

CHAPTER IV

ALCAPTONURIA

Of inborn errors of metabolism alcaptonuria is that of which we know most, and from the study of which most has been learnt. In early life it is a trifling matter, inconvenient rather than harmful, which only attracts attention because an infant stains its clothing or the urine has a peculiar appearance. As the years go on the cartilages become blackened, giving a blue tint to the hollows of the ears, brown marks develop on the conjunctivæ, and there is a great tendency to osteo-arthritic and osseous lesions. The medical man needs to be aware of its existence, and to be acquainted with the methods for its recognition, lest he mistake it for troubles of graver kinds ; but for the chemical physiologist and pathologist it is one of the most interesting of metabolic abnormalities. Not only has the study of alcaptonuria thrown much light upon the fate of the aromatic fractions of the proteins of the food and tissues, but has also helped materially to reveal a fact of far wider significance—namely, that for the individual protein fractions special metabolic paths have been evolved.

When freshly passed the urine of an alcaptonuric seldom exhibits any abnormality of tint, but it soon begins to darken in contact with the air.

This darkening, which is associated with absorption of oxygen, begins at the free surface of the liquid, and passes through various shades of brown to actual blackness. In some cases the urine, especially of infants, acquires a red rather than a brown hue. Alkalinity of reaction greatly hastens the change. Linen and woollen fabrics moistened

with the urine become stained as by a photographic developer. On boiling the urine with Fehling's solution a deep brown colour develops and copious reduction occurs, but the browning of the liquid in which the orange precipitate is suspended gives to the test a quite peculiar appearance which should be recognized by any one who has once seen it. An ammoniacal solution of silver nitrate is rapidly reduced even in the cold. On heating the urine with Nylander's solution a darkening is produced by the alkaline reagent, but no reduction of bismuth is brought about. With Millon's reagent a yellow precipitate is formed. The most striking reaction is observed when a dilute solution of ferric chloride is allowed to fall into the urine drop by drop. The fall of each drop is followed by the appearance of a deep blue colour, which lasts but for a moment, and the phenomenon is repeated until oxidation is complete. With yeast no fermentation occurs, and the polarized ray is not rotated either to the right or left.

Our knowledge of alcaptonuria is dated from the year 1858, in which year Bödeker¹ detected, in the urine of a patient with glycosuria, a second reducing substance, not a sugar, to which, on account of its behaviour towards alkalis, he assigned the name of 'alkapton', a bilingual word derived from alkali and *κάπτειν*. However, indications of the anomaly may be detected in much earlier medical writings. Thus there can be no doubt that the case of an infant who passed black urine, described by Alexander Marcet² in 1823, was of this nature. It is true that Marcet knew nothing of the reducing properties of the urine, but he describes accurately its darkening in colour on standing, the staining of napkins, and the effect of the addition of an

¹ *Zeitschrift für rationelle Medicin*, 1859, vii. 130. *Annalen der Chemie und Pharmacie*, 1861, cxvii, 98.

² *Transactions of the Royal Medical and Chirurgical Society*, 1823 xii. 37.

alkali; and he mentions that the condition was present from the earliest days of the child's life. Until the early years of the nineteenth century no distinction was drawn in medical writings between urines which were black when passed and such as darkened on exposure to air, but it is difficult to suggest any other diagnosis than that of alcaptonuria for some cases referred to in works of the sixteenth and seventeenth centuries, such as that mentioned by G. A. Scribonius³ (in 1584) of a schoolboy who, although he enjoyed good health, continuously excreted black urine, and that cited by Schenck⁴ (in 1609) of a monk who exhibited a similar peculiarity and stated that he had done so all his life. The most interesting record of this kind is to be found in the work of Zacutus Lusitanus,⁵ published in 1649. The patient was a boy who passed black urine and who, at the age of fourteen years, was submitted to a drastic course of treatment which had for its aim the subduing of the fiery heat of his viscera, which was supposed to bring about the condition in question by charring and blackening his bile. Among the measures prescribed were bleedings, purgation, baths, a cold and watery diet, and drugs galore. None of these had any obvious effect, and eventually the patient, who tired of the futile and superfluous therapy, resolved to let things take their natural course. None of the predicted evils ensued, he married, begat a large family, and lived a long and healthy life, always passing urine black as ink.

That alcaptonuria is a very rare anomaly admits of no question, and many medical men of large experience have never met with it. Of its occurrence in several members of a family, and of its mode of incidence, I have already written at sufficient length in an earlier chapter. In the great

³ *De Inspectione Urinarum*, 1584, p. 50.

⁴ *Observationes Medicæ*, 1609, Lib. iii, 558.

⁵ *Praxis Medica Admiranda*, 1649, Lib. iii, cap. 134.

majority of instances it is present from birth and persists throughout life, but has been said to have been developed as a temporary morbid sign in a very few exceptional cases.

The substance which Bodeker isolated from the urine of his patient, and which he called 'alkapton', contained nitrogen and was obviously an impure material. In some cases afterwards recorded the abnormal constituent was thought to be pyrocatechin⁶ and in others protocatechuic acid.⁷ Marshall⁸ obtained from the urine of his patient a substance which he named glycosuric acid, and R. Kirk,⁹ investigating a group of cases in a single family, isolated an acid which he called uroleucic acid, which yielded on analysis percentages of carbon and hydrogen conforming closely to the requirements of the formula $C_9H_{10}O_5$. These two investigators, Marshall and Kirk, approached very nearly to the recognition of the actual nature and composition of the abnormal constituent. There are no sufficient grounds for supposing that the reducing substances present in these earlier cases were different from that found in all the more recent ones, and where re-examination of the urine, or of material extracted therefrom, has been possible the presence of homogentisic acid has since been demonstrated.

Homogentisic acid, the excretion of which is the essential feature of the alcaptonuria, was isolated, analysed, and fully investigated by Wolkow and Baumann,¹⁰ as is set forth in their classical paper, published in 1891, some years later than the investigations of Marshall and Kirk. It was shown to have the empirical formula $C_8H_8O_4$; and the work of its discoverers, which has been confirmed by

⁶ Ebstein und Müller, *Virchow's Archiv*, 1875, lxii. 554.

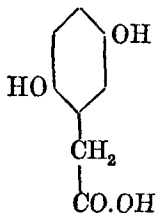
⁷ Fleischer, *Berliner klinische Wochenschrift*, 1875, xii. 529, 547.
W. Smith, *Dublin Journal of the Medical Sciences*, 1882, lxxiii. 465.

⁸ *Medical News*, Philadelphia, 1887, 1. 35.

⁹ *Journal of Anatomy and Physiology*, 1889, xxiii. 69. *British Medical Journal*, 1888, ii. 232.

¹⁰ *Zeitschrift für physiologische Chemie*, 1891, xv. 228.

Huppert¹¹ and by syntheses effected in three different manners, by Baumann and Fränkel,¹² and Osborne,¹³ and Otto Neubauer and Flatow¹⁴ respectively, has definitely proved that its constitution is that of para-dioxy-benzene-acetic acid (hydroquinone-acetic acid).



The acid was originally isolated from the urine as a lead salt, which may be obtained by a far simpler method¹⁵ than that employed by Wolkow and Baumann. The urine having been heated nearly to boiling, five grammes of solid neutral lead acetate are added for each 100 cubic centimetres of urine taken. A dense precipitate forms which is filtered off whilst the liquid is still hot, and the clear yellow filtrate is allowed to stand in a cool place. After a time lead homogentisate begins to separate out in crystalline form, and after twenty-four hours the crystals are filtered off, washed, and dried. The free acid may be obtained by passing sulphuretted hydrogen through ether in which the powdered lead salt is suspended. When the solvent, freed from lead sulphide by filtration, is allowed to evaporate, colourless crystals of homogentisic acid are left, and these melt at 146° to 147° C.

As alternative methods of extraction that of Wolkow and Baumann, in which the concentrated urine, acidified with sulphuric acid, is repeatedly extracted with ether, and the

¹¹ *Deutsches Archiv für klinische Medicin* (Festschrift), 1899, lxiv. 129.

¹² *Zeitschrift für physiologische Chemie*, 1895, xx. 219.

¹³ *Journal of Physiology*, 1903, xxix; *Proc. Physiol. Soc.*, xiii.

¹⁴ *Zeitschrift für physiologische Chemie*, 1907, lii. 375.

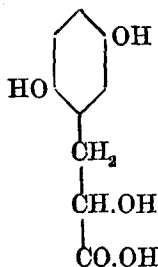
¹⁵ Garrod, *Journal of Physiology*, 1899, xxiii. 512.

lead salt is thrown down from an aqueous solution of the residue from the ethereal extracts; or that of Erich Meyer,¹⁶ which yields ethyl homogentisate, may be employed. Lastly, by benzylation of the urine the alcapton acid is obtained as di-benzoyl-homogentisamide.¹⁷

For the purpose of quantitative estimation of homogentisic acid in urine the volumetric method of Baumann¹⁸ is made use of. This somewhat tedious method is based upon the reduction of a decinormal solution of silver nitrate in the presence of ammonia, but it is necessary to employ a stronger solution (8 per cent.) of ammonia than that prescribed by Baumann (3 per cent.) in order to obtain complete reduction in the allotted period of five minutes.¹⁹

Solutions of homogentisic acid yield all the characteristic reactions of alcapton urines. They darken on exposure to air and more quickly when an alkali is added, reduce Fehling's solution on boiling, and ammoniacal silver nitrate in the cold, and yield a transient blue colour with ferric chloride.

In many accounts of alcaptonuria the statement will be found that in some cases there has been present in the urine, in addition to homogentisic acid, a second acid possessed of similar properties—viz. uroleucic acid, and that this substance is probably hydroquinone α -lactic acid.



¹⁶ *Deutsches Archiv für klinische Medicin*, 1901, lxx. 443.

¹⁷ Orton and Garrod, *Journal of Physiology*, 1901, xxvii. 89.

¹⁸ *Zeitschrift für physiologische Chemie*, 1892, xvi. 268.

¹⁹ Garrod and Hurtle, *Journal of Physiology*, 1905, xxxiii. 206.

This statement I believe to be grounded upon a misapprehension and to be incorrect, and the grounds for this belief have been fully set out in a paper written in conjunction with W. H. Hurttley.²⁰

It will be remembered that the name of uroleucic acid was assigned by Kirk to the material which he isolated from the urine of his patients at a time before homogentisic acid was known. The late Dr. Kirk never claimed that this was a second distinct alcapton acid, and, indeed, in a letter to me he expressed his opinion that his uroleucic acid was merely impure homogentisic acid. Kirk's substance, to which as the result of his analyses he assigned the formula $C_9H_{10}O_5$, melted at about $133.3^\circ C.$, whereas, as has already been mentioned, the melting-point of homogentisic acid is 146° to $147^\circ C.$ The analytical figures agreed very closely with the requirements of the above formula, and titration of a solution with alkali, under a layer of petroleum ether, gave a result which indicated a molecular weight corresponding to that of a monobasic acid of the above constitution. However, there is no room for doubt that Kirk's later surmise was correct, and that in spite of these coincidences the substance which he analysed was impure homogentisic acid. Thus we found that when Kirk's method of extraction was applied to an alcapton urine, in which a second acid had been sought for without success, the product obtained agreed with his description of the lead salt which he obtained, and the free acid isolated from the lead salt melted at 134° to $136^\circ C.$, but examination showed that it consisted mainly of homogentisic acid. It is known that the urines which Kirk examined contained this acid; Huppert obtained it from some of Kirk's original material, and we also found it in a further specimen of that material, blackened with age, but labelled 'uroleucic acid'. Moreover, when, in 1902, I was enabled by the kindness of

²⁰ *Journal of Physiology*, 1907, xxxvi. 136.

Dr. Kirk to examine fresh specimens of the urine of his patients, much homogentisic acid was obtained from them, but there was no indication of the presence of a second alcapton acid.

The view that the uroleucic acid of Kirk was a distinct substance had its origin in some investigations of the late Professor Huppert,²¹ carried out upon some of the original material sent to him in 1897. After separation of as much as possible of the homogentisic acid which it contained a residue remained which melted at 130.5° C., and this residue Huppert regarded as uroleucic acid. Further investigations led him to the conclusion that it was a derivative of hydroquinone and was probably hydroquinone α -lactic acid, which acid has the formula $C_9H_{10}O_5$ assigned by Kirk to uroleucic acid.

The accuracy of Professor Huppert's results does not admit of question, but it is evident that the scanty material at his disposal did not allow of a fresh analysis of the fraction of lower melting-point. If, as I believe, after careful comparison of Kirk's account of his investigations with Huppert's description of the material sent to him, and after personal examination of a further portion of the material, the substance examined was not a crude one, as Huppert supposed, but the actual uroleucic acid analysed by Kirk, it is obvious that the results of analyses of the material as a whole could not be applied to the fraction of lower melting-point. Otto Neubauer and Flatow,²² who have succeeded in effecting the synthesis of hydroquinone α -lactic acid, have shown that it differs from the supposed uroleucic acid in melting-point and in other respects; and it is a significant fact that with the exception of a minute residue, which melted at 133° C., but which only sufficed for the determination of the melting-point, obtained by

²¹ *Zeitschrift für physiologische Chemie*, 1897, xxiii. 412.

²² *loc. cit.*, sub 14.

Langstein and E. Meyer²³ from the urine of their patient, no indication of the presence of uroleucic acid has been found in any of the alcapton urines since described. Therefore the conclusion appears to be justified that no sufficient evidence is forthcoming of the occurrence in some alcapton urines of a second abnormal acid (uroleucic acid), a conclusion which has the advantage of making for simplicity.

Beyond the presence in it of homogentisic acid the urine of alcaptonurics shows no obvious deviation from the normal. Some earlier investigators described a conspicuous diminution of the uric acid output, but more recent work has failed to confirm this. In a number of cases the excretion of uric acid has been found not to be below the average, and crystals of uric acid stained by the brown pigment are not infrequently deposited from such urines. We may therefore confine our attention to the consideration of the parent substances and mode of origin of homogentisic acid in the human organism.

Seeing that there is no evidence that synthesis of the benzene ring ever occurs in the animal economy, Wolkow and Baumann looked to the proteins of the food and tissues as the most likely sources of the alcapton acid, and to the aromatic fractions which proteins contain—viz. tyrosin and phenylalanin—as its special precursors. This conjecture was shown to be correct by the result of the administration of tyrosin by the mouth to their alcaptonuric subject. Such administration caused a very conspicuous increase of the output of homogentisic acid. Since then this observation has frequently been repeated by other investigators, and the result has been shown to hold good for alcaptonurics in general. A corresponding increase follows an augmented intake of protein food, and especially of such proteins as are unusually rich in the aromatic fractions.

²³ *Deutsches Archiv für klinische Medicin*, 1903, lxxviii. 161.

The only exception known is recorded by Ploos van Amstel.²⁴ He had under observation a patient with combined alcaptonuria and cystinuria, an association of metabolic errors so far unique. When tyrosin was given by the mouth it appeared in the urine, and the output of homogentisic acid was not increased. This very remarkable observation will be discussed in the chapters on cystinuria.

Wolkow and Baumann were not able to test the effect of the administration of the phenylalanin, but at a later period Langstein and Meyer²⁵ arrived at the conclusion that the tyrosin of the proteins broken down in the metabolism of their alcaptonuric did not suffice to account for the quantity of alcapton acid which he excreted. This conclusion was based upon a comparison of the estimated daily output of homogentisic acid with that of nitrogen, which latter affords a measure of the protein destruction going on in the body. Hence, it appeared probable that phenylalanin also serves as a parent substance, and that it does so was afterwards demonstrated by Falta and Langstein,²⁶ who found that phenylalanin given by the mouth increases the homogentisic output just as tyrosin does.

Falta²⁷ afterwards showed, by a long series of feeding experiments with different proteins added to a constant diet, that the excretion of alcapton acid varies directly with the richness in phenylalanin and tyrosin of the proteins taken, and arrived at the conclusion that, on any given diet, the output corresponds closely with that to be expected if the aromatic fractions of the catabolized proteins are wholly converted into homogentisic acid and excreted as such.

The yield of alcapton acid after feeding with tyrosin or

²⁴ *Sammlung klinischer Vorträge*, 1910, No. 562-64. *Innere Med.*, p.193.

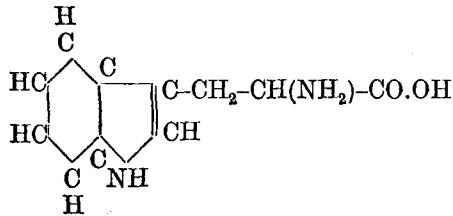
²⁵ *Deutsches Archiv für klinische Medicin*, 1903, lxxviii. 161.

²⁶ *Zeitschrift für physiologische Chemie*, 1903, xxxvii. 513; see also Falta, *Deutsches Archiv für klinische Medicin*, 1904, lxxxi. 250.

²⁷ *Deutsches Archiv für klinische Medicin*, 1904, lxxxi. 231.

phenylalanin varies with the mode of administration, and when small doses are given at short intervals, instead of a single large dose, the output is practically quantitative. This observation we owe to Mittelbach.²³

Yet one other of the known protein fractions has a benzene ring in its molecule, but in tryptophane the ring forms part of the more complex, heterocyclic, indol grouping. It appears unlikely *a priori* that in catabolism tryptophane will follow the same path as tyrosin, and that it will become converted into homogentisic acid, but in order to test the point I administered one gramme of tryptophane, for which I was indebted to the kindness of Prof. F. G. Hopkins, to an alcaptonuric boy.



Tryptophane.

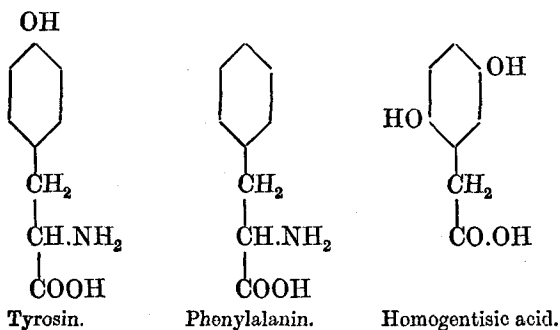
The dose was a very small one, and any effect might be expected to be slight. Therefore an equivalent dose of tyrosin (0.9 gramme) was given on two occasions as a control, the patient being kept upon a constant diet. The effect of each dose of tyrosin upon the homogentisic acid nitrogen

ratio was clearly marked, but no disturbance of the ratio was observed on the day on which the tryptophane was given. Hence I felt justified in concluding that tryptophane administered by the mouth does not increase the output of homogentisic acid, although further confirmation with a larger dose was necessary before the point could be

²³ *Deutsches Archiv für klinische Medicin*, 1901, lxxi. 50.

regarded as definitely established. I may add that Prof. Hopkins, who was good enough to examine the urine of the tryptophane day, failed to find in it any abnormal constituent the presence of which could be ascribed to an error of tryptophane catabolism. The fact that tryptophane does not yield homogentisic acid in metabolism has since been conclusively proved by Otto Neubauer,²⁹ who administered five grammes of tryptophane to an adult alcaptonuric. This dose produced neither an increased output of homogentisic acid, nor any disturbance of the H : N ratio. To sum up, it would appear that the tyrosin and phenylalanin of proteins are the only parent substances of the alcapton acid.

A glance at the respective formulæ of tyrosin and homogentisic acid suffices to show that the change from the one to the other is a complex one, for it involves the removal of the hydroxyl group from the para-position and substitution of two others in the 2 : 5 position in relation to the side chain, or possibly a shifting of the side chain itself.



Such a change presented greater chemical difficulties when Baumann wrote than it does now and could not be paralleled in animal metabolism. As, however, such successive reduction and oxidation were known to be

²⁹ *Deutsches Archiv f. klin. Med.*, 1909, xcvi. 211.

brought about by bacterial action, Wolkow and Baumann suggested that it might have its seat in the intestine of alcaptonurics, being there brought about under the influence of a rare specific micro-organism. Nowadays this infective theory, which was at one time widely accepted, has been completely abandoned, for it has been abundantly disproved.

Intestinal disinfection has no influence upon the excretion of homogentisic acid by alcaptonurics, no organism can be grown from their fæces which is able to effect such a conversion of tyrosin, nor can homogentisic acid be found in them. Moreover, it has been shown by Mittelbach, and afterwards by Langstein and Meyer and by Falta,³⁰ that abstinence from protein food does not arrest the excretion of the acid, although naturally it greatly reduces its amount. Hence we must conclude that not the food proteins alone, but also those of the tissues, contribute their quota to the total output of homogentisic acid, which would not be the case if the conversion were effected in the alimentary canal, and the fact, which we owe to Abderhalden, Bloch, and Rona,³¹ that the soluble dipeptide glycyl-l-tyrosin when injected subcutaneously into an alcaptonuric produces the same effect as tyrosin introduced into the alimentary canal, affords equally conclusive evidence that the change is not merely due to the action of intestinal bacteria.

Lastly, if the aromatic fractions of the proteins were thus changed in the intestine before undergoing absorption therefrom, seeing that they are not synthesized in the animal organism, the tissue proteins of alcaptonurics should exhibit a shortage of tyrosin and phenylalanin, if, indeed, such a deviation from normality be compatible with the maintenance of life. Abderhalden and Falta,³² who have

³⁰ loc. cit., sub 25 and 27.

³¹ *Zeitschrift für physiologische Chemie*, 1907, lii. 435.

³² *Ibid.*, 1903, xxxix. 143, and 1907, lii. 445.

investigated the blood proteins of such subjects as well as their hair and nails, have found no evidence of deficiency of the fractions in question. The same observers were able to detect the presence of homogentisic acid in the blood of an alcaptonuric, and to isolate it from the serum as the lead salt. Of some interesting experiments of Oscar Gross, which indicate the presence in normal blood serum of a ferment which destroys homogentisic acid, and the absence of this ferment from the blood of alcaptonurics, it will be necessary to speak in a later chapter.

This is the most convenient place in which to refer to the most interesting, but puzzling results of an investigation by Söderbergh³³ of the behaviour of the blood of alcaptonurics when submitted to Wassermann's test. He obtained positive reactions from the blood of three out of four Swedish alcaptonurics, men from 39 to 72 years of age, no one of whom showed any syphilitic signs or symptoms nor was conscious of having been infected with syphilis. The reaction was capricious, sufficiently so to excite suspicion as to its significance, and he found that the administration of tyrosin by the mouth, in doses of 15 grammes, was followed, within 24 hours, by the change of a negative to a positive reaction. An elaborate series of laboratory experiments showed that by the addition to normal serum of homogentisic acid, tyrosin, or phenylalanin it was possible to induce a positive Wassermann reaction. The perplexing and capricious results of some of the tests in different tubes of the same series, and with successive samples of blood from the same patient, such as are not obtained with syphilitic sera, coupled with the absence of all evidence of syphilis in his patients, led Söderbergh to the conclusion that a positive Wassermann test in alcaptonuria does not necessarily imply syphilitic

³³ *Neurolog. Centralblatt*, 1914, xxxiii. 24. *Nordiskt med. Arkiv*, 1915, xlviii, Afd. 2, Häft 4.

infection, and may be a direct outcome of the metabolic error.

The only other such observations with which I am acquainted were carried out by Schochet,³⁴ whose alcaptonuric patient gave a negative Wassermann test which became strongly positive after the administration of potassium iodide, by Debonedetti, whose patient also gave a negative Wassermann test which was not affected by administration of tyrosin, and by C. F. Cuthbert, in a family with several alcaptonuric members, in none of whom was Wassermann's test positive. Söderbergh found, on the other hand, that in one of his cases the administration of potassium iodide was followed by a change from a positive to a negative reaction, and investigated the excretion of homogentisic acid by this patient over a period of two months. A practically constant weighed diet was taken, and during the last forty days of the period three grammes of potassium iodide were taken each day. The estimations were made by C. T. Mörner's modification of Baumann's silver method, and the error from the presence of iodide in the urine was eliminated. The average output of homogentisic acid during the period in which the iodide was taken was some two grammes less per diem than that during the preceding period. If it should turn out that potassium iodide actually restrains the homogentisic output to this extent the fact will be of great importance not only for the theory of alcaptonuria, but also as offering a means of controlling the metabolic anomaly. Further experiments are greatly to be desired, including daily estimations of the ratio of homogentisic acid to nitrogen, and also of the nitrogen balance in normal controls. In Söderbergh's table the daily output of homogentisic acid varied by as much as 7 or 8 grammes on different days, in spite of the constant diet.

³⁴ Schochet, *Archives of Internal Medicine*, 1918, xxii. 82. Debonedetti, *Il Policlinico* (Sez. prat.), 1920, xxvii. 1379. Cuthbert, *Lancet*, 1923.

The darkening of alcapton urine on exposure to air is a phenomenon of considerable interest. Upon it the recognition of the anomaly most commonly depends, and the blackening of the cartilages, which is one of the most striking effects of the anomaly, is obviously due to a similar oxidation of homogentisic acid within the tissues. The tint of the darkening urine, and of the stains which it produces, varies in different circumstances, and as has already been mentioned, the urine of infant patients may develop a red, rather than a brown tint on standing. C. T. Mörner³⁵ has investigated this subject, and has shown that when the urine is rendered alkaline with ammonia, and is kept for a day or two in a stoppered bottle filled to the level of the stopper, and then exposed to the air, two other pigments are produced in addition to the ordinary brown substance. The percentage of homogentisic acid present should not be less than 0.25 nor more than 2, and that of ammonia from 1 to 4. With excess of homogentisic acid only the brown product is obtained, and the yield of the other pigments is greatest if only a small surface be exposed to the air.

One of these pigments, 'alcaptonchrome', has a beautiful violet colour in alkaline solution, and crystallizes readily in needles or hexagons which have a metallic lustre and a green reflex. Analysis showed that it contains nitrogen. The second product is red in alkaline solution, does not crystallize, and its solutions, unlike those of alcaptonchrome, are fluorescent.

Ochronosis was first described, and named, by Virchow in the year 1866 as a post-mortem finding, in a case of the clinical features of which nothing is known. Other cases were recorded from time to time, in some of which the patients are stated to have passed black urine. Albrecht³⁶

³⁵ *Zeitschrift f. physiol. Chemie*, 1910, lxi. 329.

³⁶ *Zeitschrift f. Heilkunde*, 1902, xxiii. 366.

was the first to suggest that alcaptonuria is a cause of ochronosis, and Osler,³⁷ shortly afterwards, described the development of surface pigmentation, such as had been observed in some ochronotic cases, in three elderly men who were well-authenticated alcaptonurics, and so pointed the way to the recognition of ochronosis during life. Clemens,³⁸ A. Wagner,³⁹ Gross and Allard, and Landois⁴⁰ clinched the matter by demonstrating the presence of the pigmentation of cartilages and of other structures in the bodies of individuals who were subjects of the anomaly under discussion.

In recent years, since the clinical signs of ochronosis have become more widely known, and have been carefully looked for, it has become evident that almost all, if not all, alcaptonurics who reach middle life develop ochronosis, and that some develop it in earlier life. On the other hand, alcaptonuria is not the only cause of ochronosis. The nature of most of the early cases cannot ever be known, but it is significant that all the more recent cases, with the exception of a doubtful one described by Harston and Soltau,⁴¹ fall into one of two classes. The majority of the patients have been alcaptonuric, but a considerable number, who were not alcaptonuric, had applied carbolic dressing to ulcers of the legs during long series of years. The patients of the latter class have not only black cartilages, but also exhibit the clinical features of ochronosis.⁴²

³⁷ *The Lancet*, 1904, i. 10.

³⁸ *Verhandlungen des Congresses für innere Medicin*, 1907, xxiv. 249.

³⁹ *Zeitschrift für innere Medicin*, 1908, lxxv. 119.

⁴⁰ Gross and Allard, *Mittheilungen aus d. Grenzgebieten d. Med. u. Chirurg.* 1908, xix. 24. *Archiv f. exper. Path. u. Pharm.* 1908, lix. 384. Landois, *Virchow's Archiv*, 1903, cxciii. 275.

⁴¹ *British Medical Journal*, 1908, i. 1230.

⁴² Pope, *The Lancet*, 1906, i. 24. Pick, *Berliner klinische Wochenschrift*, 1906, xliii. 478. Graeffner, *ibid.*, 1907, xlv. 1051. Reid,

In some instances there has been a conspicuous diminution of the staining after the application of carbohc acid has been discontinued. Most of the recorded cases of ochronosis with alcaptonuria have been in patients over forty years of age, but it is probable that, if looked for carefully, slight indications would be found at earlier ages. Thus Poulsen,⁴³ who has had an exceptionally large experience, detected pigmentation in a man aged twenty-three, and Kolacsek⁴⁴ in a male alcaptonuric thirty years old. These are as yet the youngest individuals in whose cases the diagnosis has been made.

The clinical picture of ochronosis is so characteristic that no one who has once seen it is likely to mistake its import. The signs are singularly uniform, but naturally are more extensive and pronounced in cases of long standing. Where blackened cartilages or tendons show through the skin a blue colour is observed, whereas superficial pigmented areas appear brown. One of the earliest signs is a blue coloration of the ears, first of the concha and anti-helix and later of the tragus and anti-tragus also. To the touch the blackened aural cartilages feel unduly rigid. Another early sign is the appearance of triangular brown patches upon the sclerotics, with their bases towards the corneæ, and the sclerotics may acquire a uniform grey tint. Later on, owing to staining of the nasal cartilages, the nose may appear blue, and a butterfly-shaped brown pigmentation of the skin of the face may appear. In the hands there may be a blue tint of the knuckles, from staining of the tendons, and rarely brown pigmentation of the thenar and hypothenar eminences and even of the nails.

Quarterly Journal of Medicine, 1908, i. 199. Beddard, *Quarterly Journal of Medicine*, 1910, iii. 329. Beddard and Plumtre, *ibid.*, 1912, v. 505.

⁴³ *Münchener med. Wochenschrift*, 1912, lix. 364.

⁴⁴ *Beiträge zur klinische Chirurgie*, 1910-11, lxxi. 254.

Poulsen⁴⁵ describes chromidrosis in the axillæ and genital region in three of his cases, and in two a bluish staining of the skin removed by washing with water. The sweat of Henze's⁴⁶ patients was said to stain their sheets, but the sweat of alcaptonurics does not usually contain homogentisic acid, and Umber and Bürger,⁴⁷ who saw a similar coloration of the skin in the axillæ of one of their patients, collected 50 c.c. of the sweat and found in it no trace of homogentisic acid. The colour, which was removed by ether, was clearly due to pigmented sebaceous secretion, and is comparable to the deep pigmentation of the aural cerumen of alcaptonurics first observed by Steir, who obtained from it a substance which yielded the alcapton reactions, and which has been noted in a number of other cases.

The post-mortem appearances in a case of ochronosis are very striking. There is seen a selective staining of the tissues, of a deep brown or black. The cartilages and fibrocartilages are the seats of election, and the staining of the tracheal rings is very noticeable, as also is the blackness of the articular cartilages and inter-vertebral discs.

The staining extends to the fibrous tissues, including tendons and sclerotics, and in advanced cases even the bones may be tinted. Patches of pigmentation are seen in the endocardium, and in the intima of the arteries wherever there is any atheromatous change. The pigmentation of the skin in advanced cases has already been described. Staining in the kidneys suggests an excretion of the pigment by these glands. The changes are identical in appearance in the alcaptonuric and carbohic cases.

It can hardly be doubted that the presence in the circu-

⁴⁵ *Beiträge zur pathol. Anatomie und zur allgem. Pathologie* (Ziegler), 1910, xlviii. 437.

⁴⁶ *Dissertation*, Leipzig, 1916.

⁴⁷ *Deutsche med. Wochenschr.*, 1913, xxxix. 2337.

lation of minute quantities of aromatic compounds is the cause of the pigmentation in both instances, and it is obvious that to produce conspicuous ochronosis the cause needs to be in operation for very long periods. The selective character of the staining suggests that it is due to the presence of an enzyme, such as a tyrosinase, in the tissues which are selected, but up till now there has been no very convincing demonstration of the presence of such an enzyme. Gross and Allard succeeded in producing blackening of cartilages by soaking them in a colourless solution of homogentisic acid nearly neutralized with sodium hydrate, whereas portions of fibrous tissue attached to the cartilage remained unstained. Poulsen,⁴⁸ who carried out a series of experiments bearing upon this question, obtained evidence of the presence of an enzyme which produced blackening with adrenalin with one specimen of human cartilage, but failed to find it in ten other series of experiments with other specimens of cartilage. The enzyme, which produced blackening of the cartilage within twenty-four hours, was destroyed by heat.

Poulsen obtained some indication of its presence in the other ten cases in quantities too small to produce any conspicuous staining *in vitro*, but these indications were so slight that he was not prepared to lay any serious stress upon them. An alternative suggestion by Pincussohn⁴⁹ that the pigment is produced *in situ* as the result of local breakdown of protein, will not explain the undoubted power of prolonged application of carbolic acid to produce ochronosis, and it is noteworthy that the gelatinous tissues are seats of election for the pigment, whereas gelatin contains no tyrosin.

Gross and Allard⁵⁰ were the first to lay stress upon the

⁴⁸ *Beiträge z. pathol. Anatomie u. z. allgem. Pathologie*, 1910, xlviii. 346.

⁴⁹ *Ergebnisse d. innere Med. u. Kinderkrankheiten*, 1912, viii. 454.

⁵⁰ *loc. cit.*, sub 41.

association of osteo-arthritic lesions with alcaptonuria. Such lesions, with fibrillation and erosion of cartilages, and formation of osteophytes are very common in elderly people, but the evidence seems conclusive in favour of a very special liability of alcaptonurics to develop such troubles in later life. Thus in a family described by Ueber and Bürger, members of two generations of which came under observation, of the eight children of an alcaptonuric father four were alcaptonuric. The surviving members, both over fifty, had ochronosis, and the father also was said to have had blue ears. And it is specially worthy of note that in this family all the alcaptonuric members suffered severely from articular lesions, whereas no single non-alcaptonuric member was so afflicted. One is tempted to regard the ochronotic change in the cartilages as the starting-point of the osteo-arthritis, and the stiffening of the aural cartilages shows that this change is not simply a pigmentation; but the cartilages of the affected joints have in some cases shown comparatively little staining, and Jankte has shown that intense blackening of the articular cartilages is consistent with a complete absence of arthritic lesions.

Moreover, it is noteworthy that there is no evidence of any special liability to osteo-arthritis in the subjects of carbolic ochronosis, possibly because in them the causes are at work over much shorter periods than in alcaptonuric cases.

Osler described the peculiar stance and gait of his ochronotic patients, and kyphosis and a stooping attitude have been described in a number of cases by Henze⁵¹ and others, but special attention has been called to these features by Söderbergh,⁵² who lays particular stress upon the changes in the vertebral column as revealed by X-ray examination. In the four cases which he studied there was kyphosis and

⁵¹ *Dissertation*, Leipzig, 1916.

⁵² *Nordiskt medicinskt Arkiv*, 1915, Abt. 2, Häft 4.

rigidity of the spinal column, as in spondylitis deformans, and osteophytes were present on the pelvic bones and on those of the shoulder girdle. There was a diffuse deficiency of lime salt in the skeleton, as shown by diminished density of the X-ray shadows, but in places there was evidence of undue density. The X-ray pictures do not show the calcareous bridges from vertebra to vertebra which are such conspicuous features of ordinary spondylitis deformans, and which bear witness to ossification of the ligaments and shrinkage of the intervertebral discs. For the above changes Söderbergh proposes the name of osteitis deformans alcaptonurica.