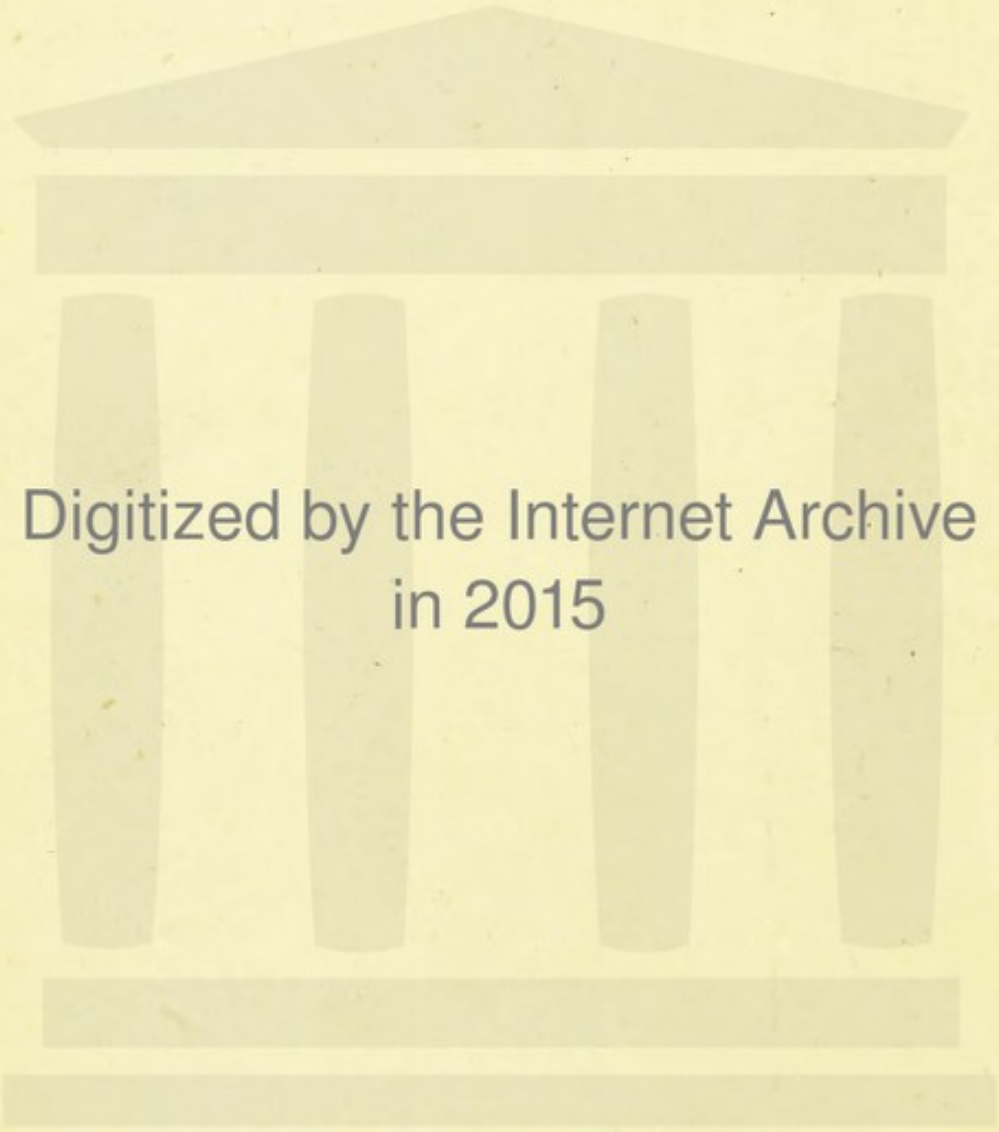
The progress of medical chemistry, comprising its application to Physiology, Pathology, and the Practice of Medicine / by J.L.W. Thudichum.

THE PROGRESS OF MEDICAL CHEMISTRY

THUDICHUM



THE PROGRESS OF

MEDICAL CHEMISTRY,

COMPRISING ITS APPLICATION TO

PHYSIOLOGY, PATHOLOGY, AND THE PRACTICE OF MEDICINE.

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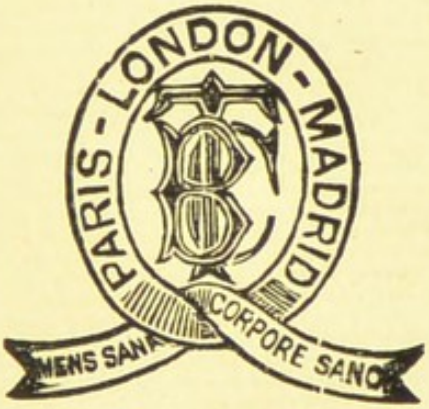

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PREFACE.

THE seventeen chapters of this volume are reproductions of a similar number of articles which were published from time to time in the *Medical Press*, mainly during 1895. They are here renewed in compliance with the wish of some friends of the science to have the matter accessible in a compendious form. This sympathy heightened the author's own wish in the matter, and induced the publishers to generously take the risk of the venture.

The most pleasing feature of the progress made during the last decade is the rise in this country of a new generation of investigators, who, by their originality and independence, have at once obtained a well-deserved success. Another phase of progress consists in the elimination of a large amount of fallacy which has disfigured and made insecure, particularly physiological chemistry. The greatest share in the production of this decadence undoubtedly belongs to the institution which was under the direction of the late Professor F. Hoppe-Seyler. This falling off was so well recognised in Germany, that during the last ten years, both his lecture room and laboratories were practically deserted.

The contents of several chapters of this volume were published by me in German as independent essays in the *Journal für Practische Chemie*, during 1895, in order that the matter might become better known in the wider chemical field. I am assured through published reports and private references that this object has been attained in a satisfactory measure.

All the critical observations which I have been obliged to make are based upon, and controlled by, original researches instituted by myself, and these have enabled me in several instances to make additions to the knowledge previously possessed. Chapters xiii and xiv are the most demonstrative proof of this assertion.

In conclusion, I recommend this little volume to the kind consideration of the profession.

11 Pembroke Gardens,
London, W., *July* 1896.

THE PROGRESS OF CHEMISTRY

IN ITS APPLICATION TO

PHYSIOLOGY, PATHOLOGY, AND THE PRACTICE OF MEDICINE.

I.—INTRODUCTORY OBSERVATIONS.

1. *The Rise of Specialism Limited.*—During the last twenty-five years the great chemical laboratories of universities and medical schools, and their leading professors, a large proportion of whom formerly were the principal promoters of medical chemistry, have furnished no contributions at all, either direct or indirect, to this branch of science. This was in so far natural, as ordinary organic chemistry so-called, free from any application to any particular science, gave them much better opportunities for successful research, while the pursuit of animal chemistry was much more difficult and quite unremunerative. Another cause of their turning their back to biological chemistry was the origination of a specialism by pathologists and physiologists, who, feeling themselves unequal to either the teaching or development by research of the chemi-

cal side of their professorships, placed first assistants then subordinate professors, representing specialists, by their side, and thus seemed actually to engender a division of their chairs into two branches. This movement, as regards pathology, found its termination in the rapid progress of the parasitic theory of bacteriology, which absorbed the available powers and means to such an extent that no chemical development whatever in the knowledge of disease has come from that side. Physiologists, on the other hand, either attempted to obtain aid from mere assistants or endeavoured themselves to add chemistry to their hitherto mainly microscopical and physico-technical accomplishments, but having, as some expressed it themselves, paid apprenticeship money and not acquired mastery they lapsed into their former frame. In some places only biological chemistry became in so far emancipated that special professorships and special laboratories were established, by means of which the teaching of the branch, if such it can be called, and its development by research was to be accomplished. But the results of this movement, predicted and therefore opposed by some weighty physiologists, have been so inconsiderable as to furnish a new illustration of the mountain-mother and the mouse-child. Some of these appointed specialists were actually uninformed as regards some of the broadest and best-established data of animal chemistry, and thus instead of leading a small number of pupils to a wider and purer knowledge of their subject, kept them only partially informed, and misdirected them to vain efforts for the maintenance of decaying doctrines and false facts: this error has been committed in a particularly glaring manner upon the subject of the first article which we shall lay before our readers, namely, the colouring

matter of the urine; but many other subjects of medical chemistry have suffered similar degradation by this want of method and thorough-going information. But happily research independent of these appointed inquirers is still living, and has lately produced results which are deserving of the utmost attention.

2. *Biochemistry outrun by Bacteriology.* — When bacteriology as a mere generality, without individualisation of its agents, took an empirical lead, and its cultivators, with the zeal usual with new creeds, imposed their antiseptic ceremonies as *a duty* upon all whom it might concern, the chemical study of particularly pathological phenomena suffered a complete eclipse. At a later period there ensued the reign of the phagocyte, and a microscopical slide became a complicated dramatic scene. Amongst the many accomplishments and astonishing feats of this creature of the bacteriological imagination none was more surprising than that by which it put all chemistry to shame, namely, *chemotaxis*. The phagocyte, if it could not get into the presence of its prey to swallow it alive, yet hated it so much that it devoured its exuviæ and excreta, for the finding of which in the blood serum or protoplastic jelly its creators endowed it with special, not otherwise defined, ability. By means of this faculty the accomplished being spotted the bacterial poison, isolated and sucked it up. But the phagocyte was too slow for these times of rapid evolution, and was drowned in antitoxic and antimycetic serum; it did not even give time to the chemist to learn something about its selective power over disease poisons; the disconsolate son of Hermes was again left to struggle on sad and alone with his slow and laborious devices.

Chemotaxis was succeeded by an approximation to really chemical ideas; the bacterial poisons remained, as they had always been unquestionable, but their removal or neutralisation was no longer left to white blood corpuscles, but confided to antidotes. And it became the greatest marvel of all times, so marvellous as to appear unthinkable to slow-minded people brought up in the belief concerning induction, namely, that these deadly poisons which destroyed and killed indiscriminately, could, by incorporation with the juices of living animals, be transformed into their own antidotes; from toxins they became antitoxins, and now saved and protected what shortly before they had endangered and destroyed.

On this field, however, also chemistry had not a chance of even raising a question or shedding a ray: its occupation as a luminiferous science seemed gone; the extraction of a few crystalloids, all derived from neurine, the most common nitrogenised nucleus of the phosphatides, called ptomaines, as the result of a facile generalisation, made no break in this fall. A dark recollection of former promise and affection caused some forlorn hopes to grope for toxins, acid, alkaloid, or albumosic; but the products did not admit of stoichiometric treatment, and antitoxiasis took the place of chemotaxis. It had not taken a lesson from the history of tuberculin, on the chemistry of which, if the manipulation bestowed upon it can be so-called, some new hopes had tried their hands and failed.

3. *A Rational Search for Antidotes.*—The hope and proposition that an antidote to disease poison could and should be found is quite reasonable, and as much amongst the possibilities as ferric oxyde hydrate as an antidote to arsenious acid is amongst the facts. But this knowledge of this antidote resulted from an

intimate study of the chemical properties of both the poison and its neutraliser. This is the example of the ideal of the medical chemist, namely, to obtain an accurate chemical knowledge of the disease-poisons, and then of their antidotes. When obtained, this knowledge can be successfully applied only with the aid of a knowledge of all the surroundings, which influence, and not rarely govern effects. Uræmia, which is so often fatal in acute fevers, is such a complication by surroundings; the chemical knowledge regarding it, and the modes in which its effects can be obviated, or avoided, is an example of the application of this information.

The direct mode of treating and healing acute bacterial diseases is, of course, the most desirable, and no so-called remedy can be called absolute or specific which does not destroy the carriers or producers of the disease, the microbes. Of this class of medicines we possess illustrious examples, both organic and inorganic, and other agents are so nearly specific as to justify the belief that they may be developed, by chemical means, into real direct healing antidotes.

4. *Organotherapy.*—Amongst the discoveries which glorify the healing art, those which refer to the cure of diseases of special organs, or at least to the counteracting of their deleterious general effects, have during late years taken an important position. The cure of senile cretinism is obviously a process of supplementation, whereby chemical substances which in health are supplied by a special organ of the body itself, are, in the case of these organs failing in their functions, supplied from without and do the duty of their natural product. Here disease arises from a deficiency, and is cured by the supply of that which was deficient. This

organotherapy is a chemical process, and the prosecution of the methods suggested by it will unquestionably lead to further important practical results. It is not impossible that the new *serum-therapy*, in its widest sense, is an example of organotherapy, even in case the toxin were transmuted into its antidote.

All these data and prospects repose upon the assumption of our possessing, respectively acquiring, complete chemical knowledge of all the bodies and substrata of processes involved. With this object in view the following articles on the "Progress of Medical Chemistry" have been composed and published. They consist of reports of the researches of all inquirers worthy of notice, of new researches made by recognised chemical methods, and of attempts to apply the knowledge so obtained to medical practice. All fundamental data will have to be patiently proved; we humbly beg our readers to consider with a lenient eye some details, which belong to the class of justifying document, or proofs absolute of chemical processes or facts. All such facts will be interpreted as results in summaries which we have endeavoured to make not only correct and useful, but also as interesting as the practical object of the matter will admit. We have tested new matter by occasional experiment, and supported data which we have been able to confirm by repetition or previous knowledge. Such data, as well as any analytical discussions, have been clearly marked out to the reader and kept distinct from other original matter by whomsoever contributed. We shall be glad to continue to receive from authors for notice in future volumes, papers or abstracts of essays on medico-chemical subjects, including therapeutical and toxicological ones, and will give to all the fairest consideration. The evolution of our plan will

also require time, as the co-ordination of a vast system of data requires the utmost caution.

II.—LATE RESEARCHES ON UROCHROME.

1. Introduction.

Dr. Archibald E. Garrod read a paper on the colouring matter of the urine before the Royal Society of London, which has been published in the Proceedings for March, 1894, vol. 55, p. 394. The author having discussed Vierordt's opinion, formed on spectroscopic data, that more than one pigment was regularly present in urine, says of *urobilin* (we leave out of sight the fallacy involved in the name) that its absorption-band in the spectroscope, when not visible in urine directly, appeared on standing or the addition of acid. Yet the quantity is at best extremely minute, and wholly inadequate to account for the coloration of the urine. Dr. Garrod is therefore convinced that the statement that "urobilin" is the chief colouring matter of normal urine *is entirely incorrect.* He has also shown that cruentin, which some years after its discovery was called hematoporphyrin, can usually be discovered by appropriate means in normal urine, but its amount is infinitesimal, and moreover it has no relation to urobilin. He is also supported by a communication from Dr. Lewis Jones to the effect that, according to some researches of the latter, he is convinced that the yellow colour of the urine *could not be due to urobilin*, because its solution was much redder than urine, because the yellow pigment was insoluble in chloroform in which urobilin is freely soluble ; urobilin had a band in F of the spectrum, which urine had not ; he allows the existence of a yellow body and is content to call it *urochrome.*

2. New Method of Extracting Urochrome.

Dr. Garrod was induced to study urochrome by experiments on uric acid. His method for its isolation is composed of the following four principal proceedings :—1. Saturation of the urine with pure sulphate of ammonium and filtration. 2. Extraction of urochrome from the filtrate with ethylic alcohol, which separates out from the saturated liquid, and carries most of the colouring matter with it. 3. Evaporation and solution of the residue in absolute alcohol. 4. Precipitation of the pigment from its alcoholic solution by excess of ether.

1. Ammonium sulphate added to urine causes a precipitate brown to pink in colour which contains urobilin. (*a*) Acidulated alcoholic extracts from the precipitate usually show a faint urobilin band, and sometimes still fainter band of *acid cruentin*. Unusually pink precipitates contain *uroerythrin* (*b*) made green by caustic alkali.

The precipitates by ammonium sulphate when washed with water yield a yellow solution of urochrome, which had been precipitated by the saturation. [This fact, and the fact that "urobilin" is obtained only by extracting this precipitate with *acidulated* alcohol, and not with alcohol only, proves that what urobilin is obtained *owes its origin to the decomposition of urochrome by the acid.*]

2. To the saturated clear filtrate *absolute* alcohol is

(*a*) S. Hoppe Seyler's method, Virchow's Archiv. 124 (1891), 30.

(*b*) The chemical properties and spectrum of *uroerythrin* were first described by me in my "Treatise on the Pathology of the Urine," second edition, p. 212, and in *Journ. Chem. Soc.*, May, 1875. Uroerythrin is reported to have been extracted by Riva and Zoja (*Gaz. Med. d. Torin*,, 1892, vol. 43, p. 925), from urine by shaking it with amylic alcohol.

added, which throws down some ammonium sulphate, and after agitation quickly separates and collects upon the surface as a clear layer, carrying with it the bulk of the urochrome. The urine is not decolorised by one treatment, but nearly so by repeated extraction.

Pour the alcoholic solution into much distilled water, and separate by renewed saturation with ammonium sulphate, at gentle warmth. By this washing pigment is lost, but urea and other crystalloids are removed, and therefore the process must not be omitted.

The golden orange-coloured extract will not mix with chloroform, and still contains water and ammon. sulph. Pour it upon some fresh solid ammon. sulph. and gently warm it ; two layers now form, a lower colourless one containing most of the water previously contained in the extract, and an upper one, being the urochrome solution.

(3) Evaporate the alcoholic extract to dryness over the water-bath, adding a few drops of ammonia from time to time, to maintain an alkaline reaction. [This is necessary to prevent the decomposition of indoxyl sulphate, which is mostly present, and would yield products of indigo-pigment in contact with acid.] A brown treacly residue remains, and solidifies on cooling ; it has a powerful urochrome odour, and still contains ammon. sulph. It has to be washed with acetic ether, which removes the bulk of the indoxyl sulphate, and a little urochrome. What the acetic ether leaves undissolved put into a stoppered bottle, add absolute alcohol and digest for some hours. Filter the orange-coloured solution and repeat the extraction. What alcohol leaves undissolved dissolves in water, and as this still contains some urochrome, this may be again extracted by renewed saturation with ammon. sulph.

4. The alcoholic solution still contains indoxyl

sulph. Concentrate it if necessary; pour it into rather more than its own bulk of ethylic ether. Urochrome falls for the greater part as an amorphous body, and may be collected on a filter moistened with ether. If any water be present, precipitation fails, and a few drops of a very concentrated watery solution of urochrome separate out and pass through the filter. The mixture of ether and alcohol is yellow, and holds much urochrome in solution.

The urochrome on the filter is tenacious, and adheres to the paper; the filter is allowed to dry in a vacuum, soaked in chloroform, ether and alcohol successively. The alcohol is intended to purify, not to dissolve, and takes but little urochrome out. On ultimate extraction with water a solution of urochrome is obtained.

This solution still contains a little urea, as is made probable [though not proved] by the fact of its yielding some gas with hypobromide in Southall's apparatus. On combustion it leaves a little sodium phosphate.

3. Properties of Urochrome and of its Solutions.

Dr. Garrod terms urochrome a colloid substance. In the dry state it is an amorphous yellowish red brown, hard, brittle, very hygroscopic substance, which may be dried *in vacuo* over oil of vitriol, or in a water oven. [It does not soften at that temperature when it is once dry. I found it so hygroscopic that a just visible trace of its dust, which had been spurted on white paper during powdering, was fused before it could be collected; the feather used for this purpose made only yellow streaks.] It is consequently readily soluble in water and in rectified spirit; less readily soluble in

absolute alcohol; when quite dry very slowly and slightly [when as a thick syrup it is treated with alcohol the latter takes up 3·54 per cent. by weight]. Acetic ether, amylic alcohol and acetone dissolve urochrome but sparingly. By repeated extraction and repeated evaporation it becomes less soluble; lastly, a darkened portion, changed, is insoluble in alcohol, though still easily soluble in water.

Urochrome is insoluble in ether, chloroform, and benzol; but a mixture of ether or chloroform with spirit dissolves a little. [When to the alcoholic solution, in which ether has produced a precipitate, a trace of hydrochloric acid is added, the entire precipitate redissolves; urochrome hydrochlorate is therefore soluble in ether and ether alcohol; free urochrome is not soluble in ether. I showed in my first research that hydrochloric acid obstinately adhered to urochrome, but that it was a base and in combination with it, I proved later.] The insolubility of pure urochrome in ether was first proved by Dr Garrod.

The reaction of a watery solution of urochrome upon litmus is amphibolic, blue paper becomes slightly reddened, and red paper taking a faint blue tint. Before the spectroscope the solution shows only an absorption of the blue end, but no detached absorptions. With zinc chloride and ammonia no fluorescence is produced. On standing the watery solution becomes brown. Addition of ammonia (even the presence of strong caustic alkali) preserves urochrome. On the other hand, it is quickly changed or decomposed by free acid even in small quantity. The solution is decolourised by nascent hydrogen; in the colourless solution no restoration of colour is effected by hydrogen peroxide.

4. Chemolysis of Urochrome with Mineral Acids.

This is the only part of Dr. Garrod's research with the conclusions from which I cannot quite agree. To decompose (chemolyse) urochrome he heated its solution with hydrochloric and sulphuric acid, and evaporated the mixture to dryness. The black residue thus obtained must have been much altered beyond the results of true chemolysis or chemical cleavage into proximate nuclei, and omicholic acid, which was missed, may well have been destroyed in this process, so as to have been represented only by the little yellow matter soluble in ether which Garrod met with. The chemolysis must be performed by boiling urochrome in relatively dilute acid, five in a hundred of dilution, for a short time, and filtering when the precipitate is well formed. If it be now filtered off, it is red or fawn-coloured, not black, easily washed and dried, and yields to ether *omicholic acid* and *omicholin*, to spirit *uropittine* (which is what has been misnamed "urobilin"), and leaves *uromelanin*. The acid filtrate may be boiled longer, but yields no more precipitate, and when subjected to distillation no organic acids in the distillate. This proves that *formic*, *acetic*, and homologous acids obtained from urine by distillation with acids do not come from the pigment, but from other bodies.

5. Bearing of Precipitants with Urochrome in Solution.

Dr. Garrod found that the precipitants behaved in the manner described by me in my various researches. But he did not use either the phosphotungstic or phosphomolybdic acid for the isolation of his urochrome. I

have produced several specimens of urochrome by this process (which excludes urea and all mineral acids at once), and found the isolated urochrome to possess all the properties described by Garrod. The process has, moreover, the advantage that it entirely excludes and annihilates the objection which Hoppe-Seyler and some of the students whom he has directed have raised to my early method, namely, that uromelanine was manufactured in the process from a carbohydrate containing no nitrogen, and urea or ammonia by long contact, in concentrated solution, in presence of acid. In my process no carbohydrate could be in the phosphotungstate or phosphomolybdate, and still less any urea, and any vestige of ammonia would be expelled by the baryta, and the combination of the urochrome with ferric oxide. As my process, now twenty years old, yields the same product as Dr. Garrod's, and is certainly not less convenient, Dr. Garrod's is a parallel confirmation of my researches in all except one chemolytic part, of which one item out of three is missing.

6. Results of Dr. Garrod's Research.

The main results of this elegant research are the following :—

The colour of the urine is almost entirely, if not entirely, due to urochrome.

The statement so commonly made in works and papers on physiological chemistry, that "urobilin" was the chief colouring matter of human urine, is entirely incorrect.

I have proved this in my paper in *Chem. Soc. Journ.* for May, 1875, quite incontrovertibly, and have shown that this so-called urobilin was a product of decomposition of urochrome by acid, namely, uropittin. Both

the physiological fiction, that it could be extracted *from bile*, as well as *urine*, and the pedigree ascribed to this matter, of which its backers knew *not a single chemical fact*, and which they only recognised by an absorption band in the spectrum, and that generally a bad one, are now once more disproved by the result of a new proceeding, which is also quite incontrovertible. Bile never yields a body having even the urochrome odour; this odour is strongest on heating or burning, is specific to the omicholin nuclei, and leaves both the uromelanin and uropittine nuclei from the moment when the omicholin is separated and removed; the specific odour remains with the omicholic bodies until they are destroyed.

I greet Dr. Garrod's research as the aurora of sunlight about to break into and through the dreary atmosphere of the physiological chemistry of this subject, its delusions, false facts, and self-destroying animosities against victorious research. If now the stoichiometric method be further applied to its products, we shall soon eliminate still more completely the carbohydrate delusion.

III.—PROGRESS OF THE STUDIES OF URINARY PIGMENTS OTHER THAN UROCHROME.

1. Cruentin as a Frequent Ingredient of Normal Urine.

After I had discovered and described *cruentin* and its spectroscopic phenomena in 1864, some years elapsed before it was rediscovered and introduced under the new name of *hematoporphyrin*. I had shown the mode of its production from hemochrome and hematine, and in 1866, the occurrence of closely related derivates, one of them exhibiting its spectrum in normal and patho-

logical urine. I shall therefore continue to describe it by the name which I originally gave it, namely, *cruentin.*

Dr. Archibald H. Garrod has made two researches on this substance (*Journ. of Physiol.*, 13, (1892) 598, and *ibid.*, 17, (1894) 349), and his results have been confirmed by Mr. F. Gowland Hopkins ("Guy's Hosp. Rep.," 50, (1893) 359). The pigment is isolated by its adhesion to earthy phosphates when they are precipitated from urine by caustic soda. Should the urine not contain a sufficiency of these earths, some calcium phosphate dissolved in very dilute acetic acid should be added. To every 100 cc. of urine 20 cc. of a solution of caustic soda of 10 p.c. strength are added, and the precipitate is collected on a filter, cautiously edulcorated, and washed off the same with water ; it is now treated with rectified spirit and hydrochloric acid, until it is dissolved, and the solution is examined before the spectroscope. Ammonia is next added, until the phosphates are reprecipitated ; and the precipitate is again dissolved, this time in acetic acid ; from this solution cruentin is completely removed by chloroform, and this extract shows the spectrum of alkaline cruentin, as do all solutions containing free organic acid. If the pink chloroform solution be evaporated to dryness, and the residue be treated with alcohol, it will frequently yield to this solvent not pigment, but impurity only. If after the removal of these the pigment be treated with alcohol containing some hydrochloric acid it will dissolve with a pink colour, and the solution will show the spectrum of acid cruentin.

Garrod found the cruentin in this manner in the urine of 20 males, one æt. 6, another 17, the others being between 18 and 35 years of age. Some attempts

at quantation point to less than a milligramme per litre, say seven parts in ten million parts of the urine. In a case of cardiac disease and nutmeg liver Garrod found 0·01 grm. per litre, or one in a hundred thousand, or fourteen times the normal amount. E. Salkowsky (*Zeitsch. Physiol. Chem.*, 15, (1891) 300) assigned to the red urine of a patient taking sulphonal a maximum of 0·871 grm. per litre, or 124 times the normal amount.

2. The Pigmentation of Uric Acid Crystals deposited from Urine.

On this subject Dr. A. E. Garrod has communicated an interesting research, illustrated by a chromo-lithograph plate (*Journ. Pathol. and Bacteriol.*, Nov., 1894, p. 100). He quotes as earliest researches on the subject those of Wetzlar ("Beiträge zur Kenntniss des Menschl. Harns," etc., Frankfort-on-the-Maine, 1821) and Duvernoy ("Chem. Mediz. Unters. über d. Menschl. Harn, Stuttgart, 1835). Both these inquirers used boiling water to extract the pigments from deposits, but their results throw little light upon the question. No further observations seem to have been made till 1881, when Künkel ("Sitzungsb. Phys. Med. Gesellsch. zu Würzburg," 1881, p. 69) found iron in sediments of uric acid thrown down by the addition of hydrochloric acid to urine.

Garrod distinguishes four groups of pigmented uric acid deposits—1, the *red*, or Cayenne pepper deposits ; 2, the yellow, or fawn-coloured ones ; 3, those coloured brown or black by abnormal pigments ; 4, brown deposits produced by mineral acids in urine.

All deposits owe their fundamental tint to the normal urochrome ; the other pigments are superadded. The pigments also influence the form of the

crystals, as recognised by Duvernoy and Ord ("The Influence of Colloids upon Crystalline Form and Cohesion," 1879, p. 52). When the latter removed the pigment by repeated solution in water, he obtained colourless crystals of the tabular form of pure acid.

Uric acid coloured by *uroerythrin* was pink, and had the razor-shell shape. The pigment has a great affinity for uric acid, and when present must have a share in any colouration of crystals.

Urobilin so-called has no share in the colouration of the deposits ; it does not influence the shape of deposits artificially produced. Cruentin also does not influence the crystals.

Deposits of urates always contain urochrome, but may be free from uroerythrin. Many lateritious deposits in health and disease contain uroerythrin. Urochrome, however, colours not only all urates, but is also the ground-tint of uric acid crystals. The red-pink crystals contain urochrome and uroerythrin.

Pigments derived from biliary colouring matter discharged in the urine colour uric acid crystals formed in it brown or black. Crystals precipitated by mineral acid are brown, from admixture of decomposition products of urochrome due to the action of the acid.

Garrod summarises his results to the effect that of the true urinary pigments which exist ready formed in urine only urochrome and uroerythrin possess the ability of colouring uric acid crystals deposited from solutions. Urochrome, being a constant constituent of the urine, always furnishes the ground-tint of the crystals, and plays the more important part in determining their form, the whetstone or canoe-shape being that which it specially tends to produce. In the majority of instances uric acid crystals, which are spontaneously and rapidly deposited from urine, contain

uroerythrin also, and it is to this pigment that the sediments owe their colour when seen in bulk. The various shades of orange and red observed in the individual crystals are due to the admixture, in varying relative proportions, of urochrome and uroerythrin; and although crystals coloured by the yellow pigment alone are sometimes met with, uroerythrin is never the sole colouring matter of the natural sediments.

3. The Presence of Iron in Natural Urinary Sediments and in Crystals of Uric Acid thrown down by Hydrochloric Acid.

It was observed by Künkel and by Garrod that urinary deposits of natural or artificial origin leave, when burnt in a platinum dish, an appreciable amount of a reddish ash; the colour is due to some ferric oxide; the amount varies much, and leaves no obvious relation to depth of colour of the crystals. The iron is not contained in either the urochrome or the uroerythrin; there is no evidence at all that it is contained in a coloured substance; if it were, such substance would have to be very rich in iron. Of course, the metal could not be found in urine in any ordinary oxidised form, but must be assumed to be present in an organo-metallic compound, in which the metal is inaccessible to the action of hydrochloric acid. This subject thus requires much more investigation than it has as yet received.

4. Late Additions to our Knowledge concerning Uroerythrin.

I gave a full description of the knowledge concerning uroerythrin in 1877 in the second edition of my "Treatise on the Pathology of the Urine," page 212.

I there described many phases of the spectrum of this body and its specific absorption bands ; also its then new reaction with caustic alkali, which turned its red colour into green, and then destroyed it permanently. These observations were specially published by me in the *Journal of the Chemical Society*, May, 1875. To these data only two new facts have been added since. One is that uroerythrin is rapidly decolourised by light, and must be kept in a dark place or protected from actinic light ; the other that it can be extracted from its watery solution by amylic alcohol, or by chloroform containing acetic acid. These data have been furnished by Riva in *Gaz. Med. di Torino*, 43, (1892), 3, and Zoja, *Archiv. Ital. di Clin. Med.*, 32, (1893), 63. The amylic alcohol does not yield the uroerythrin free from urochrome.

IV.—NEW RESEARCHES ON THE ALKALOIDS OF THE URINE AND ON UROCHROME AS AN ALKALOID, AND AN ALCOHOL.

1. The Alkaloids of the Urine.

Physiologists in general have no conception of the importance and quantities of the specific alkaloids of the urine, and as a rule give no information at all, or no adequate information concerning them.

I precipitated from a litre of normal urine of a man all that phospho-wolframic acid in acid solution did combine with. The dry rose-pink precipitate weighed 34·3477 grm. On elementary analysis it yielded 8·87 per cent. of carbon, and in two combustions 3·51 per cent. and 3·66 per cent. of nitrogen. This gives 1·229 grm. of nitrogen from the litre taken, and as the average daily excretion of this man furnishing the urine was 1,500 c.c., this gives 1·8436 grm. of nitrogen

discharged daily in the shape of alkaloids. Now as the nitrogen in a day's urine is on an average 17 grammes, it follows that in these 1·8436 grm., no less than 10·84 per cent., say nearly 11 per cent. of all the nitrogen are discharged in the shape of alkaloids.

There are, as I have shown, at least six alkaloids present in urine. On *Reducin*, $C_6H_{11}N_3O_4$, I have made no further observations.

On *Urotheobromin*, $C_7H_8N_4O_2$, the crystallised isomer of theobromin from cacao, I have made further inquiries. They show that when it is boiled in pure water *as copper oxide compound*, it reduces the copper oxide incessantly, and the green precipitate assumes a fawn colour. Owing to this affinity, it is very difficult to produce a pure cupric compound. When the cupric partly reduced compound is dissolved in dilute nitric acid carbonic anhydride escapes, so that here another oxidation is evidenced.

In a discussion on this important body, I had to defend my priority of its discovery against Dr. Salomon, of Berlin, who published some of his results, which in no respect exceeded mine in scope, but were provided with a new name of the body, four years after my original discovery and publication. Dr. Salomon has since publicly acknowledged my priority and exclusive originality in this discovery.

As regards *Kreatinin*, I have shown that when prepared from a pure crystallised aurochloride hydrochlorate, analysed as to all its constituents. it *did not reduce* alkaline copper solution ; other isomers of *Kreatinin* do, however, reduce copper.

The oxidisability of several of the alkaloids, or what is the same, their reducing action, is, however, one of the most remarkable chemical features of the urine. The solution of the united alkaloids reduces copper

oxide to suboxide, from acetate, in acid liquid; acetate of *mercuric* oxide, to acetate of *mercurous*, which salt is very insoluble and crystallises easily; *silver* precipitates are reduced and become black, also *platinum* precipitates change with reduction; *ferric* precipitates, *e.g.*, of urochrome, left in the liquid, become black, from reduction; *molybdic* and *tungstic* acids in neutral solution are quickly reduced even by phospho-compounds; I have seen *phosphate* of iron reduced to bluish *ferrous* salt, and by long boiling while air was excluded, to perfectly blue *ferrous phosphate.*

2. The Alkaloidal Character of Urochrome.

The *basic* character of urochrome is made manifest by many reactions, mainly by its precipitability from strongly acid solutions by these specific reagents for alkaloids, phospho-molybdic, and phosph-tungstic (wolframic) acid. (*a*) But the separation of the urochrome from the other alkaloids has to be effected by reagents which are independent of its alkaloidal character, and bring into play its alternative character as a cohol (alcohol) or weak acid; the best means for this purpose is ferric chloride in very dilute solution. When this is added to the hot solution of the alkaloids as they come from the baryta process for the removal of the phosphorised acids, its ferric oxide combines with the urochrome, and the compound falls down; it must be filtered off quickly while the solution is hot, for on

(*a*) These acids, which had been known only in solution in aqua regia, or in acidified solution of their soluble salts, were first prepared by me in a pure and crystallised state, and proved of the greatest practical use. The pure barium salts were decomposed with sulphuric acid, and the free acids used directly or crystallised after concentration.

cooling it deposits clouds of *urotheobromin* which are not deposited unless the urochrome be precipitated out first. From the ferric compound urochrome may be obtained by hydro-sulphuric acid, or easier by very cautious solution in dilute acid and repetition of the phospho-molybdic process. The solution on concentration yields urochrome to be dissolved in alcohol and precipitated by ether.

When the ferric process is applied to prepared urine directly, both *urochrome* and *kryptophanic acid* are precipitated simultaneously, to be separated by the phospho-molybdic process.

The alkaloidal character of urochrome is also evidenced by its complete removal from strongly alkaline solution by *animal charcoal*. Indeed, *all* alkaloids of the urine can thus be removed *absolutely*, if all acids be extracted previously by treatment with *mercuramine*.

The alkaloidal and alcoholic character of urochrome is further manifested by its combining with benzoyl and forming a series of *esters* (ethers) such as alcohols and a number of alkaloids are known to give rise to. These esters will be examined in a subsequent essay.

3. Importance of Elementary Analysis and Quantation of Non-Crystalline Animal Educts.

Dr. Garrod, in the paper on urochrome which I have analysed in a previous essay, states that he had not made any ultimate analysis of the product of his process, because, as he believed, it would be of little value without further guarantees of the purity of the product, and because such guarantees could hardly be expected to be obtained in the case of a colloid sub-

stance such as he believed urochrome to be. While fully respecting Dr. Garrod's reserve in this particular case, I do not agree with the argument so frequently used by chemists who are unacquainted with the conditions of biological science against employing elementary analysis in the case of bodies which do not easily crystallise, or are absolutely colloid. In this respect physiological chemists are unduly influenced by the mere chemists, who generally are very little acquainted with the properties of animal principles, and make demands which, if accepted, would make any effectual study of biological chemistry impossible. I hold, on the contrary, that the more difficult is the definition of a chemical principle in the pure state, the more necessary is it that it should be subjected to quantations of all its elements at different stages, in combination as well as in the free state; if now the products of its chemolysis yield simpler and easier definable substances, their analysis again advances the knowledge, and thus the problem being hemmed in from all sides, must ultimately surrender.

But in the case of urochrome elementary analyses have, in addition to their intrinsic interest and usefulness, the greatest casual importance in this, that they serve as a means of valuation of a series of hypotheses which have been imagined by a German professor, supported by him by indirect experiments, and enlarged by some students working under his directions. As the completely fallacious and erroneous nature of these hypotheses and inferences from extraneous synthetical processes is at once proved by elementary combustion, even when used only as a qualitative reaction, I here give its result with regard to the principal question involved. But to be fully understood by the reader I must put the question before

him, and as it is an important matter, request his kind attention.

4. Short Definition of the Urinary Humin Hypothesis.

Professor Hoppe-Seyler, of Strassburg, having for years in his writings opposed my teaching regarding urochrome, although, as far as one can see from his publications, he had made no experiments which in any sense were adequate even to throw some light on the subject, read in Gmelin's "Handbook of Chemistry" that this author, following Braconnot, had classed the particular black matter of Proust, uromelanin, with the so-called *humous* or *humin-substances*. As these residues of decaying vegetable matter were then generally supposed, or had even been found, to be free from nitrogen, (*a*) the professor surmised that uromelanine also was free from nitrogen naturally, and acquired the nitrogen, of which it contains, curiously enough for a supposed accidental impurity, invariably about 12 per cent., from its surroundings in the course of manipulation and chemical proceedings for its isolation. As humous substances were derived from carbohydrates, mainly cellulose, the professor further surmised that uromelanine might be derived from a carbohydrate naturally contained in urine. He now boiled *urine*, to which he added *sugar* (the carbohydrate supposed to be naturally present in urine was evidently not sufficient in quantity to prove either its own presence or the proposition constructed with its aid) with much *mineral acid* (curiously enough, *hydrochloric acid*, which I had given reasons

(*a*) Later analyses gave considerable amounts of nitrogen in humous matter, as can be seen in Gmelin's Handbook also.

studiously to avoid), and obtained a black substance, called humous, but containing nitrogen. Adopting a still more forcible proceeding, he fused *sugar* with *urea* and obtained *a caramel containing nitrogen*, which was also termed a humous substance. The properties of this product and the percentages of the elements contained in it did not fit the properties of uromelanin at all; but, probably, as he had never studied or handled this substance, he could take no lesson from it, and formulated a specious conjecture from his sugar and urea experiment and from the variations, which he induced some pupils to imitate, to the following effect.

'The normal urine contains *carbohydrates;* these carbohydrates have a tendency to be transformed into *humous substances;* they are so transformed by treatment of the urine with acids; but they may also be transformed already within the body, at least in part, and assume a little *colour;* it is such humified carbohydrate in small quantity which furnishes the *colour of urine.* There is no special or specific colouring matter of the urine at all, consequently there is no such thing as *urochrome;* what has been so-called is *mere carbohydrate*, altered in part; and what has been termed *uromelanin* is mere carbohydrate transformed into *humous substances* by acid and heat; and the nitrogen which it contains has been *inserted* synthetically, and comes from the urea with which the carbohydrate has been heated.'

Thus far the argument, which, if there had been any carbohydrate available in the urine, would have pertinently pointed to a possible danger of its nitrogenisation, namely, the presence of great masses of urea in a state of active decomposition. The presence even of several carbohydrates in urine was defended

by others, and we shall hereafter have to shortly examine their allegations and analytical data; but they were not yet humified, but supposed to be present as *carbohydrates* still. The value of the entire argument was beautifully defined by Dr. Garrod in the concluding sentence of his paper, read before the Royal Society, thus:—"Even if it be granted that the yellow pigment does yield humous substances on decomposition, any argument based upon this may well be regarded as open to the objection of explaining *ignotum per ignotius.*"

5. Analytical Refutation of the Proposition of Urochrome being a Carbohydrate.

If the yellow substance which colours urine was a carbohydrate, it *could not and should not contain any nitrogen.* To test this question finally, I prepared a specimen of urochrome by the phosphomolybdic acid process, which *excludes urea*, the ferric process which excludes all alkaloids *except urochrome*, and purified it by Garrod's process of precipitation by ether from absolute alcohol solution. Here is the result.

Nitrogen quantation in a specimen of urochrome prepared as just stated; dried at 100° in platinum boats until constant; 0·4086 grms. burnt with copper oxide, in carbonic anhydride atmosphere; gas obtained 68·0 c.c., which corrected for temperature and air-pressure is equal to 20·94 per cent. of nitrogen.

This one analysis explodes the entire carbohydrate humous argument of Hoppe-Seyler and his pupils; for *urochrome contains more than one-fifth of its weight of nitrogen.* Moreover, such urochrome yields all the products of chemolysis, which I have described, and which *all contain stated quantities of nitrogen*, and each of which in its turn destroys the carbohydrate proposi-

tion. Of these *uropittine* (falsely called urobilin) and *omicholic acid* and *omicholin* have not even any external similarity to a humous substance ; only *uromelanin* has, by its ultimately black colour, a resemblance to it, which, however, completely loses all its importance by close examination and comparison.

6. The Elasticity of Facts and Arguments employed in the Proposition concerning Urinary Humous Substances.

The humous theory having no longer any place to stand on, the readers might think that any further discussion of its details was unnecessary. As regards the subject matter this is certainly the case, but as regards the methods employed by the authors, a little further consideration may act as a timely warning.

Nitrogen and its quantation are evidently stumbling blocks in the path of the defenders of the carbohydrate proposition, and bring them to catastrophes, both in the pursuit of their alleged facts and in the exercise of their deliberative faculty. They tried hard to get a *sugar-urea-humin* which should be the equal of the black matter produced by them and termed by them *natural* (urinary) *humin*, being in reality a mixture of matters semi-carbonised by hydrochloric acid and heat. But they did not succeed ; the composition of different specimens showed fluctuations in the relative quantities of elements, which made identity impossible. The humous substances made with acid contained no nitrogen ; those made with urea and fusion contained from six to thirteen per cent. of nitrogen , but as this element was made to rise, *the carbon sank*, and the attempt to produce uromelanine was a complete failure. In the carbon their preparations varied between 65 and 53 per cent. Their specimens, therefore, completely

defeated their hypothesis. But they occupied new positions and issued bulletins, of which the following are two specimens :—

"As the 'humin' substances are able to take up different quantities of ammonia *in statu nascendi*, according to quantities which are present, their nitrogen must be considered as an accidental non-essential ingredient."

This comprehensive term of "the humin substances" was unavowedly to include uromelanin ; in order to make this quite evident to the initiated, there is the further sentence :—

"How near the artificial (sugar-urea fusion) humin is to the natural (urine-hydrochloric acid-boiling humin) is the more evident if one leaves the nitrogen out of sight."

And as regards a specimen of black matter from diabetic urine which contained nearly ten per cent. of nitrogen, we read :—

"Deducting the nitrogen, the urinary and artificial humins show a residue free from nitrogen, which has very concordant composition."

This assertion is quite unfounded ; not a single figure or element fits the data for uromelanin. But even by "*leaving the nitrogen out of sight*," or by "*deducting it from the percentage*," no uromelanin is reached. What desperate devices must men adopt who endeavour to make so-called researches on a basis of foregone false propositions !

The inquiry of Hoppe-Seyler on humin-yielding matters, such as paper, sugar, and tannin, and their humins, that is to say, black, unknown, undefined matters resulting from the treatment of the compounds mentioned with acids or alkalies, although intended mainly to discredit uromelanin and the urochrome theory, and that by a mere analogy now proved

not to exist, would have been interesting, and, perhaps in some respects valuable, had it not been disfigured by the distortions just related. Even the fact that his specimens of *hymatomelanic acid* varied in their carbon between 65 and 57 per cent., so that one would be tempted to ask which of them was hymatomelanic acid, would not necessarily have involved a rejection of the whole research, inasmuch as the fusion experiments with alkali, though made on unknown matters and mixtures, the *ignotiora* of Garrod, yielding *ignota* such as several black *hymatic acids*, yet gave as ultimate cleavage products some known substances in small quantities which may be useful in future researches. But as regards the pigmentary ingredients of the urine and their products, which this research was imagined to eliminate, wipe out and destroy, it has taught nothing at all, but left the accumulated knowledge quite unscathed, untouched, and untarnished. The animus out of which it arose, and which gave it partly its fallacious direction, is a reminiscence of another fallacy concocted in the Strassburg laboratory, namely, that kryptophanic acid was a gum, mixed with a nitrogenised matter, which nitrogen, however, baffled the inquirer in the attempt of removing it. It was therefore not indeed deducted from the percentage, for no analyses were made, but it was simply "*left out of sight!*" Such is the style of "research" to which medical science and the medical profession are now treated in some quarters.

7. Definite Results of this Research and Argument.

It is not the fact that the urine contains carbohydrates which are able to yield humous substances, either such as contain, or such as do not contain nitrogen.

It is not the fact that the urinary colouring matter is a carbohydrate in course of transformation into a humous substance.

It is not the fact that uromelanin is a humous matter derived from a carbohydrate.

On the contrary :

The yellow colouring matter of the urine, an immediate principle, is a highly nitrogenised substance, the decomposition products of which are also nitrogenised.

Uromelanin contains nitrogen when prepared under circumstances which exclude all nitrogenised matters which could furnish its nitrogen.

Any comparison of uromelanin with humous matters for the purpose of learning anything about it by analogy is therefore perfectly useless, as there is no analogy between the materials from which they arise, and even if there were the process would be perfectly idle so long as humous matters are less known and more difficult to study than uromelanin.

The proceedings adopted by Hoppe-Seyler and his followers to discredit uromelanin are therefore perfectly futile. The only true method of inquiry is the study of urochrome and its products themselves.

The whole of the statements of Hoppe-Seyler concerning urochrome and its products of decomposition are shown to be destitute of any foundation.

V.—DISPROOF OF THE ALLEGATION OF THE PRESENCE OF DEXTROSE SUGAR IN NORMAL URINE. NEW REACTIONS FOR THE DIAGNOSIS AND QUANTATION OF DEXTROSE, KREATIN AND KREATININ.

1. Introduction.

The allegation that normal urine always contained sugar was originally made by the Vienna physio-

logist Brücke, more than thirty years ago. The allegation being disputed, the discussion became a chronic controversy, in which many inquirers participated on both sides. Kühne and the late Bence Jones endeavoured to fortify the allegation by various additions, all as unconvincing as the original proposition. The opposition of Wiederhold, Friedländer, Meissner, von Babo, Seegen, and others made little impression, and to this day we can read in physiological or medical works that normal urine contained regularly half per cent. of dextrose sugar.

It had been stated by *Duhomme* (*Bull. gén. de Thérap.* 15th Feb. 1878), that kreatinin interfered with the deposition of the cuprous oxide and its dehydration in Trommer's test. He recommended, as *Bernard* had done before, to keep the suboxide in solution by excess of alkali, and therefore added four volumes of soda ley of 1·33 sp. gr. to 1 vol. of Fehling's liquid. (This could of course be used only for the volumetrical estimation of sugar, already known to be present, and not for qualitative testing of very dilute solutions, such as healthy urine to be tested for normal sugar would necessarily be.) In a later communication (*Bull.*, &c., 97, 63) the author stated that most specimens of normal urine contained some decigrammes of sugar in the litre ; cases were frequent, he asserted, in which from one to five grammes of sugar were excreted daily for years without evil effect.

In Germany the proposition has been greatly enlarged by the results of the application of a special reagent, benzoyl chloride, to the effect that the urine did not only contain sugar, but a series of carbohydrates besides, such as dextrin, gum, and others. This phase of the inquiry will be considered in a special essay.

Against most of these experiments grave doubts have been raised either by impugning the material as abnormal, really normal specimens not giving the reaction, or by explaining any actually occurring reduction of the copper test as being caused by reducing bodies other than sugar. The most important of these latter kind of objections was the statement that the kreatinin contained in and obtainable from human urine did reduce alkaline copper solution like a solution of sugar, and that thus far it might become a source of fallacy in physiological experiment or clinical medical testing (Stillingfleet Johnson in a communication to the Royal Med.-Chir. Soc.). In the present essay I propose to deal with the allegation concerning dextrose sugar only. As I have never found any evidence of the presence of dextrose in normal urine, my personal relation to this question has been clearly defined and expressed. This was necessary on account of the influence which the presence of sugar, if it had been a fact, would have had upon other important controversies in which I was involved by my researches. Thus it was maintained that the substance in urine which yielded indigo on decomposition, was a glucoside, and yielded sugar besides indigo; this indican, as the supposed glucoside was termed, was further confounded with the colouring matter of the urine, which was thus by implication alleged to furnish dextrose by decomposition. All these fallacies, including the latest phase of the carbo-hydrate humin substances, have been eliminated from the discussion one by one, by assiduous research, and controversial publications. But false facts are very long-lived, and to this day authors affected with blindness of a greatly censurable nature, and disregarding all accumulated and incontrovertible proofs, maintain the presence of sugar

in normal urine with a persistence worthy of a better cause.

It is, therefore, with great satisfaction that we are able to introduce to our readers a new and simple method by which the absence of dextrose sugar from normal or almost any non-diabetic or non-glycosuric urine can be proved. The process has been discovered by Sir George Johnson, and communicated in the *Lancet* for January 7th, 1894, and for January 12th, 1895, and is collaterally supported by the discovery of his son, Mr. Stillingfleet Johnson to the effect, that the only substance capable of giving reactions similar to some reactions of sugar, namely, kreatinin, can be removed by the application of mercuric chloride as a precipitant.

The absence of dextrose from urine, or its presence in it can be ascertained by the usual well-known tests, but most conveniently and in about two minutes' time by the new test.

2. The Test.

1. *The Reaction with Urine containing no Dextrose.*—Place a drachm of urine in a test-tube of about half an inch in diameter; add an equal volume of a saturated aqueous solution of picric acid and half a drachm of liquor potassæ, P.B. An orange red colour instantly appears as a result of the incipient reducing action of kreatinin upon picric acid at the ordinary temperature. The colour is deepened by boiling. If after the liquid has been kept at the boiling point for about a minute a bright red colour appears through the test-tube when it is held up to the light, the urine, for clinical purposes, may be confidently pronounced to be free from sugar.

2. *The Reaction with a Solution of Dextrose or with*

Diabetic Urine.—If an aqueous solution of dextrose containing not more than half a per cent. of the sugar be tested in the manner described the liquid will be rendered so dark that no light is visible through the full diameter of the tube.

3. *Quantation of Dextrose.*—In case the presence of sugar has been ascertained, its quantation may be effected by the picrosaccharometer invented by Sir George Johnson, which comprises a standard coloured solution of basic ferric acetate, and the apparatus and reagents for testing the urine and comparing its colour with that of the standard.

4. *Delicacy of Picric Acid Test.*—The picric acid test will discover 1-100th per cent. of glucose in a drachm of urine, or 6-100th of a grain.

5. *Interference of Kreatinin in Normal Urine.*—Every normal urine reduces picric acid as much as if it contained from ½ grain to 1·2 grains of dextrose in the fluid ounce, or a drachm of normal urine by its kreatinin simulates from 0·062 to 0·15 grains of dextrose.

6. *Removal of the Kreatinin and other Alkaloids (including Urochrome) by Mercuric Chloride.*—When the alkaloids have been removed by mercuric chloride, the urine, if free from sugar, exerts no reducing action either upon the copper or the picric acid test.

7. *The Mercuric Chloride Process does not affect any Dextrose which may be present.*—When to normal urine a known weight of dextrose is added, and the kreatinin, &c., are then removed by mercuric chloride, the dextrose is found undiminished.

Kreatinin, prepared as by Mr. Stillingfleet Johnson's process without the application of heat and alterative chemical agents is the only substance in normal urine that exerts a reducing action upon picric acid. This is proved by the fact that when the amount of picric

acid reduction by urine has been accurately ascertained, the corresponding indicated amount of Stillingfleet Johnson's kreatinin in aqueous solution is found to give precisely the same degree of picric acid reduction-colour as the urine, when the liquids are undiluted, and in different stages of dilution, until the amount of kreatinin is no more than one grain by weight in 200,000 minims of the liquid, the comparison being made with a pale yellow solution of picrate of potash.

8. *Special Precautions to be taken in Testing.*—To obtain the complete reduction of picric acid by an aqueous solution of S. Johnson's kreatinin containing about a grain to the ounce, more potash is required than in testing normal urine. The amount of liquor potassæ, P.B., must be one and a half drachms instead of half a drachm, and the liquid should be boiled for ninety seconds. This peculiarity is explained as follows :

The kreatinin in the urine appears to be associated with some substance which enables it to exert its full reducing action in the presence of a smaller amount of potash than is necessary when uncombined kreatinin is dissolved in water.

9. *Variations of the Test by Dilution.*--A watery solution of dextrose ceases to give any colouration with picric acid and potash when the dilution is carried beyond 1 part in 10,000 of liquid.

If, therefore, the reduction colour in normal urine were in part due to the presence of a small proportion of dextrose, the colour resulting from the analysis of the urine, and of an aqueous solution of kreatinin of the same reducing power which is equal when the two liquids are undiluted, would be unequal when the dilution is carried beyond the point of 1 grain in 10,000 minims of liquid, at which dextro-glucose ceases to exert any

reducing action upon picric acid. The urine would be paler than the equally diluted solution of kreatinin in proportion to the amount of sugar which it contained.

In testing normal urine Sir G. Johnson has invariably found that when the colours in the undiluted specimens have been equal, they have remained equal after the two liquids have been diluted beyond the point at which the action of dextro-glucose must have been eliminated. This leads him to the conclusion that S. Johnson's kreatinin is the only substance in normal urine that exerts a reducing action upon picric acid, and that not a trace of dextrose is present.

10. *Relative Reducing Power of Kreatinin and Dextrose.*—The reducing power (for picric acid) of S. Johnson's kreatinin is less than that of dextrose in the proportion of 10 to 12.

11. *The Reducing Urinary Kreatinin.*—This substance prepared by S. Johnson's process can be obtained at Apothecaries' Hall. It is prepared by the mercuric chloride process, and crystallised by evaporation in vacuo, so as to avoid the transformation into kreatin, which is always at least partially effected by evaporation at higher temperatures.

12. *Average Reduction with Picric Acid in Normal Urine.*—The average reduction with picric acid in normal urine is about equal to that which would be effected by a solution of 0·8 grain of dextrose in a fluid ounce of water, but there being no dextrose the reduction indicates practically the presence of a grain of kreatinin in each fluid ounce of urine. This would show that the methods of extracting the kreatinin from urine for purposes of ascertaining its normal quantity, yield only about half the quantity present. Fifty ounces of urine would contain 50 grains of kreatinin,

while by extraction only from 11 to 15 grains have hitherto been obtained.

13. *Reduction in Morbid Urine.*—Sir G. Johnson has observed a urine of sp. gr. 1·039, which crystallised with nitric acid, and gave a picric acid reduction equal to that effected by 1·6 grains of dextrose, indicating 1·92 grains of kreatinin per fluid ounce.

14. *Influence of Temperature on the Reduction of Dextrose, Kreatin, and Kreatinin.*—All three bodies in alkaline solution, with copper or picric acid, are oxydised on boiling almost immediately, though in slightly different degrees. But at the ordinary temperature they observe different courses. When picric acid and potash are added to a cold solution of reducing *kreatinin* or to normal urine, which always contains this body, an orange red colouration, the result of incipient reduction of picric acid, occurs immediately ; with *kreatin* at least an hour elapses before any red colouration occurs ; with *dextrose* there is an interval of at least six hours before any change of colour appears. The three tests will assume a deep colour on standing for from twelve to twenty-four hours, but the reduction of the yellow picric to the red picramic acid will not be complete until the liquid has been boiled for an hour.

15. *Influence of Dilution upon the Reducing Action of the Three Substances.*—The loss of reducing action upon picric acid effected by dilution of the test is, as regards kreatin, intermediate between dextrose and kreatinin. The limit is one grain to about 50,000 minims of water, being five times more than the limit of dextrose, and four times less than that of kreatinin.

16. *Use of the Reducing Reaction for Diagnosing a Mixture of Kreatin and Kreatinin.*—The difference in the reducing power of these substances in dilute solu-

tion may be used for ascertaining whether a specimen is a mixture or not.

17. *Separation of Kreatin from Kreatinin when mixed with each other.*—This separation has hitherto been effected by boiling alcohol, but Sir G. Johnson precipitates kreatinin by mercuric chloride; the kreatin remains in solution in the filtrate, and may be detected by the picric acid reaction commencing in from an hour to an hour and a half at the ordinary temperature; while the kreatinin is neatly isolated, the kreatin is not easily recovered by this method.

18. *Relation of the Three Substances to each other as regards the Time in which they react, and as regards the Limit of Dilution beyond which they do not react even at the Body Temperature.*—This relation is the same in both series of tests, in both kreatin occupies a position intermediate between kreatinin and dextroglucose.

19. *Relation of the Substances to each other as regards Reducing Power over Picric Acid.*—In this respect kreatin is inferior to both dextrose and kreatinin; when kreatinin reduces seven units of picric acid, kreatin will reduce only six. We have seen above that when dextrose reduces twelve parts of picric acid, kreatinin will reduce only ten, and, therefore, kreatin only 8·5 parts.

3. Quantation of Dextrose in Urine by the Picro Saccharometer of Sir G. Johnson.

A. *Solutions required.*

1. Provide a *standard solution of ferric acetate* equal in tint to that yielded by a solution of dextrose containing one grain per fluid oz., as follows :—

℞ Liq. ferri perchlor. fortior. (P.B. sp. gr. 1·42), ℥j ;
Acidi acet. glacialis (P.B. sp. gr. 1·058), ℥iv ;
Liquor. ammoniæ (P.B. sp. gr. 0·959), ℥ij ;
Aquæ destillatæ, ad ℥iv.

Mix first the iron and the acid, then add the ammonia and water up to 4 fluid oz.

2. Provide further a *saturated solution of picric acid*, prepared by boiling the crystals in proportion of six grains to the fluid oz. of water, and allowing the excess to crystallise out on cooling.

3. Have ready liquor potassæ, P.B., sp. gr. 1·058.

B. *Apparatus required.*

1. A tube of about 12 inches in length, graduated into 100 cubic centimetres, with longer division-marks at each 10 cubic centimetres, accurately stoppered and lipped.

2. A tube of half the length of the above, but of equal diameter, accurately stoppered to hold the standard solution.

3. A tube for boiling, 10 inches long, ¾ inch in internal diameter, lipped and graduated up to 4 fluid drachms.

4. A one drachm measure, preferably a pipette.

C. *Method of Performing the Analysis.*

1. *The Reduction.*—Measure 1 fl. drachm of the urine to be analysed into the boiling tube. Add 1 fl. drachm of the saturated picric acid solution, and ½ fl. drachm of liquor potassæ. Make up to the 4 drachm mark on the tube with distilled water. Heat over a spirit or gas flame and keep the liquid boiling for about a minute. Cool by dipping the tube after a minute in cold water, and ascertain that the cold liquid measures 4 fl. drachms. If it exceed that, evaporate it down to

4 fl. drachms, if it be less, fill up to the mark with water.

2. *Removal of Earthy Phosphates.*—In undiluted urine the phosphates precipitated by the potash often cause turbidity, which must be removed by filtration before the colour can be accurately estimated.

3. *Evaluation of the Amount of Reduction.*—If the colour is paler than the standard, boil with two drachms of urine instead of one, and then divide the indicated reduction by 2.

If the colour of the boiled liquid is darker than the standard, introduce it into the graduated tube until it stands at 10 divisions, place the stoppered tube containing the ferric acetate at the side of the test. Now dilute the dark red liquid in the graduated tube with water until the colour is the same as that of the standard. Each division above 10 indicates 0·1 grain of dextrose per fluid ounce. Thus 13 divisions=1·3 grains, 30 divisions=3 grains per fluid ounce.

Here it must be borne in mind that every normal urine reduces picric acid to an amount equal to the effect of from ½ grain to 1·2 grains of dextrose per fluid ounce; this reduction, due to reducing kreatinin, should be deducted when the quantity of dextrose present is very small.

If more than 6 grains per fluid ounce are indicated, dilute the urine 10 times by pouring urine up to 10 divisions in the graduated tube and water up to 100. Then analyse the liquid as before. In this case each division on the saccharometer indicates one grain of dextrose per fluid ounce. [This dilution is required because one fluid drachm of picric acid solution is sufficient for the oxydation of only one drachm of urine containing at the rate of six grains of dextrose per fluid ounce, that is to say 0·75 grains.] Thus diluting from 10 up to 48

divisions shows that the urine contains 48 grains of sugar per fluid ounce.

If the urine, when 10 times diluted, gives a colour paler than the standard, it contains less than 10 grains of sugar per fluid ounce. Another portion should then be diluted five times by filling the graduated tube up to 10 divisions with urine, then up to 50 with water, or by measuring 5 or 10 cc. with a pipette into a 50 cc. flask and adding water up to 50 cc. The analysis is performed as before. The value of the divisions now will be half that obtained with a 10 times diluted sample. Thus 18 divisions would indicate nine grains per fluid ounce. If the urine has a specific gravity of 1·035 or more, it should be at once diluted 5 or 10 times before its analysis is commenced.

The proportion of the percentage weight of sugar to the volume of the urine may be ascertained by dividing the number of grains per fluid ounce by 4·8.

VI.—THE ISOMERS OF KREATININ FROM FLESH AND URINE.

1. *Introduction on the Literature of the Subject.*

THE chemical knowledge which we possess of kreatin took its origin in an inquiry on an article of trade, namely the meat-extract or so-called bouillon, of an Association of Dutch merchants domiciled at Paris, which was conducted by Chevreul on behalf of an academic commission. (*a*) In this extract he discovered, in 1835, a crystallisable substance which he termed *kreatin*, recording by its name that it came from flesh, (Journ. d. Pharm., 21, 236). In 1844, Heintz

(*a*) Some details of the objects and results of this commission on bouillon, and on the so-called bouillon d'os, can be seen in my work "The Spirit of Cookery, etc.," p. 111, and p. 127.

(Poggend. Ann. 62, 602 ; 70, 460 ; 73, 696 ; 74, 125) and Pettenkofer, contemporaneously, but by different methods, found a substance in human urine which formed a crystallised compound with zinc chloride, subsequently ascertained by Liebig to be closely related to kreatin, namely *kreatinin* (Ann. Chem. 62, 298 ; and Chem. Research on Flesh and its preparation for Food, 1847, p. 47, a reprint of the article in the Annals). Liebig obtained both kreatin and kreatinin from the juice of the flesh of representatives of all classes of vertebrate animals, and from human urine and investigated their properties. Heintz now showed (Poggend. Ann., 74, 125) that kreatinin was partly transformed into kreatin during the processes required for its isolation, and that the muscles and excretions of animals probably contained only kreatinin. Liebig had transformed kreatin into kreatinin by the action of hydrochloric acid and heat, and now confirmed that the latter base was reconverted into the neutral kreatin under the influence of water and boiling heat, or under the influence of water and time at the ordinary temperature. After this, both substances became the object of physiological research on the one, and of more extended chemical research into their constitution on the other hand. Verdeil and Marcet (Journ. d. Pharm.,(3^{d} ser.) 20, 89) found kreatin in the blood of the ox ; Socoloff, kreatinin in the urine of the sucking calf, along with allantoin. Thudichum gave estimates of the quantities of kreatin and kreatinin obtainable from urine by the then known methods, which were continued by others (Ann. Chem., 119, 127, and Pathol. of the Urine, 1st edit. p. 126). The chemical nature of kreatinin was further inquired into by Dessaignes (Ann. Chem., 97, 343) who discovered a new base methyluramin as one of the products, and Strecker

published some considerations on the chemical constitution of kreatin. The greatest progress in our knowledge of kreatinin was made by Stillingfleet Johnson (Proceed. R. S. vol. 43, p. 493, and Med. Chir. Trans., vol. 63), by the discovery of improved methods for its isolation, and of several isomerisms and their diagnosis. It is more particularly this research which we shall analyse in the following as concisely as its practical importance will require and the volume of the subject will allow.

2. *Mode of Isolating Kreatinin of Urine as Mercuric Chloride Compound.*

Add to fresh unconcentrated urine one-twentieth of its volume of a cold saturated solution of sodium acetate; then one-fourth of its volume of cold saturated solution of mercuric chloride, and filter immediately; the filtrate deposits the whole of the normal reducing agent as mercury salt in about forty-eight hours. The precipitation is complete when the filtrate from the second mercury precipitate remains clear, even on the addition of more solution of sodium acetate and mercuric chloride. It will then be found that the permanently clear filtrate is without reducing action upon both potassium picrate and cupric oxide in boiling alkaline solutions.

(*a*) The *first precipitate* produced by mercuric chloride in normal urine is amorphous and flocculent, and has some resemblance to coagulated albumen. It had been analysed by Dr. John Green, of Birmingham (*Brit. Med. Journ.*, May 10, 1879), and gave elements corresponding to $C_{16}\,H_{30}\,N_5\,O_{16}$; per cents.: C=34·52; H=5·71; N=12·58; O=47·19. It contains uric acid; decomposed by H_2S, it gives an acid filtrate with much chlorine, no phosphoric acid, and leaves a gummy

mass on evaporation. It has no reducing action over copper or picric acid.

(*b*) *The second granular precipitate:* under quarter inch magnifying power it appears homogeneous, and the granules often have the appearance of minute crystals united in stellate groups; but under one-sixteenth the stellate groups consist of minute spherical masses. The salt is very sparingly soluble in cold water, dissolves readily in hydrochloric acid when recently precipitated, but is insoluble or nearly so in acetic acid.

3. *Chemical Reaction and Decomposition of the Spherical Salt of Mercuric Chloride.*

Aqueous ammonia does not blacken the spherical compound, but if the salt be thrown into boiling water the sediment which remains is immediately blackened by ammonia. This shows that a portion of the mercury in this compound is reduced to the mercurous condition by contact with water at 100° C. Therefore the spherical mercury salt must be washed with cold water, and must be dried *in vacuo* over sulphuric acid to avoid this decomposition.

Decomposed in cold water by H_2S, it yields mercuric sulphide and all organic matter in acid solution; this reduces both alkaline copper and picric acid solution at boiling heat. The cuprous oxide remains dissolved.

4. *Physiological Quantities of Kreatinin and of Nitrogen therein excreted.*

34,640 cc. of urine of sp. gr. 1·020, and a reducing power over picric acid equal to 0·67 grains of dextrose per fluid ounce gave 198 grams of Hg salt, or 5·7 grm. from each litre; 8·55 grm. in 1,500 per day, containing 1·71 kreatinin = 0·635 nitrogen in 24 hours..

40·625 cc. of sp. gr. 1·022, and reducing power equal to 0·86 grains of dextrose per fluid ounce gave 293·13 grm. of spherical mercury salt, or 7·19 grm. per litre. This would indicate 0·78 grains per fluid ounce of kreatinin, or 10·785 grms. Hg salt per day, equal to 2·157 kreatinin = 0·801 N. per 24 hours.

In the case of a man of 70·08 kilos. body weight there were obtained in six days 8,930 cc. urine, yielding 41·328 first Hg precipitate and 52·827 second or globular compound. The average weight of kreatinin was calculated as 1·77 grams for 24 hours, or 25 millionths of the body weight, containing 0·65 N. per day.

Average of the three sets of observations: 0·695 grm. N. per day excreted as kreatinin.

As I have shown in Article IV, "The Alkaloids of the Urine," that the amount of nitrogen excreted in 24 hours by a healthy man in the form of urinary alkaloids amounts to nearly 11 per cent. of the whole nitrogen excreted, namely 1·8436 grm. N. included in an average of daily 17 grm. N., and as of this amount 0·695 grm. is excreted in the shape of kreatinin, there remain 1·1486 grm. nitrogen discharged in the shape of the other alkaloids, urochrome, urotheobromin, reducin, parareducin, and aromin; or, in other words, 37·7 per cent. of all the nitrogen excreted in the form of alkaloids is voided in the form of kreatinin, or 4·0 per cent. of the entire daily excretion of nitrogen in all forms passes away as kreatinin.

5. *Purification and Elementary Analyses of the Precipitate.*

The spherical Hg salt is fawn-coloured, from urinary colouring matter while moist, but nearly white when dry. Decomposed by H_2S, it gives a deep yellow solution. This is digested for some days with

pure animal charcoal, and then mixed with solution of sodium acetate and mercuric chloride; largely dilute the decolourised solution before adding the mercuric chloride; separate any precipitate which falls immediately by filtration; the spherical compound will be deposited gradually in a colourless condition.

The analysis gave		Theory of $C_{16} H_{28} N_{12} O_6 Hg_7 Cl_{10}$,
C	8·588	8·575 of which the simplest
H	1·325	1·251 rational formula is
N	7·275	7·503 4 ($C_4 H_5 Hg N_3 O$. HCl)
O	4·247	4·288 3 $Hg Cl_2$. 2 $H_2 O$
Hg	62·560	62·528
Cl	16·005	15·855

The molecular weight of the compound is 2,239 and it contains 20·19 per cent. of the base $C_4 H_7 N_3 O$ (natural kreatinin).

6. *Hydrochloride of Natural Kreatinin and its Reactions.*

Produced by $H_2 S$, charcoal, and evaporation over H_2SO_4 *in vacuo*. Large well-formed prisms with pyramidal apices; to be washed with a little strong alcohol to remove some tarry dark matter; they need no recrystallisation. $C_4 H_7 N_3 O$. H Cl contains 23·74 per cent. Cl.

Mixed in aqueous solution with mercuric chloride, no precipitate ensues; but on addition of sodium acetate the spherical mercury salt separates out. The hydrochloride is greatly soluble in water and only less in alcohol.

On mixing an aqueous solution with mercuric chloride and caustic potash, a whitish precipitate occurs at first, which dissolves in excess of the potash, but after a few seconds the solution becomes turbid again

and deposits a yellow powder, which immediately becomes black by spontaneous reduction.

An aqueous solution mixed with Nessler's reagent, iodide of mercury dissolved in iodide of potassium, deposits a bright yellow matter which rapidly becomes black by spontaneous reduction, a compound ammonia being evolved at the same time.

An alcoholic solution of the hydrochloride of natural kreatinin of urine with an excess of an alcoholic solution of platinic chloride, immediately deposits a yellow crystalline platinum salt, in dendritic masses, which are anhydrous and permanent in the air, $2\,(C_4\,H_7\,N_3\,O.\,H\,Cl)\,Pt\,Cl_4$. When this salt is dissolved in water and the solution is allowed to evaporate spontaneously a platinum salt in orange-coloured prisms is formed, which loses 5·3 per cent. of water at 100°, becoming opaque and of a less yellow tint. The same orange-coloured prisms are obtained by spontaneous evaporation of a mixture of the hydrochloride of natural kreatinin with platinum chloride in aqueous solution $2\,(C_4\,H_7\,N_3\,O,\,H\,Cl)\,Pt\,Cl_4.\,2\,H_2\,O$.

7. *Preparation of the Free, Natural, or Efflorescent Kreatinin.*

The free base is obtained by treating the solution and the hydrochloride in fifteen times its weight of cold water with excess of recently precipitated lead oxide hydrate, and assiduous stirring continued for twenty minutes. When the liquid has acquired a thoroughly alkaline reaction it filters clear through paper. On evaporation in vacuo over oil of vitriol it yields the urinary base, needle-shaped crystals of *efflorescent kreatinin.* This seems to be the true natural kreatinin of urine.

8. *Preparation of two different Forms of Kreatinin, Tabular Kreatinin, Alpha and Beta.*

The washings from the lead oxychloride evaporated at 60° C, deposit anhydrous square tablets on cooling, which when dissolved in cold water yield an alkaline solution, which, on evaporation in vacuo over oil of vitriol, also deposits *efflorescent kreatinin.* When the washings from the mercuric sulphide, which has yielded the hydrochloride, are concentrated over steam, decolorised by animal charcoal, again concentrated over steam, and finally evaporated, in vacuo over oil of vitriol, the resulting crystals seem to be identical with those obtained without the application of heat, but when decomposed by lead oxide hydrate after solution in 15 parts of water and evaporated in vacuo, no efflorescent kreatinin is obtained, but anhydrous oblong or square tabular crystals are formed, similar in shape to the tabular crystals resulting from the evaporation at 60° C, of an aqueous solution of the efflorescent kreatinin of urine, but differing from them by being more transparent, and by this bearing, that their cold aqueous solution, on evaporation in vacuo over oil of vitriol deposits again anhydrous tabular crystals, and not efflorescent crystals.

This tabular anhydrous kreatinin, which recrystallises unaltered from cold solution, S. Johnson terms *tabular kreatinin alpha* (*α*) *of urine*, while the tabular and anhydrous crystals which yield efflorescent kreatinin under the same treatment are termed *tabular kreatinin beta* (*β*) *of urine.*

9. *Convertibility of the three Varieties of Urinary Kreatinin into each other.*

The *efflorescent kreatinin* crystallises in square prisms which, after isolation, lose their transparency within

less than an hour upon mere exposure to air. After complete efflorescence they retain their original form, without any tendency to crumble, and they resemble porcelain in appearance. The hydrated crystals are $C_4 H_7 N_3 O$, $2 H_2 O$. The effloresced crystals are $C_4 H_7 N_3 O$. The latter, when dissolved in boiling water, and when the solution is evaporated in vacuo over oil of vitriol, reappears as tabular kreatinin (*a*), and this weighs as much exactly as did the effloresced kreatinin dissolved in hot water.

10. *Gold Salt of the Kreatinin of Urine.*

Both the tabular kreatinin (*a*) of urine and the efflorescent kreatinin of urine, when dissolved in hydrochloric acid, and mixed with a concentrated aqueous solution of gold terchloride, yield large thin plates of kreatinin hydrochloride gold chloride, a stable salt, permanent in the air, of brilliant and golden yellow shine, darkening a little at 100°, but regaining its lustre on cooling ; its composition is expressed by the formula $C_4 H_7 N_3 O$, $H Cl$, $Au Cl_3$.

11. *Solubility in Water of Efflorescent Kreatinin and of Tabular Kreatinin (a) of Urine.*

Efflorescent kreatinin ($C_4 H_7 N_3 O$, $2 H_2 O$) requires 10·6 times its weight of water at 14° C. for solution.

The effloresced kreatinin ($C_4 H_7 N_3 O$) requires 14 times its weight of water for solution at 14°.

Tabular kreatinin (*a*) was diluted with water at 40° C. The saturated solution, kept at 17° for 12 hours, deposited crystals, but retained one grain in 10·78 grains of water in solution.

Thus it is shown that the solubility in water of efflorescent kreatinin ($C_4 H_7 N_3 O$, $2 H_2 O$, atomic weight 149) *before efflorescence*, is practically identical

with that of the tabular kreatinin (*a*) of urine ($C_4 H_7 N_3 O$) atom. weight 113.

12. *Solubility of Tabular Kreatinin (a) of Urine in Alcohol.*

Kreatinin (*a*) of urine was digested with alcohol of 0·795 sp. gr. for some hours in an extraction apparatus. The hot alcoholic solution deposited some dendritic crystals on cooling; the solution was allowed to stand for 20 hours at 17°; 362 parts by weight of absolute alcohol hold one part of tabular kreatinin (*a*) in solution.

Kreatinin from kreatin of flesh one part, requires 102 parts of absol. alcohol at 16° C. for solution (Liebig).

13. *Amount of Cupric Oxide reduced by Effloresced Kreatinin of Urine and Tabular Kreatinin of Urine in Boiling Alkaline Solutions.*

Mode of Analysis.—The reduced cuprous oxide is held in solution by excess of ammonia; 12·5 grm. of effloresced kreatinin reduce as much cupric oxide as 10 grm. of dextrose.

Twelve grm. of tabular kreatinin (*a*) of urine reduce as much cupric oxide as 10 gr. of dextrose.

$$\underbrace{2\,C_4 H_7 N_3 O}_{226} : \underbrace{C_6 H_{12} O_6}_{180} = 12 : 9{\cdot}5.$$

Hence two molecles of the reducing kreatinin of urine are about equal to one molecle of dextrose in reducing action upon cupric oxide.

14. *Reducing power of Kreatinin produced from Kreatin of Flesh, by Liebig's Method.*

329·94 parts of this kreatinin reduced as much as 180 parts of dextrose, or one molecle of dextrose is

equal to three molecles of this kreatinin in reducing action upon cupric oxide, whereas one molecle of dextrose is equal to two molecles of kreatinin of urine, efflorescent or tabular.

15. *Conclusions concerning the Urine.*

The natural kreatinin of urine causes the bulk of the reduction which normal urine effects in copper solution. The whole amount of reduction effected by that secretion is due to its uric acid and kreatinin. The weight of anhydrous kreatinin passed by a healthy man in 24 hours varies between 1·8 and 2·1 grms. The reduction effected by this is equal to that produced by 1·5 to 1·75 grains of dextrose, or from 23 to 27 grains of dextrose in 52·8 fluid ounces of urine. One-fourth of the total reduction in urine is effected by uric acid.

16. *Differences between Natural Kreatinin of Urine and the Kreatinin described by Liebig.*

(1.) As regards *reducing action*, S. Johnson found differences, but the specimen which agreed best with the description given by Liebig *was destitute of reducing action.*

Note by Dr. Thudichum.—This important observation has been hitherto not sufficiently valued, neither by abstractors nor by S. Johnson himself. I shall show below that specimens of kreatinin made from urine by, in the main, new processes showed *no trace of reducing action* on copper oxide. If, as I believe, S. Johnson has proved the occurrence or producibility of *five modifications* of kreatinin, all of which reduce copper, *a sixth* certainly exists, *which does not reduce copper*, and this particularly requires further study.

(2.) As regards *platinum salts.* Urinary kreatinin

platinum salt of S. Johnson contains 2 molecles of water of crystallisation. All others hitherto described were anhydrous.

(3.) *The solubilities in water and alcohol differ.*—Of Liebig's kreatinin, 1 part required 102 parts of alcohol at 16° for solution. One part of tabular kreatinin (*a*) of urine requires 362 parts of alcohol at 17° C. Liebig's kreatinin dissolved in 11·5 parts of water at 16°. Tabular kreatinin (*a*) of urine dissolves in 10·78 parts of water at 17°.

17. *Conversion of Urinary Kreatinin into Kreatin.*

When urinary kreatinin is boiled with a thousand times its weight of water and evaporated boiling down to crystallisation, it is gradually converted into kreatin, which may be crystallised out and separated. The mother liquor, diluted largely and again concentrated, yields a further crop of crystals, and thus by repetition of the process all kreatinin passes into kreatin. The crystals of kreatin are dissolved in boiling water, boiled with animal charcoal till colourless, filtered and concentrated. On cooling, pure *urinary kreatin* crystallises out, of the formula $C_4 H_9 N_3 O_2, H_2O$.

18. *Reconversion of Urinary Kreatin into Urinary Kreatinin.*

Acted upon by dry HCl at 100, the kreatin gives out 2 molecles of water, and becomes kreatinin hydrochlorate ($C_4H_9N_3O_2, H_2O + HCl = C_4H_7N_3O, HCl + 2H_2O$).

19. *Comparison of the Kreatinin Hydrochloride obtained by Reconversion of Urinary Kreatin with the Kreatinin Hydrochloride obtained from the Spherical Mercury Salt from Urine.*

The hydrochloride obtained from urinary kreatin by the action of H Cl at 100° dissolves in three times its weight of water ; this solution on evaporation *in vacuo*

over oil of vitriol yields transparent flat prisms, which rapidly become opaque on exposure to air, losing 7·06 per cent. in weight, and when dried at 100° C., altogether 10·9 per cent. This corresponds to a molecle of water, and the formula $C_4 H_7 N_3 O$, H Cl, H_2O, which contains 11·04 per cent. H_2O.

The kreatinin hydrochloride obtained by the same process from the kreatin of flesh has the same composition and behaviour.

But the hydrochloride of kreatinin from the mercury salt, which has never been boiled or heated, is invariably anhydrous.

20. *Nature of the Kreatinin obtained after Removal of the H Cl from the Hydrochloride obtained by Reconversion of Urinary Kreatinin.*

When the acid is removed by lead oxide hydrate, and the solution of free kreatinin is evaporated *in vacuo* over oil of vitriol, the efflorescent form of hydrated kreatinin, with two molecles of water, is sometimes obtained ; in other cases the same process yields anhydrous tabular crystals, which are crystallographically identical with the tabular natural kreatinin of urine. The cause of this variation has not been found out, but the two different kreatinins are easily convertible the one into the other.

21. *Crystallographic Form of the Tabular Kreatinin from Mercury Salt and from Kreatin.*

Both forms of tabular crystals belong to the monosymmetric system and show the same angles as those measured by Kopp and Heintz (monoclinometric or clinorhombic system.—See details of measurements in S. Johnson's essay, *loc. cit.*, and in "Pathol. of Urine," 2nd Edition, p. 125.)

22. *Crystallographic Form of the Efflorescent Kreatinin from Mercury Salt.*

The crystals are long prisms belonging to the monosymmetric system.

23. *Platinum Salts of Kreatinin from Urinary Kreatin.*

When a solution of tabular kreatinin, prepared from urinary kreatin by Liebig's process, and which has recrystallised in the tabular form from cold aqueous solution, is acidulated with hydrochloric acid mixed with aqueous solution of platinic chloride, and evaporated *in vacuo* over oil of vitriol, a crystalline platinum salt is formed. The crystals are paler in colour than the platinum salt of the tabular kreatinin of urine from Hg salt, and require nearly twice as much water, namely, 24·4 parts, instead of 14·1 parts, for solution. The salt is purified by washing away the excess of platinic chloride with strong alcohol, and then recrystallising from water.

Like the platinum salt of the kreatinin of urine it contains two molecles of water of crystallisation, which are lost at 100° C., and amount to 5·34 per cent. of the body expressed by the formula $(2\ C_4\ H_7\ N_3O,\ H\ Cl)\ Pt\ Cl_4,\ 2\ H_2O$.

Neither the efflorescent kreatinin of urine, nor the efflorescent kreatinin from urinary kreatin form platinum salts of any definite nature. The crystalline matter which remains when a mixture of chlorides is evaporated *in vacuo* over oil of vitriol, by treatment with alcohol is seen to be a mixture of a little granular matter with transparent colourless needles, probably kreatinin hydrochloride.

24. *Gold Salts of the Several Reducing Kreatinins.*

The efflorescent kreatinin, and also the anhydrous tabular kreatinin from urinary kreatin with gold ter-

chloride, give fine gold salts, crystallising in thin lustrous plates, and having the formula, $C_4 H_7 N_3 O$, HCl, Au Cl_3.

The gold salt of the efflorescent kreatinin from kreatin is darker in colour than that of the efflorescent kreatin of urine, but it has the same composition,

The gold salts of the kreatinin from urinary kreatin are decomposed by ether ; they become opaque, give out gold terchloride, and become kreatinin hydrochloride. This salt is also precipitated when ether is added to an alcoholic solution of the gold chloride salt.

But the gold salts of natural kreatinin from urine are not affected by ether. If ether be added to the alcoholic solution of natural kreatinin gold salt no change takes place.

The gold salts of non-reducing kreatinin (Thudichum) will be considered lower down.

25. *Solubility in Water and Alcohol of Tabular Kreatinin* (*a*) *from Urinary Kreatin.*

One part of tabular kreatinin (*a*) from urinary kreatin requires 10·68 parts of water for solution at 16·5° C., and 324 parts abs. alcohol at 18·5° C. One part of natural tabular kreatinin requires 10·78 parts of abs. alcohol at 17° C.

26. *Effects of Recrystallisation from Water at different temperatures upon Kreatinin obtained from Urinary Kreatin.*

There appear to be four varieties of kreatinin obtainable from urinary kreatin :—

(1.) Efflorescent kreatinin (*a*), which, having effloresced, recrystallises in the same form after evaporation *in vacuo* over oil of vitriol.

(2.) Efflorescent kreatinin (β), which having effloresced, recrystallises in the tabular anhydrous form from cold watery solution.

(3.) Tabular kreatinin (α), which recrystallises in the same form when its cold aqueous solution is evaporated *in vacuo* over oil of vitriol.

(4.) Tabular kreatinin (β), which crystallises in the efflorescent form, when its cold aqueous solution is evaporated *in vacuo* over oil of vitriol.

There is no apparent difference between the crystals of 1 and 2, nor any between those of 3 and 4. Yet when the conditions of solution and evaporation are kept as similar as possible, there is the remarkable difference described in the products of recrystallisation.

The four substances may be obtained as follows :—

(1.) Efflorescent kreatinin (α) is the product usually obtained by treating the kreatinin hydrochloride from urinary kreatin by Liebig's process with lead oxide hydrate at the ordinary temperature. After it has effloresced we may obtain from it either (3) or (4.)

The tabular kreatinin (α) is formed when the solution of the effloresced crystals is made at 100° C., even though the subsequent evaporation be conducted at the ordinary temperature.

The tabular kreatinin (β) is produced when the effloresced crystals are dissolved in water at 60° C.

The weight of both these forms of crystals is always identical with that of the effloresced kreatinin from which they were made.

(2.) Efflorescent kreatinin (β) is obtained by dissolving tabular kreatinin (α) in water at 60° C., and then subjecting the solution to evaporation *in vacuo* over oil of vitriol. Efflorescent kreatinin is never obtained by deposition on cooling from a hot solution.

From tabular kreatinin (α) tabular kreatinin (β) is

obtained by dissolving in boiling water, and then evaporating the solution at the ordinary temperature.

Example of Products of Recrystallisation of Artificial Urinary Kreatinin.

Nature of Crystals.	Dissolved in	Product.
Tabular Kreatinin	Water at 60°	Effloresecent K. (β)
Effloresecent K. (β)	Cold water	Tabular K. (β)
Tabular K. (β)	Cold water	Effloresecent K. (α)

In this case conversion of tabular into efflorescent kreatinin took place, while the efflorescent kreatinin fell back once into tabular. But the kreatinin, by repeated recrystallisation from cold watery solution *in vacuo* over oil of vitriol tends to assume the efflorescent form, and maintain that form ultimately.

By redissolving it at 60° C. we may at any time cause it to change to tabular kreatinin (β) temporarily, which swings back to the permanently efflorescent state after one or two recrystallisations from cold watery solution; or by redissolving in water at 100° C. we may produce tabular kreatinin (α), which recrystallises in the same form indefinitely from cold watery solution.

It is extremely remarkable that the efflorescent kreatinin is obtained by dissolving tabular kreatinin (α) in water at 60, and then evaporating *in vacuo* over oil of vitriol.

27. *Condition of the Two Molecles of Water in the Efflorescent Kreatinin.*

The water does not appear in the gold salt.

The water remains with the needle-shaped crystals, exactly resembling the original base, when it is recrystallised from boiling alcohol.

The water disappears when the alkaloid is dissolved at 100° C. in water, and crystallised *in vacuo* in the cold ; tabular crystals are formed.

It does not form, or does not easily form, a platinum salt.

Anhydrous tabular kreatinin has the same solubility in water as the efflorescent before efflorescence.

Anhydrous tabular kreatinin easily forms a platinum salt.

It recrystallises from alcohol in a form different from the efflorescent.

28. *Cupric Oxide Reduction effected by Tabular Kreatinin from Urinary Kreatin.*

The test is ammoniacal cupric solution.

Sixteen parts by weight of tabular kreatinin (α) reduce as much CuO as ten parts of dextrose, or

Five molecles of tabular kreatin (α) reduce as much CuO as two molecles of dextrose.

Tabular kreatinin (β) from urinary kreatin has the same reducing power as tabular kreatinin (α).

29. *Cupric Oxide Reduction effected by a Specimen of Kreatinin from Kreatin of Beef.*

The kreatinin required 390·2 times its weight of alcohol at 15° C. for solution, and its platinum salt contained water of crystallisation ; so that, though made by Liebig's process, the base did not agree in properties with that described by Liebig.

The reducing action of this base was more feeble than that of the artificial kreatinin from the kreatin of

urine. Three molecles of this kreatinin reduced as much cupric oxide as one molecle of dextrose.

By a comparison of the reducing action of several kreatinins we obtain the following order of decreasing power of deoxydation :—

Tabular and efflorescent } kreatinin from urine } 4 molecles =2 mol. dextrose.

Tabular (α) and (β) } kreatinin from urinary kreatin } 5 molecles = 2 mol. dext.

Kreatinin from kreatin of flesh } 6 molecles=2 mol. dext.

A specimen of kreatinin showed one-twentieth of the reducing power of the first (p. 495).

Kreatinin from kreatin of flesh by Liebig's process was destitute of reducing action (S. J., *l. c.*, p. 518).

Thudichum's kreatinin from urine by Pho. Mo. process passed through $Zn\,Cl_2$ and $Au\,Cl_3$ combination and analysed as gold salt was destitute of reducing action.

Thudichum's kreatinin from kreatin from urine by Pho. Mo. process and alcohol, transformed into kreatinin by $H_2\,S\,O_4$ was destitute of reducing action.

We have here to assert mainly the fact that kreatinin is a base which is capable of assuming six isomeric conditions, five of these modifications are characterised by their power of reducing cupric oxide.

One modification out of six possesses no reducing power over cupric oxide at all. And this is the modification which, with the exception of its gold chloride salt, has as yet received the least study.

The bearing of these remarkable data upon the question whether or not normal urine contains any dextrose sugar has been discussed in Article V.

30. *Summary of Data on Non-reducing Kreatinin given by S. Johnson* (*Proc. R. S., vol.* 43, *p.* 495, *June*, 1887).

A sample from Hopkin and Williams was "very deficient in reducing power." It exhibited only about one-twentieth of the reducing power of the reducing base.

The specimen of kreatinin from flesh by Liebig's process (from kreatin) was destitute of reducing action (S. J., *l. c.*, p. 578). Another specimen of kreatinin from kreatin from flesh by Liebig's process had a reducing action which was feebler than that of the artificial kreatinin from the kreatin of urine. It required 3 mol. of this kreatinin to reduce as much cupric oxide as 1 mol. of dextrose (S. J., *l. c.*, p. 529).

31. *Non-reducing Kreatin and Kreatinin prepared by Thudichum.*

Kreatin, a portion of a pure crystallised specimen, prepared from human urine by the phosphomolybdic process, &c., and alcohol, was boiled with dilute sulphuric acid, and thus transformed into *kreatinin* (sulphate). The solution-made alkaline did not change Trommer's test.

Kreatinin contained in the alcoholic solution of the mixture of alkaloids from urine also did not affect the copper test.

I had specially analysed a specimen of *crystallised kreatinin hydrochlorate gold chloride*, which had been prepared from human urine by the phosphomolybdic acid process, and the kreatinin of which had been obtained by direct crystallisation and combination with zinc chloride, and had then been combined with the gold salt, and this gold salt had been subjected

to elementary analysis as regards five of its elements with the following result :—

Quantities of Elements in Kreatinin Hydrochlorate Gold Chloride.

	Theory.		Found.
Atoms.		Per Cent.	Mean of all Analyses.
4 C	...	10·60	... 10·86
8 H	...	1·77	... 1·93
3 N	...	9·27	... 9·25
O	...	—	... —
Au	...	43·45	... 43·68
4 Cl	...	31·36	... 31·12

I removed the gold with hydro-sulphuric acid from the clear warm solution, evaporated the solution to dryness to expel excess of hydrochloric acid. redissolved the residue, and crystallised the kreatinin hydrochlorate from water.

The product was tested in three portions ; first and second crystals, and mother liquor. The solutions made alkaline with caustic soda and added to alkaline copper solution gave not a vestige of reduction.

32. *Synoptical Table of Six Isomers of Kreatinin.*

[For Synoptical Table see following pages.]

Synoptical Table, first half.

Form of Kreatinin ...	Efflorescent Kr. of Urine. $C_4 H_7 N_3 O, 2 H_2 O$.	Tabular Kr. of Urine. $C_4 H_7 N_3 O$.	Efflorescent Kr. from Urinary Kreatin. $C_4 H_7 N_3 O, 2 H_2 O$.
Solubility in Water ...	1 in 10·6 at 14° C.	1 in 10·78 at 17° C.	——
Solubility in Alcohol...	——	1 in 362 at 17° C.	——
Platinum Salt	Indefinite or decomposed by Alcohol.	2 ($C_4 H_7 N_3 O$, H Cl) Pt Cl_4, $2 H_2 O$.	Indefinite or decomposed by Alcohol.
Solubility of Platinum Salt in Water... ...	——	1 in 14·1 at 15° C.	——
Gold Salt	Unchanged by Ether.	Unchanged by Ether.	Decomposed by Ether.
Reduction of CuO compared with that of Dextrose	4 mols. $C_4 H_7 N_3 O$ = 2 mols. dext.	4 mols. $C_4 H_7 N_3 O$ = 2 mols. dext.	5 mols. $C_4 H_7 N_3 O$ = 2 mols. dext.

Synoptical Table, second half.

	Tabular Kr. from Urinary Kreatin. $C_4 H_7 N_3 O$.	Kreatinin (Liebig).	Kreatinin, (Thudichum).
Form of Kreatinin ...			
Solubility in Water ...	1 in 10·68 at 16·5° C.	1 in 11·5 at 16° C.	——
Solubility in Alcohol...	1 in 32·4 at 18·5° C.	1 in 102 at 16° C.	——
Platinum Salt	2 ($C_4 H_7 N_3 O$, HCl) $Pt Cl_4$, $2 H_2 O$.	2 ($C_4 H_7 N_3 O$, H Cl) $Pt Cl_4$.	——
Solubility of Platinum Salt in Water... ...	1 in 24·4 at 15° C.	——	——
Gold Salt	Decomposed by Ether.	——	$C_4 H_7 N_3 O$, $Au Cl_3$ and $C_4 H_7 N_3 O$, H Cl, $Au Cl_3$.
Reduction of CuO compared with that of Dextrose	5 mols. $C_4 H_7 N_3 O$ = 2 mols. dext.	6 mols. $C_4 H_7 N_3 O$ = 2 mols. dext.	No reduction of copper-test.

VII.—THE BENZOYLATED ESTERS OF UROCHROME, AND SOME BENZOYLATED ESTERS OF NEW EMUNCTORY COHOLS.

ORIGINAL RESEARCH.

1. *The Precipitate produced by Benzoyl-Chloride in Urine.*

To every litre of urine made strongly aklaline with caustic soda, and filtered from the precipitate of phosphates of earths, are added 100 further cc. of a solution of caustic soda (made by dissolving one part by weight of caustic soda in five parts by weight of water) and 50 cc. of benzoyl-chloride. The mixture is immediately strongly shaken; if it become warm, it is cooled by water, and care taken to keep it strongly alkaline. An experienced eye can recognise the loss of the alkaline reaction by a change of the colour of the mixture to a lighter yellow, the alkaline solution remaining more amber-coloured; an acid condition of the mixture is also recognised by the evolution of carbonic anhydride, which the alkaline one of course does not evolve.

The precipitation, which takes place immediately, produces a yellowish or yellow mixture of substances, of which some are *oily*, others *viscous*, others *solid*. It is best to let the precipitate settle and draw off the liquid by a syphon; filtration of the bulk is very tedious, and the oily parts of the precipitate run through the filter with the mother liquor, obstructing the paper so that filtration ceases. Therefore edulcoration may be effected only by infusion of water and decantation. In different experiments somewhat different precipitates are obtained; one set will be *viscous*, *lumpy*, like freshly-curdled milk; another will be more *pulverulent;* a third more *oily;* in a fourth the oil will

collect below the pulverulent layer. Sometimes urine, after the first precipitate has been removed, may, on renewed addition of benzoyl-chloride, give a second precipitate, but it is mostly small in amount, and does not repay the trouble of its production. This should, however, be remembered by inquirers intending to institute quantitative experiments.

2. *Separation of Proximate Constituents of the Precipitate.*

The precipitate in the moist viscid state is stirred or shaken with *strong alcohol in the cold;* a portion dissolves with a light yellow colour, while another portion remains undissolved and becomes more solid, though still retaining a certain degree of viscosity. When it yields nothing to spirit, and this remains colourless the extraction is practically complete.

We have thus divided the total precipitate into two parts; one, light yellow, *soluble in the cold alcohol* another of deep yellow colour, *insoluble* in it.

The part insoluble in cold alcohol is now boiled with new alcohol and dissolves in it with a golden yellow colour, leaving, however, a small quantity of a *slightly-coloured* substance undissolved. The alcoholic solution must be filtered hot, as it deposits the dissolved ester immediately on cooling. Sometimes the solution remains turbid, though a part of the ester be deposited; sometimes it falls as a pulverulent deposit, easily isolated; sometimes it settles as a resin at the bottom of the flask. Sometimes one has all three conditions to deal with, turbidity, pulverulent deposit, and resin. The *resin*, after isolation and drying, becomes hard and pulverisable, and seems not essentially different from the pulverulent deposit.

The *alcoholic mother liquor* contains a small amount

of the pulverulent and resinous body in solution as also any part of the body soluble in cold alcohol, which, owing to the compact resinous condition of the matter escaped the first extraction by cold alcohol. These bodies are all isolated by gradual concentration. Distillation makes the solution a little darker in colour, slow evaporation in a water-bath produces less colour, spontaneous evaporation of alcohol the least, but the latter is not practically applicable to large quantities.

We have now divided the precipitate into *three parts:*

(1) *One* insoluble in cold and hot alcohol, very small in amount.

(2) A *second* one, pulverulent, or resinous at first, pulverulent after being dried, soluble in boiling, almost insoluble in cold alcohol.

(3) A *third* part, viscous to oily, easily soluble in cold alcohol, and deposited as an oil on concentration of the alcohol.

3. *Bearing of the various Portions of the Esters with Hot and Boiling Alcohol.*

There appears to be no essential difference between the solvent effect of absolute alcohol and that of spirit of 80 to 90 per cent. strength.

The most pulverulent deposit (of esters) is obtained by allowing a hot solution of pulverulent deposit to flow into a cold saturated solution of the ester.

On slow evaporation of a solution filtered from the pulverulent deposit, or on distillation of a part of the solvent spirit, another part of the ester is deposited as a *soft* somewhat translucent *yellow resin*. Resolution of this resin in absolute alcohol, &c., does not remove the viscosity, either when the alcohol is concentrated by heat or allowed to evaporate spontaneously.

But this *yellow resin* so deposited, when removed

from the absolute alcohol or spirit, and put in a vacuum over calcium chloride, mostly becomes dense, hard, brittle, translucent ,and cracks by contraction. In thin layers, it forms a transparent varnish. It is quite insoluble in water, even on boiling, and mostly loses its viscosity on cooling.

When the spirit solution of the entire precipitate of esters, from which the insoluble in spirit part has been removed, is so treated as to allow the ingredients to become deposited in the order of their solubility, the following portions are isolated :—

A *least soluble* pulverulent part, small in quantity, and containing up to 4·5 per cent. of nitrogen. In appearance this does not differ from the next one.

A *more soluble part*, but also deposited from the spirit on cooling when saturated hot ; this is the main bulk of that part of the preparation, which appears pulverulent.. An *absolute alcohol* solution of this part, which has deposited resin by spontaneous evaporation, and was therefore saturated at 15° C., had a golden yellow colour. 5·6350 g. of solution left 0·2545 g. dry residue, equal to 4·51 per cent. This part contained only 1·540 per cent. of nitrogen, with 63·249 per cent. of carbon, and 5·100 per cent. of hydrogen.

The alcoholic solutions of these esters have a dangerous tendency to bump, and should therefore be distilled in small quantities at a time.

The esters are insoluble in dilute hydrochloric acid ; in their spirit solution neither platinic chloride nor cadmium chloride gives a precipitate. There is no alkaloidal body discoverable ; *the urochrome has completely lost its alkaloidal and acid properties.*

4. *Non-nitrogenised Crystallisable Benzoylated Esters of Cohols hitherto unknown accompanying the Yellow, Uncrystallised, and Nitrogenised Benzoylated Urochrome Esters.*

The benzoylated esters contained in the first precipitate, which are at least six in number, are divisible in two classes, firstly, such as are *nitrogenised*, and these are mainly *urochrome compounds*, and secondly, such as are *non-nitrogenised*, and contain alcohols hitherto not known as ingredients of the urine. These latter are all *crystallised*, while the urochrome esters have as yet exhibited *no sign of crystallisation.*

The esters, when dissolved in hot alcohol, *keep each other in solution*, but when isolated exhibit somewhat different degrees of solubility. This applies particularly to a small quantity of a *yellow* ester, and also a small quantity of a *white crystallised ester ;* both when isolated are quite insoluble in hot or cold alcohol, in which, when in company with the rest of the esters, they are soluble and dissolved.

To obtain the *crystallised esters* a somewhat considerable quantity of the first benzoylated precipitate is, immediately after isolation, dissolved in boiling absolute alcohol, filtered hot, and after it has deposited the less soluble urochrome ester, is evaporated in the water-bath to a low bulk ; on cooling it becomes almost solid and is allowed to stand and crystallise in a cold place. Success depends upon obtaining the degree of concentration which makes crystallisation possible

The crystalline cake is stirred with cold spirit, which dissolves the oily urochrome ester, and leaves the *white crystallised* esters undissolved. They are thrown on bibulous paper, rinsed with a little spirit, and pressed between bibulous paper. A white crystalline cake is

thus obtained, which by solution in hot alcohol may be divided into three different bodies—

1. A crystallised white part *insoluble in boiling spirit.*

2. A part *soluble in boiling spirit* deposited in masses of fine needles on cooling.

3. Part *soluble in cold spirit*, deposited *on concentration* in crystals of the lustre of mother of pearl, of the shape of cholesterin.

Of this latter body four grammes were obtained in one operation and subjected to a careful analytical study followed by chemolysis. We will term it, from its shape, *rhombic ester, free from nitrogen.*

The *mother liquor of the non-nitrogenised* esters has a deep red colour, and is, of course, yet saturated with these esters, for the isolation depends upon their being surprised in the state of crystallisation. It is, however, mainly a solution of the *viscous or oily urochrome esters.* When the solution is mixed with water, the ester is precipitated as a viscid mass and the solution becomes nearly colourless.

The viscid benzoylated urochrome ester is soluble in *ether*, in *acetone*, and in *chloroform.*

5. *Benzoylisation of Isolated Urochrome—Polybenzoylate and Spanobenzoylate.*

A watery solution of urochrome prepared by ferric chloride from baryta-treated urine, solution of the ferric salt in dilute sulphuric acid, precipitation of the urochrome by pure phosphotungstic acid, decomposition of the phosphotungstate by caustic baryta, neutralisation of the excess of the latter by carbonic anhydride is treated with caustic soda and benzoyl chloride. The yellow precipitate is immediately formed, isolated, and edulcorated; for better purification it

is extracted repeatedly with hot water; in this it fuses, but becomes hard on cooling, and pulverisable when cold and dry. It is then dissolved in hot spirit. (A mere trace of a *colourless matter*, which is powdery when dry, is left in the filter.)

Quantation of Nitrogen.—0·7686 gr. burnt with soda-lime, &c., gave 0·0966 gr. of Pt., equal to 1·77 per cent. N.

The small amount of nitrogen in this ester proves that the urochrome, which contains more than 20 per cent. of N., was highly benzoylated, and in consideration of the many radicles of benzoyl contained in it, may be termed *a polybenzoylate*. In contrast to this an ester with few radicles of benzoyl may be termed a *spanobenzoylate*. These distinctions and names are necessary to signalise the phenomena and resulting bodies which are produced during chemolysis, and also during synthesis under conditions of limitation.

The production of a diagnostic polybenzoylated ester from isolated urochrome proves that *the process* of isolating by ferric chloride, or phosphotungstic acid, or both in succession, does not alter the properties of urochrome.

6. *Urochrome contained in the Complex of Alkaloids precipitated from Urine by Pho. Wo. Acid, after Removal of Baryum Reducin by Alcohol, is precipitated from this Solution by Mercuric Chloride and from this Compound obtained as Benzoylated Ester.*

The solution of mixed alkaloids precipitated from the urine by the Pho. Wo. acid and baryta process is evaporated, and the dry residue extracted with alcohol; this leaves *baryum reducin* undissolved. The alcoholic solution gives a copious precipitate with *mercuric*

chloride, which contains *several alkaloids*, amongst them *urochrome*. The reaction must be completed carefully as much reagent is required ; the precipitate is voluminous and heavy, and undergoes a change on standing, in part by reduction of mercuric to mercurous salt ; it is turned black by caustic potash, but ammonia leaves it white at first, changing into black after a time. This precipitate, which is yellow in the dry state, is decomposed in water by H_2S, and yields an acid yellow solution, which when evaporated to a small bulk makes no deposit.

Benozyl-Chloride Treatment.—Much soda and then benzoyl-chloride added to the solution produce at once the characteristic *precipitate of benzoylated urochrome*, which soft and viscid at first, becomes firm on standing.

Result.—Urochrome extracted *as alkaloid* from urine by specific precipitants of alkaloids combines with mercuric chloride, also like an alkaloid ; when again set free it combines with benzoyl *like an alcohol* and forms *an ester*, or *several esters* differing only by the amount of benzoyl in their composition.

7. *Urochrome in the Phospho-Tungstic or Phospho-Molybdic Acid Precipitate from Urine, when dissolved in Caustic Alkali, is precipitated by Benzoyl-Chloride as Ester.*

The precipitates produced in acidified urine by the conjugated phosphorised acids named are completely soluble in caustic alkali. Benzoyl-chloride added to this solution produces immediately the characteristic precipitate of the benzoylated urochrome esters. They must be edulcorated completely from any traces of the molybdic or tungstic salts in the cold, as they react with them on application of heat in the presence of water or alcohol.

8. *Summary of Modes of Preparing Benzoylated Urochrome Esters.*

The urochrome esters have now been prepared by the following processes :—

1. Directly from alkalified *urine* by benzoyl-chloride.

2. From *isolated urochrome* by the same. As the urochrome was isolated by the phospho-wolframic and phospho-molybdic acid process, as an alkaloid, and secondarily by the ferric chloride process, as a cohol or weak acid, all carbohydrates are absolutely excluded.

3. From the *mixture of alkaloids* prepared by the phospho-wolframic and phospho-molybdic processes, after removal of reducin baryum by alcohol, precipitation with mercuric chloride, and decomposition by sulphuretted hydrogen ; from this solution benzoyl-chloride precipitates the ester.

4. From *phospho-tungstic precipitate* out of acid urine, dissolved in caustic soda, by benzoyl-chloride.

5. From *urochrome* precipitated from urine primarily by ferric chloride, and from this by phospho-molybdic acid. This process is a reversal of the succession of the precipitants used in process No. 2.

9. *Urochrome, a Manifest Alkaloid behaves as an Alcohol (Cohol).*

We have in a previous article given the evidence which proves urochrome to be an *alkaloid ;* but in its reaction with benzoyl-chloride it manifests the properties of *an alcohol*, and admits in other reactions those of a *weak acid*, for it combines with bases such as *lime*, *baryta*, and *strontia*, and these compounds are not entirely dissociated even by strong alkalies, and with *lead oxide*, separated by sulphuric acid, and with *ferric chloride* in feebly alkaline solution ; the latter com-

pound is soluble in weak acid, and in strong caustic ley. That an *alkaloid* should also manifest the properties of an *alcohol* is a fact of which chemistry records a series of remarkable instances illustrative of the formation of urochrome esters. Thus *coniine* forms esters with fatty acid aldehydes (Beilstein, p.1932); *morphine* with benzoyl forms a dibenzoylated ester (B. 1946); *codeine* the same (B. 1950); *oxychinoline* yields a benzoate (B. 2011); *kynurin* the same (B. 2012); *kynuric* (syn. *kynurenic*) *acid* is very similar to urochrome in this, that it also combines with phospho-molybdic acid as an alkaloid, with bases as a weak acid, and with *benzoyle* as an alcohol (B. 2021).

10. *Chemolysis of Benzoylated Urochrome Esters by Dilute Sulphuric Acid.*

When the benzoylated urochrome ester is treated with sulphuric acid of two per cent. strength, benzoic acid is quickly detached, and crystallises from the acid on cooling. The ester becomes less in volume, darker in colour, is liquid while hot, solid on cooling, and at one period looks deceptively crystalline when cold, from detached benzoic acid which it retains very strongly, as it forms bad contact with water. Much boiling water and long agitation are required to extract even the bulk of benzoic acid. Only little urochrome is set free, and passes into the solution precipitable by Pho. Mo. acid. If the treatment with acid and extraction with water be continued until no more benzoic acid is extracted the ester remains viscous on cooling. It is then entirely soluble in hot spirit, but this solution on cooling makes a deposit of *uropittin*. All uropittin is removed by dissolving the ester in ethylic ether, in which uropittin is quite insoluble.

11. *Chemolytic Separation of the Cohols of the Non-nitrogenised Esters from the Urochrome.*

By this chemolysis with sulphuric acid the *non-nitrogenised crystalline esters* are decomposed quicker than the urochrome, and their cohols pass into solution in the water. The spanobenzoylated urochrome, while losing a number of molecles of benzoyle, thus becomes free from these esters, which can be removed only from the pulverulent ester, but partially only from the viscous one. The acid solution now contains four principal ingredients : (1) the sulphuric acid, (2) the benzoic acid, (3) some urochrome in the free state, (4) the cohols of the non-nitrogenised esters. They are to be separated as follows :—

a. Remove the bulk of *benzoic acid* as crystals from the concentrated solution ; extract the rest by ether.

b. Precipitate *urochrome* by pure *Pho. Mo.* or *Pho. Wo.* acid in slight excess.

c. Remove excess of *Pho. Wo.* and all sulphuric acid by carbonate of baryum and a little caustic baryta.

d. Evaporate filtrate, and crystallise the *non-nitrogenised cohols* of the *crystallised esters*. If they cannot be separated as such from each other, benzoylise them again, and separate their esters as well as may be by crystallisation from alcohol.

12. *Theory of Benzoylated Urochrome Ester as suggested by the Facts of Synthesis and Chemolysis.*

Considering that this ester yields great masses of benzoic acid by chemolysis, it is necessary to assume that it is a compound of a *polydynamic alcohol, urochrome*, with many molecles of *benzoyle*. Considering further that many molecles of benzoyle may be removed gradually, in the shape of *benzoic acid*, from

any specimen of ester without its losing its insolubility or changing much in external visible properties, it is necessary to assume that urochrome may form many esters, differing only by the number of molecles of benzoyle which they contain. The benzoyle is held by the urochrome with *different degrees of affinity* (the dynamicities are unequal in power), so that, while it is easily parted with at first, under the influence of mere water, or boiling in alcohol, it is surrendered with difficulty at last; the fewer are the molecles of benzoyle present, the firmer they resist the chemolytic action of the acid. This seems also to be the case with the *soda chemolysis*, by which also the ester becomes at first firmer, and then requires stronger ley for final decomposition.

13. *Gradual Decomposition of Esters.*

But the primary compounds also seem to have a stability greater than the intermediate ones. When once in spirit they seem very stable. But when partially chemolysed, although no part may be chemolysed down to freedom from benzoyle, the decomposition seems to proceed for a while, so to say, spontaneously, *i.e.*, without the aid of any definable agent, except water.

Thus a large specimen, chemolysed by sulphuric acid of two per cent. strength, seemed near its complete decomposition, as some urochrome was free. It was boiled with water many times until it yielded no more acid to it. It was now freed from water, and dissolved in *ether*, whereby some *uropittin* was precipitated, a proof of the decomposition of a part of the liberated urochrome. The ether was distilled off, the residue was dried after droplets of water had been removed by

capillary attraction with bibulous paper. The ester remained fluid, somewhat like olive oil, but it deposited on standing slowly and gradually crystals of benzoic acid, and these increased the more the longer the ester was allowed to stand.

Most preparations of the esters *become darker* and less soluble, and in part insoluble, in every operation, n alcohol, ether, and in the free state, the *pulverulent ones* in the quite dry state ; the latter increase somewhat in weight by absorption of oxygen.

14. *Chemolysis of the Urochrome Esters with Caustic Soda.*

The viscid ester, when treated with somewhat concentrated soda solution in the water-bath becomes more fluid and floats on the surface. The solution becomes the deeper yellow or amber-coloured the longer the warming is continued ; the process is interrupted and solution filtered off. What remains of the previously viscid compound on cooling *becomes hard and brittle.*

Hyrdochloric or sulphuric acid produces immediately a copious precipitate of *benzoic acid*, soluble on boiling.

By continued boiling of the super-acid fluid, which has a pale yellow colour, it becomes brownish, evolves the characteristic smell of decomposing urochrome, and deposits *the mixed urochrome resins*, identical with those which have been described as produced in the chemolysis according to Proust, and in the chemolysis of the pure urochrome isolated by the processes which I have described. These resins are filtered off, whereupon the solution on cooling deposits more *benzoic acid*, coloured by some part of the *resins*, which are soluble in hot water, and not absolutely insoluble in old.

When a somewhat larger volume of the primary quite freshly-made mixed esters is triturated with *concentrated* soda-ley a strong reaction ensues, with evolution of much heat ; the resin is transformed into bodies, which (with the exception of one present in minute proportion) are entirely dissolved on the addition of a sufficiency of hot water. If a sufficiency of hot water be not added, *benzoate of sodium* crystallises on cooling.

To the solution dilute sulphuric acid is added, as long as it produces a precipitate of *benzoic acid.* The solution retains *the urochrome*, mixed with the sulphate and remainder of the benzoic acid.

15. *Recovery of Urochrome from the Products of Chemolysis.*

When the boiling with acid of the caustic soda solution of the esters is omitted, or opportunely interrupted, while all the benzoic acid present as benzoate is precipitated out, a *solution of urochrome in the free state* is obtained, from which the urochrome may be removed and obtained in a pure state by any of the following processes :—

(1) By *precipitation* with *Pho. Mo.* and *Pho. Wo.* acid, added to the solution acidified with excess of sulphuric acid.

(2) By *reconstitution* of the *ester*, by adding to the solution made alkaline with excess of caustic soda *benzoyl-chloride*.

In the first process the urochrome behaves as *an alkaloid*, in the second one as *an alcohol.*

But when the solution of urochrome is boiled with excess of sulphuric acid the body is entirely decomposed and yields its peculiar products, the *urochrome resins.* They are separated from benzoic acid by hot water.

16. *Isolation of the Urochrome Resins.*

The urochrome resins are dark, plastic, and adhesive while hot, brittle when cold, and may be powdered. When heated on platinum foil they fuse, give out irritating vapour, blacken, then evolve a powerful smell of burnt urine, and leave a charcoal which is difficult to burn. This bearing is diagnostic of the *omicholin bodies*, which are the fusible and odorous principles ; the charcoal is produced by the *uromelanin* and *uropittin.*

Ether extracts the *omicholin*, absolute alcohol the *uropittin ;* these remains insoluble in ether or alcohol, easily and entirely soluble in very dilute caustic soda, and reprecipitated by sulphuric acid, the *uromelanin.*

In the acid solution from which these bodies have been removed, much *omicholin* and *omicholic acid* remains dissolved, and forms oily skins and flakes on concentration, gives out the diagnostic powerful smell, adheres to any benzoic acid which may be deposited, and as it is more soluble in hot than in cold water, is deposited on cooling. It can be precipitated by phospho-molybdic acid. The filtrates from the resins, however treated for the removal of omicholin, deposit large volumes of *benzoic acid*, which must be isolated and pressed. The mother liquors yield two or three further deposits of acid and resin ; at last only sulphate of sodium remains. In this process the considerable solubility of *omicholin* in hot water becomes conspicuous, and accounts for the small quantities of the body obtained in Proust's chemolysis.

17. *Chemolysis of Benzoylated Urochrome Ester by Caustic Soda.*

The ester is suspended in strong *caustic soda* by (1

in 6 solution) and warmed in the water-bath until complete dissolution has taken place. To the cold solution sulphuric acid is added until a precipitate of benzoic acid takes place. The *benzoic acid* obtained is filtered off, pressed, dried, and weighed. The mother liquor is exhausted with ether, the ether solution distilled from a tared flask ; the residue of benzoic acid is weighed. The mother liquor thus exhausted contains the greater part of the urochrome. *The phospho-molybdate of urochrome* is dried and weighed ; in this carbon and hydrogen and nitrogen may be quantated as usual, while the phospho-molybdic acid may be quantated after fusion with nitre and separation as ammonium salt by solution in ammonia and precipitation of the phosphoric acid as triple phosphate, *i.e.*, phosphate of magnesium and ammonium.

18. *Elementary Quantation of the Benzoylated Urochrome Esters, and Conclusions derived therefrom.*

Benzoylated urochrome ester was subjected to elementary analysis and gave :—Carbon, 63·249 per cent. ; hydrogen, 5·100 per cent. ; N, 1·540 per cent. ; oxygen, 30·111 per cent. ; leading to an empirical formula of C_{48} H_{46} NO_{17}. The number of atoms of carbon and hydrogen are nearly equal ; the latter element even appears lower than the carbon ; whereas in all ordinary carbohydrates the atoms of hydrogen are *nearly double* the number of atoms of carbon. The 63·249 per cent. of C. in a carbohydrate would require 10·54 per cent. of H. If a molecle of carbohydrate were combined with several molecles of benzoyle, each replacing an atom of H, the composition of this polybenzoylated ester would be assimilated more to that of benzoyle, but the carbohydrate would influence the

spanobenzoylated esters more in the direction of its own composition, and make the hydrogen rise. The analysed ester therefore is not that of a carbohydrate, and does not contain any such as a cohol. And this result is unavoidable whether the matter analysed were a unitary substance or a mixture of esters.

A pulverulent specimen, small in quantity, was analysed for nitrogen, and gave by soda-lime and platinum 4·5 per cent. N.

A pulverulent specimen deposited from hot alcohol on cooling was analysed for all its elements, and gave: Carbon, 58·90 per cent.; hydrogen, 5·02 per cent.; nitrogen, 3·023 per cent.; giving an empirical formula of $C_{23}\,H_{23}\,NO_{15}$. To these relations between carbon and hydrogen the observations contained in the first paragraph apply with equal force.

A pulverulent specimen of ester prepared from previously isolated urochrome, treated with alcohol, by deposition from the spirit, on cooling gave 1·77 per cent. of nitrogen.

An oily or viscous ester, extracted from a primary precipitate by cold spirit, was washed with hot water, dried, incorporated with soda-lime, &c., gave 1·10 per cent. of nitrogen.

This ester was partially chemolysed by sulphuric acid, and much benzoic acid was extracted. The residue gave 1.58 per cent. of N. The nitrogen had been increased by about one-third through the abstraction of a certain quantity of benzoyl.

19. *Benzoylated Urochrome Esters arranged in the Order of their Increasing Nitrogen.*

Viscous ester	N = 1·10	per cent.
The same partially chemolysed	„ 1.58	„
More soluble portion of esters	„ 1·54	„
Pulverulent esters from isolated urochrome	„ 1·77	„
Pulverulent specimen deposited from abs. alcohol ...	„ 3·02	„
Pulverulent specimen least soluble in abs. alcohol... ...	„ 4·50	„

None of these esters were, of course, pure; they were all mixtures of urochrome esters of several degrees of benzoylisation, and contained residues of the non-nitrogenised esters described under 4. But they were all essentially urochrome esters, and yielded this body as well as its characteristic products of chemolysis. None yielded any carbohydrate, and none even yielded any reduction of alkaline copper solution. Their nitrogen is, therefore, an essential ingredient, and not a negligible impurity.

20. *The Benzoylated Non-nitrogenised Esters—The Crystallised Rhombic Ester.*

The ester is free from nitrogen, as was proved by a combustion with soda-lime in form. It is not altered by being boiled in water. It has a sweetish burning taste. It crackles when heated on platinum foil, and is thrown about, then fuses with incipient decomposition and emission of acid vapours; it then loses its liquidity and becomes solid; it now evolves peculiar smelling vapours, and the dark mass fuses a second time, but with greater viscosity, and is at last consumed. When heated in a glass-tube, it gives out

agreeably smelling vapours, not sharp like benzoic acid, then condenses to a white sublimate and an oil ; the ester does not fuse at first, but when the more volatile part is gone the residue becomes black, fuses and gives out vapours which burn with a sooty flame, and have a mild, somewhat aromatic smell. No trace of urinary colour or odour attaches to the ester or is evolved during its combustion. On combustion with lead chromate it gave carbon, 54·14 per cent. ; and hydrogen, 4·09 per cent. ; leaving for oxygen, 41·59 per cent. This gives an empirical formula of $C_{13} H_{12} O_8$.

21. *Chemolysis and Theory of the Rhombic Crystallised Non-nitrogenised Ester.*

1·9 grm. in a glass tube with 100 cc. of water containing 2 grms. of oil of vitriol was heated in a water bath for some hours. The solution deposited benzoic acid in crystals immediately after having been poured off the undissolved part. The acid was renewed several times, and the decomposition of the ester was complete ; the cohol remained as a *resinous pellet*, plastic while hot, hard when cold. The benzoic acid was collected by filtration and extraction with ether. The sulphuric acid was removed by baryum carbonate, and the *solution* evaporated. Solution and residue had neither colour nor odour of omicholine or anything else. In the highly concentrated state it produced not the slightest change when boiled with alkaline copper solution. Evaporated to near dryness, and allowed to stand, it deposited *a crystalline mass*, which, with the pellet, represented the *cohol* of the benzoylated ester.

The chemolysed ester had weighed 1·9650 grms.; there was removed *benzoic acid* pure, 0·9930 ; leaves for cohol 0·9720.

This ester was, therefore, not polybenzoylic, but contains probably only one molecle of benzoic acid upon one molecle of cohol. By this and its freedom from nitrogen it shows that it is not related to the colouring matter, and not derived from it, and contains no carbohydrate.

The most probable formulation of the facts thus far ascertained is as follows :—

$$\underbrace{C_{13}\,H_{12}\,O_8}_{\text{Crystallised ester.}} + H_2\,O = \underbrace{C_7\,H_6\,O_2}_{\substack{\text{Benzoic acid} \\ \text{M.W.} = 122.}} + \underbrace{C_6\,H_8\,O_7}_{\substack{\text{New Cohol} \\ \text{M.W.} = 192.}}.$$

22. *Body Insoluble in Alcohol remaining on Filter when the First Precipitate of Esters is dissolved in Spirit.*

Of this body only a very small quantity is obtained which might be overlooked amongst the impurities Dissolved in somewhat strong caustic soda and filtered, the solution fluoresces curiously. It is reprecipitated in white flakes by sulphuric acid, of which a slight excess extracts a little calcium phosphate. A closer investigation of the substances, which cannot be neglected by the physiologist, requires large quantities of material.

23. *Search for Carbohydrates amongst the Benzoylated Esters.*

I have applied Trommer's alkaline copper test in a great variety of ways to all the compounds and their products of chemolysis, and *never obtained any reduction whatever.*

Thus some of the *saturated spirit solution* of all the

esters was boiled with Trommer's test, but no reduction ensued.

When the alcoholic solution was, as a preliminary, boiled with sulphuric acid, and made alkaline, no reducing substance was produced. When the benzoylated urochrome, made from isolated urochrome, was decomposed with caustic soda, the alkaline dark-coloured solution gave no reaction with alkaline copper solution.

The rhombic crystallised ester did not react with the copper test on boiling. The decomposition products of this ester, obtained by chemolysis with sulphuric acid, gave no reduction with copper at any stage.

In the decomposition of the urochrome (liberated from the ester by caustic soda) by sulphuric acid, no reduction of copper was obtained at any stage with any product or mixture.

24. *Influence of Time upon the Benzoylated Esters from Urine.*

When the mixed esters, immediately after isolation, are heated with caustic soda of sufficient strength they all dissolve, under evolution of heat, without residue. When the esters have been kept for some time, they are only slowly soluble, and require renewal of the alkali. Ultimately a portion remains which is not dissolved or altered by soda.

Benzoyl urochrome is affected by warm caustic soda of 10 per cent. strength in such a manner that the oily and viscid parts are dissolved out first, and what remains at first undissolved is *soft* while hot, but *hard* when cold. This fusion is observed in other esters, *e.g.*, the inosite-benzoyl ester. This bearing also indicates the presence of *different kinds of esters of*

the same urochrome, the liquid ones with more, the solid ones with less, benzoyl added. The polybenzoylic esters are the more labile, the spanobenzoylic however the less deciduous chemical compounds. During chemolysis with soda a portion of urochrome ester becomes at last quite *insoluble in boiling alkali*, it is not changed. It is thus seen that as the research progresses it increases in complication.

25. *Separation of Benzoyl-Urochrome from the other Esters.*

The separation above described was effected by means of crystallisation and cold spirit, which dissolved the urochrome esters before the crystals. The urochrome esters, of course, contained some residue of the other esters. As the esters free from nitrogen are chemolysed quicker than the urochrome esters by sulphuric acid of 2 per cent. strength, the process offers the means of *separation*. The cohols go into solution with the benzoic acid, together with some *urochrome*, which can be precipitated out of the solution by pure phospho-molybdic and phospho-tungstic acid ; the *cohols* may be precipitated again by benzoyl chloride ; and urochrome esters which remain undecomposed will be free from the non-nitrogenised esters. To avoid the interference of these esters with the urochrome esters, it is advisable to exclude the cohols before the application of benzoyl-chloride, and to add this reagent only to the mixture of six alkaloids precipitated by phospho-molybdic acid, dissolved in soda, or liberated by baryta.

26. *Definition of an Ether and an Ester.*

The name *ester*, which I have applied to the products discussed in the foregoing, was introduced[1] by Gmelin

(Handb. 7, 170) to describe etheriform compounds produced from alcohols (cohols) by oxygen acids. It was commonly used in this country, where the theory of radicles prevailed earlier, and caused such ethers as might be termed esters to receive longer or shorter names embodying the names of the radicles (*cfr.* Watts. in Gmelin, *l.c.*, p. 190). Such ethers are also designated as *compound ethers*, but this might be considered to include the ethers with different alcohol radicles. On the whole *Esters* seems a convenient symbol.

Benzoyl-chloride has the formula $C_7 H_5 O Cl$. When this forms an ester the Cl is replaced by the cohol radicle, of which an atom of hydrogen combines with the chlorine and leaves as H Cl. A molecle of benzoyl chloride can therefore replace one atom of hydrogen and no more.

In more modern form of expression an *ether* is the product of the attachment of an alcohol (cohol) radicle to carbon in another radicle, which carbon is not oxydised ; when the carbon to which the alcohol radicle or residue is attached is oxydised the product is termed an *ester*.

VIII.—CHROMATIC REACTIONS FOR THE DISCOVERY OF SUPPOSED CARBOHYDRATES IN ANIMAL FLUIDS.

1. *Xylidin Reaction for Furfurol from Carbohydrates.*

This reaction, which was discovered by H. Schiff, and published in the Berlin Chem. Soc. Reports, 20, 540, is important in the consideration of the question whether or not certain animal matters contain any carbohydrate, even such as is not sugar, and yet may

be the antecedent of such, and, therefore, of glycohæmia or glycosuria. As several carbohydrates when boiled with somewhat concentrated sulphuric acid, some with, some without, oxydants, easily yield some *furfurol*, $C_5 H_4 O_2$, which is volatile, every reaction yielding furfurol may possibly be a reaction for carbohydrate. [Starch, cellulose, and gum arabic, when distilled with dilute sulphuric acid yield no furfurol.]

Furfurol may be recognised, even when present in extremely small quantities, as vapour by means of a colour almost identical with the colour of the oleocholide reaction to the bare eye, but differing in the spectroscope and by other properties, which it develops in contact with bibulous paper impregnated with *xylidine acetate*. Mix *xylidine* ($C_8 H_{11} N$) with an equal volume of glacial acetic acid, add some alcohol, dip strips of bibulous paper into this solution, and dry them. When such a paper is moistened with the smallest quantity of furfurol it becomes beautifully red owing to the formation of *furoxylidin* ($C_4 H_3 O_1$. CH), 2 ($C_8 H_9 N H_2$)=($C_{21} H_{26} N_2 O_2$). To prove the presence of a carbohydrate in any substance or liquid, heat the matter in a test tube with a slight excess of oil of vitriol, and let the escaping vapour pass over or through a piece of the paper impregnated with the acetate of xylidin.

Every *normal urine* yields this reaction, sometimes even a small quantity of it, or if it were very dilute it will yield it on concentration. It is, therefore, assumed that every urine yields furfurol with sulphuric acid, on boiling. As it is at present not proved whether or not the urine contains bodies other than carbohydrates capable of evolving furfurol with sulphuric acid, the reaction is supposed to indicate the presence of a carbohydrate in urine such as is capable of evolving furfurol.

When supposed carbohydrates have been *removed by benzoyl-chloride* the filtrate still gives the furfurol reaction; this has been surmised to be due to the (assumed) circumstance that the precipitation of carbohydrates from urine by benzoyl-chloride was incomplete. This would show, what is also proved by many other data, namely, that the xylidin reaction for furfurol is extremely delicate, and in its application requires great care to exclude sources of fallacy; for it may be produced by dust—paper-fibres, cotton-fibres, and a number of undefined impurities of the air.

Spectrum of Alcoholic Solution of Furoxylidin, observed by Thudichum.—On adding to some xylidin an equal volume of glacial acetic acid a turbidity ensues, which disappears on the application of gentle heat. On slow evaporation acetate of xylidin results. On addition to this of furfurol the red colour is at once produced. Furoxylidin is insoluble in *water*, and decolourised by it. It is easily soluble in *absol. alcohol.* The concentrated solution passes some red only in the spectroscope. On dilution the absorption recedes to orange; on further dilution some *violet* appears, and a broad detached absorption band remains in the middle. This on further dilution contracts, and covers *all the green;* on further dilution it dissolves without contracting any further. The solution then is only very faintly coloured. This spectrum is therefore widely different from the oleo-cholide, as well as alpha-naphthol-furfurol spectrum in oil of vitriol.

2. *Phenyl-Hydrazin Reaction for Furfurol.*

Phenyl-hydrazin gives a precipitate with furfurol (as with many aldehydic bodies), by means of which it may be quantated.

3. *Alpha-Naphthol and Sulphuric Acid Reaction for Furfurol.*

This reaction was described by Molisch, (Ber Wien. Acad. 93 (II. Abschn.), p. 912.) For this test use an alcoholic solution of *alpha-naphthol* $C_{10}H_8O = C_{10}H_7OH$) of 15 per cent. strength ; take two drops of this, and add two drops of a solution of *furfurol* of one-tenth per cent. strength (one part of furfurol in 1,000 parts of water), and then oil of vitriol, keeping the mixture cool by immersion in cold water : at first a *green ring* will appear at the point of contact of acid and mixture, which is due to the reaction between acid and alpha-naphthol ; but shortly after a *violet* ring will appear, and on agitation the whole fluid will become crimson with a shade of blue, *i.e., purple.*

As solutions of *carbohydrates* give the same reaction as furfurol, it is concluded that they in this reaction as in that with xylidin produce the colouration by evolution of furfurol under the influence of acid ; and as human urine of every kind gives the same reaction as solutions of carbohydrates, it is concluded that the urine always contains a carbohydrate.

Mix a few drops of *healthy urine* with a few drops of the solution of alpha-naphthol, and add from 0·5 to 1·2 cc. of oil of vitrol ; a *green* cloud will appear in the acid, then *a purple ring* at the plane of contact ; the top solution will be turbid from a precipitate of naphthol by the urine, but on agitation all will become *purple.*

Diabetic urine offers the same bearing. A mixture, *urochrome* and *kryptophanic acid*, from an iron salt, gives the reaction at once. The test from normal urine is very dark, and impenetrable to light ; it yields

no coloured matter to *chloroform;* on standing the mixture becomes discoloured, while the mixture without chloroform retains its colour for a long time.

The dark purple-coloured *test mixture*, to become translucent at all, requires many volumes *oil of vitriol.* Glacial acetic acid readily dissolves the test mixture, and before the spectroscope shows *a spectrum*, of which most of the blue is absorbed, while a narrow absorption band overlies about equally the D lines. This spectrum, therefore, differs essentially from that of the oleo-cholide reaction, and of the xylidin reaction, and though all may be due to furfurol, the coloured products are by no means identical. (Thudichum.)

The *delicacy* of the *reaction* is very great; it will indicate the presence of 0·00003 g. of *sugar;* of 0·00005 g. *cholic acid* dissolved in 1 cc. of spirit with one drop of a one-tenth per cent. solution of furfurol.

It has been proposed to use the test for *clinical* purposes, but in this direction its very *delicacy* is its greatest objection. Moreover, *albumen* and other *albuminous substances* yield the test, and although this can be explained by the *amylonide constitution* of these matters, the evolution of furfurol is not proved, and it is not proved whether other nuclei of the albuminous structure-molecle, not being carbohydrates, may not yield furfurol. *Urochrome* and *kryptophanic acid* both seem to give the reaction, the former being an alkaloid with at least 20·9 per cent. nitrogen, the latter an acid with 8·589 per cent. nitrogen.

4. *Differences between the Xylidin, Alpha-Naphtho and Oleo-cholide Reaction for Furfurol.*

The *xylidin* reaction terminates in the formation of *furoxylidin* ($C_{21}H_{26}N_2O_2$), a *nitrogenised* substance.

As the *oleo-cholide* reaction, which is similar in

colour, is best produced with substances *free from nitrogen* (oleic acid, cholic acid, &c)., the coloured product of the reaction must also be free from nitrogen, and differ widely from the foregoing substance.

The *violet product of the oleo-cholide reaction* is soluble in oil of vitrol, and permanent in it, soluble in glacial acetic acid, destroyed by water, insoluble in or destroyed by alcohol.

Oleo-cholide violet is *soluble in anhydrous chloroform*, and can be isolated thereby ; it remains as a violet residue on distillation, but water destroys it instantly ; *furoxylidin* is quickly destroyed by water and in alcoholic solution. Alpha-naphthol purple is insoluble in chloroform.

Oleo-cholide violet is soluble in glacial acetic acid, water being excluded, and shows three absorption bands. Alpha-naphthol violet is soluble in *glacial acetic acid*, with spectrum of one band over D.

The *alpha-naphthol reaction* is yet produced in liquids so diluted with water that the oleo-cholide reaction would not take place in them. Of the alpha-naphthol reaction, the *colour, bearing on dilution* and *spectrum* are different from the oleo-cholide reaction.

Although the *oleo-cholide* reaction is probably produced by *furfurol*, when sugar is used in its production, yet the *product* or *products* of a violet colour are not identical with those produced by *xylidin* or *alpha-naphthol*. The oleo-cholide reaction with oleic acid, sugar, and oil of vitrol, soluble in glacial acetic acid *and* chloroform gives spectrum of two bands, one between C and D, and one broader between D and G. The first band disappears first on dilution, and the broad one remains. Furoxylidin in alcohol gives only one broad band in yellow, green and blue—on dilution in green only. Therefore the spectra establish a

complete distinction between the reactions. The *oleo-cholide purple* is not identical with *furoxylidin purple*, and both differ from *alpha-naphthol purple* (Thudichum).

5. *Naphthol Reaction of Benzoylated Precipitate.*

The precipitate of mixed benzoylated compounds from urine *dissolves turbidly in oil of vitriol.* When to this solution some naphthol is added, a *red impure* (furfurol) reaction is obtained.

Isolated urochrom solution (not benzoylated) gives the same reaction. Both reactions require some time to become fully developed, but are never pure in colour. There is always present a dark admixture, something apparently charred by the oil of vitriol.

6. *Thymol Reaction with Supposed Furfurol, or with Urine.*

Thymol with carbohydrate, or urine, and oil of vitriol gives a coloured product, but the tint is uncertain, and, like the foregoing, accompanied by a discoloration.

7. *Some Conclusions to be derived from the foregoing.*

These reactions, in themselves extremely interesting and precise when performed with pure substances, are at present not available for purposes of physiological chemistry. On account of their colour they were supposed to be identical with the oleo-cholide reaction, but we have shown that their spectra and some other features negative this surmise. They are so extremely delicate as to involve the danger of erroneous conclusions as to the significance of their occurrence ; but above all their originating matters are not yet sufficiently defined or limited, so that it is not certain whether they are yielded by carbohydrates only or are

produced also by other matters not being carbohydrates. They are interesting, and may be important subjects for study in the laboratory, but for practical medical purposes they are at present not available.

IX.—ON SUPPOSED CARBOHYDRATES AS SUPPOSED SOURCES OF FATTY ACIDS FROM URINE, AND A NEW MODE OF OBTAINING AN UNEARNED INCREMENT OF LITERARY REPUTATION BY MOCK RESEARCH.

1.—*The Situation.*

It will be seen from the foregoing and several following chapters that the presence of carbohydrates in normal urine has been asserted and supported by a great variety of experiments, most being of an indirect circumstantial nature, and only few being direct in fact or intention. As the urine yields *furfurol* by appropriate treatment, the inference that this furfurol came from decomposed carbohydrate, would be acceptable, were it proved that furfurol cannot be produced out of substances other than carbohydrates. Further, as the *benzoylated esters* from urine have yielded in the hands of some experimenters reactions which do show that they possess reducing power over alkaline copper solution, it is not unreasonable to suppose that some carbohydrate was present as cohol in the ester, and *transformed* into a reducing carbohydrate by the influence of acid and boiling. But on this point the evidence is discordant, inasmuch as I have never found any carbohydrate in the esters, but always *urochrome*, an alkaloidal nitrogenised substance, and *cohols free from nitrogen* not being carbohydrates. Now such negatives are of little import opposite positive findings

In the case of the esters the positive assertions would seem superior to all doubt, were it not for three circumstances which call the relative statements in question. Firstly, the *nitrogen* in the esters was not considered an ingredient but an impurity ; Sécondly, the constant presence of the colouring matter, *urochrome*, as a cohol in the esters was not even noticed, and its products of decomposition were not eliminated from the products of decomposition of the esters supposed to be carbohydrates, and the cohols of the *non-nitrogenised esters*, not being carbohydrates, and which are the best defined ingredients of the mixture of esters, were not observed. However, the question of the nature of the urinary colouring matter was pressing some physiological chemists for a solution ; urochrome they had declined to accept, either silently or by short argument, some had even maintained that there was no special colouring matter of the urine in existence at all, but never had they supported their opposition by any direct experiment or any repetition of preceding experiments whatever. The great stumbling-block was *uromelanin*, and this had accordingly to be cleared out of the way if possible. This was attempted to be effected by the *carbohydrate hypothesis*. Of this fiction I have made an end in the fourth article, and its remains will be cleared away in a subsequent inquiry.

With the more specialised part of the carbohydrate hypothesis, namely, the *humous* or *hymatomelanic* doctrine, we shall deal hereafter ; it is easily refuted and eliminated, as all the experiments adduced in support of it are made on extraneous matters, and none on urochrome. In the present chapter we will consider some experiments on urine made by *E. Salkowsky*, Professor of Chemistry at the Pathological Insti

tute, Berlin, and published in *Zeitschr. Phys. Chem.*, 13 (1889), 264. The object of these experiments apparently was to make it probable that as the urine on distillation with an acid yielded some *fatty acid*, therefore, it contained carbohydrates.

2. *E. Salkowsky's Experiments on Fatty Acids in Normal Putrid Urine.*

The Professor omits to mention that he had treated this subject once before, twenty-six years ago, and had then found "*propionic acid*" as the main product of his operations (*a*). In 1889, however, he found no *propionic acid* at all, but *acetic acid* with an acid reducing silver solution, as he says, "*perhaps formic acid.*" In 1869 he could not confirm the presence of formic acid, and could not find acetic acid in urine (*l. c.* p. 366). Now he finds them both, but does not revoke, nor even mention, his former error, which consisted in his mistaking the mixture of these two acids for propionic acid. As his error had been corrected by others afterwards, and as it was inefficient in obliterating the better knowledge obtained before him, one might overlook this reticence as the product of a sense of humiliation ; but this would be quite erroneous, as we learn from the professor's introduction, in which *he ascribes to himself the merit of this discovery*, so far as normal urine in a state of putridity is concerned, and thus, by implication and the avoiding of all reference to former researches arrogates to himself the merit of the entire discovery. This is a new mode of proceeding for obtaining an unearned increment of literary reputation at the expense of others by shadowy researches against which I enter protest. The example is infectious, and is imitated by younger aspirants to fame in the

(*a*) "Pflüg. Arch," 2, (1869), 363.

specialistic publications, in which, as the editor of the "Annual Report on the Progress of Animal Chemistry" says, "in which alone such offal (abfälle), the result of spurious operations called 'researches,' is received."

In his publication of 1889, E. Salkowsky says that the fact of normal urine after ammoniacal decomposition containing fatty acids had hitherto (*i.e.* before his paper of 1889) been overlooked. This statement is absolutely contrary to fact, and a professor at a public institution ought to have known that it would be so before he made it, and, therefore, have avoided it. For there are no less than five publications to refute him, namely, those of the following authors :—

Louis Proust, of the year 1800, published in Spanish and French (*Ann. Chim.*, 36, 258), and of 1820 (*Ann. Chim.*, 14, 260).

Justus Liebig, of the year 1844 (*Lieb. Ann.*, 50, 161) who obtained the acetic and formic acid from putrid normal urine only, and believed that they could not be obtained from fresh normal urine.

J. L. W. Thudichum, of the year 1863, published in the *Brit. Med. Journ.*, 1864. This author isolated the acids from both fresh and putrid urine ; *id.*, more elaborately and with stoichiometic details in the 11th and 12th "Report of the Medical Officer of Privy Council" ; *id.*, in 1870, in the *Journ. of the Chem. Soc. of London*, Nov. 1870, and *Pflüg. Arch.* The first four researches of this list Professor Salkowsky ignored already in 1869, and as is done by some professors of medical chemistry in Germany, discussed the findings of *two Russian students*, one of the name of *Buliginski*, the other *Jacubasch*, on more than a page of print, as if they were serious matter of scientific import. These gentlemen were also in

ignorance of previous researches, and the last one believed that the only test which he applied to his distillate from the urine of a case of leucocythæmia, namely, a red colouration with ferric chloride, proved the presence of acetic acid, and that its presence was diagnostic of ("characteristisch," *i.e.*, peculiar to) this disease. This kind of pathology was countenanced at the chemical department of the place where leucocythæmia, *i.e.*, *Haemo-leuco-cytiasis*, was for the second time discovered and termed leukhæmia.

We will now consider Salkowsky's mode of procedure for the isolation of the acids as briefly as we can ; we insist on this resolution for brevity all the more as the Professor's harangues are long, prolix, and diffuse. His distillates were made with sulphuric acid ; 300 cc. of urine received 10 cc. of oil of vitriol, already diluted with 20 cc. of water.

All distillates contained hydrochloric acid.—This fact alone shows that Salkowsky's proceeding was faulty in many respects, and that therefore his results were not strictly comparable to those of others. But the blundering had been provided against, as the following passage from Thudichum's paper in the *Journ. Chem. Soc. Lond.*, Nov. 1870, will prove :—

"When urine or its extract is mixed with a moderate quantity of sulphuric acid and boiled, a very peculiar state of things ensues, which is not generally understood. The ingredients are not in the presence of sulphuric acid, as it were, at all, but in that of a very slight chemolytic influence only, a kind of contact action, The sulphuric acid is at once enveloped by the attraction of urea, and as long as urea is present no sulphate is formed, except that of ammonia, even on boiling. Although some benzoyl-compound is decomposed in the extract of urine, and benzoic acid

is given out, yet much hippuric acid remains in solution unchanged, and crystallises after the extract has been boiled for hours. If the hippuric acid had been in presence of free sulphuric or hydrochloric acid it must have been decomposed entirely by half an hour's boiling. To the same preserving influence of urea is due the fact that in the distillates obtained during the decomposition of urochrome according to my method, which yields the acetic and formic acid, *no hydrochloric acid is ever found.* Such acid is only evolved by the addition of a great excess of sulphnric acid to urine or its extract, and its effect and advent are unmistakably evideut in the fluid. The *mixture becomes black*, and loses entirely its previously red colour. It deposits black charred matter, which differs *toto cœlo* from uromelanine and the resinous urochrome products. From such charred urine no uromelanine and no pure products of any kind can be obtained. Now these particular conditions, more than anything else, incline me to the opinion that the acetic and formic acid are products of the decomposition of higher organic bodies, and not merely acids set free from salts by sulphuric acid. They escape simultaneously with the carbonic acid gas which is evolved from the urea, and causes a continuous slight effervescence in the fluid. The sulphuric acid does not decompose the kryptophanic acid in the extract any more than the hippuric, *i.e.*, only a portion of it, and that certainly a small one. But when free hydrochloric acid appears the kryptophanic acid disappears, forming, in fact, the great bulk of the black matters in which such decomposing extract of urine abounds. These peculiar reactions must be carefully borne in mind by every one who would successfully study or comprehend these decompositions."

The neglect of this precaution has much more serious

consequences in the consideration of other questions than appear in the present discussion. But we will show how it affects the latter incidentally. Salkowsky found *the quantities* of his products to vary greatly. In none of these experiments was the hydrochloric acid removed or deducted ; it is said to have been about equal in the distillates from fresh and from putrid urine. Yet he gives average quantities of acetic acid obtained, which are not quantated but merely guessed, Thus an entire day's fresh urine is said to have yielded 0·15 g., and a day's putrid urine 0·915 g., of acetic acid. Yet Thudichum has proved, by a series of consecutive quantations on the excretion of eleven days, with analyses of the lead salts, that the quantity of acetic acid obtained was 0·288 gr. per day, nearly double the amount alleged by Salkowsky, and that the accompanying *formic acid* might be estimated at 0·05 gr., or as equal to 5·76 per cent. of the acetic.

Salkowsky next starts the hypothesis that these acids are produced by the decomposition of carbohydrates normally present in urine, that these are decomposed by putrefaction, and yield the acids ten times greater in amount in putrid than in fresh urine. But this now lands him in a dilemma which at first sight destroys his hypothesis ; the *putrid* urine still yields as much of the *humin substance* (*i.e.*, Thudichum's uromelanin) as *the fresh ;* consequently as the humin substances are to be derived from the carbohydrates, the carbohydrates cannot have been destroyed, and cannot have yielded acids. His windings under these distressing conditions are almost to be pitied, for they seem genuine, and their expression sincere. The humin substances of putrid, he supposes, might have a composition different from those of fresh urine ! He does not only not prove this, but *he ought to know quite*

well that this is not so, and that *the uromelanin from fresh urine is identical in composition with that from putrid.* And to clench the matter, the putrid urine still gives the xylidin-furfurol reaction, and if this be due to carbohydrates they must still be there. But the *amount* of acetic acid obtained from putrid urine is incompatible with the assumption of the urine having been healthy, for it requires that 1,500 cc. of the urine must have contained at least 2·408 g. of dextrose, an amount so large as to be easily discovered by direct tests, and absolutely excluding the condition of health. Salkowsky's article contains numerous digressions without positive results of any kind. I was bound to consider it in order to enable the profession to judge of chemistry of this kind, with which the special literature is now being burdened. But the discussion has a much more important side, namely, *the ethical* one, and here a strong stand must be made against unfounded assumptions of discovery, which are really plagiarisms, the uttering of which is assumed to be facilitated and disguised by ignoring the original discoverers, and by masking them by a varnish of spurious researches, or at least entirely irrelevant and unnecessary laboratory proceedings.

3. *Summary of the Actually Existing Knowledge of these Acids.*

We summarise here what is known on the subject o these acids :—

1. Fresh normal urine contains ingredients, which on regulated decomposition yield acids of the *aromatic* and *fatty series.*

2. Of the aromatic series *benzoic acid* alone has as yet been isolated, and is known to be derived from the splitting up of *hippuric acid.*

3. Of the fatty series *acetic acid*, with an admixture of *formic*, has been isolated.

4. The *matter* or *matters* from which the fatty acids are derived is or are at present entirely unknown.

5. These matters are not amongst *the alkaloids*, the complex of which, including *urochrome* yields *no fatty acids* on proper distillation with sulphuric acid. This disproves a surmise to the effect that the acids were derived from the colouring matter.

6. There is no proof whatever that amongst the acids here in question there is any *propionic* or *butyric*, or *valerianic acid*.

7. All the acids as yet isolated are *volatile* at the heat of boiling water and with water vapour; it is not known what are the residual bodies from which the acids are derived, except in the case of *benzoic*, which leaves *glycocine* (glycocoll) in solution.

8. The acids *in putrid* urine are in a condition different from that in which they are present *in fresh*. The benzoic acid is entirely there as benzoate, and no longer as hippurate, and, therefore, falls on the mere addition of a stronger acid.

9. The *fatty acids* also seem to be present in putrid urine as *mere salts*, and are more readily evolved by stronger acids. But the share which the *absence of urea* has in the greater accessibility to acid cannot at present be accurately stated.

10. The distillates contain a *red oil*, soluble in ether, which has not yet been analysed.

11. The distillates always contain *nitrous acid*, which is introduced in food and water, and probably leaves unchanged by the kidneys.

12. The distillates also always contain a *sulphurised acid*, probably *sulphurous*.

13. The distillates do not contain any *rhodanic*

(*sulphocyanic*) *acid*, as I have proved against the late Professor Gscheidlen, who had assumed its presence on the basis of entirely erroneous statements and assumptions concerning the properties of the *lead salt* of that acid [*cfr. Pflüg. Arch*, 15 (1877), 12, *et seq.*]

X.—ON SOME UNEXPLAINED AND MISINTERPRETED REACTIONS FOR ALLEGED CARBOHYDRATES IN URINE.

1. *Introduction.*

In the sixteenth volume of *Pflüger's Archiv.* (1878), p. 551 et seq., will be found three papers, covering fifty-two pages, all directed upon the testing of human urine for sugar by volumetric analysis, by *Worm Müller*, Professor of Physiology at Christiania, in Norway. The object of *the first paper* is the discovery of a reagent for dextrose more minutely accurate than Fehling's solution of tartrate of copper in potash. We admire these efforts for transcendental accuracy, although we think them of no practical applicability in medical affairs, and by their very delicacy liable to mislead the less informed, and even to bring the adept to erroneous inferences. But our object on the present occasion is not to contravene or criticise them, but to find *some explanation* for phenomena which the author and his assistant have left entirely uninterpreted. Leaving the experiments with *sulphate* and *formiate* of copper, which were without result, out of consideration, we will treat of the effects of *acetate of copper* in *neutral* or *acid solution* only, and show their true bearing.

2. *Barfoed's Test and Reaction.*

A solution of acetate of copper is reduced by a solu-

tion of dextrose on long standing at ordinary temperature, but is not reduced by solution of dextrine, lactose, or sucrose. In given cases this would be a great advantage, but it is limited in applicability and for medical and physiological purposes absolutely useless by not being completed in less than from twelve to sixty hours. This test may not be boiled or heated as it then oxydises dextrine, and ceases to be a test for dextrose only. This reduction by dextrine is avoided by the addition of some *acetic acid* to the acetate of copper solution. Such a solution called *Barfoed's test*, after its inventor, on boiling, will be reduced by dextrose, but not by dextrine. Nevertheless, the reaction also requires several hours for completion, and thereby involves the danger of reoxydation of any suboxyde by the oxygen of the air. Another danger is that it may not be boiled for a longer period than a minute or two minutes, because by longer boiling the acetate itself is decomposed and may lead to entirely false conclusions. With all the numerous necessary precautions, dextrose may be discovered (so far as reduction is tantamount to identification) in a liquid which contains one thirty-second per cent. of it in solution. One-tenth of a milligramme, however, gives hardly any reduction.

Worm Müller modified Barfoed's solution by making it more dilute, so that it contained four per cent. of cupric acetate, and one per cent. of free acid considered as glacial. Dissolve 12 grs. copper acetate in 300 c.c. water, and add 3 gm. glacial acetic acid, or an equivalent of dilute acid.

3. *Application of the Test to the Urine, and Comparison of other Tests.*

In his *second paper* (*l.c.*, p. 562), Worm Müller relates experiments which proved that *normal urine contained*

substances which reduced solution of acetate, and very probably also of sulphate of copper at the ordinary temperature of the air when the mixture was allowed to stand during about twelve hours; under the same circumstances, and in the same copper solution *pure dextrose* solution of less than $\frac{1}{4}$ per cent. strength effected *no reduction* of the acidulated copper acetate solution. *Barfoed's* reagent, 1 c.c., mixed with 2 c.c. of normal urine and boiled during two minutes, and then allowed to stand during thirteen hours, showed a brownish red ring on the periphery of the green precipitate. Urine from which uric acid had been removed, showed the same bearing, so that the effect of this acid is excluded. This led Worm Müller to the conclusion that *normal urine contained a substance, or several substances, which reduced copper in an acid acetate solution, and which substance could not be dextrose.*

4. *Comparison of Fehling's with Knapp's Test.*

The *third paper* of Worm Müller and his assistant Hagen is a laborious comparison of the volumetric sugar test of *Fehling* with that of *Knapp*, which consists in an alkaline solution of cyanide of mercury. Dissolve 10 gr. dry cyanide of mercury in water, add 100 c.c. of caustic soda ley of 1·145 sp. gr., and make up to one litre; four parts of cyanide of mercury oxydise one part anhydrous dextrose on boiling, and the mercury sinks in the metallic state. The authors find this Knapp's reagent to be superior to Fehling's for application to the urine, and animal liquids in general. Our own experience is not in accordance with this opinion, and after many trials of Knapp's test, we have found it to exhibit so many unexpected vagaries that we return to Fehling's as the more con-

venient and reliable. In medical practice we have found that such small quantities of matter in urine as are not indicated by Fehling's test have no claim to be called dextrose, and are not of the slightest pathological importance.

5. *Experimental Explanation of the Bearing of Urine with Barfoed's Test, and of Worm Müller's hitherto unexplained Results.*

(*a*) When the urine is *freed from all acids by mercuramin*, and from *all alkaloids* by *animal charcoal*, or *phosphomolybdic acid*, it ceases to react with copper solution unless *dextrose be present.*

(*b*) When to the urine, *freed from all acids by mercuramin*, but *containing the alkaloids, including urochrom, a solution of copper*, acetate or sulphate, is cautiously added, *no precipitate* is at first produced, although the solution contains all its bases in the caustic state, and is *powerfully alkaline.* The copper dissolves, and forms *a blue solution;* more copper solution produces *a green precipitate;* on the *application of heat* the *blue solution curdles*, and the mixed precipitates sink to the bottom. When all matter yielding the green precipitate is out, the solution now yields only cupric oxyde hydrate, with more copper solution.

(*c*) In this strongly alkaline solution the *green precipitate is not reduced;* reduction only ensues when the solution contains *dextrose.*

(*d*) But when the alkaline urine solution is neutralised with any acid, preferably acetic, and *acetate of copper is added to it*, it forms at once a green precipitate, which condenses on cooling, and is *then immediately reduced*, so as to become fawn-coloured or even yellow on prolonged heating.

(*e*) This precipitate contains certainly *two alkaloids*,

namely *urotheobromin*, and *urochrom*, both in combination with *cupric*, after reduction with *cuprous oxyde*. In this manner urotheobromin *can be isolated*, if boiling be omitted, and after removal of the copper be separated from the urochrom.

(*f*) When the isolated *urotheobromin* is recombined with *copper* by addition of *acetate*, it forms a *green precipitate*; but when this compound is boiled by itself in pure water, *oxydation of the organic base takes place* and the cupric is reduced to cuprous oxyde.

(*g*) When this fawn-coloured precipitate is treated with *nitric acid* in the water in which it has been formed, it dissolves on heating, *with effervescence, due to the evolution of carbonic anhydride*.

(*h*) Again, when urine is evaporated and freed from phosphoric acid by baryta, and then treated with copper acetate and boiling, a green precipitate ensues which contains *urotheobromin* and *urochrom* as principal ingredients. It was this precipitate which *Strecker* erroneously believed to be a compound of *hypoxanthin* (*Sarkin*) with copper oxyde or acetate, containing, as he thought, perhaps *guanin*. (*Hypoxanthin* is precipitated by *copper acetate*, and was so extracted from the juice of flesh by Strecker, and called *Sarkin*.) The dry precipitate, which is fawn-coloured, consists of *urotheobromin* and *urochrom* mainly, and small amounts of other matters only. It dissolves with violent reaction in *nitric acid*, and this solution contains now several products of oxydation. Therefore, in these processes with urotheobromin and urochrome and copper, boiling, and maintenance in the wet state, and contact with nitric acid, must be carefully avoided.

(*i*) Further, when *the alkaloids* of the urine are isolated by *phosphomolybdic acid* and *baryta*, and when

to this solution acetate of copper is added, a *green precipitate* ensues, and this on standing becomes yellowish brown or fawn-coloured, quickly so when it is heated. It consists of *urotheobromin* and *urochrom*. The alkaloids, therefore, imitate the bearing of the urine in a concentrated intense form. The share of Kreatinin in this reduction has not yet been quantitatively estimated.

(*k*) When from the complex of *the six alkaloids* extracted from the urine, as described, while dissolved in hot water, urochrom is removed by precipitation with *ferric chloride*, the solution on cooling after rapid filtration, deposits almost *pure urotheobromin*, to be purified by recrystallisation, &c. Recombined with copper, this shows *the reduction* when boiled with water, as above described.

(*l*) It follows from this that *the cause* of the phenomena shown by urine with Barfoed's test, as observed by Worm Müller and Hagen, and which are not due to dextrose, are due to at least *one of the alkaloids* naturally found in the urine, and perhaps to this, namely *urotheobromin* only, or to urotheobromin and kreatinin at the same time.

(*m*) *Urotheobromin does reduce* Fehling's fluid when it is boiled in this for such a time as would be necessary to oxydise dextrose.

(*n*) Any urine, which after removal by animal charcoal of its alkaloids from its strongly alkaline solution produced by mercuramin, *is perfectly colourless*, and in that state does reduce alkaline copper tartrate, contains *dextrose*, and is a pathological object.

XI.—INQUIRIES ON SO-CALLED HUMIN SUBSTANCES AND THEIR BEARING UPON THE INTERPRETATION OF UROMELANIN, A PRODUCT FROM UROCHROME.

1.—*Introduction.*

Under the title of *Humin Substances* are comprehended a vast number of mixtures of chemical compounds which result from the gradual decay of nearly all kinds of *vegetable* organoplastic, as well as circulating substances. Few, if any, have hitherto been obtained from decaying *animal* matter, and if so, have certainly not been defined. Only one isolated substance, a product of the action of acids upon urine, *Proust's particular black matter*, showed any analogy to vegetable humous matters, and was, therefore, by Gmelin, ranged with the humin substances. It was, like the humin substances in general, believed to *be free from nitrogen*, and if it had been so, its external appearance would have justified this classification, as long as it origin remained unknown. When, however, I had shown, in the *Essay* to which the British Medical Association awarded the Hastings Medal, that this substance was a product of decomposition by acids, or by putrefaction, of *urochrom*, the colouring matter of the urine, behaved as a unitary substance, and contained always, and however prepared, a *considerable amount of nitrogen*, it had to be placed into a different category. I showed that it was a weak acid, combined with bases, and termed it *Uromelanin*. I tested its elementary composition in more than twenty different preparations, obtained by all available methods, from fresh, as well as putrid urine, and found it identical in all cases, and thus gave to its molecle a definition exceed-

ing in constancy and precision any knowledge possessed by chemists regarding humin-substances properly so-called.

During twenty-five years, both physiological chemists and physiologists failed to appreciate the bearing and contents of these researches, and turned in a desert of error round a substance which was propped up by a greater number of fallacies than any other of the numerous bogies in this field of biology. This was "*urobilin*," a matter never isolated, never analysed, a matter of which not a single chemical fact was known, and the very existence of which was only manifested by a badly defined absorption band in the spectrum. In a paper printed in the *Journ. Chem. Soc. Lond.*, Nov., 1870, I showed that the spectrum was furnished by *uropittine*, a product of the decomposition of urochrome, by the side of uromelanin, and that the matter called *urobilin* contained moreover a selection of undefined *impurities*, and that no particle of the mixture ever occurred in or could be obtained from bile.

This proof gradually acted with increasing force, and the supporters of urobilin, feeling the ground tottering under their feet, tried to make it more stabile by new argument. For this they discovered the material in *kryptophanic acid*. They made no researches upon it any more than upon urochrome or uromelanin, or uropittin, but merely fabled, hallucinated, that this acid was a kind of dextrine or gum, animal gum as they termed it; and the nitrogen ascertained by me to be contained in every specimen in regular quantity—well, the nitrogen, as in the case of the benzoylated esters, they neglected, or deducted, declared it an impurity which could not be removed, and called their alleged discovery that of *a carbohydrate*.

But it was insufficient for all the wants of the hypothesis, which required *several* carbohydrates to bear the weight of the hallucination, and the yellow colouring matter, *urochrom*, was also bereft of its nitrogen, and declared to be a carbohydrate. *Kryptophanic acid*, of which I had proved that it *yielded no uromelanin* with acids, was silently abandoned, and urochrome put in its place. The result was the last phase of the *carbohydrate hypothesis*, which I have defined thus under 4 in the Essay No. IV as the *humin hypothesis*; the latter being merely the continuation of the former is here repeated: "The normal urine contains *carbohydrates*; these carbohydrates have a tendency to be transformed into *humous substances*; they are so transformed by the *treatment of the urine with acids*; but they may also be transformed spontaneously already within the body, at least in part, and assume a little *colour*; it is such humified carbohydrate in small quantities which furnishes *the colour of the urine*. There is no special or specific colouring matter of the urine at all, consequently there is no such thing as *urochrom*; but what has been so-called is mere carbohydrate, altered in part; and what has been termed *uromelanin* is merely carbohydrate transformed into *humous substances* by acid and heat; and the nitrogen which it contains has been *inserted*, synthetically, and comes from the urea, with which the carbohydrate has been heated."

One would have supposed that the promulgators of this hypothesis would put it to the test by direct research; but they did nothing of the kind; they produced neither urochrom, nor uromelanin, nor uropittin; they did not repeat a single one of the well-known processes, but confined themselves to the cheaper and easier production of *black matter from sugar*, and a few others

carbohydrates by treatment with acids and alkalies; and when they operated on urine, which was the only object for a true research, they always maltreated it in the manner, of which I have shown the irrational nature in Essay No. viii, Sec. 2, in an extract from the paper printed in the *Journ. Chem. Soc. Lond.*, Nov. 1870. As every step failed in producing anything like uromelanine, of which they wanted to prove the descent from carbohydrates, they at last *fused* sugar and *urea* together, to force some nitrogen into the humous black; but as the nitrogen rose, the carbon sank, and the attempted synthesis of uromelanin failed.

The indirect researches which have led to this issue were mainly instituted by Hoppe-Seyler, and communicated under a title which did not indicate their scope or tendency. We here give a short abstract of their possibly useful results.

2. *Hoppe-Seyler's Essay on Humin-Substances.*

Leaving the true humous and humin substances from earth, mould, peat, wood, &c., out of sight, the author mentions the black matter obtained from *tannins* by Rochleder, Hlasiwetz, and others, and their further derivates; the black matters are not volatile and not crystallisable, and lose on being heated above 120° *water, formic* and *carbonic acid;* they form compounds with metals, which are not well defined, but are not decomposed by carbonic acid By their bearing towards acetic anhydride, these bodies, as far as examined, considered as acids, show that they contain groups of hydroxyl which can be replaced by acetyl, so that the black bodies can be transformed into acetic ethers (*Böttinger, Lieb. Ann.*, vol. 202, p. 276). When the black bodies from the tannins are *fused* with the three to five-fold weight of caustic potash and a

little water they yield *protocatechuic acid*, $C_7 H_6 O_4 + H_2O$. Some operators obtained *phloroglucin*, others not; on this latter and *other bodies* the results differ widely. To obtain comparable experiments the fusion should be effected at fixed temperatures, *e.g.*, at 240° to 250°, in a glass retort or flask, placed in an oil or paraffin bath.

3. *Products of the Fusion with Alkali of Cellulose.*

When *cellulose* in the shape of *filtering paper* is thus treated it gives out *steam*, then *hydrogen* (2·47 per cent.), with traces of *methane* (marsh-gas); the decomposition is complete at 240° in, at the outside, three hours. The residue is dissolved in water, saturated with sulphuric acid, and subjected to distillation; distil over more than half the volume, and extract the residual solution with *ether*.

The *ether solution* on agitation with solution of *soda*, yields to this *oxalic* and *protocatechuic* acid. To this soda solution add *acetic acid*, which liberates the protocatechuic acid, while leaving the oxalic with the soda. The protocatechuic acid is again extracted by ether, while the oxalic is precipitated as *calcium salt*. Cellulose thus yields 1·2750 per cent. of impure protocatechuic acid, and 2·3370 per cent. of oxalic acid; only little pyrocatechin is formed, no hydrochinone, resorcin, pyrogallol, or phloroglucin. The *distillate* contains *formic* and *acetic* acid, with small portions of higher acids. The total baryum salts from 100 grs. cellulose weighed 27·8670, and contained 15·1550 Ba = 54·38 per cent. Ba, leaving 12·7120 for the acids. The amount of water evolved is not known: the total of matter accounted for is 18·8940 per cent. This process evidently yielded no humin substances capable of further development towards uromelanin. *Cotton-wool* gave similar results.

4. *Humous Substances from Cane-Sugar by the Action of Acid.*

A kilo. of cane-sugar was mixed with 4 litres of hydrochloric acid of 20·5 per cent. strength, and heated for 24 hours in a water bath, with reflux-condenser attached to flask; *a deposit* formed, which was removed; the filtrate was distilled to 1/3 of its volume, when a a *second deposit* was removed; the residual liquid now yielded much *laevulinic acid* to ether [acetyl-propionic acid, $C_5 H_8 O_3$]; the *distillate,* after addition of baryum carbonate distilled again yielded *furfurol*, and a residue of baryum salts of *fatty acids.*

The deposited *humin substances*, treated with dilute caustic soda and extracted with much water, yield an *insoluble part*, which is swelled like mucus, and after treatment with dilute HCl water, and drying is a dusty powder, insoluble in alcohol and ether, and burns on platinum by glowing without flame. The *soluble in soda part* is precipitated by HCl; when placed in alcohol *much dissolves*, only brown *resinous drops* are deposited, which stop the filter. When the alcohol is evaporated, and the residue extracted with water and dried, a *product* remains which is *no longer entirely soluble* in soda. None of these products are even similar in bearing to uromelanine; the carbohydrate of urine, if any, is certainly not cane-sugar. The research, which is not even new as to either method or result, is entirely irrelevant to the question at issue!

When we come to consider *the yield* of the process it becomes still more uninstructive; the black matters amount to 233 grm. and the rest of those isolated do not bring the sum of the products to one quarter of the sugar used! The *insoluble* black matter called *humin* amounted to 170 g., the *soluble* (becoming insoluble in part later on) called *huminic acid* weighed 63 g. The

humin contained 63·88 per cent. carbon, and 4·46 per cent. hydrogen, and the huminic acid 64·39 per cent. carbon and 4·73 per cent. hydrogen. Sixty grammes of the *humin* fused in soda, returned 24·7 g. unchanged, and 23·855 g. of an acid easily *soluble* in very *dilute alcohol* called *hymatomelanic* (from Greek *hyma* or *hymos* in Latin *humous* or *humus*) and 0·801 g. brown protocatechuic acid; 0·1173 pyrocatechin, and 3·4655 of baryum salts of fatty acids, as well as some oxalic acid in ether extract.

A powerful acid, and a more powerful alkali have as yet produced nothing like uromelanin from the kilo of sugar, of which three-quarters are in no way accounted for.

5. *Hymatomelanic Acids from other Sources.*

Not only the acid-produced *humines* and *huminic acids*, but all *tannin-reds* and *phlobaphenes* are by fusion with potash in the above stated manner transformed into *brown acids*, which are soluble in dilute spirit, insoluble in ether and in water. These bodies Hoppe-Seyler terms *hymatomelanic acid;* their composition varies between C. = 65·08 and H = 4·22 per cent. for some, and C. = 57·24 per cent., and H = 3·34 per cent. for others! *Which is hymatomelanic acid?*

6. *Humous Matters from Glycuronic Acid.*

The chemolysis of *glycuronic acid*, $C_6 H_{10} O_7$ (obtained from dog's urine as *campho-glycuronic* acid, $C_{16} H_{24} O_8 + H_2 O$, and isolated from this by dilute acid chemolysis, by treatment with concentrated HCl, seemed a more pertinent proceeding. The acid is probably derived from a dextrose radicle, and not known ever to occur in the natural secretion, but coming into action only after the animal has been made to swallow camphor. If such a radicle could be present in urine

without the aid of its combination with camphor it might be the carbohydrate searched for, and by chemolysis yield uromelanin. Some such argument must have been present in the mind of the experimentalists, but it is extremely curious that they never searched for this glycuronic acid directly. Alas for the hypothesis! glycuronic acid gave no uromelanin ! but it gave humin matters, of which half dissolved in dilute ley, huminic acid, while the other half, humin, remained undissolved ; this swelled up, and retained much potash, though washed ; the huminic acid, freed from potash by acid, *was soluble in alcohol.*

The *humin* contained C = 60·64 p.c. ; H = 4·10 p.c.

The *huminic acid*, C = 60·64 p.c. ; H = 4·13 p.c.

These figures also differ from those for uromelanin in a forbidding manner, excluding all comparison. The huminic acid fused with soda, gave *formic, acetic,* and *oxalic,* and about half a per cent. of *protocatechuic acid,* also a body soluble in ether, and crystallising in yellow sublimable needles. This experiment also has no bearing, in fact, upon the question of uromelanin, but has reference only to the humification of glycuronic acid.

7. *Humous Matters from the Urine of Horses and Dogs which had been Dosed with Pyrocatechin.*

Humous matters were obtained from these materials by boiling with HCl. On fusion with potash protocatechuic and hymato-melanic acid were obtained, but it is not said from which of these humous matters, nor whether they were separated as in the preceding operation. The operations for ascertaining the *percentic composition* of the hymatomelanic acid *gave somewhat divergent results;* it is not stated from what standard of composition the analyses diverged, or whether they

differed from each other. The effect of the pyrocatechin is not defined nor, indeed, adverted to.

8. *Dry or Destructive Distillation of Humin Substance.*

This process yielded some small amount of distillates termed empyreumatic substances. Hymatomelanin gave carbonic acid, marsh gas, and a hydrocarbon with more carbon. Huminate of baryum gave the two gases and a residue of black coal with the baryta.

9. *Black Matters obtained from Urine by Excess of Acid.*

Having thus been beaten off on all sides in his attempt to produce uromelanin by indirect means, Hoppe-Seyler caused one of his assistants to produce black matter by the process of employing excess of acid, which I have shown to be fallacious in Essay viii. 8. 2. This *humin substance* (no plural here) showed *a considerable but fluctuating amount of nitrogen.* This *nitrogen*, which now appears for the first time on the scene, militated against the assumption of the origin of the humin from carbohydrates, *it could not be eliminated by any means except fusion of the black matter with potash.*

10. *Hypothesis of the Origin of the Nitrogen in the Black Matter from Human Urine.*

At last Hoppe-Seyler's research, if it can be called a research, seems to confront the problem for the solution of which it was undertaken ; all the roundabout ways have failed, and the experiments have yielded only information known before. Nevertheless, there remains the foregone conclusion that the black matter must come from a carbohydrate, and the nitrogen *must* have been introduced into it during its transformation into "humin." And now follow again numerous tests and indirect proceedings intended to make the pres-

ence of a carbohydrate in urine at least probable, if it cannot be proved. Thus it is stated that the HCl treatment destroys all *reducing substance* (reducing substance here means substance which reduces Fehling's solution). Of course it does, and necessarily destroys by carbonisation all organic ingredients of the urine except kreatinine. Further, it is argued the amount of humin substance obtainable is in constant proportion to the amount of reduction which the urine can effect. This copper test is really the weakest part of the entire argument, and its very occurrence is denied by many authors. It proves absolutely nothing, even if it were there. A further argument is that *the assumed carbohydrate is all removed from the urine by animal charcoal.* This is not explained (it is not a fact), but the argument breaks off, and *the nitrogen* again obtains ascendancy.

My readers will be worn out with this confusion, of which I am myself very tired, but I must complete the picture to obtain the final refutation of the entire net of fallacies.

11. *The Nitrogen is forced into the Humin-Substance made from Sugar.*

Even the urine has yielded no uromelanin, but has, as it ought to do, only given forth black, charred matter with varying amounts of nitrogen. It is, therefore, again abandoned as a material, and the argument is continued by boiling *sugar*, *urea*, and *hydrochloric acid* together. Now a humin matter with 6·73 per cent. nitrogen is obtained, but uromelanin contains above 12 per cent. of nitrogen. So the urea is increased in the mixture, and behold, the product contains 13·61 per cent. nitrogen ; but, alas ! the carbon has fallen to 53·55 per cent. from 57·97 per cent., and is by 4 per cent,

too low for uromelanin; the hydrogen is too low by 1-5th, and the nitrogen too high. No uromelanine yet! New experiments, the result of evident despair, are made: fusions by heat of mixtures of sugar and urea, but these also yield *no uromelanin*. Here the experimental ends, and *a fencing argument* begins, intended to cover the retreat. "As the humin substances are able to take up different quantities of ammonia, in *statu nascendi*, according to quantities which are present, the nitrogen must be considered as an accidental, non-essential one." (For humin substances read uromelanin.) But the nitrogen is *not accidental*, but is forced upon the sugar caramel by the strongest *chemical coercion*, and the humin substances come *from cane sugar*, and not from urinary carbohydrate!

The following shows still better the desperate nature of the argumentation:—"How near the artificial (sugar-urea-humin) is to the natural (urine-hydrochloric acid-humin) is the more evident, if one leaves the amount of nitrogen out of sight." And this is actually done in the concluding sentence:—"Deducting the nitrogen (*sic!*), the urinary and artificial humins show a residue free from nitrogen, which has a very concordant composition." This is exactly rendered, argument, style, grammar, and all. The nitrogen, so painfully forced into the carbonised sugar, has again to be deducted, and to be left out of sight to get any possible proposition at all.

Conclusions.

(*a*) The *indirect experiments* of Hoppe-Seyler have taught nothing at all as regards *uromelanin* from urine. Even the question, whether uromelanin can take up urea or ammonia in the course of its reasonable typical preparation remains unanswered.

(*b*) The allegation of *the presence* of a carbohydrate in urine remains entirely unproved.

(*c*) Consequently it is *not logical* to derive nitrogenised humin matter from a non-nitrogenised unproved carbohydrate.

(*d*) To leave the *nitrogen* out of *consideration*, or *out of sight*, is to assimilate science to the practice of the ostrich, so well known as not to require description.

(*e*) The whole of the Hoppe-Seyler's research, and of the efforts of his helpmates is *a mass of wasted efforts*, for as an inquiry into the humin substances, or any one of them, it was not only too small and partial (three-quarters of the products having been left out of consideration and wasted in the mother liquors), but misdirected by the bias of the *object to destroy uromelanin* as a chemical individual; the same motive splintered up the inquiry and prevented it from coming to any final result.

(*f*) The entire diatribe has not touched either *uromelanin* or its *origin*. *Uromelanin* is a nitrogenised product by chemolysis, of a natural regular specific ingredient of the urine, *urochrome*, which is highly *nitrogenised*, is in no sense a carbohydrate, or like one, but behaves as an *alkaloid*, or as an *alcohol*, or as a *weak acid*, according to the influence brought to bear upon it. It yields uromelanin by very gentle chemolysis besides the other products of its cleavage. *Uromelanin* when first split off from pure urochrome is *yet red* (uromelaninogen) and becomes dark only by and during the operation for its purification.

(*g*) The *constancy of the composition of uromelanin* has been proved on more than twenty preparations, and many compounds with bases. The properties and compounds do *not coincide* with those of any of the matters produced at Strassburg from sugar, &c., and

even its residue which remains after nitrogen has been deducted, does not fit the outline of the residue from the saccharine humin substances.

We implore the readers to pardon our thoroughness ; it was necessary to clear an impeding fallacy off the scientific stage, and we hope never to have to return to it again.

XII.—THE CLINICAL IMPORT OF HYPOXANTHINE AND THE MODES FOR ITS IDENTIFICATION.

1. *Practical Argument for treating the Subject.*

HYPOXANTHINE is that member of the uric acid group which contains the least oxygen. It is not an acid but an *alkaloid* and obeys the ordinary reactions for alkaloids. It is present *in the brain* in considerable amount, in *muscles*, and some other organs, but is ordinarily not excreted in the urine, or if at all, in such minute quantities that the material contained in a hundred gallons is required for its identification. There are, however, pathological conditions in which hypoxanthine is excreted in the urine in such quantities as to crystallise in the liquid on mere cooling, and such cases have been communicated by the late Dr. Bence Jones and by myself. In these and other cases which I have examined since there was always a history of *albuminuria*, temporary or persistent, and where the crystallised deposit occurred it always came from a so-called fatty enlarged kidney, never from the shrinking one. In the case of a female, who died from fatty kidney, with highly albuminous urine, the hypoxanthine was present in quantities in the liver, so that I could identify it by elementary

analysis of the product in the pure state. As a clinical object, therefore, hypoxanthine in the shape of a crystallised deposit is of importance, as indicating the gravest state of that form of kidney disease which is characterised by the discharge of *serous urine*, properly so called.

2. *New Experiments with Copper Salt lead to the Discovery of Urotheobromine.*

Under these circumstances, I thought it of importance to be able to ascertain the quantities of hypoxanthine excreted in given times by relatively easy methods; and as Strecker had isolated his *sarkine* (subsequently shown to be identical with hypoxanthine) by the aid of acetate of copper from flesh, I used this reagent, first upon pathological, later upon normal, specimens of urine, with the more confidence, as Strecker had stated, under an insignificant reserve, that he had extracted sarkine from urine by copper acetate. But by this proceeding, and from small quantities of material, no hypoxanthine was obtained; the copious precipitate by copper acetate yielded urochrome, and *a new alkaloid*, which I prepared in a crystallised pure state, found to be an isomer of *theobromine*, and called *urotheobromine*. Thus the problem was at least doubled, and I instituted a comparison of the two copper compounds, one of urotheobromine and the other of hypoxanthine, with a view to obtain an easy process for their separation, I found that the *urotheobromine compound* could not easily be obtained in a stable and pure condition, as the base, when treated with copper in combination, became oxydised, reduced the copper oxyde, and transformed it into yellow suboxyde, which remained with the altered base. When treated with nitric acid this

compound lost carbonic acid; and as Strecker had used both boiling and nitric acid in his treatment of the precipitate from urine, his non-success in this research is explained by these two oxydising reactions.

For the isolation, therefore, of urotheobromine copper acetate may be used only in the cold, and the compound must not be kept long or dried; it may not be boiled, nor treated with nitric acid. These features prevented me from producing and ascertaining the composition of a pure definite urotheobromine copper salt.

3. *Quantation of Copper in Copper-Compound from Pure Hypoxanthine.*

Hypoxanthine, on the other hand, which had been obtained from flesh by copper acetate and boiling, was found not to be oxydised; the copper compound remained bluish-green, when dry almost black, and showed no sign of reduction. Dried at 110°, it gave on the application of heat for its combustion, vapours which strongly smelled of *acetic acid*, and left a residue of copper oxyde equal to 47·60 per cent. of the compound. On the *hypothesis* that the compound consisted of a double salt of hypoxanthine-copper, with basic cupric acetate, this is represented in the following formula:—

$$\underbrace{C_5H_3N_4O}_{135} + Cu + Cu\underbrace{(C_2H_3O_2)HO}_{76}$$

63·5 63·5

and we obtain as the total of the molecular and atomic weights 338. This figure 338 is therefore the atomic weight of the salt; on combustion of this there should be left 47·07 per cent. CuO, with which the quantity found coincides.

4. *Separation of Hypoxanthine from Urotheobromine and Urochrome.*

The separation of hypoxanthine from urotheobromine and urochrome is effected as follows:—The precipi-

tates are dissolved in cold *nitric acid* in excess, and *nitrate of silver* is added to the solution. All the hypoxanthine is precipitated as *a compound with silver nitrate* $C_5 H_4 N_4 O + AgNO_3$, which is quite insoluble in excess of nitric acid, in which the urotheobromine compounds and products are soluble. The salt may be dissolved in hot nitric acid, and crystallises on cooling. This reaction, following the phosphomolybdic process, may be used for isolating hypoxanthine from large volumes of renal secretion.

XIII.—LATE INQUIRIES ON SOME IMMEDIATE PRINCIPLES OF THE BRAIN.

1. *Introductory Note on the Literature of the Subject.*

As it has been necessary for the purposes of the following essay to refer to several previous authors and researches, in order to make the observations coherent and generally intelligible, it might have been expected that they should all be quoted *in extenso*. We have, however, avoided this, mainly because the whole of the literature of cerebral chemistry has been exhaustively treated by Thudichum in his "History of Chemical Researches on the Brain," 1874, as far as regards publications up to that date; publications subsequent to that time and up to 1879 were discussed by him in an essay in "Annals of Chemical Medicine," I (1879), p. 254, under the heading of "Notes and Experiments on the Alleged Existence in the Brain of a Body termed Protagon"; also enlarged and revised up to 1886 in his "Grundzüge der Anatomischen und Klinischen Chemie," Berlin, 1886, p. 203. Of particular importance for the purposes of the present essay is, however a paper on "Phrenosin, a New Nitrogenised, Non-

Phosphorised Normal Educt of the Brain," published in Erdmann's *Journ. f. Pract. Chem.*, 25 (1887), 19; and an article in the same journal, p. 39, containing observations on an essay of E. Parcus, being an inaugural dissertation elaborated under the presidency of Dr. Drechsel, Professor of Physiological Chemistry at the Physiological Institute at Leipzig, and published in Erdmann's *Journ. f. Pract. Chem.*, 24 (1881), 310. In a foot-note to these observations Thudichum has given a complete list of his publications on brain educts and products up to 1881. The following paragraphs in a manner continue the discussion which then took place, but have reference more particularly to a publication which has issued from the Physiological Institute of the University of Berlin, entitled "On Some Ingredients of Nerve-Marrow," containing an account of the joint labours of A. Kossel, Professor of Physiological Chemistry at the Institute, and F. Freytag, and published in *Zeitschr. f. Physiol. Chem.*, 17 (1893), 431. These records will enable every reader who desires to peruse original sources, to find them and their author's names. It is the more to be desired that this should be done more frequently, as the accounts of previous researches given in a number of essays include numerous errors and representations being the contrary of fact, as will be shown in various parts of the present essay.

2. *Revival of the Protagon Proposition.*

During the last thirty years the literature of brain-chemistry has now and then echoed a name, which to the unconcerned spectator may have appeared unintelligible. To some it was so much so that they believed it to be that of an animal. It was, however, that of a matter or chemical product, and as this substance has led to some contentions on the field of the physiology

of the nervous system, it should be noted by the uninitiated reader that this so-called "protagon" is, in fact, nothing more than the solid part of an alcoholic extract of the brain, the more soluble portions of which solid part have been washed away with ether. It is the *white matter* which Vauquelin had first extracted with spirit, though he did not wash it with ether; it is nothing more or less than the cérébrote of Couerbe, which *was* washed with ether. It had, therefore, no title at all to a new name, but should have been termed *cérébrote*, but protagon, as the name for cérébrote became current. A few years later some very slight inquiries by a Russian student were the cause of a complete reversal of this popularity, and the whole physiological profession considered protagon as a fallacy to be discarded, and that with the same celerity with which it had been accepted.

The late French chemist, Frémy, having produced some white matter from the brain after Vauquelin's example, also followed Couerbe in washing it with ether; but without examining the part which remains undissolved, and which is the great bulk of the produce of the spirit decoction, he directed his attention to the ether extract; this he concentrated until it made a deposit, and then washed this deposit with only a small volume of ether, and further tested the white product which the limited amount of ether left undissolved. He assumed, not quite unreasonably, but nevertheless, quite erroneously, that this white residue from the ether extract, was identical with the white matter from which it had been extracted, tested and analysed it, and named it *cerebric acid*. The foregoing is relevant to protagon in so far as the Essay of Frémy has furnished the opportunity for one of *the fundamental errors* of the adherents of the

protagon hypothesis, namely, the assumption that the bulk of the white matter, which was not dissolved by ether, was identical with the relatively small amount of white extract which had been dissolved in ether, and this false assumption spread error in two directions—"cerebric acid" was called "protagon," and its properties were passed as those of protagon; and, on the other hand, to protagon were ascribed sundry properties of cerebric acid, *e.g.*, solubility in ether, although, in fact, it did not possess such solubility. This deplorable confusion is continued unabated by some defenders of protagon, although it was fully demonstrated as erroneous on several occasions during the last twenty years.

Some of the latest defenders, however, have now recognised the error of this attempted identification, and have begun to endeavour to extricate themselves from their untenable position; but, in doing so, they fall into a new error: they abandon the belief in the identity of the two white matters, but describe the one, cerebric acid, as a modification of the other, protagon, and thus propound *two different protagons* to begin with. But whatever work they perform is always done on the insoluble part of the white matter; the soluble part, cerebric acid of Frémy, has not been favoured by research.

The first attempted revival of protagon from the collapse above referred to was a mere repetition of the earliest known data without any advance whatever; only one kind of this very immediate principle was manipulated, and the existence of two protagons (always in the white matter insoluble in ether) which might have been constructed out of discordant analyses, was not thought of. It was alleged that the product with more than one per cent. of phosphorus was

impure. But when the matter had been discussed a little before the Royal Society and elsewhere, and after Thudichum had published his Experimental Criticism of the subject, a new kind of distinction was found i support, after all, of the surmise that there were tw protagons, one pure, another impure. No means o diagnosing or separating the one from the other were given, but from the so-called impure article, Thudichum's phrenosin was extracted and supplied with a new kind of name which might hide its identity. It was also averred, what every experience proves to be unfounded, that what was now proposed to be called pure protagon did not yield any so-called cerebrine.

Nevertheless, even most revivalists always found in their protagon a substance which they called cerebrine, and which always was a mixture mainly of phrenosin, kerasin, and sphingomyeline ; their product thus fell always under the rubric of impure protagon, and this confusion prevails in some of the latest publications. According to the revivalists protagon always perished in boiling ether ; they boiled it for many hours up to twenty-four, but made no communication, such as they had promised, concerning either the object of the process or the products of the alleged decomposition. Against this assertion concerning the destruction of protagon by boiling ether, some entered a practical protest by dissolving, boiling, and recrystallising protagon in and from ether ; but on long boiling the phosphorus in the deposit fell to 0·6 per cent., a fact which, after all, they endeavoured to explain by the erroneous surmise of decomposition. Thudichum has discussed most of the propositions and results of the revivalists in his experimental inquiry concerning protagon, and the conclusions from this research subsist unanswered in their integrity, and are, in

fact, silently adopted by subsequent writers. However, in 1893, Kossel and Freytag imagined what they call modifications of protagon, one the insoluble in ether form, on which they carry out their further operations, and the other easily soluble in warm ether, and as little destroyed by boiling ether treatment as the other. The soluble modification they identify with Frémy's cerebric acid; but instead of now defining Vauquelin's white matter as a mixture of their two modifications of protagon and some other matters (namely, those most easily soluble in ether, kephaline, cholesterine, &c.), they quite erroneously declare it to be identical with Frémy's cerebric acid. This shows again the confusion into which the protagonists all drift by their complete want of stable definitions, arising from want of acquaintance with the subject as well as its literature. Again, the varying and contrasting solubilities of the two alleged modifications are neither noticed nor used for the purposes of the research. The soluble protagon remains untreated, and the experiments which follow are all performed on the bulk of the white matter, which, though extracted with, or purified, by ether, has never been dissolved in it. For as Thudichum has shown by an exhaustive research, it is quite insoluble in ether, however long the solvent may be applied. *

The extract by ether from the white matter obtained by boiling alcohol from the brain is a mixture of many substances, the phosphorised kephalines and their congeners are the most soluble of them, and easily removed by little ether; with them goes nearly all the cholesterin, there remains a simpler mixture which is separated into two parts by lead acetate, one part com-

* "Grundzüge der Anatom. und Klin. Chemie." Berlin, 1886, p. 212.

bining with lead oxyde, the acidites, or *cerebrinacides*, another not combining with lead, basites and neutral matters, or *cerebrosides* and *amidolipotides;* of the *phosphatides* some combine with lead, some not; others combine with platinic chloride, others with cadmium chloride; neither cerebrosides nor cerebrinacides are present in any quantity, but there are amidolipotides or nitrogenised fats present in considerable proportions. All these matters are made more soluble in solvents by their association, and several become much less soluble in the same solvents when they are once isolated;* after extraction with little ether, the remaining portion (the mixture called cerebric acid) is never again entirely soluble in ether.

3. *Points of Evidence which prove that the Matter called Protagon is not an immediate Principle, but a Mixture of several immediate Principles.*

Protagon owed much of the consideration which it received on its first appearance to a certain presumption engendered by laborious preparations to which brain matter was subjected previous to its extraction by spirit. Thus the brain was to be washed, at least partly, by injection of water into the carotids of the ox. The isolated and dried, or minced or manually comminuted brain tissue was then to be treated with ether. This ether extraction process, which was also called a dehydration, was extended by some over five months of time. Yet the ether did not protect the brain matter from decomposition, according to Liebreich, and a certain undefined amount of such decomposition was even stated by him to be beneficial, inas-

* The most striking example of the dissolving influence of the more soluble over a less soluble analogous body is afforded by *paramyelin*, which when once separated from lecithin seems to have changed its character from an easily soluble to a very little soluble substance. Sphingomyelin and kerasin exhibit similar affinities.

much as it produced free fatty acids which helped to dissolve the protagon. To this we have to observe, that if there had been decomposition, the preparation ought to have been rejected entirely. But the occurrence of such decomposition has never been proved. However, the whole of the ether processes applied to brain pulp have been shown to be perfectly useless. They remove but little cholesterin, and next to no phosphatides, as these in the hydrated state are quite insoluble in ether, and are never dehydrated by it. All these preliminaries have been abandoned, since it has been proved that the brain is best dehydrated *in vacuo*, with slight heat, and that the residue yields all the same educts as brain-matter dehydrated by alcohol in a finely subdivided state.

One of the principal fallacies of the protagonists was the assumption that protagon became decomposed when heated in spirit to a temperature of above 45°. Thudichum has long since proved that the phenomenon which gave the basis to this error consisted in the *dehydration of phosphatides*, which thereby become insoluble in spirit on boiling, and in fusing enclose mechanically many other matters. Perfectly dry protagon, *i.e.*, white matter exhausted by ether, when warmed with absolute alcohol in a water bath, fuses to a stiff paste, and yields but little matter to the alcohol, even on long boiling. One can prove this dehydration of the phosphatides concerned on each of them singly, in the pure state, and also that by treatment with hot water for some time they again acquire their solubility, in alcohol, on boiling, and all other properties. This product of the dehydration of phosphatides, which is formed more easily in the alkaline and earthy salts, was termed *stearoconote* by Couerbe. Thudichum showed

that by treatment with dilute hydrochloric acid, which includes the removal of the alkalies and earths, the return of the hydrated condition was engendered and accelerated.

When this fallacy of supposed decomposition of protagon by hot spirit had been cleared out of the way, the revivalists discovered that protagon was *decomposed* by being boiled with ether. Some had "recrystallised," as they termed it, protagon from boiling ether, but notwithstanding this success, they continued the boiling in ether for hours, and obtained matters in which the phosphorus was diminished gradually to 0·6 per cent., the rise of phosphorus in other parts was not followed analytically. The performants of this curious experiment declared it to prove the decomposition of protagon by boiling ether. No single product of this process was obtained in a state of chemical isolation, or was analysed. The experiment was also without stated or discernible object.

This fallacy also of the decomposition of protagon by boiling ether has been abandoned by the latest revivalists. It was a mere invention; *no educt of the brain is decomposed by boiling ether.* The protagonists beginning with their founder were so filled with vague fear about the fragility of their material that they saw decomposition everywhere. One of them declared that the first mother liquors of protagon contained nought but decomposition products, and rejected it without any attempt to isolate any of the immediate principles contained in it. Nevertheless, he could believe that such a mixture of decomposition products had yielded a pure educt, and one only. He ignored the clearest evidence that the mother liquor was saturated with immediate principles, *lecithin* in quantity, *paramyelin*, *myelin*, *amidomyelin*, and *kephalin*, *kephaloi-*

din, and allied bodies; there were *cerebrosides* and *cerebinacides* in small quantities, but the *amidolipotides* were present in larger amount, and easy to be isolated. The operator resignedly remained in the darkness in which he had begun his labours. Even lead acetate and baryta he accused of decomposing action without any proof whatever. Thudichum's reply to his observations has remained without any answer.

So little did the protagonists know of their substance that they confounded the part soluble in ether with that which is insoluble in it, and gave such a confused account of their experiments that no one who does not know the subject independently could find his way out of the darkness. Of the matter soluble in ether, Frémy's cerebric acid, they to this day know nothing at all. Of the insoluble matter of their protagon they knew only that it yielded two cerebrosides, about which however, again, they are quite uninformed.

All the foregoing we may term *preliminary and redundant fallacies*, which might have been avoided by reflection or experiment. The following fallacies are already more intricate, and require more method for their removal, although they also arise from mere omission of elementary precautions. Such an elementary precaution is the observance of the law of Chevreul, according to which a certain mass of matter can be a unitary chemical substance only in case all its fractions have the same composition and properties. But *by fractional solution in spirit and deposition of protagon, no preparation with an invariable amount of phosphorus or any other element is obtained.* The phosphorus in all fractions examined fluctuates between 0·108 per cent., as minimum, and 2·91 per cent. as maximum. This elementary yet decisive tes the protagonists all avoid, and it may, therefore, be

said that they allow judgment to go against themselves by default.

By mere solution in and deposition from absolute alcohol, *mother liquors* are obtained which contain *kerasine*, the second cerebroside, and *sphingomyeline*, the principal phosphatide (N : P = 2 : 1) in the mixture. When to this solution cadmium chloride is added as long as a precipitate ensues, and the precipitate of *sphingomyeline dicadmium chloride* is filtered off quickly, the filtrate soon becomes turbid again, and deposits clouds of kerasin, which will sink as a gelatinous deposit. From this mother liquor, after further concentration, platinic chloride and hydrochloric acid precipitate a compound with these reagents of a body termed *assurin* in which N : P = 2 : 2.

The mere resolution in alcohol, and reprecipitation of protagon, enables the operator, by using *critical temperatures*, *e.g.*, 35° or 28°, to isolate some small portions of *phrenosine* and of *kerasine*, but not of sphingomyeline, without the employment of other reagents.

By *boiling ether*, contained in an apparatus closed on all sides, so that in contact with the protagon it actually boils while filtering, the *nitrogenised non-phosphorised amido-lipotides*, *e.g.*, *krinosin* and *istarin*, are extracted. They are absolutely insoluble in cold ether, and can be isolated only in the manner just described.

The *amounts* of all these matters stand in *no stoichiometric proportion* to each other, or to the original protagon, which would have to be the case if they were products of a decomposition.

When *precipitants* are added to solvents at the beginning of the process, the number of diagnosable matters is at once increased. Mere addition of platinic

chloride to a solution of white matter or protagon p cipitates a phosphatide which as platino-chloride compound is insoluble in boiling spirit, *apomyeline*, or sphingo-myelin, or the latter only. Mere cautious addition of alcoholic *lead acetate* precipitates *myelin* as a lead compound in which Pb replaces 2H ; *myelin* is, therefore, a *dibasic acid*. The compound is insoluble in alcohol and ether, even on boiling.

Lead acetate in larger amount and some ammonia throws down not only *myeline*, *cerebrinacides*, and *cerebrosulphatides*, but also some *cerebrosides ;* the latter compounds are neither firm, nor, at present, definite.

The *combinations of phosphatides* with cadmium chloride are many, and very useful for their separation, they are all soluble in boiling alcohol, and require special means for their isolation. One such means is treatment with hydrosulphuric acid in ether, whereby the cadmium becomes sulphide, and remaining combined with the phosphatide, dissolves in the ether with a canary-yellow colour ; another means is benzol, acting at various degrees of temperature.

Baryta precipitates at once, without loss of time and without decomposition or chemolysis, *phosphatides* and *cerebrinacides*, to which some cerebrosides adhere. All bodies are obtained unchanged from the baryta precipitate, just as from the lead precipitate. The decomposition alleged by the protagonists is a mere product of their imagination.

Protagon always contains some *earthy and alkaline salts*, which must be extracted with *mineral acid*. As the protagonists neglected this purifying process, their products were all imperfect, both in reaction and composition on this ground.

By fractional solution and deposition from alcohol a greater number of immediate ingredients could no

doubt be made evident. Thus the large rhombic crystals insoluble in ether and alcohol (described by Thudichum in "Annals," I, p. 257) are a body which has not yet been examined chemically in any respect whatever.

The inspection of protagon under the microscope shows it to be a mixture of matters of very different aspect. Phrenosin forms white opaque rosettes. *Kerasin* branched masses like a cauliflower, somewhat coloured, gelatinous of consistency, while phrenosin is chalky ; *cerebrinic* acid forms opaque balls of needles in radiary arrangement ; *myelin* yellowish balls, with concentric layers ; *krinosine* forms the wavy, long, iridescent, blue filaments, by which it is so easily recognised. *Spherocerebrin* is easily recognised in the mixture by the fan and wedge-shaped fracture and markings of its balls. As they fall at different temperatures and periods, they form horizontal strata, visible to the naked eye. All the bodies extractable from protagon have properties which show them to have been uncombined. The *cerebrosides*, and at least one of the *cerebrinacides* are *neutral glucosides ;* the *phosphatides* are partly *alkaloids*, such as sphingomyelin, which is a *dinitrogenised dipolar alkaloid ; partly acids*, *e.g.*, *myelin*, which is dibasic. The *amidolipotides* are quite neutral and have not yet been combined with any reagent whatever.

These bodies are all very *firm compounds*, and are very difficult to *decompose* in such a manner as to yield intelligible results. They are thus apt to make the adept discard the ceaseless fears of decomposition with which the protagonists surround them, mainly an involuntary result of their inability to deal with them in a logical manner.

All protagonists have promised *products* of regulated

chemical decomposition (chemolysis) of protagon, but none have given any. They all go in straight for the extraction of *phrenosin*, some for *kerasin* also, but all neglect completely the *phosphatides*. Not one of them has even as much as tried to extract the *phosphorised radicles* from protagon. These radicles actually do upset the dreamed unity of protagon yet more than other ingredients. Four phosphatides at least concur in the making-up of the phosphorus in every case; no wonder that by mere solution in alcohol *twenty matters*, all with different amounts of phosphorus, are obtained.

The protagonists speak of *crystallising* protagon, of *recrystallising* it from alcohol and ether. But these are mere presuming expressions. *There are no definite crystals ever recognisable in protagon*, Even bodies which, when isolated *crystallise well*, though only of microscopic size, like *sphingomyelin*, in the mixture are amorphous. No systematic attempts to obtain unitary crystals have ever been made by protagonists. The most one can say of the forms of protagon particles is that they are crystalline aggregates which become more characteristic when the bodies are isolated. As parts of a *physiological chemical examination* of the brain the operations of protagonists are *perfectly useless*. We have already shown that they have neglected, and in words, condemned *as decomposed*, the first great alcoholic mother liquor, containing *more than half of all the matter extracted from the brain* by the alcohol,* they have neglected the ether extract, except only that small portion answering to Frémy's cerebric acid, and to the *protagon* soluble in ether.

* Liebreich even maintained that there was decomposition under his ether treatment, and that fatty acids were produced by a decomposition of a portion of the protag n which favoured the solution of another portion of protagon.

They have neglected all *the ingredients of protagon* except what they call *cerebrin*, that is to say, Thudichum's phrenosine and kerasine ; they have not tested what the brain pulp retains when *not* boiled with spirit above 45°, and what it yields to *boiling spirit only ;* they have not tested this *brain pulp* itself, and therefore fail to judge of its remarkable associations.

On the other hand, the later protagonists have, no doubt under the pressure of criticism, abandoned already several of the earlier parts of their processes, which has been declared indispensable, *e.g.*, the perjection of water through the brain of the ox *in situ*, the dehydration so-called, truly, the treatment of the wet brain substance with ether ; they no longer maintain that protagon is decomposed by boiling with alcohol, and by heating it above 45° ; they have ceased to allege that boiling with ether decomposes the matter, and to mask this retreat they have spontaneously suggested *several forms* of protagon, but without defining them, and without showing how they could be separated ; some admit phrenosin to be an *impurity* of true protagon ; others term it a constituent separated by chemolysis by baryta, There is next to nothing left of protagon. All these derelictions show the complete want of method on the part of the revivalists ; they have only lost ground, and not advanced any part of the subject by an atom. We shall see the consequence of their want of method in the inquiry on *the chemolysis of phrenosin by nitric acid*, by which the Berlin Physiological Institute has participated in the discussion.

4. *Varieties of Elementary Analyses and their Lessons Concerning the Phosphorus, Sulphur, and Nitrogen contained in the Educts.*

The quantations of phosphorus in the various

bodies, which are now claimed to be protagon, have given results which lead to remarkable conclusions by their mere variety. Frémy had found 0·9 per cent. P in his so-called acid. R. D. Thomson 0·46 per cent. in acid made after Frémy's recipe, that is to say, just half Frémy's quantity ; Gobley had found in white matter from eggs claimed to be identical with that which Vauquelin, Couërbe, and Frémy had extracted from brain, 0·43 per cent. P. Von Babo had found 0·52 per cent. P as the mean of five analyses of cerebric acid. Liebreich found 1·5 and 1·1 per cent. P in his products ; some of his followers adopted his lower percentage, while rejecting, without explaining the higher. Kossel and Freytag, however, find in "cerebric acid," prepared after Frémy from ethereal extract, 1·35 per cent of P ; in *their* protagon, of course, the insoluble in ether form, but not fractionated and not characterised as to "modification," 1·05 per cent. P. Other protagons, again "modifications" not defined, gave a mean of only 0·97 per cent. of P.

The foregoing account shows that in all the reliable analyses of such cerebric acid as was made strictly after Frémy's precedent, the phosphorus varied between 0·46 per cent. and 1·35 per cent. The few inquirers made only one preparation, and analysed only this one preparation, and mostly only once, and mostly for phosphorus only. In no case was there any fractional solution and deposition, not a single combination, no attempt at chemolysis. Indeed the whole of the analytical work on this otherwise nondescript matter is, except as a series of qualitative tests, valueless, and has not furnished a single resultant fact that will be recorded in the future. That it is so persistently quoted at present shows that those who do so quote it have not studied the original essays, and not tried the

processes advanced in them, but instead of doing so have merely copied each other. With the exception of the finding of the bases combined with the matter called cerebric acid, which finding was at least a hint towards a necessary purification, Frémy's research has been only an injurious delusion, firstly, by his assuming an obvious mixture to be a unitary substance ; and, secondly, by assuming this *mixture of neutral* and *alkaloidal* substances to be *an acid ;* and, thirdly, by his fabulations about *oleophosphoric acid.*

The case of the white matter insoluble in ether, which alone was first termed protagon, is still more delusive than that of the white part of the ether extract from it. Any one can by fractional solution in, and precipitation from alcohol of protagon, obtain bodies with any amount of phosphorus between 0·108 per cent. and 2·91 per cent. But this obviously necessary test the protagonists persistently avoid, and thus show that they have not the ingenuousness to investigate reactions which testify against the correctness of their opinions. Thudichum has proved the invariable occurrence of these fluctuations of the phosphorus by fractionation on twenty-one specimens of fractions, and his published results have never been experimentally controverted. Some revivalists have proved the same unwittingly by their experiments on protagon with boiling ether, which destroyed the purport of their phosphorus quantations.

The cerebro-sulphatide is another of the bodies, which, if the protagonists continue to make experiments on the lines occupied by them at present, will help them to recognise their position. The white matter contains a sulphurised body, which, originally discovered by Thudichum, has by him been prepared in so concentrated a form that its soluble baryum com-

pound with 35·30 per cent. Ba contained four per cent. of sulphur. Accordingly Kossel and Freytag, without stating the origin of the knowledge, find in their protagon 0·571 and 0·507 per cent. S. This is, of course, an important complication of the question of the constitution of the brain in general, but more particularly of the protagon question, namely, if the sulphur be considered as an essential constituent of protagon, for this element stands in no proportion of an atomistic kind to either the phosphorus or the nitrogen contained in the mixture; moreover, in all former analyses of protagon the sulphur was overlooked, just as were the salts. Those who would declare this sulphur to be a non-essential accidental ingredient have to contend against positive evidence, opposite to which their negative is of no value.

It is hardly necessary to discuss the quantities of other elements found in protagon; they must, and do vary, notably *the nitrogen;* the figures for this element differ greatly in the latest essays, and one operator tries to explain the difference by the fact that some inquirers burnt their protagon with oxyde of copper, others with chromate of lead; in this writer's opinion protagon may serve as a test-object for the reliability and delicacy of analytical methods.

Most of the operators on protagon have very insufficient notions concerning *the modes* in which *qualitative tests* and *quantitative analyses* on brain-substances have to be carried out. The smaller becomes the amount of admixture to be diagnosed and removed, the greater must be the amount of material subjected to analysis; all combustions and deflagrations must be carried out in somewhat long Bohemian glass tubes, as when made in crucibles, or even short tubes, they are liable to lead to loss. Mere combustion of phosphatides

in platinum vessels, even with nitric acid, involve great loss of phosphoric acid both by reduction and volatilisation. By the non-observance of these precautions many operators have come to erroneous conclusions, *e.g.*, that their preparations were free from *phosphorus*, when they did yet contain much ; in a similar manner *the sulphur* always present has been overlooked or missed, though sought deliberately. We decline to accept analyses concerning which the method, precautions, and quantities of material analysed are not stated. And we receive no statement concerning *the nitrogen* of any cerebral substance which has not been subjected to the process of quantation of nitrogen, by an accepted method, even if the only question to be answered were whether or not nitrogen was present in it.

5. *Some Attempts to find Atomic Weights by Points of Boiling and Fusing, by Substitution and Chemolysis.*

Kossel and Freytag did not test the unity of protagon by any fractionation, which would have been a decisive test, but assumed it without any proof whatever ; they did not test protagon as we shall see they tested so-called cerebrin, to find out something about its atomic weight ; they declare the addition of such a dilute solution of baryta, as that in methylic alcohol necessarily is, to a solution of protagon in the same quality of alcohol, to effect a decomposition : but they prove no such process ; as a matter of fact, baryta, under such circumstances, effects no such decomposition, but only causes a precipitation of matter, with which it combines directly, and leaves others in solution and uncombined. If the baryta were added in sufficient quantity, and, above all, in sufficient concentration to effect a chemolysis, it would destroy both

phrenosin and kerasin, and phosphatides before them or with them, and the result would be a much more inextricable mixture than were those which Parcus, Geoghegan, and Kossel and Freytag obtained from their cerebrines. However, the latter operators having, after the application of baryta, obtained their cerebrine, and given it a formula transferred from the essay of Parcus, with the choice of three variants for option, experimentalise with three modes for fixing its atomic weight. They dissolve about half a gramme of their cerebrine in 50 cc. of acetic acid, and ascertain a rise in the boiling point of the acid amounting to 0·03, three one-hundredths of a degree of temperature. The datum was, in their own opinion, inconclusive. They therefore brominate their cerebrine, and obtain a product with 16·36 per cent. Br. This percentage they calculate, with the aid of the formula with 70 C (one out of the three of which Parcus had left the choice), to amount to the proportion of 3 Br. upon 2 N, and thus the atomic weight is found. They do not refer to Thudichum's experiment published many years before theirs, which showed that *bromination of phrenosin* gave a product with 14·43 per cent. theoretical, 14·17 per cent. actual Br. contained in it; this corresponded to a mixture of mono-brominated with dibrominated phrenosin in which Br : N = 3 : 2. We do not see how such an experiment can fix an atomic weight; on the contrary, the result of the experiment can be explained only when the atomic weight is otherwise demonstrated. It was thus that Thudichum could explain his brominated phrenosin as an accidental sesqui-compound. There is, apart from the atomic weight discussion, here not only a prior, but also a better, experiment.

All these proceedings having failed to lead to the desired results, the operators bethink themselves of

applying a process of *chemical decomposition, chemolysis* as we shortly term it. They knew that *sulphuric acid* as well as *caustic baryta* had been successfully used, but these experiments of Thudichum they did not repeat for their own information. As the agent for their own purpose they selected *nitric acid*, which seems to have been suggested to them by the precedent of Müller; but unlike Müller, who used concentrated acid, they used acid diluted with several volumes of water. In such acid they heated and boiled for some hours some of their cerebrine, and when the process was interrupted they found that they had produced *nothing but common stearic acid.*

Thudichum found as the result of his chemolysis of phrenosin, a particular acid of the composition of stearic, but only isomeric, not identical with it, as it fuses at 84°, which he called *neurostearic* acid; but the professor and his coadjutor do not test this fact; Müller had found *stearic acid*, they say, by boiling his cerebrine with nitric acid, although he did not recognise his product as stearic acid. Indeed, he could not recognise it as such, as it contained only 17 atoms of carbon, and did not even call it an acid. But they having boiled with nitric acid their cerebrine postulate an amendment of Müller's result and get it. As a collateral precaution they boil common stearic acid fusing point 69·2 with nitric acid for some hours, and do not find it visibly changed. From this they argue that their acid may be stearic, the action of the nitric acid notwithstanding. But their product fuses at 70° to 71° on fractionation a part shows fusing point at 75°, another 78°, 8°8 above stearic acid. Nevertheless, the authors maintain that this is stearic acid, but that its fusing point has been raised by an admixture of undecomposed cerebrine!

In their thermology the "fusing point" of "cerebrin" is somewhere about 176°, but they omit to say that this fusion so-called is accompanied with destruction, and forget to explain how this fusing point 176° came in any case to be lowered at all, and then to 70°, or 71°, or 78°. In case there had been a little cerebrine left in this product after three hours of boiling with nitric acid, it would have been easy to exclude it, by dissolving the acid in ether, or in alkali and water. But they accept this coarsely impure product as stearic acid, and build a theory of the cerebrin with three molecles of stearic acid upon it. Any other product or products resulting from the liberation of the *radicle of sphingosin*, containing all the nitrogen which remains from phrenosin and constituting half the amount of the insoluble product, they miss entirely, as also the third principal product, the result of the oxydation of cerebrose, namely, *mucic acid*.

6. *How History is written by the Partisans of the Protagon Hypothesis.*

The adherents of the proposition that protagon was an immediate principle, mostly preface their papers with some literary introductions, containing a sort of abstract culled from previous abstracts, and so rarely from original sources, that we have not yet met with one containing original historical information. We have seen already above the confusion to which this practice has led. Another part of their tactics is to be silent in the face of inconvenient or unimpugnable facts which are destructive of their hypothesis. Sphingosin, about which they must have read, and which as a most useful medicine, is now manufactured wholesale, and sold in trade, they do not mention, nor any seat or refuge for the nitrogen of their products. The

mother substance of sphingosin, phrenosin, they just mention in brackets, as if it were a mere subordinate synonym of their cerebrine. It therefore excites attention when they suddely become most accurate in appearance, as regards matters of literary history. Such a striking attempt at accuracy occurs in Kossel and Freytag's chronology of the discovery of cerebrine, in tables, in which some analyses of *phrenosine* and *kerasine* are attempted to be compared ; even years are given in which the publications are alleged to have been made, thus : "Parcus, 1881," "Thudichum, 1886," and this is pointedly repeated for *kerasine*.

As Kossel and Freytag knew the essay of Parcus (in the "Journ. f. Pract. Chemie.," 24 (1881), 310, they ought also to have known Thudichum's essay in the same "Journal," 25 (1881), 19, containing a full account of "Phrenosine, a New Nitrogenised Specific Cerebral Educt Free From Phosphorus." They ought also to have known Thudichum's observations on the essay of Parcus (*ibid.*, p. 39) and the subsequent correspondence Thudichum's observations on phrenosine date from 1874 ; all the products of its decomposition were fully ascertained in the next six years, and summed up in 1879-80. All the relative papers were quoted in the Leipzig Journal, p. 33, for the information of its readers. Kossel and Freytag's chronology of the discovery of phrenosin and kerasin ought therefore, to be arranged thus : Thudichum, 1874 ; Parcus, 1881 ; Kossel and Freytag, 1894. The false figures in the tables, whatever may be their origin, would, if left uncorrected, have a tendency to transfer the priority and originality of the discoveries of phrenosine, kerasine, and their products upon persons who hitherto have discovered nothing.

Thudichum's researches, therefore, have preceded

the essay of Parcus by seven years * ; the operations of Kossel and Freytag by nearly twenty years ; he might disclaim any identity of their cerebrine with his phrenosin, even if this cerebrine were not disfigured by the assumption of its being a product of chemolysis of protagon by baryta ; for this cerebrine is to bear the formula : $C_{70}\ H_{140}\ N_2\ O_{13}$, and on chemolysis with nitric acid to yield three molecles of common stearic acid upon two atoms of nitrogen contained in it. Phrenosine, on the contrary has the formula $C_{41}\ H_{82}\ NO_8$, is not obtained by chemolysis with any reagent whatever, but is an immediate principle of the brain ; yields none of the nitric acid products attributed to cerebrine, but has its formula, rôle and atomic weight, fully borne out by its products of chemolysis, all analysed and controlled by combinations and transformations, namely, cerebrose, neurostearic acid and sphingosine, and the intermediate bodies, aesthesine and psychosine ; by nitric acid it yields not stearic acid, m.p. 69·2, but neurostearic acid, m.p. 84, a principal product and two side products in small quantities from the sphingosin radicle, and mucic acid from the cerebrose ; and there is only one atom of nitrogen present in it, upon one molecle of a radicle, which in the sulphuric acid chemolysis is left as sphingosine, while the fatty acid radicle appears as neurostearic acid.

7. *The Fate of the Nitrogen in the Nitric Acid Chemolysis of Phrenosine.*

In Kossel and Freytag's operation no nitrogenised body was isolated, the products were not analysed for this element, and though they assumed two atoms of nitrogen to be present in their cerebrine and to take part in the reaction, there is no information whatever

* "Das seit sieben Jahren bekannte Phrenosin." (J. f. pract. Chem., vol. 25, (1881), p. 37.)

concerning their fate, and that of the radicle or radicles in which they would have to be proximately fixed. In this respect the essay exhibits a curious blank.

Phrenosin,e on being warmed with nitric acid diluted with water, froths and fuses, and nothing of an alkaloidal nature, notably no ammonia, dissolves in the nitric acid or is expelled in a volatile form. No alkaloid remains in the solid fused part, which consists of a fatty acid and two nitrogenised bodies in which either alkaloidal or acid properties are but feebly developed. Sphingosine, therefore, which one might have expected to have been formed, has not been formed, but two bodies which must be closely related to it instead ; one retains the faculty of yielding the oleo-cholide reaction.

This first reaction of phrenosine with nitric acid is always accompanied with a loss of weight in the insoluble matter, corresponding mainly to the loss of the cerebrose, which passes into the nitric acid. If the heating with nitric acid be cautiously performed so that hardly any red vapours are evolved, the residue is from 84 to 86 per cent. of the phrenosine used ; a small amount of matter becomes yellow, and in contact with alkalies, red.

In Kossel and Freytag's operations the loss was always greater than that just quoted, as they boiled the mixture in the nitric acid for some hours and no doubt decomposed some of the matters formed from the sphingosine radicle earlier in the reaction. The residue from their cerebrine remained 16 to 17 per cent. below the experiments just stated ; that from kerasin 10 per cent. The loss sustained by this latter, almost covers the theoretical amount of matter which would hav been lost, had it parted with all its cerebrose under Thudichum's formula.

Their formula for their cerebrin, one of the three of Parcus, $C_{70}\ H_{140}\ N_2\ O_{13}$, gives an atomic weight of 1216. If this had yielded, on decomposition by nitric acid, three molecles of stearic acid, total of atomic weights ($3 \times 284 =$) 852, this would be 70 per cent. of the phrenosine. But as the decomposition of phrenosine yields only one molecle of neurostearic acid, operations with the aid of this equivocal cerebrine-formula are quite useless.

8. *Retrospect on Müller's Operation with Nitric Acid on his Cerebrine.*

Kossel and Freytag in their chemolytic endeavours had evidently Müller's proceedings with his cerebrine in their mind's eye, and thought it a find. They praise him for his analysis of the product of the action of nitric acid upon his cerebrine, and say that it proved the product to have been *pure stearic acid;* that he analysed quite correctly but interpreted erroneously This enthusiasm for Müller's body is also quite erroneous and increases the number of their mistakes.

Müller's cerebrine had a composition which led him to the formula of $C_{17}\ H_{33}\ N\ O_3$; it could not be dried at 100°, as it decomposed at 80°, and became brownish yellow; it was soluble in boiling ether, not in cold; it gave the oleo-cholide reaction by itself with sulphuric acid alone.

Seeing that the authors accept Müller's result as correct, how can they derive stearic acid with C_{18} from a body with C_{17} by boiling with nitric acid? And that *concentrated* nitric acid, not diluted, such as they employed themselves! Müller's product of the reaction was a yellow clear oil, which solidified to a yellowish white solid fatty body; by solution in boiling alcohol, filtration of the boiling solution, and twenty-four hours'

repose, it reappeared as a white, granular fatty body, and by re-solution and re-deposition (misnamed by Müller "recrystallisation"), it was obtained as a white, waxy mass, soluble in alcohol and ether with "moderate facility"; its alcoholic solution had a slightly acid reaction, and under the microscope it consisted of minute fat globules *without a trace of crystallisation.* Müller says that it "probably contained no nitrogen," thus intimating that he did not analyse it for this element. We give in the following table the elements in per cents. for (1) Müller's cerebrine; (2) Müller's nitric acid product from this cerebrine; (3) stearic; and (4) palmitic acid.

1. Müller's Cerebrin. Per Cent. Found.	2. M.'s. Nitric Acid Product. Per Cent. Found.	÷ By At W.'s	O=1.	3. Stearic Acid. Per Cent. Required.	4. Palmitic Acid. Per Cent. Required.
C 68·46	75·52	6·293	8·71	76·056	75·0
H 11·25	12·92	12·920	17·89	12·672	12·5
O 15·66	11·56	0·722	1·00	11·268	12·5
N 4·37	?	—	—	—	—

The figures for the body obtained from cerebrine by nitric acid yield as simplest formula: $C_9 H_{18} O$, or $C_{17} H_{35} O_2$. Geoghegan, who had worked under Hoppe-Seyler's advice, had stated (in 1879) that this product of Müller's from his cerebrine by strong nitric acid had "exactly" the per centic composition of *palmitic acid*—now it is to be exactly *stearic.*

We maintain that it is perfectly useless to speculate on Müller's cerebrine with 17 C. or its product by nitric acid with 17 C (not with 18 C as Kossel and Freytag erroneously put it) which is to have gained two atoms of hydrogen, and to have lost an atom of oxygen, all effects the reverse of what nitric acid action would lead one to expect; but we must point out Müller's modesty, which exceeds that of many of his admirers,

in this that he did not even declare his product to be an acid, as he had not combined it with a base, and did not ascertain its melting point. All the considerations evoked by the study of Müller's research have been stated by Thudichum already in 1874 in his "History of Chemical Researches on the Brain," and nothing has been changed in the conclusions there arrived at.

9. *Summary of the Results of the late Inquiries.*

When we attempt to define the results of the labours employed on these inquiries we observe at once that all that which refers to "protagon" is mere repetition and reiteration, and as such, perfectly useless to the present state of biochemical science; not one of the grave objections to this hypothesis has been eliminated or even tested by analytical work; the hypothesis of "*several protagons*" is not the result of research, and is not supported by any valid fact, or even argument; it was made to meet discrepancies in the quantation of phosphorus and nitrogen, as shown above; considered from a serious scientific standpoint, it is a gratuitous invention.

The references to antecedent literature are partly deficient, partly contrary to fact, and that in a manner which seems particularly objectionable when the claims to accuracy are considered, which are put forth so pointedly.

In the matter of the treatment of protagon for the *removal of the phosphatides* (for this was the original and only object of the application of baryta) the authors mistake a mere precipitation of a body in solution for a chemical decomposition, and this is all the outcome from the application of the baryta to protagon. It appears that Kossel and Freytag obtained their "cerebrine" from the baryta precipitate, and not

from the solution from which it was made. All the rest of the products, more than half of the protagon, they left untreated.

We have isolated from this baryta precipitate *phosphorised*, *sulphurised* and *nitrogenised* bodies including *cerebrosides* and *cerebrinacides;* and the matters remaining in solution have never been free from phosphorus, although the amount remaining was relatively very small. In the lead-process applied by ourselves, phrenosine and kerasine, the matters prominently under consideration, remain in the solution, and do not pass in any considerable quantity into the precipitate.

None of the operators have ever studied protagon with a view of finding any other product except the nitrogenised non-phosphorised substance which they call cerebrine equivocally, but professedly after Müller. However, Müller had not extracted his cerebrine from previously isolated white matter, but from the entire brain-pulp treated with baryta, and the experiments of the latter day protagonists were therefore by no means identical with, and not even parallel to those of, Müller. Their products also differed in every respect.

None of the operators on protagon have ever ascertained or stated anything about the relative quantities of protagon and baryta necessary for the success of the alleged chemolysis ; none have ever said that an excess, over and above that absorbed by instantaneous precipitation, would be required, or had been applied. Chemolysis would require an excess of reagent, in default of which the operation would be left incomplete. Müller made no statement concerning the proportion of baryta to brain pulp ; as he imitated Liebig's process, which was applied to extract of meat, and not to pulp of meat, the baryta only to precipitate phosphoric acid, it is probable that he did not use the

baryta in chemolytical quantities. But no certain conclusion can be drawn from his statements, and a further investigation of them would, on many grounds, be a mere loss of time.

None of the operators on protagon ever studied any of the phosphorised principles contained in the mixture, although their study is facilitated by several interesting and instructive combinations. None have ever tried to extract the matters soluble in boiling ether, and isolated by that reagent only, such as *krinosine*, and the other solid nitrogenised compounds, which Thudichum has termed *amido-lipotides*, or *nitrogenised fats*. The removal of these substances does not exhaust the matters present, but leaves only small residues uncharacterised or unexplained.

The fact that nearlv all operators on protagon, when they have produced their material, confine their further operations to the search for *phrenosine*, though under an *alias*, shows, on the one hand, their inability to deal with the other ingredients, and exhibits on the other hand their disregard for the ethical laws of the scientlfic world, according to which, the first discoverer has the right to name his discovery. Every attempt to usurp this, in itself inalienable property, by change of name, or by suppression of name, or suppression of fact, becomes a plagiarism. For the proceeding is based on the desire of appropriating the whole or part of the discovery of others, and however skilfully it may be disguised by so-called researches, it remains unrighteous, and must be repressed. The occurrence of such an attempt is proved in every case in which any supposed or imaginable state of uninformedness being corrected, the claimants to originality do not immediately and publicly make honourable amendment.

In the case of Kossel and Freytag we object, therefore, to the use of the name cerebrine for Thudichum's phrenosine, even though they seem to allege themselves to base their use of this name on the fancied priority of Parcus, who himself had no priority of any kind, and discovered nothing, and had as little right to substitute the name of cerebrine for that of phrenosine as they themselves. They repeat the formulæ of Parcus, ignoring that they were refuted thirteen years ago, and bring no evidence whatever to support them, or even to oppose the refutation. In one matter only Kossel and Freytag are, if not absolutely, at least relatively, original, namely, in the attempt to chemolyse phrenosine by dilute nitric acid and boiling. We are thereby induced to consider this attempt a little closer.

10. *Kossel and Freytag's attempt to Chemolyse Phrenosine.*

Amongst hydrolytic agents *nitric acid* may be as useful as any other acid provided it be prevented from attacking as an *oxydant* the body to be hydrolysed, or the products of the hydrolysis. Nitric acid is, therefore, a chemolyser which is more dangerous than either sulphuric acid or baryta. This consideration was sufficiently impressed on the minds of the operators named, for they studied the bearing of stearic acid with nitric acid on boiling, and dispersed their doubts by finding that it was apparently not much altered. The experiment, however, was in itself futile by the assumption made contrary to evidence, that the acid eventually obtainable from phrenosine must be common stearic acid, and not as had been proved by Thudichum, neurostearic acid. For this, their assumption, they had no reason at all ; Thudichum's proofs are inassail-

able; nobody else had ever chemolysed successfully phrenosine under any name (Parcus stated himself that he had obtained an "inextricable mixture"); nobody had extracted any acid or any other body from it. The Berlin physiologists also obtained *as their only result, an inextricably confused mixture.* In particular they failed in isolating and diagnosing the well-known and well-characterised *neurostearic acid*, and that when they had actually in hand a product fusing at 78°, or more than 8 degrees above ordinary stearic acid, and only 6 or 7 degrees below neurostearic acid; they had no part of their product fusing below 70°, and the fusing point rose with every fractionation. They exclude the conviction, which these facts might have carried to their minds, by the forcible assumption that the product was stearic acid *made impure by "undecomposed cerebrine."* But they prove the presence of stearic acid just as little as that of undecomposed cerebrine; if undecomposed cerebrine could raise the fusing point of stearic, it could raise that of any lower or other nearly related fatty acid, margaric, or palmitic, or even elaidic, which might have been formed from an oleyl radicle by the nitrous acid evolved in the reaction. To assume that a glucoside like phrenosine, which has no real fusing-point properly so-called, but must be heated to decomposition before it fuses at 176°, would influence in the sense of raising by a few degrees the fusing point of a fatty acid melting at 69·2, is merely illogical, and so much so as not to require even the refutation afforded by a synthetical experiment.

The authors fail to diagnose or even notice the presence of the body or bodies formed from the nitrogenised radicle of phrenosine, which radicle under the

influence of hydrolysis by baryta or sulphuric acid appears as sphingosin, $C_{17} H_{35} N O_2$, and then contains all the nitrogen of the phrenosin.

The authors in their chemolysis, of course, separate the sugar *cerebrose*, $C_6 H_{12} O_6$, from the phrenosin, but give no account of its fate, and do not even mention it ; we have proved that it is mainly transformed into *mucic acid*, and thus is again manifested as *galactose*, as which Brown and Morris had diagnosed it on pure materials furnished to them by Thudichum.

We need not specially discuss the relative statements on *kerasine*, as our observations must be on them, *mutatis mutandis*, identical with those on phrenosine.

Thus Kossel and Freytag are proved to be unacquainted with a great part of the history, as well as the broad facts of brain chemistry, and to be misled by an entirely erroneous estimate of their ability to appreciate the scientific results, the purity of products, and the cogency of the theories of previous observers. The effect of their analytical operations is entirely retrograde, as they carelessly repeat indecisive data, ignore facts which have been proved for many years, iterate refuted errors, try to displace proved facts, substitute erroneous names and dates for true ones, and in the only part of their operation which might have furnished something original and new, fail in a manner which is the necessary result of disregard for the accepted principles and results of scientific research.

XIV.—RESEARCHES ON PHRENOSINE AND THE PRODUCTS OF ITS CHEMOLYTICAL CLEAVAGE.

1. *Chemolysis of Phrenosine with Dilute Nitric Acid (A) Quantation Experiments with Small Amounts.*

When pure phrenosine of the ascertained composition, $C_{41}\,H_{79}\,N\,O_8$, in a flask is mixed with five times its weight of nitric acid diluted with three volumes of water, and gently heated in the water-bath, some frothing gradually ensues, and a slight amount of red vapour is evolved. When the heating is continued by boiling over a free flame the phrenosin is soon transformed into a yellow fused mass, which is opaque and viscous, and not oily or clear, When the mixture is cooled at this stage the yellow product becomes solid and the clear nitric acid solution can be decanted without filtration. If the yellow residue in the flask be now again fused by immersing the flask in the water-bath, it oozes out liquid which is decanted, and effervesces with evolution of red vapours, to be removed by an air current. This fusion and expulsion of vapour is continued, until the matter gives out neither water nor vapour, and ceases to lose weight at 100°. It is then viscous while fused, and not oily like fused fat. It also shows itself mechanically to be composed of several bodies, one opaque, another transparent even when cold, fusible but viscous at 100°, and coloured yellowish ; the opaque body fuses below 130°, and then mixes with the one fusing at lower temperature.

1. 0·9837 grm. phrenosine, dry at 100° by this treatment, gave 0·8267 grm. residue, equal to 84·33 per cent.

2. 1·2192 grm. phrenosine, treated as before, but boiled a little longer over the free fire, left 1·0440 grm residue, equal to 85·7 per cent. This was more coloured than the residue from Exp. 1.

The residues were quite insoluble in boiling water almost entirely soluble in hot alcohol. This solution gave voluminous precipitates with baryta [the weights of these precipitates when dry were greater than would correspond to the hypothesis that only neurostearic, and no other, acid had been formed]. The experiments, therefore, showed that there was a loss of part of the weight of phrenosine, amounting in the mean to 4·98 per cent. If the loss had been that of cerebrose, and had been complete, it should have amounted almost to one quarter of the weight of the phrenosin. Consequently, assuming the cerebrose to have been all out, there was an accession of matter which would be approximately covered by the accession either of a molecle of nitric acid or of four atoms of oxygen A few tests showed that the product consisted of at least *four matters*, of which one was *neurostearic acid*, giving its baryum salt insoluble in boiling alcohol and in ether. The matter precipitated with the neurostearate, but again extracted by boiling alcohol, consisted of two matters, one soluble in ether, the other little soluble or insoluble in it. To the main precipitate of neurostearate adhered a red-coloured baryum salt, insoluble in alcohol, soluble in ether and in benzol.

(*B*) *Experiments with Larger Amounts of Phrenosine.*

The reaction of phrenosin with dilute nitric acid was thus seen to be complicated and to yield at least five products; of these the coloured ones seemed formed by a secondary action of the acid; to prevent their formation or limit it to a minimum, the reaction in the

following experiments was arrested when the first frothing ceased, and the phrenosin seemed all transformed into the yellowish, viscous, semi-fused mixture of products ; all this was effected in the water-bath only, and the mixture was never boiled with the nitric acid. The enumeration and definition of the substances thus obtained and isolated is here anticipated, to facilitate the perusal of the details of the following experiments.

Summary of Products of Chemolysis of Phrenosine by Nitric Acid.

(1) *Neurostearic Acid*, free by recrystallisation from spirit and ether, extraction by ether ; precipitation as baryum salt from absolute alcohol solution, or combined with baryta directly ; sodium and other salts.

(2) *Phrenyline*, body insoluble or very little soluble in ether, left behind by it. Soluble in boiling alcohol, deposited white and crystallised, and almost completely from it ; soft at 100°, viscid at 115°, fuses to oil below 130°. Sets to hard, cracking, pulverisable resin. Gives oleo-cholide reaction in peculiar manner, particles insoluble in anhydrous chloroform ; contains 2·0 per cent. of N.

(3) *Mucic Acid*, remaining white and crystallised on evaporation of the nitric acid solution ; identified by elementary analysis.

The foregoing are the three main products ; the following occur in subordinate quantity.

(4) *Red-coloured resinous acid* accompanying as salt neurostearates, extracted from them by ether and benzol (not alcohol) ; was colourless before contact with alkali (Na HO or Ba H_2O_2). Soluble in much ether, and extracted from neurostearates by this and by benzol ; if not extracted from salts follows the free

acid when extracted by ether ; when isolated is a red-brown resin, insoluble in hot alcohol, soluble in benzol, and deposited from this on concentration as a gelatinous mass.

(5) *Body easily soluble in ether* and in boiling alcohol, deposited white from both, not combining with Ba ; gives oleo-cholide reaction ; seems to increase solubility of (2) in ether ; it becomes diminished as (4) is extracted, and (2) is crystallised from it. The separation from (2) is somewhat imperfect.

Experiment 3, *with Larger Quantities of Phrenosine.*— 10 grm. finely powdered phrenosine were suspended in 120 c.c of water, and 30 c.c. of nitric acid (P.B.) were added thereto ; the mixture was gently heated in the water-bath, reaction ensued, manifested mainly by frothing ; very little red vapour was evolved, and the neck of the flask in which the reaction took place did not become so hot that it could not conveniently be grasped. When the reaction seemed complete as indicated by the cessation of the frothing, and the semi-fused coherent state of the solid product, the flask and contents were cooled, the solid product became firm, and the nitric acid solution could be decanted off clear. The solid product was twice boiled with water, when it was seen to become soft, and to give out some gas.

The solid product dissolved easily and completely in absolute alcohol ; while thealcohol was insufficient in quantity, a portion of the matter lay as a fused oil at the bottom, but dissolved in a sufficiency of alcohol. On cooling, the solution set into a mass of microscopical crystals of two distinct kinds, one being balls of needles (neurostearic acid), another irregular curved needles and spears (phrenylin). [The mulberry-like deposits of the soluble in ether body were not yet visible, probably remaining dissolved.]

The first white crystals, balls of needles only, when treated with soda ley, became deep yellow, and dissolved in water; excess of soda salted out a soap, which, isolated, dissolved in much water on boiling, leaving no residue on filter. The soap solution deposited flakes on cooling. This showed that the neurostearic acid was not in combination in the mixture of products, and that a nearly pure portion of it could be extracted by crystallisation from alcohol only.

The whole of the neurostearic acid was transformed into baryum salt by addition of baryta water to the alcoholic solution and boiling. To the resulting baryum neurostearate a red body adhered, insoluble in boiling alcohol, while the same boiling alcohol extracted two bodies, one almost insoluble in ether, the main product, phrenyline, and another easily soluble in ether.

The baryum salt was repeatedly dried and powdered to give access of the alcohol to matters mechanically enclosed in the heat-softened baryum soap. The spirit solutions were tested with baryta water to prove absence of free neurostearic acid. The baryum salt was decomposed with hydrochloric acid, and the neurostearic acid extracted by ether was combined with sodium. The soap solution was coloured, and could not be decolourised by animal charcoal, nor be made brilliant by filtration. It was, therefore, evaporated to dryness, and the white powder was exhausted with ether; to this it yielded nearly the whole of the coloured body (Nr. 4). The sodium soap was dissolved in boiling alcohol, filtered hot, and allowed to deposit. The deposit was white, and the alcohol retained the rest of the coloured matter, and very little of the soap.

The alcohol solution containing the principal product, phrenyline, was concentrated and evaporated to

dryness ; the residue was powdered and extracted with ether, which was boiled at each extraction and allowed to cool before filtration. A body dissolved in ether in small quantity, and remained as a white, slightly yellowish residue : it kept some phrenyline in solution, which latter crystallised out of little alcohol in which the second body remained dissolved. The phrenyline, insoluble in ether, bulky, white, retained much alcohol, and fused in that state easily to a thick oil ; it was dried *in vacuo* over oil of vitriol, and became a chalk-like, very light, porous, easily pulverisible mass, which no longer fused below 100°, softened at 115°, fused below 130°. It contained 2·0 per cent. of nitrogen, which made it probable that it was a direct derivate of phrenosine.

Experiment 4.—Ten grs. of the same pure phrenosine were treated as those in Experiment 3, but the solid product of the first reaction was not treated with alcohol, but boiled at once with baryta in slight excess, to combine all acids ; after an hour's boiling the matters changed in colour and consistency, were isolated and exhausted with boiling alcohol in the same manner as in Experiment 3. The dry baryum neurostearate was at once extracted with ether to remove the red body (No. 4). It was then decomposed, and the neurostearic acid, passed through ether, transformed into sodium salt ; this now, when dry, again extracted with ether, yielded nothing to this solvent.

The matters soluble in hot alcohol were recrystallised, dried in vacuo, and separated by ether. The insoluble matter, phrenyline, seemed to be the main product, as regards quantity.

Experiment 5.—10 grm. phrenosine were treated as in Experiment 4, and the products were added to those previously obtained.

Experiment 6.—10 grm. phrenosine were treated as just related, and the products added to the previous ones.

The products of all the six experiments made on about 42 grm. pure phrenosine were all united after their complete identity had been recognised and proved.

The nitric acid solutions, filtered, though they hardly required to be filtered, were united and tested for ammonia or alkaloid, with phosphomolybdic acid, but gave no reaction for either ; evaporated to dryness, they left a white crystalline residue, which proved to be pure *mucic acid*, $C_6 H_{10} O_8$.

5. *Special Consideration of some Features of the Process of Chemolysis and of the Separation of its Products.*

I have endeavoured to arrest the process of chemolysis as soon as the appearances indicated that all phrenosine had disappeared ; the indications were cessation of the frothing, plasticity and contraction of the undissolved products, all at temperatures just below boiling point of water, and absence of red vapours. Under such conditions, the products remained almost uncoloured, and very little of body No. 4 was formed. On the contrary, prolonged boiling set up oxydation, evolved red vapours, and produced the orange-red, resinous body. The frothing is probably due to the liberation of nitrogen, probably by interaction with nitrous acid, the latter being thus perhaps consumed in the moment of its liberation, adds its nitrogen to that set free from the phrenosine, and both together cause the frothing. When the mixture, after this first reaction, is boiled, at least two different reactions ensue. One is the oxydation of the cerebrose, another,

that of the neurostearic acid and the phrenyline, The acid is not much altered, but the phrenyline is much affected ; and this oxydation has, for the purposes of the present research at least, to be avoided. If it should be desired to continue it, the nitric acid solution, after the first reaction, containing the cerebrose sugar, should be removed at all events. For the acid must be evaporated to obtain the product, mucic acid ; and it must not be evaporated while the other products are suspended in it, as they would be oxydised and decomposed in a manner, so as to furnish a great number of products, which would, at least in part, be mixed with the products of the oxydation of cerebrose, and require much research, and large quantities of material for their separation and study.

The separation of the products as described in Exp. 4, is preferable to the early use of alcohol. The baryta binds the neurostearic acid at once, and to the compound the red-coloured resinous body adheres, and leaves the other products nearly white. But some part of the product phrenyline also adheres to the baryum salt, and is not extracted by either ether or boiling alcohol. It appears to sight as a gelatinous, transparent mass, when the neurostearic acid is extracted by ether from the magma produced by the hydrochloric acid treatment. A part remains suspended in the acid liquid, and is not taken up by ether ; it is recovered by the filter ; another part goes with the neurostearic acid into the ether ; if now the ether be shaken with small portions of water it becomes turbid, and deposits more of the gelatinous matters in the acid washing water, while itself becomes clear. By repeating this treatment with water all gelatinous matter and turbidity is removed from the ether, and neurostearic acid free from phrenyline remains after distil-

lation of the solvent. If the purification at this stage be omitted the turbidity follows into the salts and the sodium salt, *e.g.*, is not to be obtained clear, except at the cost of much trouble and loss. We have here a process of hydration. When the gelatinous body is dried, and dissolved in and redeposited from alcohol, it shows itself as crystalline, white, and when dry, chalky phrenyline. The red-coloured resinous product is extracted by ether or benzol from the baryum salt ; it is not very soluble in ether, more soluble in benzol, insoluble in boiling alcohol. A little baryum salt, dissolved by the ether, is left insoluble by the benzol ; the concentrated hot benzol solution deposits the resin as a red gelatinous mass.

I have stated these special points at the risk of appearing tedious ; but in view of the difficulty and costliness of all brain research, of the procuring of the materials and peculiarities of the phenomena, future inquirers will find that by these minutiæ I have saved them much study and labour. I have also judged it useful not to avoid too anxiously the repetition of the description of some new features of substances or processes, when they had to be viewed from different standpoints.

6. *Neurostearic Acid.* $C_{18} H_{36} O_2$.

The *baryum salt* is a white precipitate, when dry pulverulent ; it becomes soft at higher temperature, and then encloses other matters mechanically. This is the reason for which it must be repeatedly dried and powdered during the extraction with boiling alcohol. It nevertheless retains some of the neutral matters which come to sight after decomposition by hydrochloric acid and during extraction of the free acid with ether, as a gelatinous matter suspended in the acid

water below the ether. The ether must be repeatedly shaken with the water to precipitate all this matter, which seems to become separated by hydration ; if not completely removed at this stage it follows the neurostearic acid into the sodium salt, and makes its solution turbid.

The ether on distillation leaves the fluid acid, which crystallises on cooling. This is recrystallised from ether until it is deposited in white minutely crystalline granular masses.

The baryum salt after exhaustion with boiling spirit and drying must be exhausted with ether and with benzol to remove the red resinous body No. 4. The sodium salt, if coloured, should be exhausted by the same solvent. The sodium salt is completely soluble in hot spirit, and deposited from it on cooling in felted crystalline masses. When dry below 100° it is hard and pulverisable, and remains so at much higher temperatures, at 105° it seems to retain several molecles of water of crystallisation. When the neurostearic acid formed by nitric acid chemolysis had thus been combined with baryta, and exhausted with boiling alcohol, and with ether ; had been set free by hydrochloric acid, dissolved in ether and separated from the gelatinous matter by treatment of the solution with water ; when it had been combined with soda, dissolved in, and crystallised from water and alcohol successively ; when it had at last been dissolved as sodium salt in water and been again precipitated by hydrochloric acid as a white curd, it coalesced on contraction to an opaque viscid mass, which did not become fluid at 95°, nor, on boiling, cleared up. It was isolated, washed, and heated in a platinum dish for many hours, until it was quite clear ; but its colour had again changed to a red-brown, showing that it did still contain some of the

resinous body, No. 4, of the list. It was powdered, dissolved in ammonia water, and its solution, filtered while hot, remained turbid. Precipitated with Ba Cl_2, the voluminous precipitate was dried and powdered; it *yielded* again *much red-coloured matter to benzol;* it was extracted with this, dried at 115°, and then contained 21·14 per cent. Ba (theory, 19·48 per cent.). This corresponds to palmitate, which requires 21·17 per cent. of Ba. But the fusing point of the free acid was widely different from that of palmitic acid.

7. *Phrenyline, the Nitrogenised Product from Phrenosine by Nitric Acid.*

It was deposited from its alcoholic solution on cooling in white masses, which consisted microscopically of curved needles, being actually outlines of scales, like those of the wings of butterflies, The crystals are probably an *alcoholate;* for when they yield nothing more to bibulous paper on pressure, the matter will easily fuse below 95°, and the fused mass spontaneously separates into two layers, an upper alcoholic saturated solution, and a lower oily one containing more of the fused body.

Dried in vacuo over oil of vitriol, until constant, it is a white, light, soft, easily powdered, chalky mass. This fuses at 115° to 130°, gradually, passing through soft and viscous stages of rapidly decreasing intensity, until above 130° it is quite fluid; it congeals to a transparent, very hard, resinous mass, which fissures in all directions, and when cold is easily powdered.

On combustion with copper oxyde it gave 2·0 per cent. of nitrogen.

It gives *the oleo-cholide reaction* in a peculiar manner. The purple produced by sugar syrup and oil of vitriol appears slowly, and remains attached to particles. It

requires a little water for its existence, as is evident from the following facts :—Breathing upon the incipient reaction, starts, or expedites it, on the thin layers of mixture ; contact with new oil of vitriol destroys the colour by dehydration. The hydrated colour is readily soluble in *moist* chloroform ; but when to this solution oil of vitriol is added, the purple matter is discoloured and goes out of solution, and adheres to the glass as a viscid resin. When chloroform and sulphuric acid are both removed by decantation and new moist chloroform is added the coloured matter dissolves in it with the original purple colour.

8. *Considerations Suggested by the Nitrogen Quantation.*

The amount of nitrogen is very nearly that of phrenosine (in which 1·997 per cent. was found), Yet if all the cerebrose and neurostearic acid were detached the residue would have to contain about the same amount of nitrogen as sphingosine, namely, 4·91 per cent. Therefore, the new body contained less than half the amount of nitrogen which it might have contained if none had been lost. The other half must have been lost *as gas* in the chemolysis.

The body is not phrenosine, and does not contain any. Its crystallisation in needles, its rapid deposition from hot alcohol, its softening and fusing between 115° and 138°, to an oil, which becomes a hard and cracking resinous substance after fusion, and can be powdered, are all features which phrenosine shows not. Phrenosine fuses only after being changed into a black caramel, insoluble in spirit, soluble in ether ; when phrenyline is fused phrenosine is quite unchanged. The bodies also behave differently with the oleo-cholide reaction ; phrenyline requires sugar, phrenosine not ; the purple

of the latter is soluble in anhydrous chloroform ; that of phrenyline not.

Phrenyline is apparently a neutral body, and has not yet been combined with either acid or alkali.

9. *Mucic Acid.* $C_6H_{10}O_8$.

The nitric acid solution obtained by the chemolysis of phrenosine when decanted and filtered is clear and colourless. It gives no precipitate with phosphomolybdic acid, and, therefore, does not contain any ammonia or any compound alkaloid. It is evaporated to dryness and leaves a white, solid mass of small crystals. These may be recrystallised from boiling water, of which large volumes are required ; a white crystalline powder is deposited, which has the microscopic shape, and the properties of *mucic acid.*

The mother liquor was a little coloured ; neutralised with ammonia, and treated with baryum nitrate solution, it deposited mucate of baryum. This was again decomposed with hydrochloric acid, and the free mucic acid was recrystallised and added to the main portion. In all about 3 grm. of pure mucic acid were obtained. The portion of which a sample was analysed weighed 2·8655 grm. Burnt with copper oxyde and oxygen gas at the end of the analysis, 0·386 grm. gave 34·17 per cent. C., and 4·72 per cent. of H. Theory of mucic acid requires 34·28 per cent. C., and 4·70 per cent. H. The product was therefore pure mucic acid, $C_6H_{10}O_8$.

The 42 grm. of phrenosine might have yielded above 10 grm. if all cerebrose had been transformed ; but the effervescence of the nitric acid showed that much matter was oxydised. More than half a gramme was removed during recrystallisation and in various mother liquors.

XV.—THE NEW ORGANOTHERAPY.

The art of healing and assuaging diseased, sick, or feeble organs by means of *educts* and *products derived from organs* or supplying their absence or deficiency of function may be called *organotherapy*. It might be termed *isotherapy*, inasmuch as the suffering organs as well as the healing ones, however distant from their situation may be their effect, are of the same kind ; the effect of the remedy is vicarious for natural action ; thyroid acts for thyroid, adrenal for adrenal, liver for liver, stomach for stomach, brain for brain ; the vicarious body is dead, but functions for the living ; it becomes like history, a kind of temporary resurrection.

The *application* of the principle is true in all cases in which the diagnosis admits of the correct selection of the therapeutic agent. When the agent is pure, as in the case of pepsin, the effect is infallible, and commensurate with quantity.

The *selection* is the easier, the simpler is the composition of the organ to be treated or used ; it becomes the more difficult, the more complicated is the composition of the organ.

The nearest *explanation* of the action of these *educts* and *organs* is, that they supply a *material*, which owing to the diseased state of the organ to be supplemented, is either *diminished* or *absent*, either in or from the organ itself, or from the main body and its general chemistry.

Thus *thyroid* substance or extract supplies a substance which is required by the entire body, the brain and nerves, the skin, connective tissue, and other organs in descending order.

Thyroidin, as we will term the active chemical

agent, is now shown to be *a chemical substance*, and not to have anything vital about it. The thyroid body can be boiled, and has the same effect as the unboiled, or raw. The circumstance that it endured drying below 40° C, and remained active proves the same fact, but was not acknowledged to prove it. For as the presence of a ferment akin to ferments destructible by heat or boiling water was assumed, heat was avoided, The precaution is shown to be unnecessary.

There is *a functional disease of the liver* marked by the absence or diminution of *the peculiar phosphorised constituent of the bile.* When this is supplied, either as extracted from the liver or bile, or from other suitable sources, and introduced into the digestive process, the functional disorder ceases after a time, *i.e.*, as soon as the disordered absorbing villi of the tract have recovered their active condition.

The same is the case as regards *the specific biliary acids* and their derivates.

The same holds good as regards *the blood.* If the *iron salts*, which are so eminently beneficial in *anæmia* and *spanæmia*, diminution of blood corpuscles, and excess of white cells with excess of circulating albumen, be it relative or absolute, are assisted by *absorbable organo-metallic agents*, the effect is much quicker, and if to these are added other specific ingredients of the blood corpuscles the cure is made still more rapid and certain.

In *malarial conditions* this effect is particularly marked. Of such specific ingredients *the phosphorised bodies, lecithin* and *amidomyelin*, are the most important, and should always be given in combination or concurrently with iron, quinine, and organo-metallic salts.

In diseases of the *brain, spinal cord*, and *nerves*

many kinds of extracts from brain, and even brain substance itself, have lately been used. But the preparations employed had little or no power. Thus extracts made with glycerol and alcohol, and maceration extending over months, contained only traces, *i.e.*, in 2 oz., less than a few grains of specific brain educts. This was to have been used subcutaneously. But its only effect when so applied was that of the alcohol and glycerol. It could not act specifically in any way, as of all the specific brain-educts it contained only one, and that in very minute amount.

A small amount of ingredient is in itself no argument against the existence of power, as is evident from the effect of *tuberculine*. In a complex phenomenon action depends upon that necessary ingredient which is present in the smallest quantity. But mere dilution of substances required in larger quantity prevents their action or makes it dilatory, incomplete and uncertain. Even *brain* substance is too dilute ; if it is to supply deficiency, its ingredients must be differentiated, and above all must be *concentrated*, to develop all their possible action. For it is clear that of the whole amount introduced only a fraction reaches the brain, unless it has a selective power of absorption. If this be small or absent, larger amounts of organotherapeutic educts will be required to effect a cure by supplementation or by specific influence.

Therefore, concentrated brain-extracts, containing *the specific brain educts* may be given in cases at present obscure, to prove that there is a curative effect at all. When such is observed it is necessary to differentiate and find *the individual educt or educts which act specifically ;* after that therapy is plain sailing. But if the conglomerate or total extract have not a decided effect, it does not follow that all educts

are inert ; there are *antagonisms*, modified by quantity of the antagonists ; and when one is eliminated, and the other used in appropriate dose, a curative effect is certain.

The application of *orchideal substance or extract* has an undoubted remarkable effect upon the body ; it owes this mainly to ingredients akin to brain-educts. It would be specific, if the organ or extract did not contain antagonistic and at present insoluble substances. Like the educt of the tubercle bacilli it contains a healing, rejuvenescing, and assuaging ingredient, by the side of one which has opposed, or indifferent, not useful even sometimes injurious properties. To isolate the useful and eliminate the inert and impeding material is the object of *organotherapeutic pharmacy*.

Although tuberculin is a *vegetable product*, its application and effects are illustrative of organotherapeutic practice. *Rabies* furnishes a more direct illustration, although it is yet surrounded with much mystery ; the preventive or curative agent is a mere water-extract of an organ, the *spinal marrow*, a mere dead chemical matter modified by gradual decay.

Thyroid extract has undoubted *curative power*, but also undoubted *pathogenetic effects ;* these do not belong to the *curative* body. One substance cannot have two opposed effects. The effect of the pathogenetic substance, producing *thyroidism*, is one of *accumulation*.

Another power is *adrenal extract*, or *renculin*. It has the most important influence in sanguification, anæmia, spanæmia, and leucocythæmia, all malarial blood-changes, and a number of deteriorations of the blood arising from *specific diseases* (*symbioses*, because become *constitutional*), tuberculous, scrofulous, enthetic, leprous, and cancerous conditions. As aiding

the power of sanguification, renculin acts as an adjuvant alterative, raises the power of the blood to destroy *specific poisons* resulting from the chemical action of the *symbiotic parasites.*

Next in order may be placed the educt from the liver, *hepatin*, which supplies a similar hæmatopoetic material, but influences the action of the liver itself less ; the result of this action, if deficient, as above shown, of phosphorised matter is best supplemented by the phosphorised matters, lecithin and amidomyelin.

An *organotherapeutic educt from kidneys*, *nephrin*, is of great use in chronic albuminuria, with shrinking kidneys.

One of the most interesting organotherapeutic extracts is obtained *from the ovary*, not so much from the *stroma* as from the *corpora lutea ;* in conjunction with cerebral extracts it seems to attain its maximum healing effect in hysteria.

Pancreas and spleen give rise to similar considerations, and are now undergoing many experimental trials. The effects of *muscle-extract* requires no description ; the raw (uncooked) extracts have a stronger effect, as they contain soluble *myochrome*, the colouring constituent of red muscles ; but they are not easily consumed. The diseases of muscles are rare, as compared to those of other organs, but nevertheless specifics for their suppression are very acceptable.

The *heart* is treated most successfully with a specific remedy from the vegetable world, namely *digitalin*, it has a selective power for this. The caution against accumulative effects is necessary not from any collateral substances, but from mere retention of *digitalin* in the body, the kidneys discharging it only very slowly.

Organotherapeutic Pharmacy has overcome already many difficulties, but will soon be successful in present-

ing a number of excellent and active educts for practical application to the profession.

Thyroid substance or *extract* seems to act so efficiently that no great changes except purification and concentration are called for.

Brain educts admit of much development, and several are ready for use. Thus *lecithin* as such, and as *hydrochlorate*, also *amidomyelin*, can be obtained from manufacturers. These educts are obtainable in somewhat soluble, and also in insoluble, forms. Another series of educts is ready in the form of *ethereal extract* (*Extractum cerebri æthereum*) in tablets of five or more grains each. Also a body of great peculiarity and power, having the properties of an *albumen and a fat combined* in its constitution. *Cerebronuclein* is an agent of great promise ; it is prepared from *ganglionic substance* after all soluble educts have been removed, and is particularly indicated in diseases of the *cortical or grey substance* of the *brain* and *spinal marrow*.

The *non-phosphorised* substances of the brain, the *galactosides* or *cerebrosides* yield by decomposition under pharmacopoetic precautions an *active alkaloid*, which has this advantage, that some of its salts *are soluble in wate*r and fit for subcutaneous application. The most suitable of these is *sphingosine nitrate*, a white crystalline compound of remarkable properties. It seems to have a great future in nervous diseases such as hysteria, cataleptic and epileptic conditions, chorea and convulsions, not in all indeed, and sometimes not unaided by adjuvants and corrigents, but an essential effect in a systematic arrangement ; symbiotic marasm, agitant paralysis and neurasthenia have been greatly relieved by it.

The *sulphate of this alkaloid* is least soluble, but

very useful when given *per os ;* it is then assimilated and develops its effect later and in a somewhat lesser degree than the subcutaneously given nitrate, but unequivocally, particularly in cerebral diabetes.

Organotherapeutic pharmacy should now produce and have ready the following vicarious nervines :—

Lecithin and amidomyelin preparations.

The albumo-lipoid from brain.

Cerebro-nuclein from ganglionic cells.

Cerebrosides (cerebro-galactosides),

Cerebrinacides.

Sphingosine and *salts*, particularly *nitrate.*

Ethereal extract of brain (dry residue of).

And others, which may be asked for, or obtain prominence in manufacture.

The pharmacy should endeavour to obtain as many educts, such as can be sold *in a state of purity*, as in that condition alone their action can be defined and employed with certainty.

Next to the thyroid and the brain, the *adrenals* have the greatest promise of power, and should be closely studied after or by the side of these organs.

Amongst the products from the albuminous substances, some have physiological effects. *Chitine* yields an *amine*, the crystallised hydrochloride of which has eminent powers.

The new *serum-therapy* appears *as a particular branch of organotherapy.* Serum is an organ, it is the pulp or substratum of a wandering tissue, the blood. According to the alleged *rationale* of its application in the case of therapy it acts as a mere carrier, may be preserver of the active antidotes, with which it is by art impregnated.

In connection with this part of organotherapy several new and important facts and problems rose at once

before the eye of the investigator. In his endeavours to concentrate the active principle in antidotal serum he found that there are two varieties of serum in commerce differing greatly in their chemical properties, one yielding all its albumin to metallic zinc, with which, like hæmochrome, the red colouring matter of blood-corpuscles, it forms a compound in definite proportion, the other refusing absolutely to react with zinc in foil or powder. We abstain at present from any discussion of these remarkable facts, excepting only the observation that the reaction with metallic zinc proved serum-albumin to be an active oxydiser and transferor of oxygen to suitable transferees ; that in this respect it is hardly inferior to hæmochrome, and that in this region of science, as in many other chapters of physiological chemistry, the doctrines of physiologists, who hold that serum albumin is not a carrier of oxygen, and is not an oxydiser, has to be entirely corrected and reformed.

The fact inculcates an important caution, inasmuch as it explains to our mind many of the contradictions, which serum therapy has brought in its train ; it also should open our eyes to some of the dangers of empiricism which is not controlled by scientific supervision and investigation.

We therefore insist on the observation in organotherapy of the utmost definiteness and purity of the preparations to be employed. All factors must be observed and controlled, even such apparently slight matters as the sterilising or exhibiting bulk-making agents. In a case of anti-diphtheritic serum now before us, the reactive faculty of the serum had been destroyed by the steriliser which was supposed to act against bacilli only, but annihilated the chemical energy of the material at the same time, and most effectually.

The new therapy will be greatly aided by *organo-metallic compounds*, in which metals are contained in the form in which iron is contained in hemochrom. The very products of investigation of healing serums have furnished four such compounds, with zinc and copper. Of these, the zinc compounds promise to effect in the chemical forms of cortical epilepsy all that the Paracelsists had hoped. But the cerebral phosphatides are, under conditions concerning solubility, yet better carriers of metals in therapeutic forms, and we know, from relatively recent experience, that they have also powers of selection, which therapy has to take into most serious consideration.

We have, in the articles on the "Progress of Chemistry in its Application to the Practice of Medicine," &c., which have appeared in this journal, endeavoured to give proofs of the manner in which this progress ought to and can alone be effected. Many of the fundamental facts and arguments are highly technical, and appear abstruse to readers who desire quick returns for their outlay on current literature. In their interest we have stated our principles of organotherapy somewhat earlier than the course of the argument of our articles would seem to make opportune. All these readers are now in a position to co-operate in the application of the new data, and we hope may continue to take an increased interest in the building up of the scientific basis, upon which alone the ideal medical art, the healing of disease by direct antidotes, ever will be founded.

Cod-liver oil, by itself a splendid example of organotherapy as affecting symptomatic alleviation, acts so nearly specifically in many cases that the limits of its action appear to us to be the consequence of mere dilution of its active ingredients by such as are less active

or inactive. Guided by the chemical reaction of the oil we constructed a more concentrated substitute, and found that a few grains of lecithin hydrochlorate with an ounce of good cow's milk cream has an effect nearly equalling four ounces of the fish-oil. In this direction further progress is certain, as there is a difference between cod oils of the most reliable purity of manufacture, which are as striking as those of the horse-blood serums above alluded to. Organotherapeutic pharmacy can, therefore, effect progress in two directions in one by keeping away from use, or correcting, deficient oils, in another by supplying concentrated forms or strong substitutes of its action from new sources. The oil itself requires a better chemical study than it has hitherto received, even on the part of specialists.

Some cerebric extracts have been used successfully by distinguished gynæcologists in affections of the ovaries. The new soluble principles will, of course, be more active and effectual, the *ovarioluteine* in particular, aided by its proper phosphatides, will allow an almost specific medication, which in hysteria, *e.g.*, will be still more direct when hypodermic applications of the remedies, already successfully began, shall have acquired general applicability.

XVI. — A SHADY SIDE OF BIOLOGICAL LITERATURE.

The Ghosts of Spurious Researches.

The celebrated mathematician Babbage, in a work on the Royal Society published about the fourth decade of the present century, in the division relating to original scientific inquiries, discussed more particularly *spurious researches*, and divided them in four

categories: forgeries, hoaxes, cooked and trimmed papers. Of these he gave examples, and described the manner in which some of these counterfeits had been discovered. He did not, however, describe a very large class of injurious imitations, which are not manufactured and sent forth consciously, as the four categories necessarily are, but are conceived in something like good faith, yet carried out negligently without proper regard to literature, history, or method, advanced with conceit, and hailed as achievements by unintellectual sympathisers, I mean *the blunderings*. Of all the five varieties, the literature of medical chemistry, particularly the branch which is commonly termed physiological chemistry, contains a great number of very striking examples. *The forgeries*, that is to say statements for which there is no warranty in fact or experiment, while they are known to the authors to be false, are at present neither systematic nor numerous, because they are too easily discovered, but they are practised as collateral supports to slight inquiries, and particularly a good many of the formulæ attributed to bodies with new names, such as "urobilin," are mere equivocal inventions, destitute of any, even the slightest, analytical basis, and put forth to garnish hypotheses exploited as facts, and thus giving to false facts an appearance of genuineness, which, under the circumstances, is fraudulent. The hoaxes appear under varying aspects, according to their intention, but as they could not benefit their authors are rarely malevolent, but on the contrary, by their frequently satirical tendency and humorous manner, act as correctives to undoubted evils, and as they are discoverable by scrutinising minds, avoid the turmoil of literary dispute, but stand smiling on their eminence, and have not as yet, as far as I am aware, caused any mischief to any of those who

were confiding in their reality. *The cooked researches* are always compounds in which known data are skilfully blended with more or less new matter in such a manner as to make the whole appear as new. This class includes a good many plagiarisms which have to be disguised, mainly by giving new names to well-ascertained matters, and a good many more repetitions of established data, which it is pretended to have made more precise or more useful. It also includes that kind of appropriation which consists in an author seizing another man's data, the result of much thought, work, and expenditure, and altering without research or experiment, the cardinal data, squeezing them into an hypothetical frame of his own, and representing them as the efflux of his own thought and work. *The trimmed researches* are mostly new data of an inferior kind to which an appearance of greater precision is given by clipping or adding, particularly to analytical figures, to make them fall in with preconceived hypotheses ; in this process formulæ even, are employed, which express corrections to be applied, the exponents of which are regulated by probabilities, or by mere presumptions, and which lend themselves, therefore, to great abuse. Some of this trimming tendency has been engendered by unreasonable demands for impossible precision put forth by judges of papers in connection with some learned societies, which judges were quite inexperienced in this kind of research. Inversely this trimming so engendered has produced the curious result, that researches which are absolutely genuine and yield mathematically correct results, are suspected of being produced with the aid of such trimming, and are, therefore, liable to be rejected. I knew a chemist, who having obtained by most careful preparation and analysis absolutely theoretical figures of elements of

lactate of zinc from the human brain, was proceeding to alter his figures in the second decimal, as he was apprehensive that the accurate data actually found by the balance would be disbelieved and rejected as trimmed. But the most dangerous class of spurious researches in the present day are a particular kind of blunderings, which have for their authors either students or specialistic professors, or a combination of the two, are elaborated in specialistic laboratories or so-called institutes, published in specialistic periodicals, and then abstracted by professional penny-a-liners for the advantage of so-called central journals, or annual reports on progress, or appendices to the journals of societies, the authorities of which consider the production of this kind of literature a highly meritorious occupation. Such abstracts then become the sources from which inquirers or authors of systematic works take their inspiration and their matter, and the result is that the fatal facts, the incorporate blunderings are further embodied with the system of teaching, corrupt science, pervert the honest student, impede the inquirers in a twofold manner either by suggesting to him a false basis whence to start, as did Frémy's cerebric acid to many inquirers, or by producing mistrust in his results which necessarily differ from the false facts of the handbooks and abstracts. This blundering in the beginning is generally not tainted by malevolence but not rarely becomes dishonest when its consequence, have to be warded off by whomsoever they concern. If any man correct the blunders, even from the sheer necessity of preserving his own reputation, the corrected blunderer generally becomes an enemy of the corrector, and all relative printed matter that issues from the blunderer afterwards is tainted by a revengeful animus.

In the face of these evils the medical profession, and a good many members of the chemical profession also, are perfectly helpless ; they have neither the materials nor the literary means at their command to test the credibility of alleged facts and theories laid before them, which are to pilot their views or influence their practical proceedings. Finding the evil effects of these false facts and false theories, or their perfect uselessness, they naturally lose confidence in so-called physiological and pathological chemistry (when the product should be called spurious or blundering chemistry) and naturally cease to give to it any consideration. And yet it is this true science alone which can place into their hands the antidotes to disease-poisons in such forms, that they must not only produce mysterious immunities or vicarious pathogenies, but directly counteract the disease, remove the pathic phenomena, and that not only in a selection of cases, but in all cases, in accordance with the ancient proverb : Once a remedy always a remedy.

Errors committed in the prosecution of research with the best intentions are pardonable faults, and will always be so, as long as they are not defended but withdrawn by their authors as soon as their erroneous nature is recognised. Then, if not withdrawn by the authors, they have to be cleared out of the way by those who would like, or are by their position obliged to make progress in the study of the subjects in question. The inquirer is thus compelled to become a critic, in the first place, of course, for his own information and security. But he will soon be compelled to make his critical results public, for if he publish his researches without having previously cleared the opposition out of the field, he will soon find himself the object of attack by anonymous critics, who feel

themselves eclipsed or neglected, and his results surreptitiously denounced as fallacious, or, as we have heard it termed, he will find the validity of his new data doubted and his acquisitions ignored. This is not by any means a personal matter affecting the weal or woe of an individual, but it concerns the progress of the entire body of medical chemistry, as I will now show on the great subject of the chemistry of the brain.

The research of Vauquelin in the year 1811 was the beginning of our modern methods, the principles soluble in hot alcohol were for the first time extracted and termed "peculiar white matter," but were not examined or separated any further. Their isolation and distinction was first attempted by Couerbe in 1834. He found stearoconote (being according to my researches anhydrides of phosphatides produced by strong alcohol, therefore a product), cephalote, the soluble in ether phosphatides of the kephalin group; further éléencéphole, the nitrogenised fats diffusible in water; and lastly cérébrote, being the bulk of the cerebrosides, &c.. insoluble in ether and soluble in boiling spirit. These substances were no doubt very impure by admixture of analogous matters, but they represented fundamental distinctions, and thereby engendered a progress. Couerbe was the first to apply organic analysis to brain-products, and thereby established the composition of the only brain-ingredient, which he obtained pure and recognised as a normal constituent, namely, cholesterine. Couerbe's finding of sulphur in all his four preparations in quantities varying between 1·959 per cent. and 2·138 per cent. was an error, which Frémy laid hold of and tried to explain by another error of his own, namely, that the sulphur was derived from admixed albuminous sub-

stances. If explanation there be, the sulphuric acid came, in my opinion, from impure nitric acid, which he employed to oxydise his substances. Frémy's cerebric and oleophosphoric acid (1841) were the result of mere blundering as great as that manifested in the work on fermentation which he launched against Pasteur ; but, unfortunately, on the earlier matter there was no weighty authority to correct him. By wiping out Couerbe's distinctions and substituting his own erroneous selections he retarded brain research by at least thirty years. Von Babo's laborious efforts, and those of others I have quoted, only excite our regretful sympathy, for that trusting in Frémy they entirely lost their labours and failed in their object.

The researches of Gobley (1846 to 1851), first made on eggs, later on brain and fish roe, yielded the idea and name of *lecithin* and the discovery of *glycerophosphoric acid* as a product obtained by the decomposition of some of the phosphorised substances. The advance made by these researches was again nearly arrested and made retrograde by Frémy's misinterpretation of them, which Gobley justly refuted and resented.

Müller's research (1857) was excellent as long as it adhered to the comparison of watery brain extractives, with muscular extractives. When he came to specific brain substances he completely failed to find any direct educt of any kind, but extracted a matter which no inquirer since his time has been able to find again, and which I must believe to have been an accidental product of his baryta process. He threw doubt upon glycerophosphoric acid, which Gobley had so well proved to be a product of the chemolysis of phosphorised substances. He had evidently not studied any of the French researches, and neglected even

Vauquelin's white matter, which has been the basis of all progress made since his time. It was, however, a curious result of this sporadic find, that it started the surmise of the possible presence in the brain of peculiar nitrogenised, non-phosphorised principles. However, as a matter of fact, the finding of this product did not lead him to the discovery of a single educt; and as regards the great mass of educts, phosphatides, as well as cerebrosides, cerebrinacides, and cerebrolipotides, the research of Müller was a blank. Liebreich (1864) renamed Couerbe's cerebrote, but omitted the sulphur; by omitting the application of Chevreul's law, as well as all other tests for the unity of the preparation to which he gave the pretentious name of protagon, he initiated another period of twenty years of error. All these futile efforts I comprise under the category of blunders, for they were caused solely by the neglect of well-known principles, and those principles or rules so evident that they might be called axioms.

Gobley had found a phosphorised body, believed to be lecithin, in ox bile, and Strecker had obtained an alkaloid, called cholin, from it by chemolysis. This cholin was now considered, and by accident correctly, to be a constituent of lecithin, and with its aid, and that of glycerophosphoric acid, and the two fatty acids found by Gobley, namely, margaric and oleic, a formula for lecithin from eggs was constructed by Strecker, which was in the main correct. But the phosphatide in ox bile was not lecithin, as I proved at a later period. Now Liebreich having propounded cérébrote as protagon, and decomposed the phosphorised body contained therein, and probably also some cerebrosides, discovering cholin, called it neurin, and obtained by his baryta process a mixture of fatty acids, from which he extracted what he termed impure stearic acid. A

mixture of more than a dozen substances was thus mistaken for unitary, and multiplied the confusion of protagon, but in the representations of writers and teachers this fallacy superseded all other views and facts.

Now came the counterblast to protagon by a Russian student of the name of Diakonow, who having isolated from brain a kind of lecithin, analogous apparently to that described by Gobley and Strecker, as obtained from egg-yolk (said, however, to contain two molecles of stearic acid, a form which has not since been observed again), surmised what had also been suggested by Strecker that protagon was a mixture or compound of lecithin and (Müller's) cerebrine. At once all authors and teachers abandoned protagon and taught the new fallacies. No one as much as guessed, much less tested, the complicated nature of the cerebral white matter, not even Strecker, who had observed without explaining the varying proportions between nitrogen and phosphorus in the phosphorised matters of eggs.

Meanwhile, I published the first series of my researches, in which I proved the multiplicity of the phosphorised and nitrogenised substances, and described many new ones with sufficient accuracy so that anyone could reproduce and study them. These researches became the object of most absurd attacks on the part of Dr. Gamgee, then Professor of Physiology at Owens College, Manchester, preparatory to his plunging in association with a Mr. Blankenhorn into a defence of the protagon doctrine, of which he revived the variety with the lowest percentage of phosphorus. In this effort the blundering so characteristic of modern physiology reached a climax. Even the testimony of Sir Henry Roscoe to the effect that protagon was an

immediate principle could not save it from its unavoidable fall. For Gamgee himself, by using my method for the preparation of phrenosine, extracted this principle from protagon, and to hide its identity called it pseudocerebrine. This phase, the earlier part of which was discussed before the Royal Society, was terminated by me by an experimental critical essay, in which twenty-one fractions of protagon so-called, gave twenty-one different percentages of phosphorus. This proved the fundamental blundering in this particular treatment of protagon to have consisted, as already related above, in the complete neglect of the law first enunciated by Chevreul, namely, that a substance, in order to satisfy the claim for its unitary condition, must, on fractionation, have the same composition in all its fractions. But protagon split up into different fractions, all of which had different compositions. In addition to the neglect of this law, all authors except Frémy, and the later ones notwithstanding Frémy, had overlooked the mineral constituents, being for them impurities in their products.

Protagon, however made, contains no lecithin, in the true sense, and none of Diakonow's lecithin so-called. Further, protagon contains none of Müller's cerebrine, and none has as yet been obtained by decomposition. But all the handbook makers copied the blunders, and negligently inflicted them upon their readers.

Now came Geoghegan's blundering about cerebrine, which has not left a single available fact, although Hoppe Seyler, in whose laboratory Liebreich's and Diakonow's and Geoghegan's operations had been carried out, called the latter's product the "true pure cerebrine." This was five years after the discovery and complete characterisation of phrenosine. This essay contained contradictions and analytical and arithmetical blunders so

broad that even the formula was painfully miscalculated, and had to be corrected before its intrinsic vanity could be proved.

The error of protagon received a new edition at the hands of Professor Baumstark, of Greifswald. He heeded not my criticism but remained confined in the circle of fallacy, and increased it by the surmise which was not supported by the slightest experimental proof, and is in fact totally unfounded, that the mother liquors of protagon contained only decomposed matters. As in the description of all his predecessors so by him protagon was portrayed in contradictory terms, being now insoluble in ether, and on another page "recrystallised" from ether. In this case two different protagons ought to have been defined, but were not distinguished. Gamgee, however, after he had produced my phrenosine from his protagon, proposed two varieties of protagon, the pure and impure; the impure was to have been the one which yielded phrenosine, misnamed pseudocerebrine. As there is no protagon so-called free from phrenosine, all protagon, is, therefore, necessarily impure. Lately, Kossel and Freytag revived this quality and increased it to an undefined multiplicity, but without a tittle of proof, by a mere frivolous improvisation, to cover their retreat from an untenable proposition, and save them the necessity of contradicting a professor of their own faculty and university. Protagon is a dead fallacy, and no person of reason or sense can honestly maintain any longer the doctrine concerning it. That it could have been twice revived as described, shows all the deficiencies of study, method, and assiduity in practical work which I have above proved to characterise modern laborants. And the less they know and do, the greater is the conceit with which they put forth their fallacies, and the incompetence with

which they treat, if they know them at all, the researches recorded in literature.

Such modern essays are sometimes published under the name of a pupil or of a laborant of the more advanced kind, but are really portions of the professor's own stock of information, a fact sometimes made evident by the professor's name appearing in conjunction with that of the pupil. To this proceeding none would object if it were not that the papers signed by students only are abused for putting forth the professor's criticisms, which he does not dare or does not like to put forth in his own name. The latest and most puerile of such performances was put in scene recently by the lately deceased Professor Külz, of the physiological Institute of Marburg, and was published in the "Archiv für Biology," edited by Professors Voit and Kühne. The laborant working on a brain from the deadhouse, came to the absurd result that none of the constituents of the brain did contain any *sulphur*. He found no true fact of any kind, made no progress whatever, but when he had, as he thought, arrived at this negative, which was evidently the sole object of the proceeding (by an unquestionable process of blundering in common analysis), he straightway sent it to be recorded in an "Archive," the editors of which strangely enough accepted and printed this trash.

In my "Historical Account of Researches on the Chemistry of the Brain," which had been published previous to 1874 by twenty-two authors, I had proceeded so objectively that no one has, and, I believe, no one could, justly take exception to my digests and judgment. Indeed, I had rather restored to credit researches previously neglected or condemned, for example, the acceptable parts of those of Couerbe, Gobley, and Köhler, distinguishing those parts which

my researches did not enable me to confirm or accept, but explaining, as far as I could, in every case, the causes which probably had led the authors to erroneous conclusions. My work on "The Chemical Constitution of the Brain," was kept entirely free from all and any critical observations. But in return for that studied moderation I received only anonymous and acknowledged misrepresentations, which I had to correct and silence by a number of critical publications. Thus, in a special essay I have demonstrated the abuse which different authors made of the word cerebrine. The latest abuse is by Kossel and Freytag, who employ it to hide the discovery of phrenosine, for the same purpose for which we have seen above that in a pseudoresearch the term pseudocerebrine was used.

Some essays which followed after the publication of the protagon hypothesis, but made no reference to it, were indecisive, but not misleadingly erroneous ; parts were even progressive, such as Köhler's discovery of the solubility of some of the phosphatides in water, and of the lead-salt of myeloidin. But not being a chemist, and limited in his analyses to the aid of a friend, he was unable to pursue his research, otherwise so original, to a clear issue.

XVII.—THE QUANTATION OF UREA WITH HYPOCHLORITE OR HYPOBROMITE.

The late Dr. E. W. Davy, brother of Sir Humphry, proposed in 1854 as an alternative to the then novel and frequently employed process of Liebig for the quantation of urea a mode of evolving the nitrogen of the urea of urine by hypochlorite as gas, and ascertaining its weight from its volume. I made many experiments

with this process soon after its publication, and compared it with that of Liebig in different relations ; I even constructed an apparatus without any corks, caoutchouc tubes, joints, or indeed any possibility for leakage, an apparatus which is absolutely secure, and, therefore, eclipses all the arrangements which have been invented during the last 25 years for the application of the rival reagent hypobromite. The results of these trials and comparisons proved to me that the process was much too uncertain for scientific work, and liable to indicate lesser amounts of urea than were actually present. As a means of estimating the total quantity of nitrogen in urine the method was still more uncertain, and having fully convinced myself of its unsuitability for practical research, I abstained from giving a lengthened exposition of it in my Treatise on the Urine, and from any criticism which the statement of my experiments would have involved, but merely recording the principle, referred the reader to the original publication. It was the Leipzig chemist, Knop, who later on recommended to substitute hypobromite for hypochlorite on the ground that the reaction was more rapid, and completed in a shorter time. Nevertheless, the method found little application until Hüfner, professor in Tübingen, constructed a special apparatus to be used in the process. By various modifications, the latter, and contemporaneously with him Schleich, improved their manipulation between 1871 and 1878, so that the error of the method, which always consisted in a deficit, became diminished from 7 to 2 per cent. of the required volume of gas ; but to perform such an analysis required "a longish time and anxious care" even in the hands of Schleich. In 1877 Maxwell Simpson and O'Keefe described a new apparatus, of which the connecting materials were

corks and caoutchouc tubes. These possible sources of error were eliminated by Falck, of Kiel, who in 1881 constructed an apparatus made of glass exclusively, but with three stopcocks, and three ground glass joints, and set to work. Though very complicated and fragile, it had at least this security over previous contrivances, that no nitrogen could be lost, even in case, as sometimes happened, some of the urine to be analysed penetrated with the nitrogen into the eudiometer, for this vessel also was filled with the brominated soda liquor. Falck employed mercury to secure the bottom of his reaction bottle, just as I had done with the process employing hypochlorite. And this gives the opportunity to explain to the readers, that most difficulties of apparatus which caused so many inventions were produced by the operators endeavouring to avoid the use of mercury at any cost, as Davy had originally proposed, to save expense. But the cost in accuracy was so enormous that the method found no application for the production of scientific constants, and has not been so applied to this day. This want of accuracy was so well understood that most operators supplied formulæ for correction of the results, and relegated the process to application for what they termed "ordinary purposes," or "clinical application," because, as was implied, or stated, a process with a deficiency of many per cents. was good enough for the doctors.

As notwithstanding the numerous well-founded objections to the use of this process which have accumulated during forty years, it has lately been again put forward, particularly as being good enough for the practical purposes of physicians, and as this proposal though immediately opposed, is liable to mislead the profession, I have thought it useful to pass in review

both the alleged virtues of, and objections to the process in as short a manner as is consistent with intelligibility.

Davy truly said that his method was convenient and simple, and could be carried out by many, as the reagent, *liquor sodæ chlorinatæ*, was present in almost every well stocked chemist's shop. Instead of mercury he used salt water to fill the eudiometer, and here the first source of inaccuracy arose. But this is avoided at once by using mercury. When hypobromite was substituted, two hindrances arose, firstly, the solution had to be specially procured, and when procured, did not keep; it had, therefore, to be freshly manufactured almost daily by mixing liquid bromine with caustic soda ley. Into the battle of the apparatuses we must not enter, for as was the case with the replies of the gymnosophists to Alexander, each is better than the other. One part of urea contains 0·466 parts of nitrogen; if, therefore, all the nitrogen could be evolved as gas by any reagent, 0·1 gramme of urea, to specialise a quantity suitable for practical work, would give 37·14 cubic centimetres of nitrogen, measured at the temperature of 0° C., and a pressure of air equal to 760 millimetres. This result is practically never obtained, because the varying conditions all introduce modifications of the process, which influence the result in the sense of diminution of the nitrogen liberated. These conditions are the following, mainly:

1. *Varying Concentration of the Hypobromite.*—Knop's solution contained 15·6 cc. bromine in the litre, and gave only 92·3 per cent. of the nitrogen due; when the bromine was raised to 45 cc. per litre, the deficit sank to 2 per cent.; no further improvement is attainable by making the solution stronger. Falck used a solution of 60 cc. Br. in the litre, to no advantage.

2. *Varying Concentration of the Solution of Urea.*—The foregoing data resulted from a solution of urea of 2 per cent. strength, or about the strength of normal average healthy urine of adult males. When a solution of urea of 1 per cent. strength is used, the deficiency is again diminished, and when it is of ½ per cent. strength it is further diminished; up to a certain point the diminution of the deficit ensues in the inverse proportion to the dilution.

3. *Varying Concentration of the Mixture of Urea Solution and Hypobromite.*—In this relation dilution with water increases the evolution of gas by 0·48 per cent. for every 25 cc. water added.

Of course, to every volume of gas the corrections for temperature and air pressure have to be applied, but the results of the corrections are not important, and of less amount than the inherent errors of the process. Falck has compared the results of eighteen series of data obtained by twelve observers, and shows that with all precautions and great care urea may be practically quantated, when it is in a pure dilute solution; the error is about 1 per cent.

4. *Variation in the Time given for the Evolution of the N gas.*—Some read off the volume of N after the reaction had lasted five minutes, and applied a corrective for the deficiency which was about 6·5 per cent.; the same conditions with waiting for all evolution of gas, which lasted for hours, reduced the deficiency to 1·2 per cent.

5. *Variations in the Contents of the Eudiometer.*—Mercury gives the best, salt brine the worst, hypobromite medium results.

6. *The Apparatus* and *personal Care of the Observers* come in for much, for in some forms of apparatus the urea solution can in part escape decomposition by

rising through the hypobromite, and being forced into the eudiometer undecomposed ; this is the reason for filling the eudiometer with hypobromite.

If the urine were, and were always, a chemically pure solution of urea of not more than 2 per cent. and not less than 0·5 per cent. strength, the hypobromite would probably give sufficiently accurate results. But urine is a mixture of many ingredients which modify the results of the reaction in many ways, and of these influences only a few are known at all, and fewer still are recognisable during an analysis.

When hypobromite acts upon urine nitrogen is evolved, which Falck has calculated to be properly composed as follows :—

	Per cent.
Of the nitrogen of urea are assumed to be evolved...	99·90
Of ammonium salts	99·70
Of kreatin (if such be there, which is questionable)	67·40
Of uric acid	47·78
Of kreatinin	37·43

These per cents. are parts in 100 contained in each substance, but the actual amount of nitrogen evolved by each substance in the course of actual analysis is quite unascertainable.

In urine, therefore, the hypobromite process gives an excess of nitrogen, if only the amount of urea be wanted ; and it leaves a deficiency if the total nitrogen of the urine be wanted. It is, therefore, unsuitable for the solution of either problem. Under the best conditions hypobromite yields from 88·94 to 92·3 per cent. of the total nitrogen. The urine of strong healthy men leaves an even greater deficit than 12 per cent. ; this deficit is mainly produced by the resistance of the

alkaloids to decomposition ; they are partly brominated, and retain a large portion of their nitrogen. Liebig's method yields, with all precautions, nearly 99 per cent. of the total of nitrogen in urine.

If the urine be treated with mercurammonium, and the alkaloids removed by charcoal, the remaining liquid is, as regards nitrogen, a solution of pure urea.

Liebig's method, therefore, gives us *the total nitrogen* is one operation, the *ureal nitrogen* is another, and if we apply it after mercurammonium and before charcoal, it gives us *the ureal and alkaloidal nitrogen* combined less *the acidic one*. We can therefore ascertain by its means the amounts of the three principal forms of nitrogen, and most accurately the ureal one. No doubt hypobromite may be applied after the preparation of the urine by mercurammonium and charcoal, and the amount of urea ascertained within one per cent., but the manipulations will be more laborious and take much more time than the continuance of Liebig's process. But this application is my emendation and not that of the advocates of the hypobromite process.

In the practice of medicine the hypobromite process is of the smallest use. Total quantity of urine in 24 hours and specific gravity yield useful and accurate information ; a bad analysis of a fragment of excretion leads to merely erroneous conclusions. We, therefore, recommend to our readers Liebig's process, with the addition of treatment by mercurammonium and charcoal, not only for serial work, or the ascertaining of constants, but for diagnostic work in single cases also. The results frequently throw unexpected light on the course of well ascertained sickness, no less than upon the factors of obscure organic disease.

The hypobromite process has been discussed by at

least twenty authors, and ultimately been left in the condition above described. All these inquiries have produced some valuable data, but most of them had only approximations to accuracy in view, and from these we dissent on principle. As Falck says properly, "Allow an error of only three per cent., and what good is an analysis?" It is, in fact, no analysis at all, but a ceremonious delusion.

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