continue the brandy every two hours; to take beef-tea in small quantities frequently; to have turpentine applied to the side, and the stump poulticed.

June 20th, noon. He was decidedly better; pulse 108; respiration, 40; tongue much furred; skin cooler and moist. The stump was rather inflamed. He had slept a little. 9 P.M. He was still improving; and had taken a little bread and milk.

June 21st. Pulse 108, rather weak; tongue dry and brown. His breathing was still oppressed. A friction-sound was distinctly audible on the right side. The face was congested; the neck still rather emphysematous. He had slight delirium. The stump was suppurating freely, but sloughy in parts. He was ordered to omit the opium, and continue the ammonia and ether. A small blister was applied over the seat of pain; and half a grain of muriate of morphia was given at bedtime.

June 22nd. Respirations 44; pulse 120. He had slight delirium. The skin was hot; the tongue brown and dry. He had not slept well. An ounce of brandy was ordered to be given every hour.

June 23rd, 11 A.M. Pulse 132; respirations 32. He had slept better. There was great discharge from the stump. His respiration was very easy and natural; appetite better. In the evening, he seemed still a little better; but, about 3 A.M. on the 24th, the stump began to bleed; and, before this was found out, a very great quantity of blood was lost, and the patient died about a quarter of an hour after the house-surgeon had reached him.

POST MORTEM EXAMINATION, thirty hours after death. (From notes by Dr. Roberts, Junior House-Surgeon.) The stump was very sloughy. The axillary artery was found firmly ligatured and appeared quite healthy; it contained a firm clot, partially decolourised and undergoing organisation; the inner coat of the artery was tinged with the absorbed colouring matter. The exact source of the bleeding was not determined; but it came from the neighbourhood of the posterior circumflex artery, which lay in a mass of bloody slough. There was fracture of the third, fourth, fifth, and sixth ribs, on the right side. The broken ends of the fifth projected inwards, and