A NEW CASE OF ALKAPTONURIA

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Further records of individual cases of alkaptonuria are still to be desired as materials for the elucidation of certain points in the hereditary incidence and chemical features of this rare error of metabolism. It is on this account that notes of observations which we have recently made upon a case hitherto unrecorded are here given.

Minnie L., a remarkably fair-haired little girl, aged three years, was recently brought to the Hospital for Sick Children, Great Ormond Street, on account of the peculiar colour of her urine. There she came under the notice of Dr. Still, who, having diagnosed the case as one of alkaptonuria, kindly transferred her to Dr. Garrod's care.

The child's general health was excellent, and the only other symptom noticed besides the colour of the urine and its staining properties was occasional dysuria. This symptom was present in a case described by Stange,¹ and was also experienced by H. Embden² when he took a dose of eight grammes of homogentisic acid by the mouth, and so rendered himself temporarily alkaptonuric.

No history could be obtained of any similar urinary anomaly in the family of either parent. The patient is an only child, and there is no blood relationship between her parents.

The district nurse who attended to the mother at her confinement stated that she had noticed that the infant's urine stained her apron on the second day of its life. This statement is in complete accord with what was noticed in another case recorded by one of us,³ in which also the staining was noticed on the second day of life.

1. *Virchow's Archiv.*, Vol. CXLVI, p. 86, 1896.

2. *Zeitschr. f. physiol. Chemie*, Vol. XVIII, p. 304, 1893.

3. *Lancet*, 1901, Vol. II, p. 1484.

The urine, which was of natural colour when passed, darkened on exposure to air, and yielded all the usual reactions of alkapton urines.

Thus the addition of a few drops of a dilute solution of ferric chloride produced a transient deep blue colour. Alkalies caused rapid darkening, especially when the liquid was warmed. The urine reduced Fehling's solution readily with the aid of heat, and the peculiar chocolate tint produced by the suspension of the orange-coloured precipitate in the dark brown liquid was characteristic.

Ammoniacal silver nitrate solution was rapidly reduced in the cold. The polarimeter showed the absence of any rotatory power of the urine.

On some days abundant uric acid crystals, tinted by the brown pigment, were spontaneously deposited.

Some of the urine was heated nearly to the boiling point, and to it was added solid neutral lead acetate in the proportion of six grammes for each 100 c.c. After filtration the clear yellow filtrate deposited, on standing, a copious crop of crystals of lead homogentisate.

Of the washed and air-dried crystals 1.6047 grammes were exposed to a temperature of 100° to 110° C. for two periods of half an hour each. The loss of weight was 0.145 gramme, which represented a loss of water of crystallisation of 9.05 per cent. The water of crystallisation in lead homogentisate $(C_8H_7O_4)_2 Pb, 3H_2O = 9.08$ per cent.

A portion of the lead salt was finely powdered in a mortar, and was suspended in anhydrous ether through which a stream of sulphuretted hydrogen was passed. After filtration from the precipitate of lead sulphide the ether was allowed to evaporate, and in this way the free acid was obtained in colourless crystals. It melted at 146° C., which is the melting point of homogentisic acid.

Ethyl homogentisate was prepared as follows by Erich Meyer's method¹ :—

A portion of the free acid was dissolved in alcohol, and the solution was saturated with gaseous hydrochloric acid. After standing

1. *Deutscher Archiv. f. klin. Med.*, Vol. LXX, p. 443, 1901.

for some hours the alcoholic solution was freely diluted with water, rendered feebly alkaline with sodium carbonate, and repeatedly extracted with ether. The ethereal extract was dried over calcium chloride, and on evaporation deposited crystals of the ethyl ester, which melted at 120°C. , the melting point of ethyl homogentisate.

Lastly, 500 c.c. of urine were submitted to benzoilation with benzoyl chloride and sodium hydrate. The precipitate was extracted with alcohol, and the alcohol was thrown into water, when a precipitate immediately formed. The product, once recrystallised from hot alcohol, melted at 201°C. (This agrees with the melting point of di-benzoyl homogentisamide, which is so obtained,¹ but after repeated recrystallisation from alcohol the melting point of that compound is raised to 203° or 204°C.)

It was thus established beyond question that the properties of the urine were due to the presence of homogentisic acid.

With a view to ascertaining whether a second alkapton acid (uroleucic acid) were also present, $2\frac{1}{2}$ litres of the urine were subjected to Wolkow and Baumann's process² for the extraction of homogentisic acid. After acidification with sulphuric acid the urine was evaporated to a small bulk, and was thrice extracted with ether. From the collected ethereal extracts the ether was distilled off, the syrupy residue was dissolved in hot water and warmed, and a solution of basic lead acetate was freely added.

The liquid was filtered, and on standing deposited crystalline lead homogentisate. After forty-eight hours the crystals were filtered off, the excess of lead was removed from the filtrate by a stream of sulphuretted hydrogen, and the filtrate from the precipitated lead sulphide was repeatedly extracted with ether. After standing for forty-eight hours over calcium chloride to remove water and any alcohol which might be present, the ether was distilled off from the extract. A small syrupy residue which remained became crystalline on standing in a vacuum exsiccator. However, the amount of crystalline substance was very small, and we did not succeed by the

1. *Journal of Physiology*, Vol. XXVII, p. 89, 1901.

2. *Zeitschr. f. physiol. Chemie*, Vol. XV, p. 228, 1891.

use of any solvent in obtaining the crystalline substance free from the brownish syrup with which it was mixed, nor melting at so high a point as 100°C .

Two further attempts with fresh quantities of the urine were attended with no better success, and we can only state that if a second alkapton acid was present the quantity must have been extremely minute.

The child was placed upon a constant mixed diet, and after three days the urine of eight individual days was collected for the determination of the quotient $\frac{\text{homogentisic acid}}{\text{nitrogen}}$.

The results of these determinations, which were carried out by T. Shirley Hele and one of us, are recorded elsewhere,¹ and here it need only be mentioned that the quotients obtained were in accord with those got in other cases of alkaptonuria in which such determinations have been made.² They tend to confirm the conclusion that there is only one degree of alkaptonuria, and that the homogentisic acid excreted represents all the tyrosin and phenylalanin of the proteins broken down.

The average daily excretion of homogentisic acid, as determined by the silver method of Wolkow and Baumann (8 per cent. ammonia being used instead of 3 per cent.),³ was 1.832 grammes.

The noteworthy points in this case are :—

1. The fact that the patient is a female, for females are few among congenital alkaptonurics.
2. The absence of consanguinity of the parents, blood relationship being common in such cases.⁴
3. The absence of any second alkapton acid at any rate in quantities capable of being detected by the method recommended for that purpose.
4. That the H : N quotient was in accord with those which have been obtained in other cases of alkaptonuria.

1. *Journal of Physiology*, Vol. XXXV, p. 15, Proc. Physiol. Soc., Dec. 15th, 1906.

2. *Ibid.*, Vol. XXXIII, p. 198, 1905.

3. *Ibid.*, Vol. XXXIII, p. 206, 1905.

4. *Lancet*, 1902, Vol. II, p. 1616.