

CHAPTER V

THE THEORY OF ALCAPTONURIA

It will be obvious, from all that has gone before, that the error of metabolism which is at the back of alcaptonuria is a failure to deal with the aromatic fractions of proteins in the ordinary way, and that both the proteins of the food and those of the tissues are implicated in the error. Before proceeding further it is necessary, therefore, to consider what is known as to the ordinary way of dealing with these fractions.

It is an unquestionable fact that the great majority of aromatic compounds when introduced into the human organism, escape with their benzene ring intact and are excreted in the urine in combination with sulphuric acid—as aromatic sulphates, or with glyccoll—as the acids of the hippuric group. Not so tyrosin and phenylalanin, which are in no sense foreign substances but important constituents of proteins, for these suffer disintegration of the aromatic nucleus and are completely destroyed. It is true that they do not stand quite alone in this respect, nor is it to be expected that they should do so, for any aromatic substance which is an intermediate product of their catabolism will obviously be in like manner broken up, as will also compounds so closely allied to them in their molecular structure that when exposed to the same destructive influences they share their fate. Experimental investigations have supplied a clue to the seat of the destruction of such substances and the nature of the changes which they undergo. Thus, G. Embden, Salomon, and F. Schmidt¹ found that when certain protein fractions are perfused through the liver acetone is formed.

¹ Hofmeister's *Beiträge*, 1906, viii, 121, 129.

Glycocoll, alanin, glutaminic acid, and asparagin led to no such formation of acetone, but with leucin, tyrosin, and phenylalanin, as well as with phenyl- α -lactic and homogentisic acids, a conspicuous yield of acetone was obtained. The aromatic acids on this list are all broken up in the normal organism, whereas phenyl- β -lactic, phenyl-propionic, and phenyl-acetic acids, which are not so broken up, do not yield acetone in the perfused liver. Furthermore, J. Baer and L. Blum² found that substances which figure in the above list of acetone-yielding compounds cause an increased excretion of β -oxybutyric acid when administered to diabetics. But apparently it is not only in the liver that such changes can be brought about.

By addition to normal human and animal sera solutions of homogentisic acid in normal saline solution, with all due precautions against oxidation and bacterial contamination, Oscar Gross³ obtained evidence of the presence, in such sera, of a ferment which has the power of destroying homogentisic acid. The quantity of alcapton acid was conspicuously diminished, but only when the amount added was small did it disappear completely. Prolonged heating to 56° to 60° destroyed the ferment, and its activity was diminished when the serum had been kept for 24 hours.

No such results could be obtained with sera from two alcaptonuric patients. Hence it would appear that normal serum of men or animals contains a ferment which has the power of destroying homogentisic acid, probably with formation of acetone, whereas this ferment is not present in the serum of alcaptonurics.

The question which next calls for consideration is whether in alcaptonuria the failure to deal with tyrosin and phenylalanin is or is not complete. In diabetes we are confronted

² *Archiv für experimentelle Pathologie und Pharmakologie*, 1906, lv. 89.

³ *Biochemische Zeitschrift*, 1914, lxi. 165.

with a failure to deal with glucose in the normal way, a failure which may be of any degree, from such as leads to a small excretion of glucose only after a meal rich in carbohydrates, to one so nearly complete that the proteins are called upon to contribute to the urinary output of glucose. The amounts of homogentisic acid excreted by congenital alcaptonurics differ but little, provided always that the food of the subjects is similar in kind and in proportions. There is no evidence that the alcapton acid ever occurs in traces in the urine; the output amounts to several grammes in the day or none is found. Four or five grammes a day is an average excretion on an ordinary mixed diet poor in milk. The evidence afforded by the ratio of homogentisic acid to nitrogen is of far greater value in this connexion than that derived from the measure of the daily output of homogentisic acid alone. A mere increased supply of a given protein in the food does not disturb this ratio, for with the increased destruction nitrogen and alcapton acid in the urine increase *pari passu*. But though unaffected by quantitative changes in the diet the ratio is profoundly disturbed by changes in the quality of the proteins taken, as must needs be the case, seeing that some proteins are far richer in tyrosin and phenylalanin than others, whereas their yield of nitrogen is comparatively constant. Thus the substitution of casein, which is rich in the aromatic fractions, for egg albumin, in which they are much more scantily present, will greatly increase the output of homogentisic acid relatively to that of nitrogen, as Langstein and Meyer and Falta have demonstrated.⁴

If in alcaptonuria the error were complete and maximal, all subjects of that anomaly when fed upon a given standard diet should excrete equal quantities of homogentisic acid, and changes in the quantities consumed, although they will

⁴ *Deutsches Archiv f. klin. Med.*, 1903, lxxviii. 161. *Ibid.*, 1904, lxxxi. 231.

conspicuously affect the total output of the acid, should leave the ratio of homogentisic acid to nitrogen undisturbed. In order to obtain conclusive evidence upon this point it would be necessary to place a number of alcaptonurics upon a standard diet, in which not only are the proteins the same but in which also the several proteins are represented in uniform proportions. These conditions would be best fulfilled by a diet of milk alone, but unfortunately, observations so carried out are not available. The best available standard, although far from a uniform one, is a mixed diet, and the figures quoted are drawn from the observations of Langstein and Meyer and Falta upon one alcaptonuric, of Schumm upon another, and of Hele and myself upon yet three others. All the patients were, at the times to which the figures refer, upon a mixed diet, and the results serve to show that under roughly uniform conditions of diet the ratios show a striking similarity.⁵

Other estimations published in more recent years, by Cronvall,⁶ Ravold and Wheeler,⁷ Gibson and Howard,⁸ Fromherz,⁹ and Katsch,¹⁰ have yielded very similar figures, and the conclusion cannot be avoided that in the great majority of cases of alcaptonuria the H:N ratios vary within comparatively narrow limits, even with diets which are only approximately uniform.

This fact, coupled with Falta's estimate that the output of homogentisic acid corresponds roughly to the calculated amounts of tyrosin and phenylalanin in the proteins broken down, suggests that the failure to deal with the aromatic fractions of proteins in the ordinary way is complete.

⁵ *Journal of Physiology*, 1905, xxxiii. 198, and 1906, xxxv (Proceedings of the Physiol. Soc., p. xv).

⁶ *Upsala Läkareförenings Förhandlingar*, 1907, xii. 402.

⁷ *Journal of Biological Chemistry*, 1910, vii. 465.

⁸ *Archives of Internal Medicine*, 1921, xxviii. 632.

⁹ *Dissert.*, Freiburg. i. Br., 1908.

¹⁰ *Deutsches Archiv f. klin. Med.*, 1918, cxxvii. 210.

TABLE SHOWING THE DAILY H : N RATIOS IN FIVE CASES OF ALCAPTONURIA, DURING PERIODS
IN WHICH MIXED DIETS WERE TAKEN.

<i>Anton M., in Basle, aged 50 years.</i>		<i>Falta.</i>	<i>Male, 20 years. O. Schumann.</i>	<i>Thomas P., aged 8 years.</i>	<i>Albert P., aged 4 years.</i>	<i>Minnie L., aged 3 years.</i>
<i>Langstein and E. Meyer.</i>						
46 : 100	36 : 100	48-1 : 100	44-5 : 100	43-8 : 100	48-4 : 100	42-7 : 100
44 "	42 "	44-9 "	42-2 "	39-9 "	47-6 "	27-0 "
42 "	38 "	40-3 "	47-6 "	35-9 "	44-9 "	48-3 "
42 "	34 "	44-3 "	42-6 "	40-0 "	52-7 "	39-6 "
43 "	34 "	50-4 "	49-5 "	37-7 "	48-8 "	39-1 "
46 "	40 "	44-5 "	42-9 "	44-4 "	50-7 "	34-5 "
46 "	53 "	40-9 "	45-8 "	35-9 "	45-4 "	44-8 "
44 "	40 "	40-8 "	41-3 "	38-7 "	54-3 "	—
—	45 "	41-8 "	—	43-9 "	53-5 "	—
—	54 "	—	—	—	—	—
Averages :—						
44 : 100	41-6 : 100	44-0 : 100	44-0 : 100	40-0 : 100	49-6 : 100	39-4 : 100

Note.—The above ratios are all some 5-8 per cent. too low, owing to the use of 3 per cent. ammonia in estimating homogentisic acid. The error so caused is fairly constant. The figures for Minnie L. are obtained by subtracting 5-8 per cent. from each ratio, 8 per cent. ammonia having been used in this case. The first four columns relate to the same individual.

However, in face of the evidence now available, this view must be abandoned, and some other explanation has to be found for the uniformity of the H:N ratios in so many cases of the anomaly.

Gross and Allard¹¹ obtained very high H:N ratios, 60-70:100, which they regarded as showing a want of uniformity in different cases, but their patient was taking large quantities of milk, on one day as much as three litres, and changes in the protein content of the diet appear to have been chiefly brought about by the addition or withdrawal of milk. Their ratios are comparable with those worked out by Langstein and E. Meyer during a period of milk and plasmon diet (average 62.6:100), and that the above explanation is correct is shown by the fact that even the addition of plasmon (sodium casein) to the diet scarcely affected the ratio, whereas when superposed upon an ordinary mixed diet it greatly increases the homogentisic output relatively to that of nitrogen. But, as Fromherz points out, the conspicuous rise of the H:N ratio during a febrile attack which occurred within the period covered by the observations, suggests a diminished power of dealing with the aromatic fractions at that time. In a case recorded by Zipmer¹² the output of homogentisic acid reached as high a figure as 18 grammes per diem. The nature of the diet is not stated, and nitrogen was not determined, but judging by sulphur estimations the H:N ratio should have been about 92. Umber and Bürger¹³ made a very complete study of one of their cases, including determinations of nitrogen balance, and give full details of dietaries. The investigations extended over 22 consecutive days. On the first day the H:N ratio was 49.7, and on the last two days, when a diet rich in meat and eggs was taken, and the

¹¹ *Zeitschr. f. klinische Medizin*, 1907, lxiv. 359.

¹² *Inaug. Dissertation*, Würzburg, 1903.

¹³ *Deutsche med. Wochenschrift*, 1913, xxxix. 2337.

nitrogen intake amounted to some 26 grammes per diem, the ratio was between 50 and 60. From this it would appear that on an average mixed diet the H : N ratio was not far removed from the average for cases of alcaptonuria. The first six days formed a period of nitrogen retention, the diet contained 2 litres of milk and six eggs, and the nitrogen intake was 19.1 grammes. The average H : N ratio was 92.9. During the second period of six days the nitrogen intake was slightly over 7 grammes, and the average H : N ratio was 119.5. In the third six days the nitrogen intake was about the same, but the average H : N ratio had fallen to 76.6. During the last period of four days, with a nitrogen intake of 25.76 grammes, the average ratio was 60.6. The output of homogentisic acid varied between 4.5 grammes on the first day and 19.95 on the twenty-first, and the highest H : N ratio was 151.4 on the nineteenth. Such figures had never previously been obtained in any case, and it would be of great interest to repeat the observations upon other adult alcaptonurics, with exactly the same diets. It is obvious, as Umber and Bürger point out, that the wide variations in the Homogentisic acid : Nitrogen ratios are to a large extent due to the excretion of some products of protein breakdown before others after a period of nitrogen retention.

It appears to me that only by examining a series of cases with absolute uniformity of diet can it be decided whether in some cases of alcaptonuria wide departures are made from the average H : N ratio.

Mittelbach¹⁴ tried the effect of three days fast upon an adult male alcaptonuric patient, and found that the output of homogentisic acid fell to 1.6 gramme in the day, but the withdrawal of food did not cause it to disappear, presumably because a part of the output was derived from the tissue proteins. The nitrogen output was not determined. Far

Deutsches Archiv f. klin. Med., 1901, lxxi. 50.

more elaborate observations upon the effect of fasting were carried out by G. Katsch¹⁵ upon an alcaptonuric child of three and a half years. After five days upon an ordinary diet during which the average H : N ratio was 46.8, a weighed diet specially rich in tyrosin was given, and the average ratio rose to 51.3. Then followed three successive hunger days, on the third of which homogentisic acid disappeared completely from the urine, and did not reappear during the four following days, during which a diet of fat and carbohydrate was taken. When protein was again given the ordinary H : N ratio was restored. During another period of carbohydrate and fat diet with very little protein the ratio fell to a very low figure, namely 2.8. It was obvious that in this child the error was not maximal, seeing that during the hunger period tissue proteins were broken down but no alcapton acid was excreted. The fall of the homogentisic output corresponded fairly closely with a conspicuous excretion of acetone, and Katsch thinks that the difference from Mittelbach's results may be due to the fact that the patient was a child, and that during the hunger period the aromatic protein fractions were converted directly into acetone, owing to the great liability of children to acetonæmia. He suggests as an alternative that, in the special conditions of hunger metabolism, another path than that through homogentisic acid is followed by the aromatic fractions of proteins. Katsch suggests further that by administering homogentisic acid to an alcaptonuric during a hunger period valuable information might be obtained, as it would be shown whether it were excreted unchanged, or caused an increase of acetone output. Unfortunately this experiment was not tried.

That there is an alternative path open to the aromatic protein fractions, besides that through homogentisic acid, is no longer open to doubt.

¹⁵ *Deutsches Archiv f. klin. Med.*, 1918, cxxvii. 210.

The evidence of this, which we owe to the researches of Dakin and of Fromherz and Hermanns, will be discussed later. Whether this second path is in constant use, or is only resorted to as an emergency exit, we do not yet know. If the latter be the case we have an explanation of what may be spoken of as the normal H:N ratio, but, on the other hand, this may, as Fromherz suggests, represent the metabolism of some definite proportion of the aromatic fractions of the proteins broken down. It may be that there is a definite quantitative relationship between the portions which follow the two paths respectively, which is liable to be disturbed by morbid processes.

Two explanations are possible of the fact that alcaptonurics excrete homogentisic acid whereas normal persons do not. Either the alcapton acid is a strictly abnormal product formed by a perverted metabolism of tyrosin and phenylalanin, or it is an intermediate product of normal metabolism which in alcaptonurics escapes further change.

It may be premised that the behaviour of homogentisic acid in the organism is rather that of a normal product than that of an interloper. None of the chemical protective mechanisms are called into play to cope with it in alcaptonuria, save that which is called out by any acid which is not destroyed in metabolism. It is excreted in simple combination with bases and not as an aromatic sulphate or in combination with glycocoll. Its next homologue, gentisic acid, on the other hand, although it is for the most part destroyed in passage through the body, is in part excreted as aromatic sulphate, as Likhatscheff¹⁶ showed and as Neubauer and Falta¹⁷ also found.

As an acid, homogentisic acid is in part combined with ammonia, for the protection against acids is in no wise

¹⁶ *Zeitschrift für physiologische Chemie*, 1895, xxi. 422.

¹⁷ *Ibid.*, 1904, xlii. 92.

selective. Several observers have obtained evidence of an increased excretion of ammonia by alcaptonurics.¹⁸ In no instance was the increase of urinary ammonia comparable with that observed in some pathological conditions, such as grave diabetes, for, as we have seen, the output of the acid itself is never large as compared with that of the acids of the acetone group in some morbid states.

It stands to reason that an intermediate product of catabolism which is normally absent from the excreta must needs be completely destroyed under ordinary conditions, and that homogentisic acid conforms to this requirement there is abundant evidence to show. Thus, although H. Embden¹⁹ succeeded in producing a transitory alcaptonuria in himself by swallowing eight grammes of the acid, he found that smaller doses had no such effect. Falta,²⁰ too, failed to induce an excretion of the acid in himself and two others by taking quantities of from four to six grammes in repeated half-gramme doses at short intervals. Hence it is evident that homogentisic acid is a member of that small group of aromatic compounds of which the benzene ring is broken down in their passage through the body, and, as has been mentioned, further evidence of this is afforded by the fact that it yields acetone when perfused through the liver. In these respects it behaves as a normal intermediate product might be expected to do. That this destructive power may be overtaxed is shown by Embden's experiment upon himself, and by those of Wolkow and Baumann upon dogs.²¹ In normal persons such overtaxing can never occur, since the maximum daily output taken at a single dose will hardly give rise to experimental alcaptonuria; but in

¹⁸ See E. Meyer, *Deutsches Archiv für klinische Medicin*, 1901, lxx. 463. Schumm, *Münchener med. Wochenschrift*, 1904, li. 1599. Umber and Bürger, loc. cit., sub. 13.

¹⁹ *Zeitschrift für physiologische Chemie*, 1893, xvii. 182, and xvii. 304.

²⁰ *Deutsches Archiv f. klin. Med.*, 1904, lxxxi. 264.

²¹ *Zeitschrift f. physiol. Chemie*, 1891, xv. 282.

disease the power of destroying homogentisic acid is sometimes conspicuously lowered—for example, in grave cases of diabetes, as Langstein,²² Falta,²³ and others have demonstrated.

Garnier and Voirin,²⁴ who were the first to suggest that homogentisic acid is a product of normal metabolism, were inclined to ascribe its presence in the urine to excessive production, the power of destroying it being overtaxed. Whether the power of destroying it were merely overtaxed or wholly lost it might be expected that homogentisic acid given by the mouth to an alcaptonuric will be excreted nearly quantitatively, being added to the ordinary output, and H. Embden showed that this is the case.

But there is no evidence of excessive formation, whereas the failure to destroy homogentisic acid is undoubtedly a feature of alcaptonuria.

Dakin²⁵ is one of the chief opponents of the intermediate product theory, and his objections are chiefly based upon the effects of administration to normal and alcaptonuric individuals of two substances which, for reasons to be explained later, could not presumably be converted into homogentisic acid by an alcaptonuric. These substances, para-methyl-phenylalanin and para-methoxy-phenylalanin, are completely destroyed in passage through both normal and alcaptonuric organisms, without causing any formation of homogentisic acid. Fromherz and Hermanns²⁶ confirm these observations of Dakin's, and in their experiments a fall of the H:N ratio bore witness to the destruction of the substances in question by their alcaptonuric patient. However, it

²² *Deutsche medicinische Wochenschrift*, 1905, xxxi. 457.

²³ *Ibid.*, 1904, lxxxii. 265.

²⁴ *Archives de Physiologie*, 1892, 5^e s., iv. 225.

²⁵ *Journal of Biological Chemistry*, 1911, ix. 151.

²⁶ *Zeitschrift f. physiol. Chemie*, 1914, lxxxix. 113; *ibid.*, 1914, xci. 194.

appears to me that Dakin's results supply an argument for the existence of an alternative path, rather than a proof that homogentisic acid is a product of abnormal metabolism.

Grutterink and Hijmans van den Bergh²⁷ have also argued against the theory of a normal intermediate product, but on quite different grounds. To patients with diabetes or hepatic disease who were found to have greatly impaired powers of destroying homogentisic acid taken by the mouth, these observers administered tyrosin in doses of 10 to 15 grammes. They argue that after such large doses of tyrosin sufficient homogentisic acid should have been formed to overtax the limited destructive power, supposing that it were a normal intermediate product, and that some should have been excreted in the patient's urine, whereas in no instance did such a result follow. This evidence cannot, certainly, be lightly set aside. However, we cannot be sure that at any moment sufficient alcapton acid would be in existence to overtax the destructive power, which although diminished was undoubtedly not abolished. Nor is it certain that when such impairment results from disease the formation of homogentisic acid, as well as its destruction, is not interfered with. Grutterink and van den Bergh satisfied themselves that the tyrosin given was well absorbed, but they do not state whether tyrosin itself, or derivatives thereof, other than homogentisic acid, were sought for in the urine. Knoop²⁸ had previously raised a similar objection. He fed dogs with phenyl- α -lactic acid, a compound which, like tyrosin, increases the homogentisic output of alcaptonurics. He, too, argued that, as the power of dogs to destroy homogentisic acid is known to be limited, if it were a normal product it should have appeared in the urine. However, the fact that some of the

²⁷ *Nederlandsch Tijdschrift voor Geneeskunde*, 1907, ii. 1117.

²⁸ Hofmeister's *Beiträge*, 1905, vi. 150.

phenyl- α -lactic acid was recovered as such from the urine suggests that a block occurred at an earlier stage in the catabolic series.

One of Abderhalden's²⁹ laboratory assistants took 50 grammes of tyrosin by the mouth in the course of 24 hours, and the urine and fæces of three days were examined. Six grammes of tyrosin were recovered from the fæces, and the urine of the first 24 hours contained homogentisic acid. Its nature was proved conclusively by the properties of the crystalline lead salt, and of the acid obtained therefrom, and by elementary analysis of the latter. Unfortunately the assistant declined to repeat the experience, and Abderhalden himself passed no homogentisic acid after no less a dose than 150 grammes of tyrosin, nor did Strasser after 25 grammes of phenylalanin. Nevertheless a man apparently normal, undoubtedly passed homogentisic acid after a large dose of tyrosin, and this is the strongest evidence yet forthcoming that this acid is an intermediate product of normal metabolism.

It appears to me that the evidence in favour of the theory of an intermediate product far outweighs that which can be brought against it; and apart from Abderhalden's experiment, perhaps the most serious objection which can be raised to the view that homogentisic acid is an abnormal product, peculiar to alcaptonurics, is that such a view involves the assumption that the alcaptonuric, who alone has the power of forming homogentisic acid, is also exceptional in having no power of destroying it when formed.

The impaired destruction of the alcapton acid which results from certain morbid conditions has interesting bearings upon the question of temporary or intermittent alcaptonuria. The records of such cases are very few, only four or five in all, and further work upon such cases is much to be desired. In some of them the evidence that the condition was temporary is not conclusive, and in others

²⁹ *Zeitschrift f. physiol. Chemie*, 1912, lxxvii. 454.

the evidence of the nature of the abnormal excretory product is not sufficiently complete for the establishment of so important a point. In none of them save in that of Zimnicki,³⁰ whose paper appeared in a Russian journal which I have not been able to obtain, have quantitative estimations been carried out. His patient, whose alcaptonuria was intermittent, suffered from hypertrophic cirrhosis of the liver. In Geyger's³¹ case, also intermittent, the patient was a diabetic. Of strictly temporary cases that described by Carl Hirsch³² is the most remarkable. A girl, aged 17 years, with a febrile gastro-enteric catarrh, passed, on three days only, a urine which darkened on standing, contained indican, and also yielded the alcapton reactions. From it Professor Siegfried extracted an acid which formed a sparingly soluble lead salt, but neither the melting-point of the acid nor any analytical figures are given.

A temporary or intermittent excretion of homogentisic acid seems more compatible with the theory that it is a normal metabolic product than with the opposite theory. It is noteworthy that Geyger and Zimnicki's patients suffered from diseases in which the power to destroy homogentisic acid is apt to be impaired, and it is conceivable that in rare instances the impairment may proceed further and become practically complete.

Assuming it to be a fact that homogentisic acid is a product of normal metabolism, the result of the administration of various aromatic acids to alcaptonurics may reasonably be expected to throw light upon the intermediate steps between the parent protein fractions and that substance. Any compound, which represents a link in the chain should, on the one hand, be destroyed in the normal organism, as tyrosin and homogentisic acid are, and, on the other hand, should increase the output of homogentisic

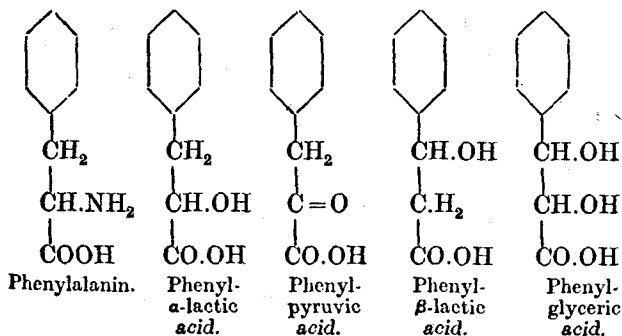
³⁰ Jeshenedelnik, 1899, No. 4; abstract *Centralblatt für Stoffwechsel- und Verdauungskrankheiten*, 1900, i. 348.

³¹ *Pharmakologische Zeitung*, 1892, p. 488.

³² *Berliner klin. Wochenschrift*, 1897, xxxiv. 866.

acid by alcaptonurics. Any substance which does not behave in the manner indicated cannot form such an intermediate link.

Many aromatic acids have been administered to alcaptonurics at various times, but very few have been found to fulfil the above conditions. The most complete set of observations of the kind were carried out by Otto Neubauer and Falta,³³ and their findings have been fully confirmed by Grutterink and van den Bergh.³⁴ It was found that compounds, such as phenylacetic and phenylpropionic acids, which have simple side chains, have no effect upon the output and do not raise the H : N ratio. Those which increase homogentisic excretion resemble tyrosin and phenylalanin in having an easily attacked grouping in the α position upon the side chain, whereas when the substituted group occupies the β position no such result follows. Thus phenyl- α -lactic and phenylpyruvic acids are excreted as homogentisic acid, whereas phenyl- β -lactic acid is not. Even the presence of a second substituted group in β position, as in phenylglyceric acid, suffices to prevent the transformation.



Again, when the substituted grouping in the α position is rendered more stable by benzoylation, the formation of

³³ *Zeitschrift für physiologische Chemie*, 1904, xlii. 81.

³⁴ *loc. cit.*, sub 27.

homogentisic acid is prevented, for as L. Blum has found, benzoyl-phenylalanin does not increase the alcapton output.

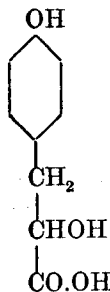
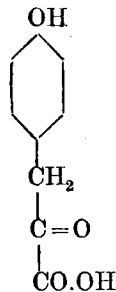
There is good reason to believe that desamination—that is to say, the removal of the amino group—is a very early stage in the catabolism of the amino-acids of which the molecules of proteins are built up, and of the aromatic fractions amongst others. After a meal rich in proteins the resulting excretion of homogentisic acid was found by Falta³⁵ to commence more promptly and to come to an end sooner than the corresponding increase of the output of nitrogen, and this has been confirmed by Langstein and Meyer. Mittelbach placed the maximum excretion of homogentisic acid within the two or three hours following the protein meal, but in some observations which I made³⁶ upon specimens of urine passed at short intervals throughout the day, although an obvious increase occurred shortly after a meal rich in protein, the excretion was still larger during the second period of four hours than during the four hours immediately following the meal. It is easy to imagine that desamination is effected by substitution of hydroxyl for the amino-group, and that the formation of phenyl- α -lactic acid is the first step in the breaking down of phenylalanin. Not only does phenyl- α -lactic acid fulfil the conditions laid down above, but it is one of the compounds which have been found to yield acetone when perfused through the liver; but an observation of Otto Neubauer³⁷ apparently negated this supposition. This observer found to his surprise that paroxy-phenyl- α -lactic acid, which stands to tyrosin in the same relationship as does phenyl- α -lactic acid to phenylalanin, failed entirely to increase the output of homogentisic acid by an alcaptonuric. Hence he con-

³⁵ *Verhandlungen der naturforschenden Gesellschaft in Basel*, 1903, vol. xv, Heft 2.

³⁶ *Transactions of the Royal Medical and Chirurgical Society*, 1903, lxxxv. 69.

³⁷ *Deutsches Archiv f. klin. Med.*, 1909, xcv. 211.

cluded that the intermediate product is probably the ketonic acid, and the probability is greatly increased by his further observation that paroxy-phenylpyruvic acid does increase the alcapton output—

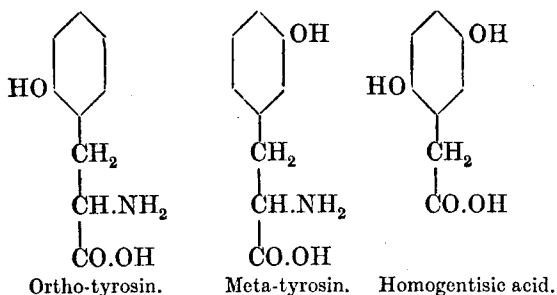
Paroxy-phenyl- α -lactic acid.

Paroxy-phenylpyruvic acid.

Between paroxy-phenylpyruvic acid and homogentisic acid other stages must intervene, and the appearance of two hydroxyl groups in the 2:5 positions upon the ring must precede or coincide with the final oxidation of the side chain. Were this not the case paroxy-phenylacetic acid would be formed, and this acid has not the properties requisite for an intermediate product, and is not converted into homogentisic acid by an alcaptonuric.

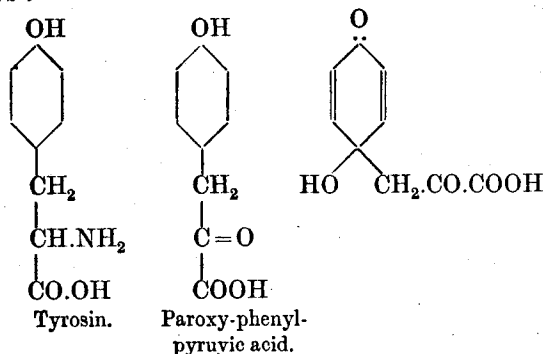
The structure of the benzene ring itself is of no less importance than that of the side chain. Falta found that di-brom-tyrosin and halogen proteins do not increase the homogentisic excretion in alcaptonuria, and we owe to L. Blum³⁸ the important observation that ortho-tyrosin and meta-tyrosin are equally inert in this respect, although each of these compounds has a hydroxyl group in the position of one of the hydroxyls of homogentisic acid. Otto Neubauer has shown that the same holds good for the allied compounds, ortho- and meta-oxy-phenylpyruvic acids.

³⁸ *Archiv f. exper. Path. u. Pharmacol.*, 1908, lix, 283.



Hence it would appear that the presence of a hydroxyl-group in the para-position is not only no hindrance to the change but is rather essential to it, and that the change in the ring is not brought about, as was originally supposed, by removal of the hydroxyl in the para-position, but rather by shifting of the side chain. Such a shifting was first suggested by Erich Meyer,³⁹ on the analogy of Bamberger's⁴⁰ observations on the conversion of toluyl-hydroxylamine into tolu-hydroquinone, and is presumably brought about by the formation of a compound with the quinone grouping.⁴¹

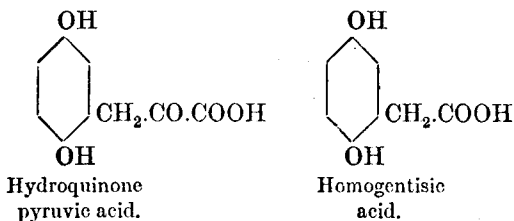
Otto Neubauer represents the probable series of changes as follows :



³⁹ *Deutsches Archiv f. klin. Med.*, 1901, lxx, 446.

⁴⁰ *Berichte d. deutschen chemischen Gesellsch.*, xxviii, 245.

⁴¹ See Friedländer. Hofmeister's *Beiträge*, 1908, xi, 304.



He has administered hydroquinone-pyruvic acid, the third intermediate product in this series, to an alcaptonuric, and has found that it has the requisite power of increasing the homogentisic output, although the increase was less than might be expected.

If such a path, through a compound having the quinone linkage, be actually followed, it is necessary to assume that phenylalanin acquires a hydroxyl group in the para-position; and is converted into tyrosin as a preliminary to the further changes which it undergoes.

It is interesting to note that Blum found that when ortho- or meta-tyrosin is given to a normal man it is in part excreted as the corresponding oxy-phenylacetic acid, which shows that the shortening of the side chain, such as occurs when homogentisic acid is formed, is within the powers of the normal metabolic processes.

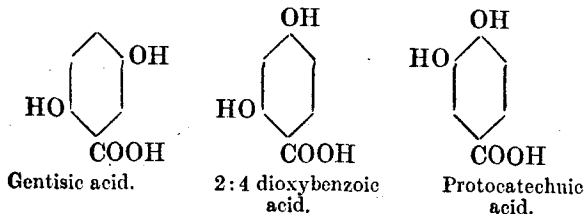
So far we have been discussing changes which, if homogentisic acid be a product of normal metabolism, are carried out in the normal and alcaptonuric organisms alike. Where the alcaptonuric differs from the normal individual is in having no power of destroying homogentisic acid when formed—in other words, of breaking up the benzene ring of that compound.

Apparently the factor which determines the disruption of the ring is the presence of the two hydroxyl groups in the 2:5 position upon it.

Thus, Neubauer and Falta⁴² found that of the three

⁴² loc. cit., sub 33.

isomeric dioxybenzoic acids gentisic acid alone was to a large extent burnt in the human organism, although some 15 per cent. of the dose given appeared in the urine as aromatic sulphate. In gentisic acid, as in its homologue the alcapton acid, the hydroxyl groups occupy the 2 and 5



positions. When the alcaptonuric took gentisic acid by the mouth Neubauer and Falta found that the reducing power of his urine was conspicuously increased, not owing to a greater output of homogentisic acid, which could hardly have resulted, but to the excretion of gentisic acid as such.

This indicates that in alcaptonuria the failure to break up the benzene ring extends to acids with hydroxyl groups in the 2:5 position other than homogentisic acid, and that the essential error resolves itself into an inability to destroy the ring of acids so constituted. Homogentisic acid is apparently the only compound formed in normal metabolism which offers itself for such disruption, and accordingly the alcaptonuric excretes it.

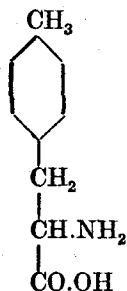
This conception of the anomaly locates the error in the penultimate stage of the catabolism of the aromatic protein fractions, which is in accord with the fact that both exogenous and endogenous tyrosin and phenylalanin, contribute to the excreted homogentisic acid in alcaptonuria.

We may further conceive that the splitting of the benzene ring of homogentisic acid in normal metabolism is the work

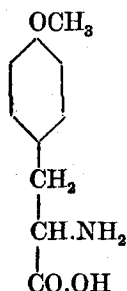
of a special enzyme, that in congenital alcaptonuria this enzyme is wanting, whilst in disease its working may be partially or even completely inhibited.

We know little as yet concerning the second path, by which the aromatic fractions may be broken down without passing through the stage of homogentisic acid, beyond the fact that it exists.

As has already been mentioned, Dakin showed⁴³ that certain aromatic compounds when administered to an alcaptonuric were completely broken down, but caused no increase of the homogentisic acid output. These compounds are para-methyl-phenylalanin and para-methoxy-phenylalanin.



Para-methyl-phenylalanin.



Paroxy-methyl-phenylalanin.

Fromherz and Hermanns⁴⁴ confirmed Dakin's results with para-methyl-phenylalanin and gave a much larger dose, viz. 16 instead of 5 grammes.

Dakin selected these substances because their structure excluded the formation from them of a substance with the quinone linkage, such as Neubauer suggested as a precursor of homogentisic acid.

In Fromherz's experiment a slight fall of the H:N ratio on the day on which the methyl-phenylalanin was taken excluded any conversion into homogentisic acid, whilst the

⁴³ loc. cit., sub 25.

⁴⁴ loc. cit., sub 26.

fact that the amino-acid was destroyed was demonstrated by the absence of any increase of ether-soluble acids in the urine. Fromherz and Hermanns also found that the corresponding meta-compound was equally well destroyed, and that it too produced no increased output of homogentisic acid, although the formation of a quinone derivative is not prohibited by the methyl-group in the meta-position. With meta-methyl-tyrosin, a compound which fulfils Neubauer's requirement, in having an hydroxyl group in the para-position, only a slight increase of homogentisic acid resulted from a dose of 12 grammes, and the bulk of that compound was broken up in some other way.

Again paroxy-phenylpyruvic acid, which was found by Neubauer to increase conspicuously the output of alcapton acid, yielded only about one-third of the estimated quantity of homogentisic acid, and there was evidence that of the acid administered the larger part was burnt completely in some other way.

Fromherz and Hermanns conclude that there is undoubtedly a second, and are perhaps even more paths for the catabolism of aromatic substances, and suggest that the second path may lie through a pyrocatechin derivative, whereas the homogentisic path lies through derivatives of hydroquinone, and in support of this adduce their experiments with 3·4-dioxy-phenylalanin, which is destroyed in the animal organism as readily as is paroxy-phenylpyruvic acid. How far this second route is utilized for the burning of tyrosin and phenylalanin in normal metabolism is quite uncertain, but to me it is easier to suppose that the ordinary H : N ratio which is fairly uniform in so many cases of alcaptonuria, is rather due to its being used as an emergency route, than that there is a uniform quantitative relation between the portions dealt with in the several ways. However, if it can be shown that on absolutely the same diet the H : N ratio is far above the average in some

cases, and that in fever the H:N ratio is conspicuously raised, independently of any change of dietary and of the working of such causes as nitrogen retention and its after effects, it will be proved that the uniformity of the H : N ratio does not indicate complete conversion of tyrosin and phenylalanin into homogentisic acid. We shall be driven to conclude that the catabolism of some part of these aromatic fractions of the proteins of the food and tissues *habitually follows some other path than that through homogentisic acid.*