A CONTRIBUTION

TO THE

STUDY OF ALKAPTONURIA

BY

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THE rare anomaly which is known as alkaptonuria possesses great interest alike for the physiologist and the physician; for the former because the study of the abnormal constituent or constituents which are present in the urine of alkaptonuric individuals, and of the conditions which control their excretion, promises to throw important light upon the metabolism of proteids and upon the fate of tyrosin in the organism; for the latter because the condition is one liable to be mistaken for glycosuria, and because of the peculiar coloration of the urine which develops on standing, and on account of which a considerable proportion of the cases come under observation.

The following are the essential features of the urine of alkaptonuria:

1. Although of normal appearance when passed, the urine rapidly acquires a deep brown colour and ultimately becomes black on exposure to air.

2. The brown colour is greatly intensified by alkalies, its development being accompanied by absorption of oxygen.

3. The urine reduces Fehling's solution with the aid of heat, and actively reduces ammoniacal silver nitrate solution in the cold.

4. The bismuth test for sugar yields negative results (one recorded example alone offered an exception to this rule).

6. The urine has neither dextro-rotatory nor lævo-rotatory power.

7. The fermentation test yields negative results.

8. Fabrics moistened with the urine become deeply stained on exposure to air.

The Clinical Aspects of Alkaptonuria.

In the table on page 392 I have entered all the recorded cases of alkaptonuria which I have been able to find, and in which the necessary details are forthcoming, commencing with the case recorded by Bödeker[1] in 1859 in a paper in which he described the earliest attempt to isolate the substance to which the reducing power of such urines is due.

In the fourth edition of Bowman's 'Medical Chemistry,' edited by Bloxam (1862), it is mentioned that Dr. Johnson had observed the occurrence of alkaptonuria in an infant, his attention being called to it by the staining of the napkins. No further details are given, and I have been unable to find any fuller record of the case, which would rank second in chronological order. It is therefore not included in the table.

Nor has a search in much earlier literature proved wholly fruitless. In 1822 Dr. Alexander Marcet read before the Medico-Chirurgical Society an account of the urine of an infant, aged seventeen months, which turned

[1] References to the authors quoted will be found in the bibliography at the end of the paper.

black soon after being passed, and which stained napkins of a dark purple hue. The condition was noticed almost immediately after the child's birth. The darkening effect of alkalies was specially noted. The chemical properties of the pigment, which were investigated by Prout, agreed with those of the alkapton pigment, and, although evidence of a reducing action of the urine is wanting, it seems hardly possible to doubt that this was a case of alkaptonuria.

Schenck, in his 'Observationes Medicæ,' published in the seventeenth century, in a chapter dealing with the passage of black urine by healthy persons, quotes from Johan Bellfortis, the case of a monk who passed black urine during the six years that he was under observation, and stated that he had done so all his life. This also may have been an example of the condition under consideration.

In addition to the twenty-four recorded cases, there are included in the table seven cases hitherto unpublished. For the notes of five of these I am greatly indebted to Dr. Pavy, who also kindly supplied me with some specimens of urine.

Of the remaining patients one is an infant who came under the care of my colleague Dr. Voelcker, at the Hospital for Sick Children, and to him I would here express my gratitude for his kindness in handing over the case to me.

The last patient is under the care of Dr. C. E. Baker, to whom also my best thanks are due for the particulars given, and for furnishing me with several litres of the urine for examination.

It is upon an analysis of these thirty-one cases that the following account of the clinical features of alkaptonuria is based.

Alkaptonuria would appear to be considerably commoner in males than in females, the series of thirty-one

cases including twenty-three males and only eight females.

In the great majority of instances the anomaly dates from early childhood, but it has occasionally appeared as a temporary phenomenon during an illness, or has apparently developed in later life. In a certain number of the recorded cases no data bearing upon this point are forthcoming, or it is merely stated that the alkaptonuria was of long standing. In seventeen cases it had been observed from infancy, but it is probable that the above figure does not at all represent the full proportion of life-long cases. In five cases only is the phenomenon definitely stated to have been merely temporary or of later development.

In the case of my own patient the staining of the napkins was noticed on the day following birth, and in Ebstein and Müller's case (No. 3) the alkaptonuria was traced back to the early days of life.

In one instance, Stange's (No. 18), the phenomenon was apparently intermittent, the urine losing its abnormal properties for long periods, and this observation renders it most important that such intermittent alkaptonuria should be, as far as possible, excluded in cases apparently of later development. Evidence upon this point is not always easy to obtain, and in at least one case, Ogden's (No. 17), the patient, an adult man, was unaware of any peculiarity of his urine.

In this connection, evidence supplied by the mothers of patients as to staining of the napkins in infancy is of special value.

Life-long alkaptonuria is sometimes met with in several members of a family, but, although brothers and sisters are apt to share this peculiarity, I know of no instance in which it has been transmitted from one generation to another. When it occurs in families some members are apt to escape, and a child born between two alkaptonuric members may pass normal urine.

Kirk's patients (Nos. 7, 8, and 9) were three out of

four brothers of whom the eldest escaped. Marshall and Futcher's patients (Nos. 10 and 22) were brothers. Walter Smith's patient (No. 5) has an alkaptonuric brother (No. 26) whose urine I have had the opportunity of examining. An intermediate brother has escaped. Baumann and Embden's patients (Nos. 12 and 13) were a brother and sister born out of wedlock. Both parents subsequently married and both had children, none of whom were alkaptonuric.

Dr. Pavy has met with a family of fourteen, amongst whom were four alkaptonuric members (Nos. 28, 29, 30, and 31). These were the ninth, eleventh, thirteenth and fourteenth children. The tenth and twelfth had escaped.

As Wolkow and Baumann pointed out, life-long alkaptonuria is apparently without any injurious effect upon the health of its subjects. Baumann's patient had reached the age of sixty-eight, and his sister, whose urine was studied by Embden, that of sixty years.

The staining action of the urine is usually the only source of annoyance, unless the patient presents himself for life insurance and, as has repeatedly happened, is rejected as diabetic.

Stange's patient, who excreted an exceptionally large amount of the abnormal constituent, suffered from dysuria and herpes of the penis, but my patient, although his urine is concentrated, scanty, and very rich in the same substance, has no dysuria, although frequent scanty micturition is present. In one instance (No. 5) slight ailments were apparently attended by more intense darkening of the urine, and in one of Dr. Pavy's cases the urine was noticed to be darker than usual before the menstrual periods.

The temporary cases are of great interest, and, curiously enough, of the five such cases recorded no less than four have been in females.

Carl Hirsch's case (No. 21) is specially striking because it was definitely stated that the urine had never previously shown the same characters, and there was no other known

case in the family. The patient, a girl aged 17, who had suffered from no previous illness except measles, passed urine having the characteristic features of alkaptonuria on three successive days, during an attack of gastro-enteric catarrh with diarrhœa and vomiting. The phenomenon did not recur during convalescence. A chemical examination of the urine was made by Professor Siegfried, and, although conclusive analytic data are not furnished, there is practically no doubt as to the nature of the abnormal substance present.

W. von Moraczewski's patient (No. 19) was a woman aged 43, whose urine was of natural appearance on her admission to hospital with phthisis and tubercular peritonitis. Alkaptonuria developed shortly before death, and no history of previous alkaptonuria could be obtained.[1]

Geyger's case (No. 14), of which hardly any clinical details are given, was very remarkable. A diabetic man passed alkapton urine on one day only, and the diagnosis was fully confirmed by chemical examination. The abnormal constituent was afterwards sought for in vain for weeks, but we learn from a statement of Embden's that it was again found on a subsequent occasion after the sugar had disappeared from the urine.

In Slosse's case (No. 16) the development of alkaptonuria apparently coincided with an aggravation of the patient's malady. The patient was a woman, whose age is not stated, who suffered from pyonephrosis for which an operation was performed. The case ended fatally.

Maguire's case (No. 6) is in some respects unique. The patient, a female, who had suffered from gastric ulcer two years previously, developed alkaptonuria at the age of 27, six months before Maguire's examination of the

[1] Galloway recently mentioned, in a discussion at the Dermatological Society of London, the case of a woman, aged about thirty-five, who developed what was in all probability alkaptonuria as a præmortal symptom in the course of general desquamative dermatitis. The urine reduced Fehling's solution, yielded no osazone, and developed a brownish tint on exposure to air.

urine was made. Apparently its development did not coincide with any illness, and when developed it continued at any rate for months.

The Chemistry of Alkaptonuria.

Bödeker, who in the year 1859 first described alkaptonuria, isolated the peculiar reducing substance in a crude condition, and, on account of its behaviour with alkalies, assigned to it the name of alkapton (alkali κάπτειν). He believed it to be a nitrogenous compound. In Bödeker's case, as in one subsequently recorded by Geyger, sugar was also present. Although, as the result of the later researches of Kirk, Wolkow and Baumann, Huppert and others, we now possess a far more accurate knowledge of the chemistry of the condition, the name "alkaptonuria" persists, and is likely to persist, as a convenient designation for the anomaly which Bödeker described.

In 1875, Ebstein and Müller, whose patient was an infant, concluded from the reactions of the urine that the reducing substance present was pyrocatechin, and Fleischer arrived at a similar conclusion from his investigation of an adult case.

In 1882, Walter Smith, who examined the urine of an alkaptonuric child, concluded that the abnormal constituent was not pyrocatechin but more probably protocatechuic acid.

In 1886, Kirk, who had the opportunity of studying two cases in a family of which a third member had also been alkaptonuric, isolated a substance which he then called urrhodic acid, but which he subsequently showed (1888) to contain two distinct substances, to which he assigned the names of uroleucic and uroxanthic acids respectively. Ultimate analyses of uroleucic acid led him to assign to it the formula $C_9H_{10}O_5$.

In 1887, Marshall isolated from an alkaptonuric urine,

by a different process, a crystalline acid which he called glycosuric acid.

In 1891, Wolkow and Baumann extracted from a similar urine a crystalline acid to which the reducing action of the urine in question was apparently wholly due, and to which they assigned the name of homogentisinic acid. Analyses showed that it had the formula $C_8H_8O_4$, and it was apparent, from an elaborate study of its reactions and decompositions, that it must be regarded as hydroquinone-acetic acid. They devised a method for its quantitative estimation in which advantage was taken of its power of reducing ammoniacal silver nitrate in the cold. Baumann and Fränkel afterwards synthesised homogentisinic acid from gentisinic aldehyde.

We learn from Embden that Baumann obtained homogentisinic acid from some of Marshall's material, and Huppert, whilst confirming Kirk's observations as regards the presence of uroleucic in his cases, found that in some crude material sent to him by Kirk homogentisinic acid was present in still larger amount than uroleucic.

From his investigation of uroleucic acid Huppert arrived at the conclusion that of two possible compounds corresponding to the empirical formula arrived at by ultimate analysis of this substance, viz. trioxybenzene-propionic acid and dioxybenzene-lactic acid, uroleucic acid is in all probability the latter.

Geyger, who was unacquainted with Baumann and Wolkow's results, then recently published, obtained an acid which he regarded as identical with the glycosuric acid of Marshall, but the results of analyses of the lead salt which he gives agree very well with those of homogentisinate of lead. All other subsequent observers who have investigated cases of alkaptonuria have demonstrated the presence of homogentisinic acid, and have supported their identification of this substance by evidence of varying degrees of cogency.

Thus we find two distinct aromatic acids, homogentisinic and uroleucic acids, occurring in the urine of

alkaptonuric subjects. Of these, homogentisinic acid has been found in every case in which it has been looked for, and has been shown to have been present in some of the cases described before the classical research of Baumann and Wolkow. Uroleucic acid, on the other hand, has hitherto been met with only in Kirk's cases, but, except in Baumann's case, its presence does not appear to have been excluded. It is not improbable that this acid, which resembles homogentisinic acid in its properties, and which is much less readily detected, may have been present in other urines besides those examined by Kirk, and it is desirable that all future cases should be investigated with a view to demonstrating or excluding its presence.

The Nature of Alkaptonuria.

Our knowledge of the nature of the anomalous process which leads to the excretion of the alkapton acids in these rare instances is still scanty, but Wolkow and Baumann demonstrated the remarkable fact that the administration of tyrosin, by the mouth, to an alkaptonuric individual greatly increases the excretion of homogentisinic acid in the urine, although tyrosin given by the mouth does not cause alkaptonuria in a healthy individual. Indeed, by far the greater part of the tyrosin given to their patient reappeared in the urine in the form of homogentisinic acid. Embden repeated this observation upon a second patient, a sister of the preceding one, and obtained a similar, although not quite so conspicuous, increase.

In both patients, as in Ogden's and Stange's cases also, a meat diet conspicuously increased the excretion of homogentisinic acid.

Wolkow and Baumann produced alkaptonuria in a dog by the administration of 4·5 grammes of pure homogentisinic acid by the mouth, but a considerable portion of the acid administered was apparently broken up into

tolu-hydroquinone and carbonic acid, and there was a conspicuous increase of the aromatic sulphates in the urine. Embden's experiments upon himself showed that 4 grammes of homogentisinic acid taken by the mouth did not appear at all in the urine, whereas 8 grammes caused a temporary alkaptonuria and intolerable dysuria, but only a small proportion of the amount taken appeared in the urine. In the case of his alkaptonuric patient, on the other hand, 75 per cent. of a dose of homogentisinic acid (10 grammes) given by the mouth reappeared in the urine.

Unlike tyrosin, phenyl-acetic and phenyl-amido-acetic acids when administered to Embden's patient caused no increased excretion of homogentisinic acid. Ogden obtained a similar negative result with benzoic acid.

The above observations seem to show conclusively that tyrosin is the parent substance of the homogentisinic acid in the urine, but, as Wolkow and Baumann point out, beyond the fact that they are both aromatic acids these two substances have nothing in common, and the conversion of the former into the latter involves a shifting of the hydroxyl groups upon the benzene ring, in other words a simultaneous oxidation and reduction within the molecule. Such a change is, as they further point out, unknown as a metabolic process within the tissues, whereas it is observed as the result of fermentative changes. Hence, by a line of argument for which I must refer my readers to the original paper, they arrive at the conclusion that it is probable that alkaptonuria is due to the bacterial activity in the intestines, and perhaps to the presence of a special bacterion in such cases. The difficulty presented by the rarity of the condition they meet by a comparison with that known as *Diaminuria*, a condition almost equally rare and which also may persist for long periods. Embden and several more recent observers have initiated experimental investigations with a view to testing the correctness of this theory, but hitherto the results obtained, if

not directly opposed to Baumann and Wolkow's view, have failed to lend it any support.

Neither Baumann and Wolkow nor several more recent observers have succeeded in detecting any trace of the alkapton acids in the fæces, even in the diarrhœal stools after castor oil, which may be supposed to represent the contents of the upper portions of the intestine.

Embden, Ogden and others have tested the effects of various intestinal antiseptics, including β-naphthol, oil of turpentine and kefyr, but although conspicuous diminution of the ethereal sulphates of the urine was observed, the excretion of homogentisinic acid remained unaltered.

Culture experiments from the ordinary stools and from those passed after the administration of castor oil have yielded negative results, no alkapton acid being formed either by cultures in broth, meat juice or tyrosin broth.

It must therefore be acknowledged that the weight of evidence is at present opposed to the views of Wolkow and Baumann; and Embden's observation, that when an alkaptonuric individual took homogentisinic acid by the mouth a far larger proportion reappeared in the urine than when the same substance was administered to a healthy individual, would seem to point to abnormal metabolism in the tissues, in spite of the difficulty of reconciling the chemical processes here involved with the known facts of tissue-metabolism.

Stier obtained from the abundant aural wax of his patient a substance which had the properties of an alkapton acid, but no other observations which are recorded point to the occurrence of homogentisinic acid elsewhere than in the urine. That it is absent from the fæces has already been mentioned, and Fürbringer failed to obtain the characteristic reactions in the pericardial effusion, decolourised blood, or in a watery extract of the kidneys in one of the earliest observed cases.

I must here mention that it has been repeatedly observed that alkapton urines do not deposit crystals of uric acid even after the addition of an acid, and some

observations by Embden, Ogden, and Stange, seemed to show that in these cases the excretion of uric acid is conspicuously diminished. As all silver methods are inapplicable to these urines, the observers mentioned employed Fokker's method for the estimation of uric acid.

More recent estimations by Futcher, Stier, and Noccioli and Domenici, who all employed the more accurate method introduced by Hopkins, showed no such diminution, and an observation of my own points in the same direction, as will be presently shown.

Stier's observations are specially conclusive, as he made daily comparative estimations of the uric acid excretion of an alkaptonuric boy, aged 8, and that of his brother, aged 9, whose urine was normal. Sometimes the one and sometimes the other passed the larger amount of uric acid.

Moreover, alkapton urines *may* deposit crystals of uric acid which are deeply stained by the brown pigment. Ebstein and Müller noticed this and Stange observed crystals on some days and not on others; again, one of the urines which I have examined deposited dark brown crystals of uric acid on standing.

After this preliminary summary of the present state of our knowledge of alkaptonuria I will proceed to the description of my own observations.

Case 1. Thomas P— came under Dr. Voelcker's care, at the Hospital for Sick Children, at the age of three months. He was brought to the hospital on account of the peculiar appearance of his urine, which acquired a deep reddish-brown colour and stained the napkins deeply. The stains became developed on exposure to air. The mother stated that the woman who nursed her in her confinement called her attention to the staining of the napkins on the day following the birth of the child.

At that time the urine and the stains had a much redder or more purple colour than they have at present (the child being now more than a year old), and a similar

change of tint after the earliest months of life was observed by Ebstein and Müller in their case.

The child was fed entirely by the breast to the age of ten months, was fairly well nourished and exhibited no impairment of his general health. He was stated to have suffered from inflammation of the lungs when two months of age. He is the youngest of four children ; the mother is fairly strong, but the father, a drayman, is delicate and complains of his chest. No other case of similar peculiarity of the urine has occurred in the family, and I have examined the urine of the three elder children with negative results.

After weaning, at the age of ten months, the child was admitted as an in-patient, and I am indebted to Dr. Barlow for kindly allowing him the use of a cot in his ward.

After a stay of three weeks in hospital the child was discharged, but was shortly readmitted with a severe attack of summer diarrhœa which at one time threatened to prove fatal, but from which he made a good recovery, and has since enjoyed excellent health, save for troubles incidental to teething.

Careful inquiry from the mother failed to elicit any account of staining of the body clothing of the patient, such as might be expected to occur if homogentisinic acid had been present in the sweat, and no such staining was observed in the hospital, although the urine stained deeply any fabric wetted with it.

The fæces were repeatedly examined, but neither alcoholic nor watery extracts ever gave any alkapton reactions. Dr. Drysdale, who was good enough to make a bacteriological examination of a specimen of fæces obtained just before the child left the hospital, reports as follows:— "Gelatine and agar cultures were made from the fæces, but very few organisms grew. In the second and third dilutions only one organism grew. This was a diplococcus which grew slowly on gelatine, forming round, raised colonies of a Chinese-white colour. It grew

equally well under aërobic and anaërobic conditions. It did not form gas nor coagulate milk. When grown in ordinary broth to which tyrosin had been added no substance yielding the alkapton reactions was formed either in aërobic or anaërobic cultures. No animal experiments were performed. This diplococcus is not, in my experience, a familiar organism of fæces; its predominance in the cultures was remarkable, but, as only one specimen was examined, its presence may have been accidental."

Cultures made on a subsequent occasion showed only the ordinary intestinal organisms with an abundant growth of *Bacillus coli communis*.

The urine of the patient is unusually scanty in amount, always acid in reaction, and highly concentrated. On some occasions the specific gravity has been as high as 1035. When the urine is freshly passed its colour is natural, but it quickly darkens, assuming a deep brown tint. It reduces Fehling's solution with the aid of heat, but the reduction is capricious and is considerably masked by the blackening due to the alkaline reagent. Alkalies greatly intensify the brown colour. Ammoniacal silver solution is readily reduced in the cold. The urine does not rotate the polarised ray in either direction, and no reduction of specific gravity or evolution of gas is produced by yeast. The bismuth test for sugar gives a negative result.

Micturition, though scanty and unusually frequent, is apparently not painful.

The youth of the patient precludes for the present any systematic analyses of the daily excretion, but I hope to make such observations at a later period.

No uric acid crystals are deposited from the urine on standing, even after the addition of an acid, but the amount of uric acid excreted is certainly not unduly small. On one occasion, when almost the entire quantity of urine passed during twenty-four hours was collected, 100 c.c. were found by Hopkins's method to contain 0·08075 grm. of uric acid. As the total amount of urine

was only 120 c.c., this corresponds to a minimum day's excretion of 0·0969 grm. of uric acid.

From a healthy child, aged one year, 400 c.c. of urine were collected in twenty-four hours, and the total amount of uric acid contained therein was found to be 0·063 grm.

For the detection of homogentisinic acid in the urine two different methods were employed. Firstly that of Wolkow and Baumann, as modified by Ogden, which may be briefly summed up as follows:[1] Acidification of the urine with dilute sulphuric acid; repeated extraction with equal volumes of ether; distillation of the ether; solution of the syrupy residue in water, and addition to the solution when heated nearly to boiling of a concentrated solution of basic lead acetate. On cooling, acicular or prismatic crystals of lead homogentisinate are deposited and continue to form for at least twenty-four hours.

On applying this method to the urine in question a rich formation of crystals took place, which had the characteristic form, and were, as is always the case, tinted by included pigment. When the crystals were dissolved in hot water the solution gave the alkapton reactions, and yielded, as solutions of homogentisinic acid should do, a transitory dark blue colour with a dilute solution of ferric chloride.

0·1923 grm. of the crystalline product lost, at 100° C., 0·0175 grm. of water of crystallisation = 9·10 per cent. The calculated loss for the formula $(C_8H_7O_4)^2Pb.3H_2O$ = 9·08 per cent.

Again, 0·2268 grm. of the dehydrated crystals yielded, after repeated treatment with sulphuric acid in a platinum crucible to constant weight, 0·1263 grm. of lead sulphate, corresponding to 38·03 per cent. of lead; whereas the calculated percentage of lead in dried lead homogentisinate is 38·25.

A specimen of the lead salt was decomposed, under water, by means of a stream of sulphuretted hydrogen,

[1] For details of the process I must refer my readers to the original papers.

and, after the excess of sulphuretted hydrogen had been boiled off, the filtrate was evaporated to dryness at a low temperature. Crystals of homogentisinic acid were obtained which were purified by re-crystallisation from ether. The colourless silky prisms of the free acid were dried in the exsiccator, which caused them to become white and opaque, and were found to melt between 145° and 146° C. (The melting point of homogentisinic acid is 145° to 147°.)[1]

The free acid is somewhat hygroscopic and, in order to obtain satisfactory determinations, it requires to be thoroughly dried, as otherwise the presence of water materially lowers the melting point.

The above observations showed beyond all question that homogentisinic acid was abundantly present in the urine of Thomas P—.

Considerably larger yields of lead homogentisinate were obtained by an extremely simple method of extraction, which I have described in a recent number of the 'Journal of Physiology.' I found that when the urine itself was heated nearly to boiling, without any preliminary treatment, and for each 100 c.c. at least five or six grammes of solid neutral lead acetate were added, after filtering off the bulky precipitate which was formed, the clear yellow filtrate deposited crystals of lead homogentisinate on standing.

The formation of crystals under these circumstances usually commences within two or three hours, and is complete in twenty-four hours. Cold greatly hastens the separation, but does not materially increase the yield.

By this simple process the urine of Thomas P— yielded fairly constantly about 0·5 grm. of the lead salt for each 100 c.c. In applying the process to urines less rich in homogentisinic acid larger quantities of lead acetate may require to be added, and in summer weather artificial

[1] I am indebted to Dr. Orton, Demonstrator of Chemistry at St. Bartholomew's Hospital, for the loan of apparatus, and for kindly checking the various determinations of melting points.

cooling will perhaps be necessary to start crystallisation.

A comparison with the urine of a second patient showed that homogentisinic acid was present in this urine in unusually large amount, although owing to the scanty excretion the daily total was not remarkable. A specimen in which the reducing power was estimated by Wolkow and Baumann's silver method was found to reduce a quantity of silver nitrate equivalent to 0·0577 grm. of homogentisinic acid per 10 c.c., and this also is an unusually high figure. However the reducing power of uric acid introduces, as Morner has shown, a considerable error, and, as has been mentioned, this urine was by no means deficient in uric acid.

It remained to be ascertained whether or no the uroleucic acid of Kirk was also present in this urine. In searching for it I followed a plan similar to that recommended by Professor Huppert in the tenth edition of Neubauer and Vogel's work, and would take this opportunity of thanking Professor Huppert, not only for his kind replies to questions, but also for some valuable suggestions derived from his most recent observations which are not yet published.

Professor Huppert's plan is based upon the fact that uroleucic acid is not thrown down as a lead salt by the addition of such quantities of lead acetate as are employed in the processes described above, although Kirk succeeded in obtaining the lead salt by rubbing up an excess of neutral lead acetate with the solution until a thin paste was formed.

Any uroleucic acid which may be present is therefore to be looked for in the mother liquor from which the crystalline lead homogentisinate has been deposited. Unfortunately the precipitation of homogentisinate is not *complete* when either Wolkow and Baumann's or my simple method is employed, and consequently a complete separation of the two acids cannot be brought about by such means.

Uroleucic, like homogentisinic, acid gives the various alkapton reactions, but with ferric chloride a green instead of a blue colour appears, and uroleucic acid melts at 130° to 133° C., whereas the melting point of homogentisinic acid is 145° to 147° C. If, then, the remainder of the alkapton acid can be extracted from the mother liquor, the melting point will supply a means of detecting uroleucic acid, seeing that the melting point of a mixture of the two acids should be lower than that of either of the constituents of the mixture.

The chief difficulty met with is in the adequate purification of the small specimens of residual acid obtained, for the crystals obtained by evaporation of the ethereal extracts are always embedded in a brown syrupy material which is taken up by the same solvents as the alkapton acids.

In my earlier attempts extracts obtained by Baumann and Wolkow's method were employed. I found that even when all crystallisation had ceased and further addition of basic lead acetate caused no more crystals of lead homogentisinate to form, the mother liquor, which should contain any uroleucic acid present, still contained traces of alkapton acid, but it still gave with ferric chloride a dark blue colour, whereas uroleucic acid is described as yielding a transitory green tint with that reagent. After removal of the lead by means of a stream of sulphuretted hydrogen, and evaporation of the filtrate at a low temperature, no adequate amount of crystalline acid was obtained for a satisfactory purification and determination of the melting point.

The filtrate obtained in the simple process, after lead homogentisinate had ceased to crystallise out, was next treated as follows :—Sulphuric acid was added in sufficient quantity to precipitate the excess of lead in the form of sulphate and to leave the filtrate acid ; the acidified liquid was then repeatedly shaken with equal volumes of ether ; the ether was distilled off, and the brown syrupy residue was placed in a small dialyser immersed in water

with a view to separate the alkapton acid from the brown syrupy material with which it is mixed. The watery solution so obtained gave a dark blue reaction with ferric chloride, and on evaporation at a low temperature left a very scanty crystalline residue which was purified to some extent by re-crystallisation from ether. The crystals so obtained, although by no means satisfactorily pure, did not melt below 140°, whereas the melting point of uroleucic acid is 130° to 133° C., and a mixture of the two acids should have a still lower melting point. Hence it was concluded that the urine of Thomas P— did not contain uroleucic acid in any considerable quantity, or that if this acid were present it was only in very minute amount as compared with homogentisinic acid, and, indeed in quantities too minute to be detected by the methods employed in such small volumes of urine as were here available. The small ultimate residue apparently consisted of homogentisinic acid which has escaped precipitation as the lead salt.

Case 2.—The patient is a school-boy, aged 14, in good health, who has been alkaptonuric from infancy. He is a brother of the patient whose case was investigated by Drs. Armstrong and Walter Smith, of Dublin, in 1882, of whom the latter arrived at the conclusion that the abnormal urinary constituent was probably protocatechuic acid. The birth of the present patient and the fact that he had alkaptonuria were mentioned by Dr. Kirk in one of his papers.

By the kindness of Dr. C. E. Baker I was supplied with some three litres of the urine, but have had no opportunity of making any quantitative estimations of the excretion on successive days.

The urine had all the characteristic features already described, was acid in reaction, and had a specific gravity of 1025. On standing, even without the addition of an acid, deep brown crystals of uric acid were deposited. As estimated by Baumann's silver process,

10 c.c. of the urine had a reducing power equivalent to that of 0·0363 grm. of homogentisinic acid, *i. e.* about half the reducing power of that of Thomas P—.

By Wolkow and Baumann's method crystalline lead homogentisinate was readily obtained.

The urine also yielded crystals of this salt by the simple method above described, the yield being about half that obtained in the previous case.

The following figures were obtained with the product of the simple process, and they not only afford conclusive proof that here again one was dealing with lead homogentisinate, but they also show that the product obtained by my process is no less pure than that extracted by the process of Wolkow and Baumann :—

0·3127 grm. of the salt lost at 100° C. 0·0288 grm. of water of crystallisation = 9·21 per cent. (calculated = 9·08 per cent.)

0·2710 grm. of the dehydrated crystals yielded 0·1510 grm. of lead sulphate, equivalent to 38·05 per cent. of lead (calculated = 38·25 per cent.).

No special examination of free homogentisinic acid from the lead salt extracted from this urine was carried out, but a specimen obtained from a mixture of the lead salts from this and the previous case, in each instance extracted by my method, melted between 146° and 147°.

The mother liquor from which the crystals had been deposited (simple process) was treated as in the previous case, viz. by the addition of sulphuric acid, filtration from the precipitate of lead sulphate, and repeated extraction with ether. In the residue left after distilling off the ether a considerable mass of crystals of free acid formed, and these were transferred to a porous tile, which absorbed nearly all the coloured syrup and left the crystals nearly pure. After drying over sulphuric acid a specimen melted at 143° C., thus behaving as a slightly impure specimen of homogentisinic acid.

Seeing that uroleucic acid, if present in the urine,

should have constituted at least a large part of this scanty final product, and should have manifested its presence by a conspicuous lowering of the melting point, it seemed evident that this acid was not present in this urine in any appreciable amount.

Case 3.—The specimens of urine which Dr. Pavy was kind enough to hand over to me for examination were passed by three members of the family already referred to. They had been standing in bottles for no less than eight years, were alkaline in reaction, and were for the most part almost black in colour. It was evident that in the lapse of time a considerable part of the alkapton acid originally present in them had undergone oxidation. However, the examination of these specimens promised to be of considerable interest, as one hoped that it might still be possible to detect the presence of homogentisinic acid in them.

The first specimen was passed by a youth aged 18 (Case 31 in the Table); 300 c.c. of the urine were available, consisting of a darker and a paler portion. These were mixed together, filtered from a bulky black sediment which had formed, and treated according to Wolkow and Baumann's process.

The addition of sulphuric acid caused much effervescence. From the aqueous solution of the residue from the ethereal extracts a crop of microscopic acicular crystals was deposited, apparently consisting of lead homogentisinate. The solution itself showed a transitory dark blue colour with ferric chloride. 0·2349 grm. of the crystalline deposit lost, at 100° C., 0·0152 grm. of water of crystallisation = 9·04 per cent., which agrees closely with the calculated figure, 9·08 per cent. 0·3042 grm. of the dried lead salt yielded 0·1718 grm. of lead sulphate = 38·57 per cent. lead. This too high percentage was probably due to incomplete removal of lead acetate. The estimation could not be repeated for lack of material.

It was, however, evident that, although so long kept, the urine still contained homogentisinic acid.

Case 4.—This was another of Dr. Pavy's cases (No. 30 in the Table). About 300 c.c. of this urine, which also consisted of a darker and paler portion and was alkaline in reaction, yielded by Wolkow and Baumann's method a fair crop of crystals, which had all the appearance of those of lead homogentisinate. The mother liquor yielded a transitory dark blue colour with ferric chloride.

0·3085 grm. of the crystals lost, at 100° C., 0·0282 grm. of water of crystallisation = 9·14 per cent.

The lead salts from both this and the previous specimen melted, with complete blackening, at about 215° C., which is given as the melting point of lead homogentisinate, but the exact temperature at which melting occurs is very difficult to determine, and the change which takes place at about 215° appears to be rather of the nature of a decomposition than simple fusion.

Here also the presence of homogentisinic acid was beyond doubt.

Case 5.—The greater part of the specimen from this case (No. 29 in the Table) was unfortunately lost, and although from the small amount remaining a small quantity of crystalline lead salt was obtained, this did not suffice for any satisfactory confirmatory tests, and therefore no safe conclusions can be drawn from the incomplete examination, although the appearance of the crystalline deposit left very little doubt that here also homogentisinic acid was present.

Leaving aside this fifth case, I am thus able to add four fresh cases of alkaptonuria to the list of those in which homogentisinic acid has been found in the urine.

These observations lend additional support to the view that homogentisinic acid is a constant constituent of

alkapton urines, and plays the chief part in the production of alkaptonuria. It has indeed been found in every case in which it has been specially looked for, and even in some of the cases examined before the publication of Wolkow and Baumann's researches re-examination of the material has led to its detection.

On the other hand, in two of the cases here dealt with, I endeavoured to ascertain whether uroleucic acid was also present, in both instances with negative results. Wolkow and Baumann equally failed to find any other reducing substance than homogentisinic acid in the urine of their patient, but in many of the cases since recorded no special search for uroleucic acid would appear to have been carried out.

It seems probable that uroleucic acid is rather of the nature of a bye-product, and that the urines examined by Kirk were peculiar in containing considerable quantities of this substance in addition to homogentisinic acid which, as Huppert has shown, was present in them in even larger amount.

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SERIES I.—*Published Cases.*

Date of publication and number.	Sex.	Age at time of observation	Duration of alkaptonuria.	Family history.	Associated maladies, &c.	Results of chemical examination of urine.	Observer.
1859 1	M.	44	Not stated	Not mentioned	Carcinoma of vertebral column	"Alkapton." Sugar also present	Bödeker
1875 2	M.	29	„	„	Phthisis pulmonalis	Alkapton	Fürbringer
1875 3	M.	4–15 months	First noticed in second week of life	„	Noticed after icterus neonatorum	Pyrocatechin	Ebstein and Müller
1875 4	M.	—	Not stated	„	Fractured rib and other injuries	„	Fleischer
1882 5	F.	3 years	Life-long	Sister of No. 26	— —	Protocatechuic acid	Armstrong. Walter Smith
1884 6	F.	27	Noticed at age of 27	Not mentioned	Gastric ulcer two years previously	Probably protocatechuic acid	R. Maguire
1886 7	M.	8	Life-long	Brother of Nos. 8 and 9. An elder brother, aged 10, not alkaptonuric	Healthy	From the urine of these patients Kirk isolated two acids, which he named uroleucic and uroxanthic respectively.	R. Kirk
1886 8	M.	—	„	Brother of Nos. 7 and 9	Died at age of three years of whooping cough	Huppert extracted homogentisinic and uroleucic acids from Kirk's material (1897)	„
1886 9	M.	Infant	„	Brother of Nos. 7 and 8	Healthy	— —	„
1887 10	M.	—	Not stated	Brother of No. 22	Insurance case	Glycosuric acid — homogentisinic acid found by Baumann (quoted by Embden). Probably uroleucic acid, also (Huppert)	John Marshall
1886–7 11	M.	Young	„	Not mentioned	„	No protocatechuic acid—a crystalline acid obtained	Barton-Brune

1891 12	M.	68	Life-long	Brother of No. 13	Carcinoma of prostate	Homogentisinic acid first demonstrated in this case	Baumann and Kraske. Wolkow and Baumann.
1892 13	F.	60	„	Sister of No. 12	Morbus cordis	Homogentisinic acid	Embden
1892 14	M.	—	Observed on a single day—recurred later (Embden)	Not mentioned	Diabetes	Glycosuric acid. The analytical figures agree with those of homogentisinic acid. Sugar also present	A. Geyger
1892 15	M.	—	Long standing	„	Regarded as diabetes	Homogentisinic acid, not sugar	Garnier and Voirin
1895 16	F.	—	Appearance concurrent with aggravation of malady	„	Pyonephrosis—operation—death	Homogentisinic acid	A. Slosse
1895 17	M.	45	Unknown	No other known case in family	Healthy	„	H. V. Ogden
1896 18	M.	18	Life-long, but apparently intermittent	No other case in family	Herpes of p nis. Dysuria	„	P. Stange
1896 19	F.	43	Apparently as a præmortal symptom	Not mentioned	Phthisis and tubercular peritonitis	„	W. von Moraczewski
1897 20	M.	About 50	Probably long standing	„	Had previously suffered from sciatica and facial neuralgia	„	G. Denigès
1897 21	F.	17	For 3 days only during an attack of gastro-intestinal catarrh	No other case in family	Gastro-intestinal catarrh. No previous illness, except measles	„	C. Hirsch
1898 22	M.	57	Not stated	Brother of No. 10	Insurance case	Not yet published	T. B. Futcher
1898 23	M.	8	Life long	No other case in family, only brother, aged 9, not alcaptonuric	Healthy	Homogentisinic acid	Ewald Stier
1898 24	F.	47	Life long	No other case in family	Inguinal hernia—operation.	„	Noccioli and Domenici

SERIES II.—*Unpublished Cases.*

Number.	Sex.	Age at time of observation.	Duration of alkaptonuria.	Family history.	Associated maladies, &c.	Results of chemical examination of urine.	Observer.
25	M.	3 months onwards	Noticed on second day of life	No known case in family — brothers and sister not alkaptonuric	Healthy. Inflammation of lungs at 2 months; diarrhœa at 10 months	Homogentisinic acid; uroleucic acid not found	Dr. Voelcker's case.
26	M.	—	Life-long	Brother of No. 5. 3rd child	Healthy	,,	Dr. Baker's case.
27	M.	30	,,	No known case in family	Sacro-iliac and lumbar pain; floating kidney	— —	Dr. Pavy's case.
28	F.	30	,,	9th of 14 children. Sister of Nos. 29, 30, and 31	Healthy	Homogentisinic acid—proof incomplete	,,
29	M.	—	,,	11th child. Brother of Nos. 28, 30, and 31	Healthy	— —	,,
30	M.	22	,,	13th child. Brother of Nos. 28, 29, and 31	Urine supposed to contain sugar	Homogentisinic acid	,,
31	M.	18	,,	14th child. Brother of Nos. 28, 29, and 30	Refused at an Insurance Office as glycosuric	,,	,,

(For report of the discussion of this paper, see 'Proceedings of the Royal Medical and Chirurgical Society,' Third Series, vol. xi, p. 133.)