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PART I.

ORIGINAL COMMUNICATIONS.

ART. XXI.—*On the Occurrence of Protocatechuic Acid in Urine.*

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WITHIN the last few years the application of chemistry to physiology has been pushed with vigour by an active school of workers, and novel and interesting results of considerable importance to biological problems have been already obtained. A little more than two years ago Dr. Purser gave a lucid summary of our knowledge as to the occurrence of indigo-producing substances in the urine, and showed that their origin is due to certain processes of putrefaction which take place in the alimentary tube, chiefly in connexion with pancreatic digestion (*Dublin Journal of Medical Science*, Jan., 1880, p. 55).

These indigogenous bodies belong to the "aromatic series" of chemical compounds, and in my present paper I propose to deal with the occurrence in the urine of another member of this series, the physiological relations of which group have been investigated with remarkable success during the past five years.

In December last Dr. J. W. Moore submitted to me a specimen of curious urine which he had received for examination from Dr. George C. Armstrong, Blackrock, Co. Dublin. It was exhibited and discussed at a meeting of the Medical Society of the College of Physicians (*Dublin Journal of Medical Science*, Jan. 1882, p. 53).

^a Read before the Dublin Biological Club, April 18, 1882.

The urine was passed by a little girl, aged three years, apparently in perfect health, and not taking any medicine whatever. Dr. Armstrong had attended the mother three years previously in severe puerperal convulsions. I examined the mother's urine and found it normal in every respect. It was not darkened by liquor potassæ even after boiling. The mother remarked that the child's urine, although perfectly normal in appearance when first passed, on being allowed to stand, deepened in colour and stained the child's linen. This was more noticeable whenever the child had a cold or other slight ailment. Some of the urine was sent to Dr. Tichborne, who noted the following points:—Sp. gr. at 60° F., 1.025. Reaction acid; slight deposit of urates. Neither bile nor albumin was present. Urea = 1.2 per cent.; *i.e.*, 5.25 grs. per fluid ounce. Dr. Tichborne also remarked that "this urine presented a great peculiarity; it contained a substance which is only met with occasionally, and which has been termed alkapton." The term "alkapton" was introduced by Bödeker in 1861 (*Ann. Chem. und Pharm.*, Vol. 117, Jan., 1861) to designate a peculiar body occurring rarely in urine, and which he distinguished by these characters:—1. Strong alkalies darken the urine without the application of heat, and the coloration proceeds *from the surface of the liquid downwards—i.e.*, oxidation cooperates with the alkali;^a hence, alkapton (alkali, ἀπταω). 2. Reduction, at least partially, of the copper test. 3. Non-fermentation with yeast.

In all of these respects the results upon testing the child's urine agreed with Bödeker's statements. [Experiments shown with a sample of the fresh urine—*i.e.*, four months after first observation.] The urine afforded no indigo reaction; it quickly reduced nitrate of silver, and exerted slight action on Löwe's bismuth test for sugar, but with neutral Fe_2Cl_6 it gave a distinct green colour, turned reddish-brown by subsequent addition of alkali.

I may observe at once that the word alkapton conveys no real information, and was given at a time when the physiological chemistry of the urine was very imperfectly understood. Bödeker's experiments simply showed that the urine contained a reducing substance with a strong affinity for oxygen.

From various considerations it appeared probable that the pecu-

^a Several substances confer upon urine the property of darkening with an alkali from above downwards, *e.g.*—1. Pyrogallol. 2. Pyrocatechol. 3. Protocatechuic acid. 4. Hydroquinol. 5. Carbolie urine sometimes, owing probably to oxidation of free hydroquinol, derived from the gradual splitting up of hydroquinol-sulphuric acid.—(Maly; Virchow, and Hirsch. *Jahresber.* 1879, I., p. 144.)

liar substance or substances in the urine belonged to the aromatic series. Through Dr. Armstrong's courtesy I was furnished with additional supplies of the child's urine, and proceeded to investigate it in the laboratory of Trinity College, Dublin, by permission of Dr. Emerson Reynolds, to whose assistance I am much indebted.

Before entering into the details of the analysis, it may not be unacceptable to give some account of the recent literature bearing upon the subject of my communication. The excellent *Zeitschrift für Physiologische Chemie*, started by Hoppe-Seyler in 1877, represents much of the best work of the chemico-physiological school in Germany, and from it and from Hoppe-Seyler's *Physiologische Chemie* (1877-81) most of what follows is taken.

It may be well to premise that the term "aromatic compounds" is applied in chemistry in a technical sense. It includes a very large class of bodies, containing each at least 6 atoms of carbon, and represented by the fundamental hydrocarbon, benzene, C_6H_6 , with its homologues, and their numerous derivatives. The group obtained its name from the fact that many of its members occur in bodies possessing an aromatic odour, such as essential oils, gum-resins, &c. Carbolic acid (phenol) is one of its best known members, and the relations of a few of them to each other and to benzene (benzol) are easily seen from the subjoined formulæ:—

Benzene, - - - - - $C_6H_6 = C_6H_5H$

(a). Phenol (carbolic acid), - - - $C_6H_5(HO)$

(Cresol and thymol are homologues of it).

(b). $\left\{ \begin{array}{l} \text{Pyrocatechol (oxy-phenic acid),} \\ \text{Hydroquinol,} \\ \text{Resorcinol,} \end{array} \right. \begin{array}{l} - \\ - \\ - \end{array} \left. \right\} C_6H_4(HO)_2$

(c). Pyrogallol^a (pyrogallic acid), - - - $C_6H_3(HO)_3$

This chemically-associated group of bodies has also much in common in relation to their physiological actions, and to their practical and therapeutical applications.

The occurrence of aromatic bodies in the urine is chiefly, but not altogether, dependent upon the food, for the urine is not quite free from them even after long-continued fasting. When aromatic compounds are introduced with the food into the intestinal canal, or into the blood, directly or indirectly, or even when merely applied to the skin (carbolic acid), they, as a rule, suffer

^a It is proposed to restrict the termination "ol" to hydroxyl derivatives of hydrocarbons—i.e., to compounds of alcoholic character.—(Suggestions for Chemical Nomenclature. Watts' Dict. of Chem., 3rd Suppl., p. 1420.)

only partial decomposition after absorption, although they may be of an easily oxidisable nature. For the most part they pass into the urine either unaltered or partially oxidised, sometimes in combination with other bodies. In this respect there is a marked difference between the fatty and the aromatic compounds. When large quantities of fat, sugar, or albumen are introduced into the system they are almost completely decomposed into CO_2 , H_2O , and urea; while, on the other hand, aromatic substances, even so easily oxidisable as pyrocatechol and pyrogallol, pass through the organism unchanged in great part, so much so that de Jonge was able to demonstrate the existence of pyrocatechol in the urine of a rabbit, to which only 4 milligr. of pyrocatechol had been administered. The aromatic substances which occur in urine may be classed under four groups:—

1. Acid sulphuric ethers, of which phenol-sulphuric acid ($\text{C}_6\text{H}_5\text{HSO}_4$) is the simplest representative.
2. Glycocol-combinations of aromatic bodies, of which the best known and most important representative is hippuric acid.
3. Combinations with glycuronic acid ($\text{C}_6\text{H}_{10}\text{O}_7$), of which few are as yet known.
4. Uncombined aromatic compounds—*e. g.*, coumarin.

With the exception of hippuric acid and a few analogous compounds our knowledge of the other aromatic bodies is of very recent acquisition, and to E. Baumann largely belongs the merit of having successfully investigated the behaviour of aromatic compounds in the animal economy, and the alterations which they undergo in their passage into the urine. Thirty years ago Städeler showed that the urine of cattle and of men contained substances which, upon distillation with diluted sulphuric acid, yielded phenol along with other aromatic products, and in 1876 Baumann proved that phenol and its allies were excreted in the urine in the form of ethereal combinations with sulphuric acid. Combinations of hydrochinol, pyrocatechol, and resorcinol with sulphuric acid, as compound ethers, were first observed by Baumann and Herter in the urine of dogs, to whom these derivatives of benzol had been given. After the administration of benzol, pyrocatechol and hydroquinol are found in human urine. Phenol also partly passes into these bodies, and benzol is partly oxidised into phenol. Baumann and Preusse ascribe the dark colour of carbolie urine to hydroquinol. Traces of pyrocatechol-sulphuric acid frequently occur in human urine (Baumann), and occasionally

more abundantly,^a independently of the admixture of pyrocatechol with the food. Pyrocatechol was first found by Ebstein and Müller (Virchow, *Archiv.* LXII., p. 554) in the urine of a child, and additional cases have been recorded by Fürbringer and Fleischer. Neubauer and Vogel (7te Aufl., 1876) suggest that Bödeker's alkapton was probably pyrocatechol, and Eichhorst (*Lehrbuch der physikal. Untersuchungsmeth.*, Th. II., 1881, p. 279) also is of the same opinion in regard to Bödeker's and Fürbringer's cases. But Ebstein and Müller (*loc. cit.*) do not allow that what they describe as pyrocatechol agrees with Bödeker's alkapton, which was said to contain nitrogen. When pyrocatechol is administered along with the food, it escapes freely as a conjugate in the urine, which shows a corresponding diminution of sulphuric acid in the form of sulphates.

Now each of these bodies above referred to would be liable to reduce alkaline copper solutions, and when present in any considerable quantity may, it is easy to see, give rise to fallacies in testing for sugar.

Moreover, there are now and then found in the urine, under very different circumstances, substances which reduce copper in alkaline solution, are *lævogyrate*,^b rarely *dextrogyrate*, and which have usually passed for sugar, but which have not hitherto been isolated. Upon the interesting and much-vexed question whether grape sugar occurs in normal urine, Hoppe-Seyler holds that, as a rule, sugar is not present, but that substances occur in normal urine, in small and variable amount, which are closely allied (*e. g.*, glycuronic acid) to grape sugar, and which are so easily decomposed that their identification is attended with great difficulty.

To return now to the method of analysis pursued in the present case.

After a preliminary trial by another method, a sample of the urine was examined in the following manner:—

Dec. 19th, 1881.—About eight ounces were cautiously precipitated with neutral acetate of lead, and filtered.

^a Constantly in the urine of horses. The dark colour of the urine of herbivora, as well as the brownish colour of their blood plasma and serum, are probably due to decomposition products of aromatic substances, such as pyrocatechol and pyrogallol.

^b Lævogyrate bodies occur in the urine of man and animals after the administration of chloral hydrate, nitro-toluol, camphor, and phenetol. After the administration of brom-benzol the fresh urine is strongly lævogyrate, and this optical activity is very easily destroyed by dilute acids and alkalis, or by heat.

The lead precipitate was decomposed by H_2S , and tested by ferric chloride and alkali, but exhibited a mere tinge of coloration, probably due to incomplete washing of the precipitate.

The filtrate from the acetate of lead reacted strongly (brown) with NaOH , and was then treated with an excess of basic acetate of lead, and filtered.

This filtrate gave no colour reaction with ferric chloride and alkali—*i.e.*, the substance sought for was entirely carried down by the basic acetate.

Basic acetate of lead precipitate. Diffused in water, and passed in H_2S , filtered.

Filtrate perfectly colourless. Turned brown with KOH , and green with Fe_2Cl_6 .

Distilled in an atmosphere of CO_2 .

DISTILLATE.	RESIDUE.
No reaction with Fe_2Cl_6 .	Diluted. Bluish green with Fe_2Cl_6 , turned clear dark-red by excess of ammonium car- bonate.

I was at first disposed to assign this case to the same category as Ebstein and Müller's case of pyrocatechol, but Preusse has shown (*Zeitsch. für physiol. Chem.*, II., p. 324), that pyrocatechol has been frequently confounded with protocatechuic acid ($\text{C}_7\text{H}_6\text{O}_4$).^a

The two bodies can be separated and distinguished from each other as follows:—

$\text{C}_6\text{H}_4 \begin{Bmatrix} \text{OH} \\ \text{OH} \end{Bmatrix}$	$\text{C}_6\text{H}_3 \begin{Bmatrix} (\text{OH})_2 \\ \text{CO}_2\text{H} \end{Bmatrix}$
PYROCATECHOL.	PROTocatechuic ACID.
Upon distillation passes over with watery vapour.	Does not.
In alkaline solution is soluble in ether.	Is insoluble.
Green with Fe_2Cl_6 , turned violet by alkalis.	Green with Fe_2Cl_6 , reddened by addition of alkali.

I concluded then that the substance I had to deal with was protocatechuic acid, (1) because it did not volatilise upon distilla-

^a One of the forms of dioxybenzoic acid. Best got by fusing East Indian kino with caustic soda (Watt's Dict., Vol IV., and 2nd Supplement).

tion,^a and (2) on account of the characteristic colour reaction with ferric chloride and alkalis obtained (a) directly with the fresh urine, and (b) with the isolated product of analysis.

I greatly regret that it was not possible, from the material at my command, to prepare enough of the pure product to submit it to elementary analysis.

Upon mentioning the subject to my friend, Professor W. N. Hartley, he suggested that optical examination would aid in the identification of the substance, and he was good enough to take the trouble to examine the urine, and to send me the following laboratory note :—

“The urine, unmixed with other substances, gave a fine green colour, with a neutral solution of ferric chloride, which turned to a red on the addition of ammonium carbonate. The same reaction was observed with a freshly-prepared solution of protocatechuic acid, the peculiar tints being the same in each case. The urine had a slightly right-handed rotation. In order to separate protocatechuic acid, if possible, acetate of lead was added to the urine, the phosphates were filtered off, and the liquid was evaporated by boiling *in vacuo*. Subsequently cooling and standing caused crystals to separate. The crystals were examined under the microscope; they formed stellate groups. On treatment with ferric chloride, only a transient green coloration was seen; subsequent treatment with ammoniac carbonate precipitated the ferric compound, but on standing for an hour this redissolved, with the production of the striking colour before observed. Alcohol (absolute) was added in about double the volume of the urine; the liquid was filtered, and evaporated spontaneously *in vacuo* till reduced sufficiently in volume to admit of spectroscopic examination. The spectroscopic method was that devised by me, described in the *Phil. Trans.*, Part I., 1879. This enables one to detect any aromatic derivative by means of the characteristic selective absorption that such bodies exert on the ultra-violet spectrum. The absorption bands have their position and width registered by photography, the entire spectrum being focussed on one plate. For a description of the instrument and process, see *Science Proc. Roy. Dub. Soc.*, 1881. About fourteen photographs of the spectra yielded by protoca-

^a In Ebstein and Müller's case the pyrocatechol (?) did not come over upon distillation of the urine, either alone, or acidulated, or rendered alkaline. Ten cc. of the urine, with caustic potash, under mercury, absorbed 6–8 cc. of oxygen. The peculiarity of the urine, first noticed when the child was four months of age, was still observed a year later.

techuic acid in solutions of different strengths were taken. These were compared with photographs of the urine concentrated to $\frac{1}{20}$ th its original volume, and diluted with various proportions of water. Only a general result confirming the colour reactions was obtained, because no weighable quantity of the crystallised substance was separated from the urine. Otherwise accurate quantitative results and an absolute proof of the identity of protocatechuic acid with that of the body in the urine could be obtained by constructing what I have called dilution curves for registering the relative positions and intensity of the absorption bands in solutions of different strengths. From a comparison of the two series of photographs, I should say that the proportion of protocatechuic acid in the urine was about 1 part in 10,000, and was certainly not so much as 1 in 5,000 parts of the liquid."

When protocatechuic acid, which, according to Hoppe-Seyler (*Phys. Chem.*, p. 840), is widely diffused in the green parts of plants either free or in an easily-decomposed combination, is administered to animals with their food, the urine is found to contain pyrocatechol-sulphuric acid along with protocatechuic-sulphuric acid and free protocatechuic acid, but, so far as I am aware, no case has hitherto been recorded of the occurrence of protocatechuic acid in human urine.

ART. XXII.—*On the Therapeutic Properties of Quinine Iodate and Bromate; and on the Physiological Activity of Superoxidised Molecules.*^a By CHARLES A. CAMERON, M.D., M. Coll. Phys.; Fellow and Professor of Chemistry and Hygiene, Royal College of Surgeons in Ireland; Vice-President of the Society of Public Analysts of Great Britain and Ireland, &c.

THE researches of Arthur Gamgee, Priestly, and Larmuth have shown that the three forms of phosphoric acid and of vanadic acid have very different degrees of physiological activity. The salts of orthophosphoric acid are almost inert when their bases are inactive, whilst the pyrophosphates and metaphosphates are poisonous. The orthovanadic acid is poisonous, but pyrovanadic acid and metavanadic acid are still more poisonous.

The high physiological activity of the pyro and metaphosphates has been attributed to the unsaturated condition of their nuclei.

^a Read before the Medical Society of the King and Queen's College of Physicians, Wednesday, May 3, 1882. [For the discussion on this paper see page 530.]