XVIII. PROTEIN METABOLISM IN CYSTINURIA.

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With regard to the anomaly of protein metabolism as seen in cystinuria, two schools of thought exist. On the one hand, Folin and his co-workers believe that there is but one type of the derangement, although it is capable of exhibiting varying degrees of severity. On the other hand, Neuberg considers that the condition is of three kinds, and that, whilst the presence of cystine in excessive quantities in the urine is characteristic of all its phases, yet it may be accompanied in some cases by tyrosine and leucine, and in others by putrescine and cadaverine. These very divergent views give the subject a complex aspect, and create, moreover, the difficulty of evolving a working hypothesis which might serve as a basis for the re-investigation of a problem not only of practical but of theoretical importance in that its elucidation would probably throw light on our present conceptions of normal protein metabolism. For these reasons further studies of individual cases appear necessary.

The present study arose from the admission to a ward of the Royal Infirmary, Edinburgh, of a patient with the following clinical history.

"Patient when seen was aged 23. In June, 1926, early one morning she was seized with a very acute pain in the left side of the abdomen. It was so severe that she had to get up from bed and walk about. This pain lasted for three or four hours very severely, and gradually passed off during the next two days. A good deal of vomiting was present when the pain was at its worst. Micturition caused an exacerbation of the pain. So far as she is aware she has never passed any calculi or gravel. No jaundice was present during the attack.

"Special interest was taken in the case because of the strong family history of the disease, several members having already died from the consequences of this disturbance.

"Apart from an operation for appendicitis in 1925, the patient had had no other illnesses. She was to all appearances a normal healthy girl."

Cystinuria is classified by Garrod as one of the "inborn errors of metabolism" and in this connection the reference in the above clinical notes to the occurrence of the derangement in other members of the family is highly interesting. The genealogical tree of the patient has been investigated so far as it was possible to do so, and it was found to illustrate very clearly the

hereditary aspect of the anomaly. Although there are, it is to be regretted, some gaps in the tree about which no information can be gained, nevertheless, since such records in the literature are not numerous, it is thought worthy of inclusion here (Fig. 1). The position of the subject of the present study in the family tree is indicated by the encircled figure, while crosses show the existence of members concerning whom no information is available.

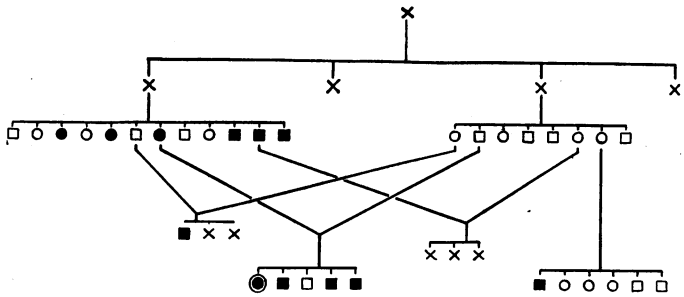

Fig. 1.

Aim of the investigation.

The original object of the present investigation was to test by urinary analysis the dietary methods advocated by various workers in this field for the treatment of cystinurics in an endeavour to ameliorate the condition of the patient in whose family several deaths, apparently attributable, directly or indirectly, to the derangement, had occurred. While the work was in progress, however, the patient displayed such keen interest in her case that the scope of the investigation was greatly extended and additional feeding experiments carried out in order to gain further information regarding the location of the disturbance. The experiments herein described may be briefly summarised as follows.

1. Hospital diet—control.
2. Hospital diet plus sodium bicarbonate.
3. ,, ,, disodium hydrogen phosphate.
4. ,, ,, cystine.
5. ,, ,, glutamic acid.
6. Low protein diet containing a minimum amount of cystine.
7. High protein diet.

Each experiment was followed by an interval during which the patient was placed on the normal hospital diet in order to stabilise her metabolism so far as possible before the next experiment was carried out.

Method.

Throughout the whole of the period of investigation the protein content of the diet was carefully controlled so that the results obtained from the analyses of the urine during the different experiments might be comparable. Even under such conditions fluctuations occur and are difficult to explain. The urine was collected under toluene in 24-hour samples and on these estimations of the total nitrogen (micro-Kjeldahl), ammonia (aeration), urea (urease), inorganic, ethereal and neutral sulphur (Fiske), amino-nitrogen (Folin) and cystine (Looney) were performed. The method of estimating the cystine requires comment. In most of the recorded investigations of cystinuria the amount of urinary cystine was determined either by its actual isolation from a concentrated urine [Gaskell, 1907] or by calculation based on the assumption that the increase either in the neutral sulphur itself [Alsberg and Folin, 1905] or in the ratio of neutral sulphur to total sulphur [Mester, 1879] was due to the excretion of cystine as such. Such methods can scarcely be regarded either as being simple or accurate. For our present purpose, however, the colorimetric method devised by Looney [1922] was available, and, since preliminary attempts at the quantitative recovery of purified cystine added to both normal and cystinuric urines gave satisfactory results, the method was used throughout the investigation. While the later stages of the work were in progress a modification by Hunter and Eagles [1927] of the original method of Looney was published. The replacement of sodium carbonate by sodium hydroxide as one of the reagents in the method certainly eliminates the possibility of "cloud" formation which occasionally occurs when the original method is used. Nevertheless the results obtained by both methods on the same solutions of cystine do not differ outside the limits of colorimetric methods of estimation.

At intervals, estimations of the non-protein-nitrogen, urea and chloride were carried out on samples of blood taken from the patient.

In addition to this quantitative work, qualitative tests on the urine, sometimes concentrated under reduced pressure, for tyrosine, leucine, cadaverine and putrescine, were performed. On numerous occasions, moreover, the patient's faeces were examined for the presence of the last-named two substances.

Analysis of results.

1. *Hospital diet.* Before the main feeding experiments were attempted it was essential to discover how far the patient's metabolism could be stabilised, especially with regard to the excretion of cystine. For this purpose the patient was kept in bed and given a convalescent diet yielding on an average 1750 calories. The results obtained during the latter end of this period, given in Table I, show that the cystine output varied considerably from day to day and bore no relation to the total nitrogen in the urine. A possible reason for

Table I.

Date (1926)	Protein in diet g.	Urine cc.	Nitrogen g.			Cystine g.			Sulphur g.			Amino-N g.	Remarks
			NH_3-	Urea-	Total	Aq.	Ppt.	Total	Inorg.	Neut.	Total		
10. xi.	69	1885	0·26	4·64	9·05	0·08	0·02	0·10	1·05	0·47	1·52	0·29	
11. xi.	75	1300	0·28	4·16	8·18	0·15	0·05	0·20	0·81	0·33	1·22	0·20	
12. xi.	75	1060	0·31	3·08	6·68	0·54	0·13	0·67	0·74	0·80	1·56	0·17	
13. xi.	69	1380	0·29	2·61	7·73	0·58	0·21	0·79	0·96	0·89	1·94	0·27	
14. xi.	66	1463	0·34	2·00	7·90	0·21	0·15	0·35	0·91	0·80	1·82	0·26	

Table II.

6. xii.	75	1390	0·20	3·25	6·81	0·26	0·06	0·32	0·80	0·50	1·32	0·21	
7. xii.	85	1060	0·15	2·71	6·84	0·27	0·07	0·34	0·74	0·47	1·24	0·21	23·4 g. $NaHCO_3$
8. xii.	63	1830	0·23	2·34	9·15	0·56	0·09	0·65	0·96	1·06	2·04	0·27	,,
9. xii.	74	1700	0·20	3·55	8·33	0·78	0·02	0·81	1·04	0·92	1·97	0·24	,,
10. xii.	72	1400	0·20	3·58	8·54	0·38	0·15	0·53	0·99	0·65	1·66	0·22	,,
11. xii.	67	1250	0·23	3·15	7·00	0·27	0·07	0·34	0·74	0·64	1·44	0·17	
12. xii.	62	900	0·16	3·26	6·48	0·12	0·07	0·19	0·74	0·29	1·18	0·15	
13. xii.	65	800	0·26	2·94	6·36	0·23	0·05	0·28	0·65	0·34	1·13	0·16	

Table III.

21. xi.	60	1234	0·36	3·09	7·16	0·48	0·09	0·57	0·84	0·64	1·59	0·20	
22. xi.	67	1340	0·23	3·12	7·10	0·64	0·17	0·84	0·58	0·78	1·45	0·27	
23. xi.	78	895	0·35	3·05	6·48	0·20	0·13	0·33	0·43	0·25	0·72	0·21	6 g. Na_2HPO
24. xi.	67	1240	0·36	3·36	6·95	0·36	0·15	0·51	0·47	0·47	0·99	0·20	,,
25. xi.	72	1670	0·35	3·32	8·18	0·13	0·18	0·31	0·51	0·40	0·95	0·26	,,
26. xi.	76	1140	0·30	2·09	7·18	0·35	0·07	0·42	0·36	0·50	0·88	0·27	,,
27. xi.	72	1170	0·39	2·54	6·98	0·40	0·37	0·77	0·38	0·59	0·99	0·27	

Table IV.

15. xi.	66	1050	0·21	2·25	6·32	0·08	0·14	0·22	0·61	0·45	1·10	0·18	
16. xi.	65	1530	0·18	4·34	8·26	0·19	0·23	0·42	0·98	0·77	1·78	0·38	2 g. cystine
17. xi.	70	1200	0·23	3·84	8·52	0·18	0·37	0·56	1·90	0·70	2·70	0·37	4 ,,
18. xi.	74	1700	0·61	4·72	9·01	0·11	0·01	0·13	3·03	0·51	3·66	0·34	6 ,,
19. xi.	74	1000	0·54	5·00	9·40	0·26	0·02	0·28	3·74	1·26	5·02	0·16	8 ,,
20. xi.	74	1707	1·01	7·75	10·41	0·38	0·03	0·41	4·53	0·70	5·25	0·32	
21. xi.	60	1234	0·36	3·09	7·16	0·48	0·09	0·57	0·84	0·64	1·59	0·20	

Table V.

27. xi.	72	1170	0·39	2·54	6·98	0·40	0·37	0·77	0·38	0·59	0·99	0·27	
28. xi.	42	1210	0·25	1·80	4·92	0·50	0·11	0·61	0·23	0·22	0·47	0·17	
29. xi.	69	785	0·32	1·88	6·20	0·08	0·18	0·27	0·64	0·33	1·00	0·18	4 g. glutamic acid
30. xi.	68	1250	0·49	4·63	9·50	0·26	0·17	0·43	0·91	0·39	1·40	0·26	6 ,,
1. xii.	59	1270	0·33	4·00	9·21	0·56	0·19	0·75	0·81	0·54	1·36	0·25	8 ,,
2. xii.	70	1460	0·31	2·54	5·78	0·27	0·04	0·31	0·77	0·40	1·27	0·29	8 ,
3. xii.	64	1020	0·41	2·40	6·56	0·20	0·06	0·26	0·79	0·53	1·40	0·26	

this fluctuation was thought to lie in the varying quantity of cystine in the diet, and although it is impossible to eliminate entirely this amino-acid from the food, a dietary containing the minimum amount of cystine was fed during the remainder of the investigation. Reference will again be made to this point when the results of the experiments with the low and high protein diets (Exps. 6 and 7) are considered.

2. *Hospital diet plus sodium bicarbonate.* One of the methods advocated by different workers from time to time for the treatment of cystinurics has been the administration *per os* of sodium bicarbonate, the purpose being to render the urine strongly alkaline and so diminish, owing to the increased solubility of cystine, the liability of the formation of concrements in the urinary tract. Instances, however, are on record where such treatment even resulted in the actual reduction of the amount of cystine excreted. Thus Klemperer and Jacoby [1914] found that on feeding sodium bicarbonate the excretion of cystine gradually fell to zero only to recur when the use of this drug was stopped. More recently Looney *et al.* [1923] showed that the administration of 15–20 g. of sodium bicarbonate per day produced a definite fall in the amount of cystine excreted, and that this was accompanied by a marked disturbance in the distribution of the sulphur in the urine, indicating that the alkalosis produced had a definite influence on the course of protein metabolism.

As will be seen from Table II, which gives the results of one of several feeding experiments with sodium bicarbonate (23·4 g. per day), the findings of the latter workers could not be confirmed. The immediate effect was an increase in the amount of cystine excreted followed by a decrease to a figure a little lower than the original one. An increase in the neutral sulphur fraction took place though this is possibly related to the increased elimination of total nitrogen. It may be considered that the preliminary increase was due to the solution of cystine previously lodged in the urinary tract, but such an idea cannot be seriously entertained since similar rises occurred in three different experiments following one another at short intervals.

3. *Hospital diet plus disodium hydrogen phosphate.* The use of disodium hydrogen phosphate in preference to sodium bicarbonate for the purpose of producing an alkalosis is advocated by some workers, and an experiment was carried out on the lines of the preceding one with this compound. 6 g. were fed per day. On November 26, four days after the experiment started, the urine was definitely alkaline. At that point, however, there was a distinct fall in the amount of urea and total nitrogen excreted, a fact which is difficult to understand since the alkalinity produced by the sodium bicarbonate in the previous experiment was accompanied by a rise in the excretion of both of these components. Moreover so far as the excretion of cystine is concerned the use of disodium hydrogen phosphate offers no advantages over that of sodium bicarbonate. Results obtained during this experiment are given in Table III.

4. *Hospital diet plus cystine.* It has long been known that the cystinuric could oxidise a certain amount of ingested cystine to inorganic sulphate.

Little work of a quantitative character, however, has been done on this aspect of the problem. Moreover, with the view of attempting to find whether there was a peak in the ability of the patient to oxidise cystine given in this way, increasing amounts were given starting with 2 g. on November 16, and ending with 8 g. on November 19, so that a total of 20 g. of cystine were fed during the period of 4 days.

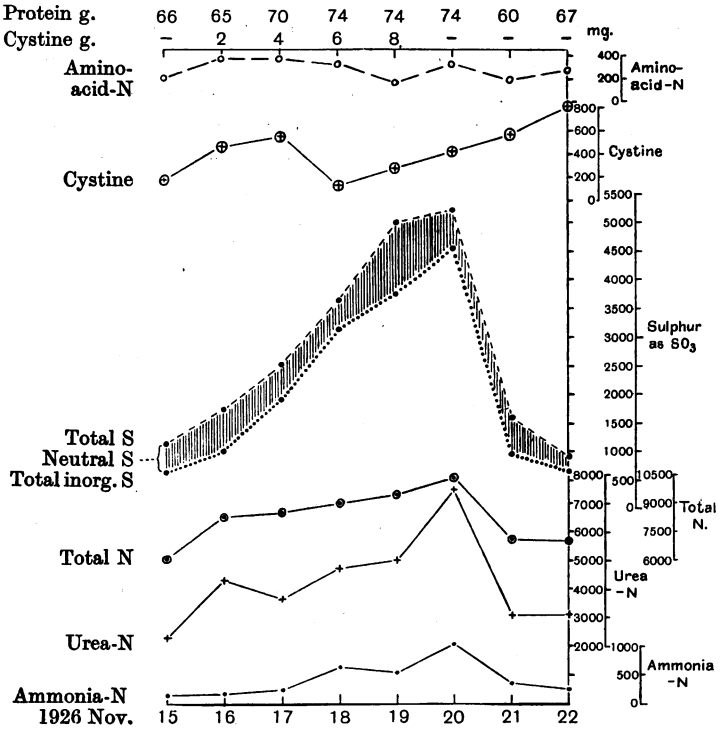

Fig. 2.

Analysis of the results obtained during the period covering this experiment (Table IV) shows that, assuming 0·60 g. (excreted on the days immediately preceding and following the period of the experiment) as the average amount of inorganic sulphate normally excreted, the additional inorganic sulphate eliminated during the feeding experiment was 11·5 g. This could only have arisen from the 20 g. of cystine fed, an amount which, theoretically, should yield 13·5 g. of inorganic sulphate. Hence some 85 % of the cystine administered must have been decomposed, a result which definitely shows that in this case at least the mechanism of the processes of oxidation of the cystine-sulphur is not at fault. Moreover, from the shape of the curve (Fig. 2) of the excretion of inorganic sulphate, it is apparent that the peak of the ability of the patient to oxidise cystine fed by the mouth had not been reached. It is to be noticed that there is no increased elimination of cystine as such during the period in question.

As will be seen from the results recorded, the increase in the inorganic sulphate in the urine is accompanied by a definite increase in the amount of ammonia excreted, a coincidence which follows from normal kidney function.

5. *Hospital diet plus glutamic acid.* One of the many peculiar features of the derangement of metabolism exhibited by cystinurics is the low ratio of urea-nitrogen to total nitrogen in the urine, and this is clearly seen from the results in the various tables to be characteristic of the patient under study. This together with the fact that there is an abnormal amount of cystine in the urine would make it appear at first sight that the processes of deamination are not functioning normally. The question then arises whether this abnormality is closely confined to the cystine molecule or is suffered generally by the whole of the amino-acids of the protein molecule. In order to study this aspect of the problem it was decided to feed a second amino-acid, glutamic, of which a small quantity was available. Following the lines adopted in the previous experiment, this was added to the diet in quantities of 4, 6, 8 and 8 g. on successive days.

From the figures recorded in Table V, it will be seen that the immediate effect was a rise in the total nitrogen and urea-nitrogen excreted, the rise in urea-nitrogen easily accounting for the additional nitrogen fed as amino-acid. It may thus be concluded that the power of general deamination is not lacking in this patient.

6 and 7. *Feeding experiments with a low protein diet* (6) *and with a high protein diet* (7). The feeding experiments with the amino-acids cystine and glutamic acid show beyond doubt that in the case under investigation the processes of oxidation and deamination are functioning normally, and that a search must be made elsewhere for an answer to the question as to what is the origin of the urinary cystine and how it escapes the normal processes of metabolism. It has already been shown (Table I) that in attempting to stabilise the patient's metabolism, it was necessary, on account of the large fluctuations in the amount of cystine in the urine from day to day, to eliminate so far as possible the cystine-rich proteins from the diet. Despite the fact that the concensus of opinion appears to favour the view that there is no connection between the diet and the amount of cystine in the urine, the results of our preliminary feeding experiments did not agree with such a conception. To gain further evidence on this point, feeding experiments were carried out in which the diet contained firstly only some 30 g. of protein and secondly 65–75 g. of the same proteins. The results of these experiments are given in Tables VI and VII respectively. That a larger amount of protein was absorbed on the high protein diet is readily seen in the higher values of the urinary total nitrogen. Accompanying this rise in the amount of protein absorbed, however, is a rise in the amount of cystine excreted from 0·71 g. (average for 9 days) to 1·1 g. (average for 9 days). This rise is large and is in almost direct ratio with the additional nitrogen eliminated, so that it is difficult to see how the conclusion that the urinary cystine is related to the protein

of the diet can be avoided. So long, therefore, as the cause of the anomaly remains unknown, it would appear that from the point of treatment cystinurics at times of crises should have their dietary protein cut down so far as is practicable, due regard being paid to the question of nitrogen equilibrium.

Table VI.

Date	Urine cc.	Nitrogen NH_3- g.	Nitrogen Urea- g.	Nitrogen Total g.	Chlorides g.	Phosphates g.	Cystine Aq. g.	Cystine Ppt. g.	Cystine Total g.	Diet
17. vi.	1050	0·159	1·395	5·25	7·5	1·198	0·495	0·105	0·600	*Low cystine*
19. vi.	1160	—	—	7·77	4·76	1·455	0·399	0·200	0·599	Protein 30 g.
20. vi.	995	0·229	3·19	5·97	3·72	1·248	0·580	0·209	0·789	Calories 1500
21. vi.	800	0·232	2·48	4·92	2·92	0·816	0·576	0·134	0·710	
22. vi.	1060	0·175	2·86	4·88	3·52	1·059	0·493	0·144	0·637	
23. vi.	1080	0·230	3·24	5·56	4·04	1·256	0·599	0·133	0·732	
24. vi.	1320	0·158	3·3	6·54	6·13	1·49	0·785	0·175	0·960	
25. vi.	1050	0·229	4·2	6·62	4·68	1·01	0·644	0·105	0·749	
26. vi.	835	0·164	2·97	5·05	3·42	1·10	0·574	0·059	0·633	

Table VII.

28. vi.	1150	0·245	4·26	7·02	6·77	1·44	0·573	0·114	0·687	*Convalescent*
29. vi.	1740	0·254	5·85	9·13	11·2	2·3	1·296	—	1·296	Protein 65/75 g.
30. vi.	1310	0·183	5·11	7·73	7·26	1·66	1·04	0·088	1·128	Calories 1900
1. vii.	1420	0·168	4·68	7·95	8·8	1·68	1·19	0·143	1·333	
2. vii.	1630	—	—	9·78	11·92	2·1	1·157	0·141	1·298	
3. vii.	700	0·147	3·01	4·52	4·50	1·03	0·544	0·189	0·733	
4. vii.	1625	0·205	6·5	9·75	11·9	1·82	1·38	0·182	1·562	
5. vii.	990	0·111	3·47	4·85	5·12	0·971	0·724	—	0·724	
6. vii.	1395	0·165	6·55	8·44	8·22	1·91	1·18	—	1·18	
7. vii.	1165	0·792	3·49	6·11	7·07	1·14	0·65	—	0·65	

Occurrence in the urine of tyrosine and leucine, and the diamines, putrescine and cadaverine. Although various workers have also reported the presence of the amino-acids, tyrosine and leucine, and the diamines, putrescine and cadaverine, yet the search for these two substances has by no means been uniformly successful. Nevertheless, the number of cases reported as showing that the excretion of abnormal quantities of cystine is only one of the manifestations of this derangement of protein metabolism was considered sufficiently large to warrant the examination of the patient's urine for the presence of these compounds. On three separate occasions during the progress of the investigation 3–5 litres of urine were concentrated under reduced pressure. In each instance the results were as follows.

(*a*) Neither leucine nor tyrosine could be isolated from the concentrate, while the colour reaction obtained with Millon's reagent was no more intense than that obtained with a similarly concentrated normal urine.

(*b*) Treatment of the concentrate with β-naphthalenesulphonic chloride according to the method of Abderhalden and Shittenhelm [1905] gave a gummy precipitate which could not be made to crystallise.

(*c*) Both the benzoylation process of Baumann and Udransky [1889] and the phenyl *iso*cyanate method of Loewy and Neuberg [1904] gave amorphous precipitates which would not crystallise after repeated attempts at purification.

It was concluded, therefore, that none of the above amino-acids or amines was present in sufficient amount to permit of the isolation of a crystalline derivative.

Blood estimations. Estimations of the blood urea-nitrogen and non-protein-nitrogen carried out at different intervals on the patient's fasting blood gave results as follows: urea-N 9·5, 10·0, 9·8 mg. per 100 cc.; N.-P.-N, 20·0, 20·5, 20·3 mg. per 100 cc. All these figures are decidedly subnormal.

Discussion.

The fact that the presence of neither the amino-acids, tyrosine and leucine, nor the diamines, putrescine and cadaverine, could be detected in the urine of the subject of the present study adds yet one more case to the growing number of cystinurics in whom the derangement of protein metabolism is limited strictly to the excretion of an abnormal quantity of cystine in the urine. Moreover, the results of the feeding experiments with the isolated amino-acid, cystine, which show that this compound is quantitatively oxidised to inorganic sulphate, confirm the findings of earlier workers in this field, *e.g.* Hele [1909] and Looney *et al.* [1923], and support the conclusion that the cystinuric oxidises cystine fed as such by the mouth in a perfectly normal manner. Such a conclusion stands in strong contradistinction to the statement of Lewis [1924] that "the failure of the organism to oxidise one specific amino-acid in cystinuria would support the theory that the processes of deamination and oxidation are not the same for all the constituent amino-acids of the protein molecule," for which little, if any, evidence exists at the present time. Moreover, the fact that the peak of the curve of the ability of the patient to oxidise cystine had not been reached, even when so much as 8 g. per day were administered, makes it difficult to understand the suggestion of Hunter and Eagles [1927] that "one of the errors of cystinuria lies in the incapacity of the liver to handle the cystine normally in reserve there." That the cystinuric can oxidise large quantities of cystine administered orally, together with the results of the final feeding experiments showing that, contrary to general opinion, the quantity of urinary cystine varies directly with the amount of protein fed, would make it appear that "the error" is not one connected either with the normal process of oxidation or with the organ in which that process takes place.

Rather would it appear that the disturbance is one closely related either to the digestive processes of the organism or to the permeability of the intestinal wall. The fact that cystine fed as such is completely oxidised while some of the cystine arising from dietary protein escapes oxidation would suggest that the amino-acid in the latter case is actually passing from the intestinal tract into the portal system, not in the isolated form, as it does normally, but as a peptide, and as such escapes the normal oxidative processes of the liver. Fission of the peptide might result when it reached the tissues

and encountered the intracellular enzymes. Cystine would then be liberated only to be excreted by the kidney owing to its presence in abnormal amount in the blood-stream.

Such an idea receives other experimental support apart from that included in this paper. Blum [1903] found that experimental cystinuria could be produced by the injection of cystine into the peripheral circulation but not when it was injected into the mesenteric venous circulation. Such facts would be met if, in the cystinuric, the passage of the liver was effected by the cystine in the form of a peptide, in which it was so linked up that it escaped the normal processes of deamination and oxidation.

A possible solution of the problem of metabolism presented by the cystinuric lies in the feeding of complex peptides containing cystine, and it is probable that a more exact knowledge of the action of enzymes on the protein molecule might give a clearer indication of the source of the urinary cystine in this type of condition.

As regards the more practical question of the treatment of cystinurics, it would appear that the only safe method is that of cutting down the dietary protein of the patient so far as is consistent with the maintenance of nitrogen equilibrium. From the results obtained there is nothing to recommend the continual use of sodium bicarbonate.

Summary.

Evidence is advanced that the cystinuric oxidises cystine to inorganic sulphate normally. Neither the amino-acids, tyrosine and leucine, nor the diamines, putrescine and cadaverine, could be found in the urine of the patient who formed the subject of the present study.

The amount of cystine in the urine appears to bear some relation to the quantity of dietary protein.

The administration of sodium bicarbonate, but not of disodium hydrogen phosphate, to the cystinuric is accompanied by a marked disturbance of the sulphur fractions of the urine. Neither of these compounds affects the excretion of cystine to any extent.

In reporting this work, the author desires to take this opportunity of expressing his deep indebtedness to Dr Robert Thin of Edinburgh for facilitating the carrying out of this research, and also for providing the exceedingly useful information regarding the genealogical tree of the subject. The work was carried out with the help of a grant from the Medical Research Council which is hereby acknowledged.

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