CONCERNING CYSTINURIA AND DIAMINES.

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It is only of recent date that the occurrence of these substances in the excreta has ceased to be regarded as due to abnormal bacillary or mycotic action in the alimentary canal, and has come to be recognised as evidence of the occurrence of some anomaly in proteid metabolism whereby primary proteid cleavage products are excreted unchanged or after slight change in the urine and fæces. Attention has been especially drawn to the condition wherein cystin is excreted because of its easily recognisable crystalline form. It however has now been shown that the anomaly may not only affect the sulphur containing group of the proteid complex but that the other amido-acids may be affected similarly to the cystin. It may further be possible that there are some cases in which the sulphur containing group may not be affected whilst the other amido-acids are. Thus leucin and tyrosin either singly or together have been described in the urine of cystinurics by Abderhalden and Schittenhelm[1], Moreigne[2], Percival[3], and Piccini and Conti[4]. The anomaly may also affect the di-amido cleavage products and lead to the presence of cadaverin and putrescin in the excreta. These bodies were first demonstrated in the excreta by Baumann and Udranzsky[5] in a case of cystinuria and since then have been noted by other investigators in similar cases. The presence of these substances, like that of the other amido-acids, is not constant in cases of cystinuria, and further these bodies may be present on one occasion and not on others, as has been shown in the cases investigated by Udranzsky and Baumann[5] and Garrod and Hurtley[6]. In the former case occasionally no diamines were found for several days, and once when investigated by Garcia[7] only putrescin was found in the urine. In the latter case in the majority of specimens examined no diamines were detected, in the rest at times only putrescin was found and at others both. As a rule cadaverin is usually found in the urine and

putrescin in the fæces. These diamines have only been discovered in the minority of cases. Cohn[8] examined a family of seven and found no diamines in any of them, Pfeiffer[9] examined a similar family with like results, in Bödtker's[10] case no diamines occurred, whilst in two cases investigated by Stadthagen and Brieger[11] only cadaverin was noted in the urine. Diamines have not been found in the excreta of normal individuals, but Roos[12] demonstrated them in cholera dejecta, W. Hunter[13] found traces by treating large quantities of urine from cases of pernicious anæmia, Dabrowski[14] however obtained traces of cadaverin by evaporating 100 litres of normal urine. The true origin of the diamines was first put forward independently by Moreigne[2] and C. E. Simon[15] who regarded them as due to the same anomaly of arrested proteid metabolism as cystinuria, but affecting the hexon bases. Thus cadaverin is derived from lysin by the loss of CO_2,

$$\underset{\text{lysin}}{CH_2.NH_2.(CH_2)_3CH.NH_2.COOH} = \underset{\text{pentamethylene diamine (cadaverin)}}{CH_2.NH_2(CH_2)_3CH_2.NH_2} + CO_2$$

Putrescin is derived from the ornithin fraction of arginin which then loses CO_2

$$\underset{\text{arginin}}{C(NH)NH.CH_2-(CH_2)_2-CH.NH_2.COOH}$$

$$= \underset{\text{ornithin}}{NH_2.CH_2.(CH_2)_2.CH.NH_2.COOH} + \underset{\text{cyanamide}}{CN.NH_2}$$

$$NH_2.CH_2(CH_2)_2.CH.NH_2.COOH = \underset{\text{tetramethylene diamine (putrescin)}}{NH_2.CH_2(CH_2)_2.CH_2.NH_2} + CO_2.$$

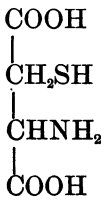
This view has gained support from the experiments of Neuberg and Loewy[16] who administered to their patient, who did not spontaneously excrete diamines, lysin and arginin by the mouth and found that with the former cadaverin and with the latter putrescin appeared in the urine. Garrod and Hurtley[6] however tried a similar experiment on the case investigated by them with negative results. This is the more remarkable because, although at the time of experiment this patient did not spontaneously excrete diamines he at a previous period of observation had done so. When arginin was administered to him putrescin did not appear in the urine.

Just as the presence of diamines is by no means constant in the urine of cystinurics, in the same way the other amido-acids are also very variable in their occurrence. Besides the cases mentioned above in which leucin and tyrosin appeared others have been recorded in which no such substances were present, as in those of Loewy and Neuberg[16], Alsberg and Folin[17], C. E. Simon[15] and Garrod and Hurtley[6]. Cystinuric patients again appear to differ in their reaction

to mono-amido-acids when administered by the mouth, as they do to the di-amido-acids when similarly administered. Thus Loewy and Neuberg[16] found that leucin, tyrosin and aspartic acid given in this way to their cystinuric patient, whose urine was free from such substances, could almost be wholly recovered in the urine; whereas in the cases of Alsberg and Folin[17], C. E. Simon[15] and Garrod and Hurtley[6] tyrosin given by the mouth did not appear in the urine. When cystin is fed, similarly, diversity of results has been noted.

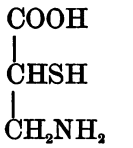
In Loewy and Neuberg's[16] case the results varied according to the nature of the cystin administered. When cystin from a calculus was thus given there was no increase in excretion of cystin in the urine, the whole amount administered was broken up and passed in a more or less completely oxidised form, whereas when protein cystin was given by the mouth the whole amount was added to the ordinary cystin output. In the case investigated by Alsberg and Folin[17] ordinary protein cystin given *per os* was completely passed in the urine as sulphates and less oxidised sulphur compounds.

Loewy and Neuberg[16] concluded from their experiments that they had obtained confirmation of their view that there are two isomeric cystins and that the difference in the results of administering calculous and protein cystin was due to differences in the two varieties. They found that the ordinary deposit of cystin in cystinuria is identical with protein cystin and that its constitution is as has been shown by Friedmann[18] a-amino-b-thio-propionic acid, whereas peculiarly the cystin forming cystin calculi is an isomer having the composition b-amino-a-thio-propionic acid. According to them this isomerism can be explained by supposing that the two isomers originate from a carboxycystein in the albuminous molecule. Carboxycystein having the formula:



from this protein cystin can be derived by the loss of the "a" acid group, thus

and the isomeric calculous cystin by losing the "b" acid group,



They concluded that their patient was incapable of breaking up the "a" amido cystin but could break up "b" amido cystin and that this was in accordance with the results that they obtained in the same case on feeding leucin, tyrosin and aspartic acid, which also passed unchanged into the urine, these also being "a" amido-acids. The "b" amido cystin was broken up because it was a variety which did not appear normally in the tissues, and so far only "a" amido-acids are known to occur in nature.

These results are however at variance with the observations of other investigators on other cases of cystinuria, who have shown that in their cases ordinary protein cystin was completely broken up and oxidised and that other "a" amido-acids share the same fate when administered to these patients by the mouth. Finally Emil Fischer and Suzaki[19] throw some doubt on the occurrence of an isomeric cystin in cystin calculi and suggest that the difference in the calculous cystin and protein cystin is due to the presence of impurities in the calculous cystin, since they were able to demonstrate tyrosin in some calculi obtained from the same source as Neuberg. Latterly Abderhalden[20] has come to the same conclusion as regards the identity of the two cystins as Fischer and Suzaki.

It is of interest to note that other bodies of as yet imperfectly known constitution have been found in the urine of cystinuric patients. Thus Bödtker[10] in addition to benzoyl cadaverin and putrescin found another crystalline body on benzoylation of the urine of a case of cystinuria examined by him. This substance contained 2·88 % of N and melted at 203°—204° C. Garrod and Hurtley[6] in a case recently investigated by them, in which at the time of observation the urine was free from diamines, found on benzoylation a crystalline substance in the form of fine colourless needles melting at 205° C., containing 4·8 % N. They came to the conclusion that this body might be possibly in some way derived from tryptophan. When tyrosin was administered to the same patient another crystalline substance was obtained on benzoylation of the urine. Its melting point was 253° C., and was thus not di-benzoyl-tyrosinamide which melts at 246° C.

It will thus be seen that cases of cystinuria differ very much from one another. In view of the great physiological interest attaching to this condition with regard to the fate of the amido-acids in the intestinal mucosa and tissues, I was very glad to be able to have the opportunity of investigating a few points in connection with this anomaly.

The points investigated were the effects of:

1. Abstinence from food for a day.
2. An almost pure carbohydrate diet.
3. An excessive meat diet.
4. The administration of tyrosin.
5. The administration of some of the patient's own purified cystin.

The patient's urine under ordinary conditions was pale yellow in colour and had a large deposit of cystin with some oxalates. The results of the analysis of a 24 hours' specimen were as follows:

Quantity	1150 c.c.	
Total nitrogen	13·6 grammes	
Uric acid	0·78	,,
Total sulphates as SO_3	1·3	,,
Aromatic sulphates as SO_3	0·163	,,
Total sulphur less cystin as SO_3	1·68	,,
Cystin	0·532	,,
"Neutral" sulphur less cystin	0·38	22·8 % of total sulphur

The urine did not give a red colour with Millon's reagent. Amido-acids were tested for by the method of Abderhalden and Schittenhelm[1]. The urine was first filtered free from cystin, which was then dissolved in ammonia, re-precipitated by glacial acetic acid, collected and weighed. A measured quantity of the urine was then taken, acidified with glacial acetic acid and evaporated at 48° C. under diminished pressure. A precipitate of cystin occurred which was then removed. The urine was then still further concentrated till it gave no more precipitate when placed in the cold. The resulting precipitates were collected, dissolved in warm 10 % ammonia solution, cooled; no precipitate occurred from this solution. The alkaline reaction of the solution was then gradually diminished with glacial acetic acid till just short of neutralisation. The solution was then cooled but no precipitation occurred. The absence of a precipitate as the result of this treatment showed the absence of tyrosin. The solution was then made strongly acid with glacial acetic acid, when the cystin was precipitated. This precipitate was collected, dried and weighed. From this the total

amount of cystin was calculated and added to that spontaneously precipitated. The cystin had been thus removed from the urine and if any tyrosin had been present it would have been detected as well. The presence of other amido-acids could not be accurately investigated, since the solubilities of the b-naphthalin sulphochloride[21] compounds of the various amido-acids have not been worked out sufficiently to allow of their separation from one another. The solubilities of the leucin compound have however been ascertained, and these were made use of to see if any of this substance could be separated from the di-amido compound. For this purpose the urine was taken after the removal of the cystin as mentioned above, made alkaline with normal caustic soda and shaken with an ethereal solution of b-naphthalin sulphochloride after the method of Abderhalden and Schittenhelm[21]. After removal of the ether the urine was treated with 5/N solution of HCl, the precipitate collected and treated with a small quantity of cold absolute alcohol, which on evaporation was found to have extracted nothing from the precipitate, thus showing that there was no leucin present, since the b-naphthalin sulphochloride compound of leucin is very easily soluble in cold absolute alcohol. The corresponding compound of the diamines is apparently not soluble in cold absolute alcohol.

The total sulphur and sulphates were estimated in some of the urine from which the cystin had been removed by acidification and evaporation.

By these methods the total cystin, sulphates, sulphur less the cystin and the presence of mono-amido-acids were determined. On applying Salkowski's test for thio-sulphates to the original urine they were found to be present in considerable amount. Some of the original urine was then treated by the benzoylation method for diamines. A copious precipitate occurred which after purification proved to be benzoyl cadaverin. The purified precipitate was dissolved in a small amount of warm alcohol and was not precipitated when added to a large excess of ether. The melting point of the crystals was about 130°C. and ·1 grm. of the substance contained ·00895 grammes of N estimated as ammonia, giving a percentage of 8·95 of N, the calculating percentage being 9·03. The unprecipitated benzoyl cadaverin in the urine was also separated and added to the other. From these results the total amount was estimated after drying and calculated to be 0·968 grammes for the 24 hours.

A specimen of fæces was also treated after the method of Udranzsky and Baumann[5]. A benzoyl compound was obtained

which after having been dissolved in a little warm alcohol was found to be in greater part precipitated by excess of ether, only a small part remaining in solution. The precipitated part was therefore benzoyl putrescin, its melting point was 174° C. and it was found to yield 9·24 % of N, the calculating percentage being 9·46.

The soluble part was benzoyl cadaverin, but was in only very small amount. The quantity of benzoyl putrescin was 0·54 grammes.

The features of this urine were therefore; 1. The presence of notable amounts of cystin. 2. The absence of any other mono-amido-acids. 3. The excess of neutral sulphur, this in addition to the cystin. 4. The presence of the derivatives of the di-amido-acids in the urine and fæces.

1. *Abstinence from food for a day.* The patient having had to undergo an operation no food was taken for over 24 hours. The urine for 24 hours was collected on the day of operation from 8 a.m. to 8 a.m. on the following day. The patient had had no food after midnight on the day of operation. Chloroform was administered.

The results of the analysis of the urine were as follows:

Quantity passed	800 c.c.
Total nitrogen	5·152 grammes
Uric acid	0·4 ,,
Total sulphates as SO_3	0·31704 ,,
Total sulphur less cystin	0·6775 ,,
Neutral sulphur less cystin	0·35946 ,,
Cystin	0·486 ,,
Benzoyl cadaverin	0·318 ,,

2. *Effect of an almost pure carbohydrate diet.* On another occasion the patient was given a diet consisting of bread, butter, rice, fruit, tea, one pint of milk and no meat. The urine was collected after the passage of the morning urine on the day of the experiment for the next 24 hours.

The analysis of the urine was as follows:

Amount	1550 c.c.
Total nitrogen	9·114 grammes
,, uric acid	0·412 ,,
,, sulphates	0·8235 ,,
,, sulphur less cystin	1·1626 ,,
,, neutral sulphur	0·3391 ,,
,, cystin	0·57 ,,
,, benzoyl cadaverin	0·366 ,,

3. *The effect of an excessive meat diet.* The patient was given 1 lb. of

meat in addition to his usual food. The urine was collected as before mentioned.

Amount	1625 c.c.s	
Total nitrogen	17·29 grammes	
„ uric acid	1·16	„
„ sulphates as SO_3	2·04	„
„ sulphur less cystin	2·4681	„
„ neutral sulphur less cystin ...	0·4281	„
„ cystin	0·514	„
„ benzoyl cadaverin	1·108	„

No other amido-acids were discovered.

4. *Effect of administration of tyrosin.* Five grammes of tyrosin were given in addition to the ordinary diet. The tyrosin given was optically active. No trace of tyrosin was found in the urine by the above mentioned methods, nor was any trace of the crystalline substance, obtained by Garrod and Hurtley[6] in their case, found after benzoylation of the urine. The benzoyl compound obtained consisted wholly of benzoyl cadaverin.

5. *Effect of administration of cystin.* In addition to the ordinary diet 4·6 grammes of the patient's own cystin were administered. In order to obtain this amount the urine was collected for a prolonged period and treated with glacial acetic acid and a little chloroform to preserve it. The urine was kept in the cold and the resulting precipitates of cystin collected. These were subsequently purified by dissolving in ammonia and re-precipitating with glacial acetic acid.

In order to investigate accurately the urine for the purposes of this experiment, it was collected for three days; (1) for the 24 hours before the administration of the cystin, (2) and (3) for the two successive 24 hours following.

The cystin was estimated by Abderhalden's[1] method. The spontaneously precipitated cystin was collected on a filter and dissolved in ammonia. The resulting solution was then added to the urine and the whole was accurately measured. A definite quantity was then treated by this process. The b-naphthalin sulphochloride method of Abderhalden and Schittenhelm[21] was no good for this purpose, because there is as yet no method of separating the b-naphthalin sulphocystin from the similar di-amido compounds. The urine was however also treated by this method and the resulting precipitate treated with a large quantity of hot absolute alcohol and allowed to cool, when there separated out a crystalline material which was in excess of the amount of cystin calculated by the other method. However the

amount did not in any way approach the quantity of cystin taken, so that the results obtained by this method are really confirmation of the other one.

The results of the analysis were:

	24 hours spec. preceding administration	1st 24 hours after administration	2nd 24 hours
Amount	1400 c.c.	1450 c.c.	1400 c.c.
Total nitrogen	12·186 grms.	12·612 grms.	11·897 grms.
,, sulphur (SO_3) less cystin	1·9686 ,,	4·3217 ,,	1·9198 ,,
,, sulphates	1·6317 ,,	3·15724 ,,	1·6502 ,,
,, neutral sulphur less cystin	0·337 ,,	1·1634 ,,	0·2696 ,,
,, cystin	0·577 ,,	0·604 ,,	0·561 ,,

The results brought out by these experiments with regard to the cystin part of the anomaly are:

1. The amount of cystin excreted was practically independent of the diet.

2. The ability of the patient to break up cystin administered by the mouth, even though it was cystin that had been previously excreted by the patient; his tissues having been incapable of breaking it up.

3. The large amount of imperfectly oxidised sulphur in the excreta besides the cystin.

These results tend to show that in the case experimented upon practically the whole of the cystin excreted was derived from tissue katabolism since it was independent of taking food and its nature and amount. Since a day's abstinence from food had no effect on the excretion it follows therefore that the greater portion by far of the cystin excreted must have been derived from tissue katabolism, and that being so there must have been in this case a marked deficiency in a ferment which normally removes the sulphur group from the thio-amido acids. Moreover food had no influence on the cystin excretion; it appears to follow that if the cystin excreted was in part derived directly from the thio-amido part of the food taken it would be necessary to conceive of the absorption of the thio-amido-acid complex as such by the intestinal mucosa, and since there is already evidence from the starvation experiment that the tissues were deficient in the sulphur removing ferment, then it would follow that the excretion should have been influenced by diet, since a greater or less amount of these bodies would have been brought to tissues imperfectly capable of dealing with them. This however was not the case in this experiment.

Physiologists now growingly hold the opinion that the amido-acids are not absorbed as such but are de-nitrified by the intestinal mucosa

and converted into the corresponding simple fatty acids before absorption. These experiments appear to lend additional support to this view because diet and even the administration of cystin had no effect on the cystin excretion. Now if the thio-amido complex were under ordinary circumstances absorbed as such, then alterations in diet should have had a marked influence on the cystin excretion, seeing that there is in addition evidence of a deficiency in the sulphur removing ferment in the tissues. The most important evidence however appears to be given by the cystin experiment. The cystin given was some that the patient had previously excreted. If now the cystin part of the proteid were absorbed, without the removal of the sulphur, directly by the intestinal mucosa, either because this is the ordinary course of events, or because in this anomaly the intestinal mucosa might have been deficient in the sulphur removing ferment, then the tissues being, as has already been pointed out, incapable of dealing fully with the thio-amino-acids, a large amount of the absorbed cystin should have been excreted unchanged in the urine. This however was not the case, there was no increase in the cystin output but the whole amount taken was excreted in the urine as sulphates or less completely oxidised compounds. This shows that the absence of increase of cystin in the urine was not due to non-absorption by the intestinal mucosa and passage unchanged in the fæces. It would thus appear from a consideration of this case that the intestinal mucosa ordinarily has a ferment which breaks up the thio-amino part of the proteid complex and that this is not absorbed as such but is broken up before absorption. The tissues as well possess a ferment which similarly breaks up the thio-amino complex resulting from tissue katabolism. The explanation that the thio-amino-acids are absorbed as such and broken up in the tissues does not seem probable, for then it would have to be considered that the tissues were incapable of dealing with the thio-amino-acids derived from tissue katabolism but capable of breaking up those derived from the food. The anomaly in this case would therefore seem to be due wholly to an error in the tissue ferments and not due to any abnormality in the intestinal mucosa.

This explanation of the anomaly is supported by the cases of Alsberg and Folin[17], and Garrod and Hurtley[6]. Leo[22] has previously shown that diet has practically no effect on the excretion on the cystin in cystinurics. In Loewy and Neuberg's[16] case the farther possibility is opened out that there may be persons with this anomaly in whom there may be not only a defect in the tissue ferments but also one in those of the intestinal mucosa, since in their case cystin

administered by the mouth reappeared almost completely in the urine. It might have been possible in this case to note variations in the cystin excretion corresponding to variations in the diet. The peculiarity of this case however which is difficult to explain is in the diametrically opposing results when protein cystin and calculous cystin were administered.

4. These experiments also show that in this patient no other mono-amido-acids were present in the excreta even after administration by the mouth.

These results are parallel with those of Garrod and Hurtley[6], Alsberg and Folin[17] and C. E. Simon[15] who also found no trace of amido-acids after administration by the mouth. These results also lead to the same conclusions concerning the fate of the amino-acids in the alimentary canal as was brought out by the consideration of the cystin experiment. It would also appear that the de-nitrifying ferment cannot act on the thio-amino-acids till the sulphur group has been removed.

Loewy and Neuberg's case[16] differed in that mono-amido-acids similarly administered were passed almost completely unchanged in the urine, and this appears to point to a defect in the intestinal mucosal ferments as well as in those of the tissues.

5. This was one of the rarer cases of cystinuria in which diamines were present in the excreta. Diet appeared to have a greater effect on the diamine excretion than on the cystin, which was also previously noted by Garcia[23]. Unfortunately the experiment of administering arginin and lysin could not be performed. So far there are only two observations on this point; Loewy and Neuberg's[16] and Garrod and Hurtley's[6]. In the latter case no diamine appeared in the urine after the administration of arginin even though the patient had at a previous period passed putrescin and cadaverin spontaneously. In the former case peculiarly, although the patient did not spontaneously pass diamines, administration of the di-amido-acids was followed by the appearance of the corresponding diamine in the urine. No observations were apparently made as regards the fæces.

The anomaly of the occurrence of the diamines in the excreta does not appear to admit of the same explanation as the occurrence of cystin and the mono-amido-acids. The di-amido-acids would appear to be acted upon in a different way.

There may possibly be a different de-nitrifying ferment for them, since in some cases it may be that the mono-amido-acids only are affected and

the di-amido-acids normally broken up and *vice versa.* If there were only one variety of ferment acting on both mono- and di-amido-acids alike then it would be expected that when diamines appeared in the excreta mono-amido-acids would appear as well (or the different amido groups may have differences in resistance to ferment actions). A further peculiarity lies in the observation that cadaverin usually appears in the urine and putrescin in the fæces, as if the latter were excreted by the intestinal mucosa in the majority of cases. Loewy and Neuberg's[16] case is again peculiar in this respect, since their patient could break up the hexon bases in the food but not when given as such. It may be as suggested by Garrod and Hurtley[6] that there are cases which can deal perfectly with the amido-acids when given in the ordinary combination as proteid, but not when given as such.

CONCLUSIONS.

It appears justifiable to draw the following conclusions from a consideration of the cases of cystinuria:

1. There is strong evidence that the amido-acids are de-nitrified by the intestinal mucosa.

2. In cystinuria cases a defect may be present in the

a. sulphur removing ferments;

b. de-nitrifying ferments;

c. or a defect in both.

The defect appears to be most usual in the tissues but may also occur in the intestinal mucosal ferments.

3. De-nitrification of the thio-amino-acids does not occur till the sulphur has been removed.

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