ON THE SUPPOSED OCCURRENCE OF UROLEUCIC ACID IN THE URINE IN SOME CASES OF ALKAPTONURIA.

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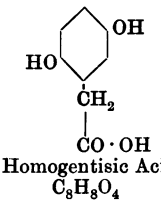
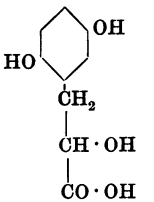
In all the more recent accounts of Alkaptonuria it is mentioned that in some cases there has been found in the urine, in addition to homogentisic acid (hydroquinone-acetic acid), a second abnormal aromatic acid of like properties, called uroleucic acid, and which is probably hydroquinone-α-lactic acid.



Homogentisic Acid
$C_8H_8O_4$



Uroleucic Acid
$C_9H_{10}O_5$

The grounds for this statement are as follows:

Homogentisic acid was isolated, and its composition was established, by Wolkow and Baumann[1] in 1891. Previously to this, in 1886-88, the late Dr R. Kirk[2] had investigated the urines of two alkaptonuric brothers, and extracted therefrom an acid, which he submitted to analysis and found to have a composition which agreed very closely with the requirements of the formula $C_9H_{10}O_5$.

	Uroleucic acid (Kirk)	Calculated for $C_9H_{10}O_5$	Homogentisic acid $C_8H_8O_4$
C.	54·457 %	54·54 %	57·14 %
H.	4·985	5·05	4·76
O.	40·558	40·41	38·10

[1] *Zeitschr. f. physiol. Chem.* xv. p. 228. 1891.

[2] *Journ. of Anat. and Physiol.* xxiii. p. 69. 1889.

A titration of the acid with sodium hydrate, under a layer of petroleum ether to prevent oxidation and browning, showed that 40 parts of NaOH were required to neutralise 198 parts of the acid, figures which supported the above formula for a monobasic acid.

To this acid, which melted at "about 133°·3," and gave the alkapton reactions, Kirk assigned the name of uroleucic acid. It will be shown later, that the urines in question undoubtedly contained the then unknown homogentisic acid, and that homogentisic acid is thrown down as its lead salt, when Kirk's method of extraction is employed. Hence, the conclusion cannot be avoided that the substance analysed consisted in large part of that acid; in a word that although Dr Kirk came very near to anticipating by some years the discovery of homogentisic acid, the alkapton acid which he obtained was not a pure substance. Indeed in a letter to one of us he himself wrote, "I have no doubt that my uroleucic acid must have been simply impure homogentisic acid."

The view that the uroleucic acid of Kirk was a second alkapton acid, distinct from homogentisic, had its origin in some investigations of the late Professor Huppert[1], who examined some of the original material sent to him by Dr Kirk. In it he found much homogentisic acid, thus demonstrating that the alkaptonurics in question conformed to the ordinary rule in this respect. However after as much of the homogentisic acid as possible had been precipitated as the lead salt, first by neutral, and afterwards by basic lead acetate, he obtained a residue which melted at 130°·5. (The melting point of homogentisic acid is 146°—147°, and that of uroleucic acid, as determined by Kirk, about 133°·3, the figure 130°·3 given in Huppert's paper is obviously a misprint.) This fraction of lower melting point was looked upon as consisting of the uroleucic acid of Kirk, with the formula $C_9H_{10}O_5$.

As an acid having this formula might be either a trioxybenzene-propionic acid, or a dioxybenzene lactic acid, the scanty material available was submitted to further tests, with a view to determining which of these formulæ was the more probable.

With Millon's reagent it behaved like homogentisic and not like gallic acid. Moreover when the hydroxyl groups were methylated, and the side chain was oxidised with permanganate, both the homogentisic fraction and that of lower melting point yielded the same oxidation product, which melted at 116°. Hence it was inferred that uroleucic acid had two hydroxyl groups upon the benzene ring, and was probably hydroquinone-α-lactic acid.

[1] *Zeitschr. f. physiol. Chem.* XXIII. p. 412. 1897.

The material at Prof. Huppert's disposal did not permit of further investigations.

Since 1888 uroleucic acid has been sought for in vain in alkapton urines by Wolkow and Baumann and others. Only Langstein and E. Meyer[1], employing a method suggested by Huppert, obtained in one case a minute residue, melting at 133°, after homogentisic acid had been removed, but the quantity only sufficed for the determination of its melting point. At a later period Falta[2] failed to find this substance in the urine of the same subject.

The formula $C_9H_{10}O_5$ had much to commend it, and Otto Neubauer and Falta[3] showed that hydroquinone-α-lactic acid falls naturally into place as an intermediate stage in the conversion of tyrosin and phenyl-alanin into homogentisic acid. However it should be optically active, whereas Kirk's material did not rotate the polarised ray.

This argument from probability has recently been deprived of its force by the important work of Otto Neubauer and Flatow[4], who have synthesised hydroquinone-α-lactic acid, and have clearly shown that this substance, which melts at 87°, is not the supposed uroleucic acid.

The accuracy of the observations of Prof. Huppert, who accomplished so much with so little material, is not for one moment in question. Everything turns upon the nature of the material which he examined, and of which he speaks as "rohe alkapton-säure." The fact that it was grey in colour shows that it cannot have been the crude, dark red substance, which Kirk obtained at the early period of his work[5] by simply evaporating the ethereal extracts of the acidified urine. A careful study of Dr Kirk's writings upon the subject suggests that it can have been nothing else than the product obtained by his method of precipitation by lead acetate, in other words the uroleucic acid which he analysed.

If this be so, it is evident that Prof. Huppert was under a misapprehension as to its nature, and that the analytical figures of Kirk could only be applied to the substance as a whole, and not to that portion which remained after as much as possible of the homogentisic acid had been removed. Clearly the material available did not suffice for a fresh analysis of the fraction of lower melting point.

This conclusion having been reached, it was obviously necessary to

[1] *Deutsches Archiv f. klin. Med.* LXXVIII. p. 165. 1903.
[2] *Zeitschr. f. physiol. Chem.* XLII. p. 82. 1904.
[3] *Ibid.* XLII. p. 81. 1904.
[4] *Ibid.* LII. p. 379. 1907.
[5] *Brit. Med. Journ.* II. p. 1017. 1886.

study the working of Kirk's method of extraction when applied to a known alkapton urine, and we accordingly obtained several litres of the urine of the patient Thomas P. who has been under observation for some ten years, and in which urine uroleucic acid had been sought for in vain.

The method differs from that afterwards employed by Wolkow and Baumann for the extraction of homogentisic acid, in that the urine is acidified, after concentraction, with hydrochloric instead of sulphuric acid. The use of hydrochloric acid may lead to the formation of considerable amounts of ester when the ethereal extracts are evaporated, unless the ether used be alcohol free.

A further difference is that instead of the addition to a hot watery solution of the residue from the ethereal extract of a moderate quantity of a lead acetate solution, the solution is fractionally precipitated with lead acetate in the cold, and is finally saturated by grinding it up with an excess of solid lead acetate.

With Kirk's description before us we followed out his method in all its details. The precipitate of the lead salt was obtained in seven fractions. The earlier, coloured fractions were rejected and the final precipitate from the saturated liquid, which was colourless and minutely crystalline, answered in all respects to the description of the lead salt of uroleucic acid.

After filtration the precipitate was washed with water and was freed from lead by suspension in water and the passage of a stream of hydrogen sulphide. The filtrate from the precipitate of lead sulphide was repeatedly extracted by shaking with ether. On evaporation of the ether, which had been dried over calcium chloride, a crystalline deposit of colourless acid was obtained. The crystals softened at 134° and were completely melted at 136°.

The alkapton acid thus obtained in abundance from the urine with which we were working could be nothing else than homogentisic acid, with some impurity or impurities which lowered its melting point to a figure very near to that assigned by Kirk to his uroleucic acid. That this was indeed so was proved in various ways, as by precipitation of lead homogentisate from a solution of the product, isolation of the free acid and determination of its melting point.

One gramme of the material was dissolved in water, and was titrated against $\frac{N}{10}$ NaOH, under a layer of petroleum ether, phenolphthalëin being used as indicator. The result corresponded to a

molecular weight of 163·9, that of homogentisic acid being 168, and differed widely from Kirk's figure (198).

It is therefore evident that the material obtained by the above method from a urine which contains homogentisic acid consists mainly of that substance, but that saturation with lead acetate yields a less pure product, than the slower crystallisation of the lead salt from less concentrated solutions.

In 1902, by the great kindness of the late Dr Kirk, one of us was enabled to examine several litres of the fresh urine of each of his patients, who since his investigations had passed from childhood to manhood[1]. In no obvious respects did these urines differ from those of seven other alkaptonurics examined at various times. They contained homogentisic acid in ordinary amounts, and when this had been precipitated as lead salt, as far as is possible by the simple direct method[2], after removal of lead the small remainder of alkapton substance in the mother-liquors, was converted into the ethyl-ester by Erich Meyer's[3] method. The crystalline ester obtained melted at 120°, which is the melting point of ethyl-homogentisate. Hence it was concluded that these alkaptonurics were no longer excreting any appreciable quantity of a second alkapton acid. Dr Kirk kindly sent, at the same time, the last remainder of his original material. It had become perfectly black, but was labelled "Uroleucic Acid." It was found to contain much homogentisic acid, and the conclusion was reached that it also contained the lactone of that acid.

We recently examined the last scanty remains of this material, then some twenty years old. As an attempt to recrystallise the unchanged portion from ether was not successful, we dissolved it in water, boiled the solution with animal charcoal, and so obtained a clear solution of a yellow colour. On evaporation in vacuo this left a crystalline residue, which softened at 125° and was completely melted at 129°·5.

Titration with $\frac{N}{10}$ NaOH, under a layer of petroleum ether, indicated, for a monobasic acid, a molecular weight of 182·3. The liquid assumed a rich green colour as the neutral point was approached, suggesting the presence of lactone. From another small portion a few crystals of lead homogentisate were prepared. It was evident from the change from white to black, from the melting point, and from the

[1] *Lancet*, II. p. 1616. 1902.
[2] *Journ. of Physiol.* XXIII. 512. 1899.
[3] *Deutsches Archiv f. klin. Med.* LXX. p. 456. 1901.

titration that the material had undergone changes in the course of twenty years.

The above experiments, taken in conjunction with the synthesis of hydroquinone-α-lactic acid by Otto Neubauer and Flatow, seem to us to justify the conclusion that the evidence of the occurrence of a second alkapton acid in some cases of alkaptonuria is insufficient.

If this be so, and the supposed existence of uroleucic acid as a distinct chemical individual arose from a misconception, as has been suggested above, it must be acknowledged that a series of very remarkable coincidences have contributed to ensure its acceptance.

The first of these is the very close agreement of Kirk's analytical figures with the requirements of the formula $C_9H_{10}O_5$, a formula which remained a purely empirical one until after the discovery of homogentisic acid.

Secondly, there was the coincidence that the residue obtained by Huppert, after the removal of as much homogentisic acid as possible from the original material, had a melting point so near to that assigned by Kirk to uroleucic acid.

Thirdly, that Langstein and E. Meyer obtained a residue, when searching for uroleucic acid in the urine of their patient, which had the correct melting point.

Lastly that it should have been shown, many years after Kirk's investigations, that an acid having the formula $C_9H_{10}O_5$, and the structure of hydroquinone-α-lactic acid, was a likely intermediate product in the catabolism of the aromatic fractions of proteins.