NOTES ON URINARY CHEMISTRY.

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I.—ON LEUCIN AS A BULKY URINARY SEDIMENT.

IN December, 1901, I was asked by a lady to examine the urine of her daughter, aged 23 years, whom I have never seen as a patient. The young lady had to be careful about her diet, but is, I am informed, in excellent health in every other way, except in one particular. For about two years, on and off, she has suffered from hypogastric pain and a frequent desire to pass water. These symptoms are specially troublesome at night, and the pain sometimes wakes her three or four times. The pain is, perhaps, worse after eating certain things, *e.g.*, rhubarb or uncooked apples, or if she has had claret for dinner.

The first specimen of urine which I received in December, 1901, showed no peculiarity of colour. It was acid, and free from albumen or sugar. It deposited a considerable white sediment, about $\frac{1}{3}$ -inch in depth, which resembled in appearance an ordinary phosphatic deposit.

When examined under the microscope no crystals were seen either of uric acid or of octahedral calcium oxalate, and no tubecasts or organic structures of interest. The sediment was found to be entirely made up of —

- (a) Minute clear spheroids ;
- (b) Larger bright spheroids ;
- (c) Discoid bodies.

These last bodies were marked with fine concentric lines,

CASE IV.—BEFORE TREATMENT.

CASE IV.—AFTER TREATMENT.

and the periphery appeared granular, or else exhibited fine radial striæ. (See Fig. 1.*)

The urine was examined upon several occasions subsequently : twice again in December, and once in April, 1902.

None of the discoid bodies were observed a second time ; but in the urine passed on December 4th and December 31st a few small clear spheroids were noticed, in association with ordinary crystals of uric acid and octahedra of calcium oxalate. In two specimens (April, 1902) only amorphous urates, free uric acid, and calcium oxalate were detected. After the morning's urine had stood for two days a few groups of bright stellar, acicular crystals were seen.

I submitted the crystals to the inspection of several of my medical friends, and am much indebted to Dr. Alcock (at that time assistant to Dr. Purser, Professor of Institutes of Medicine), not only for his drawings of the crystals, but also for the trouble he took in the preliminary chemical examination.

However, as there were some points of difficulty regarding the solubility, and behaviour to reagents, of the crystals, I handed over the sediment (purified by washing with dilute alkali in order to free it from uric acid or urates) to Dr. Emerson Reynolds, who most kindly investigated the matter and supplied me with some valuable notes embodying his observations. The preliminary examination showed that the deposit did not contain uric, oxalic, or phosphoric acid ; and had no reducing action upon copper salts.

The spheroids polarised light, and exhibited a central black cross.

The precipitate was insoluble in dilute alkalies, but was dissolved by HCl (25 per cent.), and by strong H_2SO_4 .

* Cf. drawings in Gamgee's *Physiol. Chem.*, II., p. 240. Sheridan Lea, *Chem. Basis of the Animal Body*, p. 149. Neubauer and Vogel, *Analyse des Harns*. 1898. Tafel I, Fig. 5 (several forms).

From Dr. Emerson Reynolds' notes I extract the following data :—

A portion of the deposit was dried and examined.

(a) Heated in a melting-point tube it partially fused below 180° C., but did not completely liquefy even at 210° , indicating that it consisted of a mixture of two substances of different fusibility.

Ordinary leucin melts about 170° , but an isomer is known which does not melt at 210° .

(b) A portion heated more rapidly in a rather wider tube gave a feathery sublimate (a leucin character); and the residue charred with an unpleasant smell.

(c) The purest portions gave off ammonia freely when heated with soda-lime.

(d) A portion tested for sulphur did not afford any trace of that element.

The purified deposit was very slightly soluble in alcohol, the solution when filtered off and evaporated to dryness yielding a very faint residue. Ordinary leucin requires 1,040 parts of 98-per-cent. alcohol for solution at mean temperature. Impure leucin is more soluble in water and alcohol. Perfectly pure leucin crystallises in snow-white, glistening plates.

When another portion was boiled for some time with water, it partially dissolved, and the solution on evaporation left a residue which included some of the granules, and some more distinctly crystalline groups.

When the boiling aqueous solution was very carefully treated with neutral copper acetate and evaporated over sulphuric acid, a few perfectly distinct, rich deep blue (with purple shade) crystals were obtained, similar to those afforded by leucins.

All these properties point to the conclusion that the deposit included at least *one* leucin.

It is known that the amido-acids of the leucin type can furnish both chemical and physical isomers, which differ materially in melting-points, solubilities, and optical properties.

Leucins are amido-derivatives of caproic acid ($C_6H_{12}O_2$), the sixth term of the series of fatty acids. Leucin = $C_6H_{11}NH_2O_2$. There is reason to believe that, as a physiological compound, leucin is either :—

α-amido-(normal) caproic acid ; or,
α-amido-isobutylacetic acid.

This latter form is known to exist in three modifications, two of which are optically active, owing to the presence of an asymmetric carbon atom :—

It evidently, therefore, may be a difficult matter, in a given case such as the present one, to decide whether only one leucin is present or not,

Schulze and his pupils have shown that optically inactive leucin (prepared from vegetable albumen) is *α*-amido-isobutylacetic acid ; and Dr. Bernhard Gmelin has carefully investigated the constitution of three leucins prepared from (a) yeast, (b) casein, (c) albumen of hæmoglobin. Their solubilities in water and alcohol varied considerably. Thus, at 19°, yeast-leucin and casein-leucin required 29 parts of water for solution, and hæmoglobin-leucin 45·8 parts. At 17°, casein-leucin required 1,385 parts of alcohol (99 per cent.). All three forms were dextro-rotate.

Schulze converted the leucins into their corresponding oxyacids, and then reduced these to the fatty-acid series, and his results appear to establish the identity of the three fatty

acids so obtained with isobutylacetic acid, and not with normal caproic acid.

The causes of the differences in the properties of leucins from different sources may lie in :—

(1.) Chain-isomerism : *i.e.*, differences in the constitution of the fundamental fatty acid.

(2.) Position-isomerism : position of the NH_2 group relative to the carbon atoms.

(3.) Physical isomerism : *i.e.*, spatial relations of the atom or atom-groups, around an asymmetric carbon atom.

Now there are, theoretically, eight possible caproic acids, and seven of these are known. From these may be derived thirty-one isomeric amido-acids.

But if we accept Schulze's and Gmelin's proposition that isobutylacetic acid is the parent acid, we may exclude the derivatives due to chain-isomerism.

From isobutylacetic acid four amido-acids are possibly deducible in virtue of position-isomerism. But three (β , γ , δ) of these seem to be improbable from the known reactions of leucin, and hence we are restricted to the conclusion that leucin represents α -amido-isobutylacetic acid.

There remains then only one adequate factor in explanation of observed differences in properties, viz., physical isomerism. Gmelin considers that the three dextrogyrate leucins which he has prepared realise the complementary form of the lævogyrate modification which Schulze obtained by the agency of *Penicillium glaucum*, from inactive α -amido-isobutylacetic acid. Accordingly we are led to the belief that differences in the properties of leucins may be concerned not solely with *one* modification thereof, but may depend upon a varying mixture of optically active forms. For example, inactive leucin is the least soluble in water (1 : 160 parts). Is it merely a molecular mixture, or, is it a compound molecule formed of two optically active moieties ?

From his cryoscopic determinations Gmelin concludes that inactive leucin is only a molecular mixture, and is not a true compound, such as :—

Similarly, with alanin and glycocin. (*Zeitsch. für physiolog. Chem.*, XVIII., 1894, 21.)

Of the physiological or pathological significance of leucin little can be said. All are agreed that it is a decomposition-product of proteids; and, as regular end-products of albumen-disintegration, we find leucin, tyrosin, and asparaginic acid. The two last compounds are aromatic derivatives. The peculiar odour from the axilla and between the toes has been thought to be associated with decomposition-products of leucin (Hammarsten), but this is doubtful.

According to Radziejewski most organs of the body yield a little leucin (Neumeister, *Physiolog. Chemie*, I., 254), whereas tyrosin is not found normally in perfectly fresh tissues.

Leucin is very rarely found in urinary sediments, and I do not know of any previously-recorded case in which it has appeared as a macroscopic sediment in bulk, and unassociated with tyrosin.

Neubauer and Vogel (*Analyse des Harns*, 10 Aufl., 1898, 280) go so far as to state that it is not to be looked for in urinary sediments. Nevertheless, leucin and tyrosin have occasionally been observed to separate in the crystalline form spontaneously from pathological urines, but, as a rule, special procedure is needed to secure their crystallisation. Tyrosin is less soluble than leucin.

In regard to prognosis we recognise that the amido-acids, leucin and tyrosin, have been discovered in the urine chiefly in cases associated with rapid destruction of liver-cells, *e.g.*, acute yellow atrophy of the liver. Yet Herter states that he knows of instances “ of obscure febrile disease in which these

bodies have appeared in the urine for several days in succession, and in which the patient has made a good recovery.” (*Lectures on Chem. Pathology*, 1902.)

In the *Brit. Med. Jour.*, Sept. 4, 1880, p. 381, there is an abstract of a paper by Dr. E. C. Anderson, who states that he had detected leucin and tyrosin in the urines of a large number of cases representing most diverse conditions. Sometimes he found leucin alone, sometimes tyrosin only, and sometimes both. He recorded only cases in which leucin and tyrosin existed in the urine in very appreciable amount; and he believes that leucin is probably present in small amount in the urine of most people, except those in the highest state of health who lead temperate active lives.

I do not know of any confirmation of these somewhat surprising statements.

APPENDIX.

Leucin has been reported as having been found, pathologically, in :—

- (1.) Acute yellow atrophy of the liver. In the blood, liver, and urine.
- (2.) Acute phosphorus-poisoning. Liver and urine.
- (3.) Cirrhosis of the liver. Liver and urine.
- (4.) Continued fevers : typhus and variola. (?)
- (5.) Leuchæmia. In the blood.
- (6.) Cholera-stools.
- (7.) Gangrene of the lung. In the sputum.
- (8.) Pus and dropsical transudates.
- (9.) Atheroma.

Some of these observations need confirmation.

II.—ON A REACTION BETWEEN UREA AND FORMALDEHYDE AND ITS CLINICAL SIGNIFICANCE.

Some time ago, wishing to examine microscopically a patient's urine, which was turbid with amorphous phosphates,

I cleared it with acetic acid, and added a little formalin for preservation until I had leisure to examine it next day. The urine was free from albumen and sugar. Next day I noticed that a white deposit had formed in the urine. This was separated by the centrifuge and when examined under the microscope was found to consist wholly of :—

(a) Discoid bodies.

(b) Very small oblong and oval bodies, single, or arranged in pairs ; somewhat resembling large bacteria.

The discoid bodies appeared granular with a radial striation, and many of these were split at one side, probably owing to pressure. They stain with methylene blue (Earl), and are doubly refractive, showing a dark cross similar to that of a starch-granule (W. H. Thompson).

At first sight the discs recalled to mind the microscopic characters of leucin, but closer examination soon dispelled this idea.

Since neither I nor any of my medical friends had seen anything exactly similar previously, the question arose : What was the nature of the deposit ? One naturally thought of a possible interaction between formaldehyde and some constituent of the urine, the most likely being urea.

Dr. Earl found that by adding formalin to healthy urine a similar deposit could be obtained, and I ascertained that an aqueous solution of pure urea, when mixed with commercial formalin, slowly deposits a white sediment which consists entirely of the discoid bodies above described, about twice the diameter of a red blood-disc. The deposit is very insoluble in water and alcohol, and is not easily attacked by acids or alkalies in the cold.

I next consulted Dr. Emerson Reynolds, Professor of Chemistry in the University of Dublin, and I am much indebted to him and to his assistant, Mr. E. Werner, for information, and for references.

It is well known that aldehydes, especially the lower members of the series :—

- (a) Readily polymerise.
- (b) Are prone to form condensation-products with other substances.
- (c) Act as reducing agents.

A familiar example of polymerisation is afforded by the substance known as *paraform*, which is sold in the form of tablets for purposes of disinfection.

The concentrated aqueous solution of formic aldehyde not only contains gaseous formaldehyde, CH_2O , but also the hydrate, $\text{CH}_2 \begin{Bmatrix} \text{OH} \\ \text{OH} \end{Bmatrix}$ and probably other hydrates.

On complete evaporation of the solution the solid anhydride, paraformaldehyde, is left—possibly diformaldehyde $(\text{CH}_2\text{O})_2$.

As an example of a condensation-product may be cited the preparation known as urotropin. If a little liquor ammoniæ be added to commercial formalin, and the liquid cautiously evaporated to dryness, the two gaseous bodies unite, with exit of water, and a white solid remains, which is urotropin :—

= hexamethylene-tetramine.

Chemists have long been acquainted with the fact that urea unites with aldehydes in the cold, and Dr. Emerson Reynolds investigated some of these reactions 30 years ago. At that time formaldehyde did not come under consideration. Now methylene-, ethidene-, and ethylene-ureas have been prepared.

Within recent times several communications have appeared in respect to the combinations of urea with formaldehyde, and I will briefly refer to them.

In 1889 Hölzer and Lüdy prepared from formaldehyde and urea the simple combination $\text{C}_2\text{H}_4\text{N}_2\text{O}$.

The constitution of this compound may be taken as either

or as,

This latter formula would correspond to methylene-urea, and the reaction for its production is—

This compound decomposes at 200° with evolution of ammonia and at 240° melts to a brown liquid, which solidifies on cooling, and the residue is soluble in hot water. (*Ber. der Deutsch. chem. Gesellsch.*, 17,659 : 18,3302 : 29,2751. Cf. Lüdy, *Wien. Akad. Ber.*, 118, II., b. 191.)

In 1896 and 1897 Goldschmidt further investigated the products of the interaction of carbamide (urea) with formaldehyde. He obtained a compound, $\text{C}_5\text{H}_{10}\text{N}_4\text{O}$, which corresponds to 2 molecules of carbamide + 3 molecules of formaldehyde, and its probable constitutional formula is :—

He suggests that this reaction might possibly be used for determination of urea, since the precipitation seems to be almost quantitative.

The urea was dissolved in dilute HCl and treated with excess of formaldehyde. The precipitate is not acted on by alkalies, but is decomposed by strong acids. (*Ber. der chem. Gesellsch.*, 1896, bd. III., 2,638.)

In another communication Goldschmidt states that carbamide, when allowed to remain along with the excess of formaldehyde (40-per-cent. solution) in alkaline solution, yields a white precipitate, $\text{C}_3\text{N}_2\text{O}_3\text{H}_8 = \text{CO} \cdot (\text{NH} \cdot \text{CH}_2 \cdot \text{OH})_2$, which is soluble in hot water, and is precipitated by alcohol as a gelatinous mass. After two recrystallisations from water it appears to be transformed into $\text{C}_6\text{N}_4\text{O}_5\text{H}_{14}$.

In a neutral solution he finds that the chief products are the above, $C_3N_2O_3H_8$; and a body, $C_5N_4O_5H_{14}$, insoluble in water (*Jour. Chem. Soc.*, April, 1898, 178; from *Chem. Zeit.*, 1897, 21,460).

In this conflict of formulæ I will adopt as the simplest Lüdy's expression for methylene-urea, $CO \begin{smallmatrix} \text{NH} \\ \text{NH} \end{smallmatrix} CH_2$, which is accepted in Richter's *Organic Chemistry*, 1900, p. 400.

I could not detect any reaction, at least no precipitate was found, upon mixing aqueous solution of thio-urea (sulpho-urea, Reynolds) with formalin.

F. v. Hemmelmayr, however, has obtained the parallel compound methylene-thiocarbamide, $CS \begin{smallmatrix} \text{NH} \\ \text{NH} \end{smallmatrix} CH_2$ by mixing together equal weights of thiocarbamide and chlor-methylalcohol. A white precipitate is formed which is almost insoluble in all ordinary solvents. (*Monatsh.* 12.89.)

I likewise failed to observe any evidence of reaction between uric acid (urate of potassium) and formaldehyde in the cold, but, according to Weber, Pott, and Tollens, diformaldehyde-uric acid ($C_5H_4N_4O_3 \cdot 2 CH_2O$), can be readily formed, and it behaves as a weak monobasic acid. (*Jour. Chem. Soc.*, February 1898, 66; from *Berichte*, 1897, 30.2514.)

Since formaldehyde is now so frequently employed as an antiseptic, and has probably often been added to urine as a preservative, it is somewhat surprising that the reaction now described has not been, so far as I know, recorded.

To the clinical physician the relationship of formaldehyde to urine presents three points of practical interest :—

(1) It yields with urea a white precipitate (probably methylene-urea), which might easily be mistaken for leucin.

(2) If present in urine formaldehyde will reduce the copper-test, and so introduces a fallacy in testing for sugar.

(3) Formaldehyde interferes with the detection of small amounts of albumen by means of heat and acetic acid.