

## CHAPTER VII

### CYSTINURIA AND DIAMINURIA

THE foundation of the wider conception of cystinuria as an error of protein metabolism of which the excretion of cystin in the urine, although the most constant, is only one of the manifestations, was laid by Udranszky and Baumann<sup>1</sup> in 1888. These investigators found diamines, cadaverin and putrescin, both in the urine and fæces of a cystinuric patient under their observation; and the significance of this find was greatly enhanced when, shortly afterwards, Stadthagen and Brieger<sup>2</sup> detected cadaverin in the urine of two other cystinurics. In Baumann's case the diamines were readily isolated from the urine of twenty-four hours by benzoylation in the presence of sodium hydrate,<sup>3</sup> and the daily yield varied between 0.2 and 0.4 gramme of benzoyl-diamines. The major part of the urinary diamine was cadaverin, whereas in the fæces putrescin was the more abundantly present. In 1893, when Garcia<sup>4</sup> had the same patient under investigation, putrescin was alone found in the urine,

<sup>1</sup> *Zeitschrift für physiologische Chemie*, 1889, xiii. 562.

<sup>2</sup> *Berliner klinische Wochenschrift*, 1889, xxvi. 344.

<sup>3</sup> 1,500 cubic centimetres of urine are shaken with 200 cubic centimetres of 10 per cent. sodium hydrate solution and from 20 to 25 cubic centimetres of benzoyl-chloride, till the smell of the last disappears. The precipitate is filtered off, washed, and treated with hot alcohol. The filtered alcoholic extract is thrown into excess of water. If diamines be present their benzoyl compounds will in a short time separate in minute crystals. The melting-point of benzoyl-cadaverin is 129° C., that of benzoyl-putrescin 176° C. Two other methods have been employed for the detection of diamines in urine—viz., the picric acid method of Stadthagen and Brieger (loc. cit., sub 2) and the phenyl-isocyanate method of Loewy and Neuberg (*Zeitschrift für physiologische Chemie*, 1904, xliiii. 355).

<sup>4</sup> *Zeitschrift für physiologische Chemie*, 1893, xvii. 577.

but in 1897, nine years after their original discovery, both diamines were once more excreted as at first.

In no condition other than cystinuria have these diamines been found in urine in quantities which could be detected by the ordinary methods in the excretion of twenty-four hours. By evaporating down as much as 100 litres of normal urine Dombrowski<sup>5</sup> was able to demonstrate the presence of traces of cadaverin therein, and after evaporation of large volumes of the urine of patients with pernicious anæmia William Hunter<sup>6</sup> obtained small yields of benzoyl-diamines. Roos<sup>7</sup> also found cadaverin in the fæces of a patient with dysentery, and putrescin in those of a sufferer from cholera nostras, but the urine was not examined for diamines. I have myself benzoylated the urine of twenty-four hours of large numbers of patients suffering from various maladies, but such searches for diamines have invariably proved fruitless, save in cases of cystinuria.

Even in cystinuric cases the search for diamines in the urine and fæces is far from being uniformly successful. In a number of cases they have been found by Bödtker,<sup>8</sup> C. E. Simon, Riegler, Marriott and Wolf, Thiele, Cammidge, Schölberg, Desmoulière, and myself, but in others Cohn,<sup>9</sup> Baumann,<sup>10</sup> Alsberg and Folin, Loewy and Neuberg, Hurtle, Hele, and I have been unable to detect their presence, although in some instances the examinations were persisted with over considerable periods.

My own experience relates to nine cases of cystinuria. In

<sup>5</sup> *Archives polonaises des Sciences biologiques et médicales*, 1903, ii.

<sup>6</sup> *Transactions of the Medical Society of London*, 1890, xiii. 386.

<sup>7</sup> *Zeitschrift für physiologische Chemie*, 1892, xvi. 192.

<sup>8</sup> *Norsk Magazin for Lægevidenskab*, 1892, liii. 1220; *Zeitschrift für physiologische Chemie*, 1905, xlv. 393.

<sup>9</sup> *Berliner klinische Wochenschrift*, 1899, xxxvi. 503.

<sup>10</sup> Pfeiffer, *Centralblatt für Krankheiten der Harn- und Sexual-Organ*, 1897, viii. 173.

four of these diamines were found in the urine at one time or another and in one in the fæces also ; in several cases no opportunity of examining the fæces presented itself. My impression is that the likelihood that diamines will be detected in any given specimen of cystin urine is comparatively small, but that if in any case the examination be continued over sufficiently long periods they are likely to be found eventually.

Of the two diamines cadaverin has been much the more frequently found in the urine, whereas there is evidence that putrescin is more often present in the fæces. In no other case have cadaverin and putrescin, or either of them, been shown to be present so continuously, in quantities easy of detection, both in urine and fæces, as in the original one of Udranszky and Baumann ; but even in that case the quantities and relative proportions of the diamines present varied considerably, and at one time they were almost absent from the urine for several days. In the case investigated by Cammidge and myself<sup>11</sup> cadaverin was found in the urine of two days only out of 41, and putrescin in the fæces at one of six examinations. In another case, in which specimens of urine were sent up to us in gallon jars, one such specimen was rich in cadaverin which was readily extracted by benzoylation from each separate fraction treated, but in no other sample from this patient was any diamine found. From the urine of yet a third patient Schölberg and I<sup>12</sup> got putrescin and cadaverin on several occasions, but five years later, when the same patient was under continuous observation for several weeks, Hurtleley and I<sup>13</sup> were uniformly unsuccessful in our search for diamines in his urine and fæces. Desmoulière<sup>14</sup> found

<sup>11</sup> *Journal of Pathology and Bacteriology*, 1900, vi. 327.

<sup>12</sup> *The Lancet*, 1901, ii. 526.

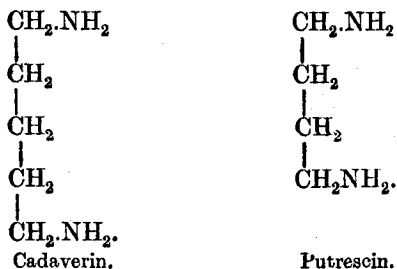
<sup>13</sup> *Journal of Physiology*, 1906, xxxiv. 217.

<sup>14</sup> *La Cystinurie. Thèse de Paris*, 1911, no. 328.

cadaverin and putrescin in both the urine and fæces of his patient.

These results indicate that in some cases of cystinuria the presence of diamines, in quantities which can be detected in the urine of twenty-four hours, is a very intermittent phenomenon which may only be manifested at long intervals. It is evident that it cannot safely be asserted that a given cystinuric never excretes them, even when they cannot be found in the urine for days or even weeks together. It is possible that the apparent intermittence is merely due to varying amounts, for a number of experiments, in which cadaverin was added to normal urine in different proportions, have convinced me that failure to detect diamines by the methods in use cannot be held to exclude their presence in quantities, small indeed, but much greater than the normal traces found by Dombrowski. Hele,<sup>15</sup> when working with a urine in which cadaverin was sometimes present, frequently noticed the odour of cadaverin during evaporation of the alkaline urine before fusion, preparatory to total sulphur determinations, even when no diamine could be detected by benzoylation.

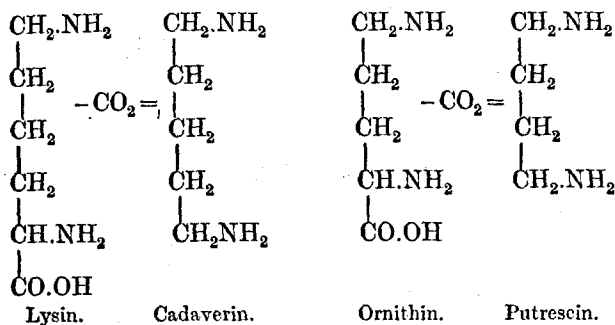
The substance known as cadaverin is penta-methylene-diamine, and putrescin is tetra-methylene-diamine. They are two members of a series of such compounds, of which other members are known.



<sup>15</sup> *Journal of Physiology*, 1909, xxxix. 69.

An obvious explanation of the occurrence of these two particular members of the diamine series, both in the excreta of cystinurics and when proteins undergo decomposition under the influence of bacteria, is afforded by the fact that they are intimately related to two of the protein fractions, the diamino-acids lysin and ornithin.

Lysin is converted into cadaverin by the elimination of carbon dioxide, and in like manner ornithin, which enters into the composition of the important protein fraction arginin, yields putrescin by a similar change.



When Udranszky and Baumann wrote, our knowledge of the structure of protein molecules was far more imperfect than it now is, and, as the known source of the diamines in question was the bacterial decomposition of proteins, they naturally suggested that their presence in the urine and fæces of cystinurics might result from a similar decomposition carried out in the alimentary canal, and that cystinuria itself was probably indirectly due to an intestinal infection. The absence of cystin from the fæces weighed strongly against its being itself a product of intestinal decomposition, but it was thought that the diamines thence absorbed might in some way preserve the cystin from its usual fate. However, the administration of diamines to dogs was found not to produce cystinuria in them, intestinal

disinfection was found to have no influence upon the excretion of cystin or diamines, and cultures from the fæces of cystinurics have failed to reveal the presence of abnormal bacteria having the power of forming diamines from proteins. In the absence of any positive evidence in its favour the infective theory of cystinuria, which at one time met with wide acceptance, has now been abandoned, and opinion has veered round to the view that the diamines which cystinurics excrete are themselves products of metabolism, derived from the lysin and arginin of proteins broken down in the organism, a view which was independently advanced by Moreigne<sup>16</sup> in France and by C. E. Simon<sup>17</sup> in America.

This hypothesis receives very strong support from the observations of Loewy and Neuberger<sup>18</sup> upon their exceptional patient already frequently referred to. Although no diamines could at ordinary times be detected in his urine, when lysin was administered to him by the mouth he excreted cadaverin in large quantities, and when arginin was so administered he excreted putrescin. In two other cases of cystinuria Hurtley and Hele and I<sup>19</sup> could find no putrescin in the urine after the administration of 5-gramme doses of arginin carbonate, although one of the patients had spontaneously excreted putrescin five years previously, sometimes alone and sometimes in association with cadaverin. As we shall see, these results are fully in keeping with those obtained with other protein fractions also, in Loewy and Neuberger's case and other cases of cystinuria respectively.

It would be very interesting to know whether in Loewy and Neuberger's case the taking of diamino-acids by the mouth was followed by the appearance of diamines in the fæces as well as in the urine, for, although the diamines have

<sup>16</sup> *Archives de Médecine expérimentale et d'Anatomie pathologique*, 1899, xi, 254.

<sup>17</sup> *American Journal of the Medical Sciences*, 1900, cxix, 39.

<sup>18</sup> *Zeitschrift für physiologische Chemie*, 1904, xliii, 355.

<sup>19</sup> *loc. cit.*, sub 13, p. 220.

comparatively seldom been found in the fæces of cystinurics their occurrence therein is beyond question, and if in cystinuria they are products of metabolism we must assume that they are in part excreted by way of the alimentary canal.

It does not necessarily follow that the diamines present in the fæces in cases of grave intestinal infection, such as Roos examined, have the same origin as those excreted in cystinuria, for it is quite possible that they are formed in the intestine by bacterial action, and even that the normal traces in urine have such an origin; but in Udranszky and Baumann's case the abundant diamine yield from the fæces was undoubtedly connected with the urinary output, and with the underlying cystinuria, for diamines are not to be detected in normal fæces, and their patient had no intestinal disorder. It is worthy of note that, in the case upon which Cammidge and I worked, the urine of the only day on which putrescin was found in the fæces yielded no diamine.

It is conceivable that the change from diamino-acids to diamines might occur after excretion, both in urine and fæces, from the diamino-acids excreted as such. Neither Hele nor Cammidge and I could obtain any evidence that diamines were formed in urine which was kept, either with or without exposure to air, or even allowed to decompose, although the patients whose urine was examined had excreted diamine previously.

Hele<sup>20</sup> also sought, by Wohlgemuth's method, for unchanged diamino-acids in the same urine, but without success, although four to six litres were treated, a fact which detracts from the importance of his decomposition experiments, for in the absence of diamino-acids diamines could hardly be formed.

More recently Ackermann and Kutscher<sup>21</sup> have found lysin

<sup>20</sup> *Journal of Physiology*, 1909, xxxix. 52.

<sup>21</sup> *Zeitschrift für Biologie*, 1912, lvii. 355.

in the urine of a cystinuric in which diamines had repeatedly been sought for in vain. From 80 litres of the urine they obtained 2.6 grammes of lysin mono-chloride, and proved conclusively that they were dealing with the diamino-acid in question by ultimate analysis of its platino-chloride and picrate, and by the melting points of its compounds. They convinced themselves that the quantity isolated represented only a part of the lysin present. In explanation of the failure to find cadaverin in this urine Ackermann and Kutscher suggest that a cystinuric may destroy cadaverin more quickly than he forms it from lysin, and point out that if this is the case, the urine of a cystinuric who is actually excreting cadaverin should yield far larger amounts of lysin than they obtained in their case. Investigations of the diamino-acid excretion of patients with diaminuria are much to be desired.

It is to be expected that the quantities of diamines, or at least of their parent diamino-acids excreted will be influenced by the intake of protein foods, and Garcia<sup>22</sup> thought that the excretion of diamines varied with the amount of protein in his patient's diet, and Thiele<sup>23</sup> more recently expressed the same opinion. The latter observer obtained a far larger yield of benzoyl-cadaverin from the urine of a day of excessive meat diet than from that of a day of abstinence or of a diet rich in carbohydrates. However, his observations are too few to allow any definite conclusion to be drawn from them, seeing how widely the diamine excretion varies, apart from changes of diet. In the case which Cammidge and I investigated the diet of the days in which diamines were excreted did not differ from that of other days. Other influences undoubtedly come into play, and it is by no means certain that the output of diamines stands in any direct quantitative relation to the excretion of diamino-acids.

<sup>21</sup> loc. cit., sub 4.

<sup>22</sup> *Journal of Physiology*, 1907, xxxvi. 68.



To sum up, the excretion, in some cases of cystinuria, of cadaverin and putrescin both in the urine and fæces, in quantities such as have not been met with in any other condition, is an established fact. The easier explanation which ascribes their presence to an intestinal infection offers no adequate explanation of their association with the excretion of cystin, and receives no support save from its inherent plausibility. The alternative theory, which regards the diaminuria as an outcome of the same error of metabolism as the cystinuria, has much more in its favour, despite certain obvious difficulties, for other primary protein fractions besides cystin and lysin are undoubtedly present in the urine of some of the subjects of this anomaly.

Of the other amino-acids which enter into the composition of the molecules of proteins only leucin and tyrosin have, as yet, been found in the urine of cystinurics. In 1891 Piccini and Conti<sup>24</sup> detected crystals of tyrosin, together with those of cystin and of uric acid, in the urinary sediment in their case. Percival<sup>25</sup> spoke confidently of the presence of both leucin and tyrosin in small amounts in the urine of his cystinuric patient. Moreigne<sup>26</sup> also described the presence of tyrosin, but the method which he recommends for its detection suggests that he mistook for crystals of that substance the prismatic crystals of cystin hydrochlorate.

The presence of these amino-acids in the urine of one cystinuric at least has been established, beyond all possibility of doubt, by Abderhalden and Schittenhelm,<sup>27</sup> who extracted from the urine of their patient both tyrosin and leucin in considerable quantities, and fully proved their

<sup>24</sup> *Lo Sperimentale*, 1891, xlv. 353.

<sup>25</sup> *Archivio Italiano di Clinica Medica*, 1902, xli. 50.

<sup>26</sup> *loc. cit.*, sub 16.

<sup>27</sup> *Zeitschrift für physiologische Chemie*, 1905, xlv. 468.

nature by the ultimate analysis of the tyrosin which separated after evaporation of the urine, and of the naphthalene-sulphonic-derivate of leucin obtained therefrom. Emil Fischer and Zuzuki<sup>28</sup> also detected tyrosin in a cystin calculus. Leucin and tyrosin would appear to be rarer constituents of such urines than are the diamines. In no case which I have had under observation have they been detected, although sought for. Millon's reagent lends valuable aid in the detection of tyrosin, for although all urines yield some pink colour with this reagent, even in the cold, the presence of tyrosin in any considerable quantity conspicuously intensifies the colour on heating. It may safely be concluded, indeed, that any urine which does not yield more than the normal reaction on heating with Millon's reagent does not contain tyrosin in appreciable amount.

The urine of Loewy and Neuberg's patient contained no tyrosin, but when tyrosin was given to him by the mouth he excreted it unchanged and almost quantitatively. After a dose of 6.2 grammes of tyrosin no less than 4.82 grammes were recovered from the urine, and its nature was confirmed by ultimate analysis. On the other hand, C. E. Simon,<sup>29</sup> Alsberg and Folin,<sup>30</sup> Thiele, Hele, Hurtley, and I have failed to find any tyrosin in the urine of other cystinurics after the administration of similar doses. Hurtley and I obtained from the urine of our patient, on the days on which tyrosin was given, a benzoyl compound which melted at 253° C. The yield was small and its nature was not determined. Thiele obtained no such product by benzoylation of the urine of the tyrosin day in his case. Loewy and Neuberg also found that aspartic acid, another protein fraction, was excreted by their patient when administered

<sup>28</sup> *Zeitschrift für physiologische Chemie*, 1905, xlv. 405.

<sup>29</sup> *Ibid.*, 1905, xlv. 357.

<sup>30</sup> *American Journal of Physiology*, 1905, xiv. 54.

by the mouth, but in Alsberg and Folin's case aspartic acid so given did not reappear in the urine.

The question arises whether the implication of the several protein fractions follows any definite sequence, according to the extent of the error, or whether, in different cases, now one and now another fraction is implicated. To this question no certain reply can yet be given. It may even be that the excretion of cystin is not essential, and that in some cases of the anomaly which we speak of as cystinuria, some other protein fractions may escape destruction whilst cystin is dealt with in the normal way; but the cystin fraction alone appears to escape destruction in so many cases that it is highly probable that it is the first to be involved in the error.

Not a few cystinurics have excreted cystin and diamines, but no leucin or tyrosin. Thus Desmoulière could find no leucin or tyrosin in the urine of his patient whose urine and faeces contained both cadaverin and putrescin. On the other hand Percival, who found leucin and tyrosin in the urine of his patient, failed to detect any diamine, and Abderhalden and Schittenhelm make no mention of the presence of diamines in their case in which leucin and tyrosin were undoubtedly excreted. The evidence available suggests that the incidence of the error upon the several protein fractions is capricious, rather than that the amino- and diamino-acids are involved in any definite order.

The nitrogenous metabolism of cystinurics deviates from the normal in certain ways not fully explained. When diamines and amino-acids are present an excess of undetermined nitrogen in the urine—i. e. of nitrogen not contained in urea, uric acid, kreatinin, and ammonia—is to be expected, but Alsberg and Folin, and Wolf and Shaffer, whose observations upon this aspect of the subject are the most complete yet carried out, found an abnormally high nitrogenous residue, although the urine of their patients

contained no diamine or recognizable quantities of amino-acids other than cystin. This excess could not be ascribed to the small quantity of cystin excreted. Marriott and Wolf also found an excess of undetermined nitrogen, but their patient excreted diamine. Alsberg and Folin suggest that the excess of undetermined nitrogen in the urine in their case, which was conspicuously increased when cystin was given by the mouth, may have been due to an incomplete destruction of cystin, the sulphur of which was excreted as sulphate, whereas the nitrogen was apparently not eliminated in urea. Wolf and Shaffer are inclined to ascribe it to amino-acids, in quantities too small to be detected by the available methods. In their case with biliary fistula the proportion of undetermined nitrogen remained unduly high, even when cystin was no longer being excreted in the urine.

It will be clear, from all that has gone before, that we are still far from being in a position to formulate a satisfactory theory of cystinuria. Before this can be done it will be necessary to accumulate many more data by patient investigation of individual cases, and, above all, quantitative data. Obviously the anomaly is a very complex one, of different range in different cases and even of distinct natures. No theory which will explain the ordinary cases of cystinuria can apply to such a case as that studied by Loewy and Neuberg, in which any protein fraction given by the mouth appeared in the urine, the monamino-acids as such and the diamino-acids as diamines, whereas these same fractions, when given in combination as polypeptides or in the more complex form of proteins, were dealt with in the ordinary way. This raises important side issues relating to the degree of disintegration which proteins normally undergo in the alimentary canal, and suggests that, if they were broken down into their component amino- and diamino-acids before absorption, this patient should have

excreted the fractions unchanged, as, indeed, he did excrete cystin and the mixed products of advanced tryptic digestion. When the patient took glycocoll by the mouth some 20 per cent. of the quantity administered was apparently burned, whereas when glycyl-glycin was given, a dipeptide which is not split up by the pancreatic ferment but is split by the intestinal juice, only 10 per cent. escaped destruction. Polypeptides were completely burnt.<sup>31</sup>

The varying extent of the error, as regards the number of protein fractions involved in cases of cystinuria, suggests that it is manifested at an early stage of the catabolic series, and concerns a mechanism which deals with a number of amino- and diamino-acids in common. In this respect cystinuria stands in conspicuous contrast to alcaptonuria, which involves a late stage in the catabolism of two closely allied protein fractions, a stage so late, indeed, that the tyrosin and phenylalanin derived from the breaking down of food and tissues are alike implicated. The fact that the abnormal substances excreted retain their amino-groups intact points in the same direction, for there is strong evidence that desamination occurs at an early stage of the breaking down of proteins. It is a process to which all the protein fractions are normally subjected and, as Lang<sup>32</sup> has shown, desaminating enzymes are widely distributed in the tissues. Moreover, it would appear that the several amino-acids are desaminated with various degrees of ease, and that different members of the group are specially attacked in different organs and tissues. Lang found that phenylalanin was the most difficult of desamination of the protein fractions, and under some conditions cystin and tyrosin were refractory. The maximum yield of ammonia, by which the change was estimated, was obtained when glycocoll or leucin was exposed to the action of the liver or the pancreas.

<sup>31</sup> *Biochemische Zeitschrift*, 1907, ii. 438.

<sup>32</sup> Hofmeister's *Beiträge*, 1904, v. 321.

This complex process of desamination, carried out in a variety of tissues, by the action of enzymes which appear to differ somewhat in their modes of action, may possibly afford a clue to the differences observed in individual cases of cystinuria. Thus the observation of Lang that the power of removing the amino-group is specially conspicuous in the intestinal mucous membrane on the one hand, and in the liver on the other, gives a hint of a possible explanation of such different types of cystinuria as are exemplified in the ordinary cases and in Loewy and Neuberg's respectively; an explanation based upon absence or inhibition of the enzyme which effects the change in the one situation or in the other. It would seem, also, that when, for any reason, the amino-acids escape this early change no alternative mechanism is available for dealing with them, and they are excreted unchanged or in the case of lysin and arginin as diamines.

Ploos van Amstel's<sup>33</sup> extraordinary case, which was referred to in an earlier chapter, is of special interest in this connexion. The patient, a woman aged 42 years, passed a cystin calculus under observation, and showed the pigmentary signs of ochronosis. Her urine had the ordinary characters of alcapton urine, and deposited hexagonal crystals of cystin. Neither diamines, leucin, nor tyrosin could be found in it. The case offers the only known example of this combination of anomalies, and also of an alcaptonuric whose output of homogentisic acid was not increased by tyrosin taken by the mouth.

The experiment described by Ploos van Amstel in his published paper was incomplete, but I am indebted to that observer for an account of a second experiment afterwards carried out. He states that when the patient swallowed five grammes of tyrosin there was no notable increase of homogentisic acid in the urine, and that the tyrosin taken

<sup>33</sup> *Sammlung klinischer Vorträge*, 1910. *Innere Med.*, 193.

was recovered, almost quantitatively, from the urine. Obviously in this case, as in that of Loewy and Neuberg, the tyrosin of the food and tissue proteins was dealt with, but in the imperfect manner of alcaptonurics, as witness its absence from the urine, and an excretion of six or seven grammes of homogentisic acid in the day ; whereas tyrosin taken as such escaped destruction.

The most probable explanation of the observed facts would appear to be that having failed to be desaminated at the outset, the tyrosin taken by the mouth was unable to follow either the ordinary catabolic path through homogentisic acid or the alternative route which is known to exist. If this be the true explanation it lends support to the hypothesis that the essential error in cystinuria is a defect of desamination of the amino-acids of which the protein molecules are built up, and that cystinuria, like alcaptonuria, may be classed as an arrest rather than a perversion of metabolism.