METABOLISM IN CYSTINURIA.

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Preliminary Note on Cases.

A larger knowledge of cystinuria has revealed the far-reaching importance of this subject. To Wollaston[1], as his name for the substance, cystic oxide, indicates, cystin was no more than a local product dependent on unknown local conditions. Some years later cystin was shown to contain sulphur[2]. In 1879 Baumann and Preusse[3] caused an experimental cystinuria in dogs by feeding them with brom-phenol and this suggested that cystin was an intermediate product of normal metabolism, and, as it contained sulphur, necessarily of protein metabolism. The identification of cystin as di α amino α thio-propionic acid by Goldmann and Baumann[4], subsequently altered to di α amino β thio-propionic acid by Friedmann[5], and Mörner's[6] preparation of cystin from hair, decided its position among the cleavage products of protein.

In 1888 diamines were discovered in the urine and fæces of a cystinuric by Udranszky and Baumann[7], and for a time the cause

of the disorder was referred to bacterial activity in the alimentary tract. This theory was soon found to be untenable. Since Baumann's time diamines have been shown to occur frequently but not always in the excreta of some cystinurics, and in many instances tyrosin and leucin have been clearly identified. It is thus usual to regard cystinuria as a disorder of protein metabolism of unknown origin, and as cystinurics are not alike in the degree to which the protein fractions are involved, it has been pointed out that the differences which exist may be referred to the absence of particular enzymes of particular tissues, employed in the catabolism of protein. In the Croonian Lectures for 1908 Dr Garrod[8] has presented a complete summary of the work on Cystinuria, and has described very fully its relationship to normal metabolism.

In the present paper there are recorded observations on three cases of cystinuria, in which special account is taken of the daily output of cystin &c. in the urine, and the presence or absence in the excreta of protein fractions other than cystin.

Case No. 1.

C. W., female, age 22 years. This subject was under close observation for a period of five weeks (March—April 1907). The case in its clinical aspect had been recorded elsewhere by others[9]. I take this opportunity for expressing my thanks to Mr Southam of Manchester for his kind help both with this case and also with W. S., the third case. The patient had been under observation for severe lumbar pain, though there was no direct evidence of renal calculus. The urine was generally slightly alkaline, normal in colour, did not contain albumin, blood or pus, and deposited the hexagonal crystals of cystin only on standing. On three occasions the urine was acid, and showed a rich deposit of cystin.

Search for Diamines. The benzoylation process of Baumann and Udranszky[7] was employed in the search for diamines, cadaverin and putrescin. On a few occasions the phenyl-isocyanate method of Loewy and Neuberg[10] was also utilised. The urine was examined daily for five weeks, and on no occasion were diamines obtained. The fæces were similarly examined on eleven occasions with negative result. The body melting at 205° C. described by Garrod and Hurtley[11] was not encountered.

Search for Tyrosin and Leucin. On no occasion, save following the administration of tyrosin, did the reaction of the urine with Millon's reagent suggest the presence of that body. On two occasions a quantity of urine (1000 c.c. and 4000 c.c. respectively) was examined with negative result for tyrosin and leucin by evaporation under reduced pressure and treatment with β-naphthalene-sulpho-chloride in accordance with the directions given by Abderhalden and Schittenhelm[12].

Search for Glycocoll. 500 c.c. urine were precipitated with phospho-tungstic acid in the presence of sulphuric acid. The filtrate was freed from the reagent by baryta, and the excess barium removed by carbon dioxide. The filtrate was shaken for 6 hours with 4 gm. β-naphthalene-sulpho-chloride in ethereal solution, the reaction being kept just alkaline. The liquid was then separated from the ethereal solution of the reagent, made acid with 5N. HCl, and shaken out several times with ether. The residue obtained by distilling off the ether was extracted with boiling water. A product crystallised out, which after several re-crystallisations from hot water gave a melting point of 156° C. The amount was ·09 gm. The melting point was unaltered by the addition of a little pure naphthalene-sulpho-glycocoll. From a second 500 c.c. urine there was obtained ·02 gm. of the same product. These figures for glycocoll fall within the normal limits, so that in this case there is no apparent derangement in the catabolism of this amino-acid.

The administration of Arginin Carbonate. On April 19th the cystinuric took 5 gm. of arginin carbonate in solution, nearly neutralised by hydrochloric acid. No trace of putrescin could be detected in the urine by the benzoylation method either on this or the following day.

Professor W. H. Thompson kindly supplied the arginin carbonate.

The administration of Tyrosin. On April 6th and on April 24th the cystinuric took 5 gm. tyrosin. The tyrosin was administered in cachet, one gram every two hours. The urine on the day of the administration gave a Millon's reaction slightly more marked than that seen with normal urines. On each occasion a product was obtained by benzoylation, which after repeated re-crystallisation from alcohol gave a melting point of 253° C. This product was only obtained after the administration of tyrosin in accordance with the observations of Garrod and Hurtley[11] in another case.

The administration of Cystin. On April 12th 4 gm. of cystin, prepared from horse hair, were administered by the mouth in cachet. This was almost entirely excreted as sulphate.

The daily output of Cystin &c. On certain days quantitative estimations were undertaken. Details of this work are furnished below.

CASE No. 2.

T. N., male, age 63 years, first exhibited symptoms of calculus in 1893, and was recognised at that time as a cystinuric. In the following year a suprapubic cystostomy was performed, and a cystin calculus removed. After the operation there were no further vesical symptoms. Previously the patient had had several severe attacks of gout, but since the operation only two slight attacks. I am indebted to Dr T. Armstrong Bowes of Herne Bay for this clinical note, and for his kind help in obtaining and sending samples

of urine from this case. The urine was passed into stoppered bottles containing toluene, and quantitative estimations undertaken as soon as received. The urine was faintly alkaline, pale in colour, always contained a small though variable amount of albumin, but no blood or pus. There was usually a rich deposit of cystin crystals.

In the first instance samples of urine were obtained in large bulk and independent of any particular period.

Sample 1, received April 1907. Volume 4630 c.c. Total nitrogen 31·11 gm. Total cystin 1·56 gm. C/N ratio 4·9. Tyrosin or leucin could not be demonstrated in 1500 c.c. Diamines were absent.

Sample 2, received July 25th. Volume 10550 c.c. Benzoyl cadaverin was easily obtained.

Sample 3, received Dec. 18th. Volume 10440 c.c. Total nitrogen 76·0 gm. Cystin 1·73. C/N 2·28. Diamines were not found.

Sample 4, received Jan. 27th, 1908. Diamines were not found. Diamino-acids not found.

Sample 5, received March. Diamines and diamino-acids were not found.

In Oct. and Nov. 1907 observations were made to test as far as possible any alteration in the output of cystin &c. with variation in diet.

CASE No. 3.

W. S., male, age 32 years. This patient's condition was first recognised in 1906, when a cystin calculus was removed from the bladder. The clinical history of the case is recorded elsewhere by Mr Southam[9]. Samples of urine were obtained from this patient with precautions similar to those employed in the preceding case. The urine was clear, was neutral to litmus, contained no albumin, and yielded a rich deposit of cystin on standing.

Sample 1, received May 11th, 1907. Volume 4760 c.c. Total nitrogen 57·31 gm. Total cystin 1·20 gm. C/N ratio 3·5. Diamines not found.

Sample 2, received July 11th. Volume 4220 c.c. Total nitrogen 53·17 gm. Total cystin 3·08 gm. C/N ratio 5·8. Diamines not found.

Sample 3, received Dec. 5th. Volume 3840 c.c. Total nitrogen 44·82 gm. Total cystin 2·15 gm. C/N ratio 4·8. Diamines, tyrosin, and leucin not found.

In Jan. and Feb. 1908 estimations on day and night urines were carried out.

THE EXCRETION OF CYSTIN.

1. *The daily output.* There is no satisfactory method of estimating cystin in urine. The quantity usually present is very small, so that the percentage error in estimation is considerable. The older method of

precipitation by acetic acid is valueless. Any method based on sulphur determinations assumes that the neutral sulphur in a cystinuric in excess of the normal figure occurs wholly as cystin. The β-naphthalene-sulpho-chloride method as employed by Abderhalden[13] and evaporation under reduced pressure as practised by Abderhalden and Schittenhelm[12] are complicated and therefore not free from objection. The method employed in this instance—Gaskell's[14] method, precipitation in the presence of acetone—is not absolute, and in the case of dilute urines the error is large. In such instances it was found necessary to evaporate the urine under reduced pressure, before proceeding with the estimation.

In the Croonian Lectures Garrod[8] has collected some 19 cases, in which it is possible to form an estimate of the cystin excreted. It is there seen that with one or two exceptions the average output of cystin is about 0·3 to 0·5 gm. per diem. In the later cases, where less inaccurate methods have been employed, the numbers approximate very closely. In the three cases recorded the daily excretion falls within these limits.

Case	Days	Average	Highest	Lowest
1	16	0·34 gm.	0·47 gm.	0·14 gm.
2	8	0·41	0·55	0·32
3	4	0·45	0·55	0·40

2. *The effect of diet.* In tracing the influence of diet on the output of cystin, observers have been led to rather contradictory results. Alsberg and Folin[15], relying on the absolute increase of neutral sulphur above the normal average on the same diets, came to the conclusion that on a protein-free diet the daily excretion of cystin was 0·5 gm., on a protein-rich diet 1·0 gm., *i.e.* the cystin is much increased by an increase in nitrogenous food. Abderhalden and Schittenhelm[12] by precipitation of cystin after concentration and acidification of the urine, found in one instance that the cystin was unaltered as the result of increasing the nitrogen of the food. Thiele[16] arrived at the same conclusion, but in this case also observations were restricted to single days on each diet. Recently Wolf and Shaffer[17] have confirmed the observations of Alsberg and Folin[15]. Relying on the neutral sulphur determinations without any direct cystin estimations, they conclude that the cystin of the urine varies directly as the amount of food protein.

On this occasion an attempt was made to test the effect of diet in two of the patients. Unfortunately no very wide variations in diet

could be employed. The estimations carried out in the urine were:— total nitrogen (Kjeldahl), urea nitrogen (Mörner-Sjöqvist), total sulphur, sulphate, and ethereal sulphate (Folin[18]), cystin (Gaskell[14]), and, in the second case only, creatinin (Folin[19]), uric acid (Hopkins), ammonia (Baussingault-Shaffer[20]); estimations were done in duplicate throughout.

CASE 1. C. W. The patient's appetite was extremely variable, especially in the first week during catamenia. From April 4th onwards, the diet consisted approximately of: fish 150 gm. or chicken 100 gm., potatoes 100 gm., bread 200 gm., butter 50 gm., one egg, rice pudding 150 gm., milk 500 c.c., water, tea, &c. 1000 c.c.

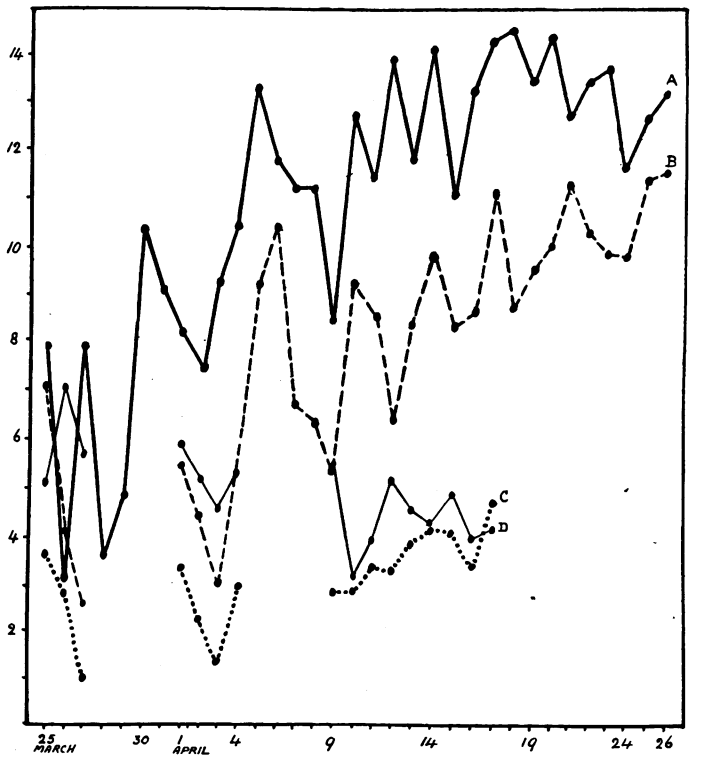

Fig. 1. C. W. The curves express the nitrogen of the food (A), the total nitrogen of the urine (B) in grams, the cystin of the urine (C) in decigrams, and the C/N ratio (D). (See Table I.)

The first diagram shows the nitrogen of the diet, the total nitrogen, cystin of the urine, the ratio $\frac{\text{Cystin} \times 100}{\text{Total Nitrogen}}$ or the C/N ratio. The

curves for food-nitrogen, and total nitrogen, of the urine undergo great variation but show partial correspondence. The cystin output, though greater on a high nitrogen diet, does not increase so much as the nitrogen output. With a sudden rise in the nitrogen as on April 10th there is a fall in the C/N ratio. The output of neutral sulphur, as given in Table I, is similar. The variation is not so wide as in the output of sulphate. Mr J. F. Gaskell very kindly undertook the cystin estimations in this case.

CASE 2. T. N. In October 1907 with the co-operation of Dr Bowes I was able to obtain twenty-four hour specimens of urine on four consecutive days. During this period the patient's diet consisted of milk 1 pint, mutton ½ lb., potatoes ½ lb., bread 1 lb., butter, a herring, a rasher of bacon, and whiskey. In November I obtained a similar series. The diet was rather more generous and consisted of milk 1 pint, beef ½ lb., fish ½ lb., potatoes ½ lb., bread 1 lb., butter, 2 eggs, cheese 1 ounce, Plasmon 2 ounces. The values obtained are given in Table II. The cystin output remains very constant in spite of variations in the excretion of nitrogen.

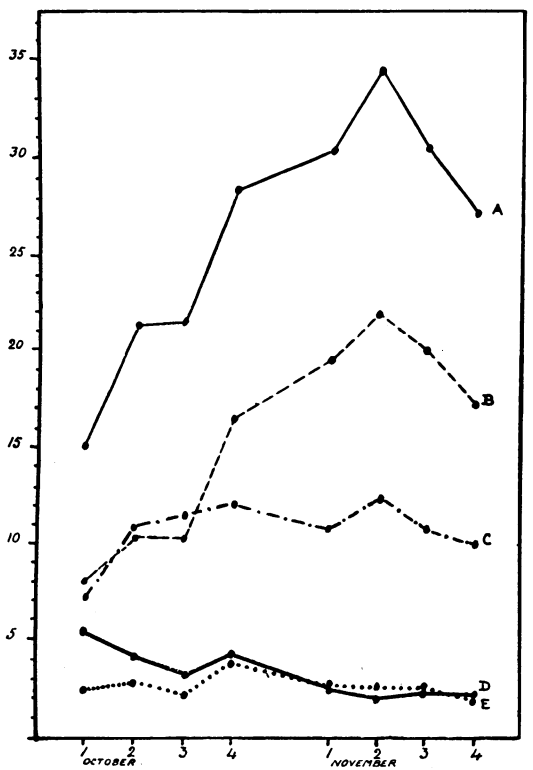

Fig. 2. Case 2. The curves express the output of total sulphur (A), total sulphate (B), neutral sulphur (C), cystin sulphur (E), in centigrams, and the C/N ratio (D). (See Table II.)

In both of these patients the neutral sulphur output is large, similar to that recorded in other cases of cystinuria. In the second patient however the output of neutral sulphur is much the higher, although the cystin is almost the same. This difference would suggest some abnormality of sulphur metabolism other than that involved in the excretion of cystin, provided there is no great error in

estimation. The influence of food on the neutral sulphur is not clear. Unfortunately no great variation could be effected. These figures therefore are not strictly comparable with those given by Alsberg and Folin[15], Wolf and Shaffer[17], who were able to employ very wide extremes of diet. Here it would appear that although the neutral sulphur tends to be higher on a high protein diet, the increase is not proportional.

Averages.	No. of days	Total nitrogen	Cystin	$\frac{\text{Cystin} \times 100}{\text{Nitrogen}}$	Neutral sulphur
Case I. C. W.	8	4·85 gm.	·26 gm.	5·5	·448 gm.
	8	8·83	·37	4·3	·578
Case II. T. N.	4	10·03	·41	4·3	1·034
	4	16·47	·38	2·3	1·098
Extremes.					
Case I. C. W.	—	2·60	·15	5·7	—
		4·06	·28	7·0	—
		11·16	·47	4·2	—
Case II. T. N.	—	6·88	·38	5·5	·702
		9·61	·44	4·3	1·090
		18·71	·39	2·1	1·250

3. *The administration of cystin.* The administration of cystin as such to cystinurics has not always been attended with a constant result. Loewy and Neuberg[21] found that while their patient was able to burn cystin obtained from calculus, he excreted unchanged cystin obtained from hair. In the first instance the increased output of sulphur was observed in the neutral sulphur fraction.

Alsberg and Folin[15] could not confirm these results. Cystin administered by the mouth was excreted as sulphate in all instances. Thiele[16] found the increase after giving cystin prepared from the patient's urine chiefly in the form of neutral sulphur other than cystin. More recently Wolf and Shaffer[17] have thoroughly investigated the problem. On all occasions cystin or cystein given by the mouth was fully oxidised, and when given subcutaneously was excreted partly as sulphate and partly as neutral sulphur.

It was only found possible to give cystin to the first patient C. W. On April 12th 4 gm. cystin (prepared from horse-hair) were administered by the mouth in cachet, one gram every two hours. Unfortunately on this day some of the urine was lost. Owing to the large variations in the diet and therefore in the excretion of sulphur-containing bodies, the effect of administering cystin is not obvious, until the percentages of

TABLE I. *Case* 1.

Date 1907	Food nitrogen	Urine vol. c.c.	Total nitrogen, gm.	Urea nitrogen, gm.	Cystin, gm.*	Urea N. ×100 / Total N.	C/N×100	Sulphur, gm. SO_3: Total	Total sulphate	Neutral	Cystin	Residual	Per cent. of total sulphur: Total sulphate	Neutral	Cystin	Residual	Per cent. of total nitrogen: Total S.	Total sulphate S.	Neutral S.	Cystin S.	Residual S.	Remarks
March 23	7·9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
24	7·9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
25	7·9	1014	7·12	5·88	·37	82·5	5·1	—	—	—	·24	—	—	—	—	—	—	—	—	1·4	—	
26	3·1	520	4·06	3·60	·28	88·5	7·0	—	—	—	·19	—	—	—	—	—	—	—	—	1·9	—	
27	7·9	525	2·60	2·41	·15	92·6	5·7	—	—	—	·10	—	—	—	—	—	—	—	—	1·5	—	
28	3·6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Catamenia.
29	4·9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
30	10·40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
31	9·1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
April 1	8·2	730	5·74	4·62	·34	80·4	5·9	1·198	0·614	0·584	·22	·36	51·2	48·7	18·8	29·9	8·35	4·27	4·08	1·6	2·5	
2	7·4	580	4·30	3·70	·22	86·0	5·2	—	—	—	·15	—	—	—	—	—	—	—	—	1·4	—	
3	9·3	475	3·03	2·66	·14	87·7	4·6	0·525	0·295	0·230	·09	·14	56·3	43·7	17·7	26·0	6·93	3·89	3·04	1·2	1·8	
4	10·4	845	5·61	4·83	·30	86·1	5·4	—	—	—	·20	—	—	—	—	—	—	—	—	1·4	—	
5	13·3	1360	9·17	7·88	—	85·9	—	1·754	1·213	0·541	—	—	69·2	30·8	—	—	7·65	5·29	2·37	—	—	
6	11·8	1620	10·43	8·80	—	84·4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5 gm. Tyrosin by mouth.
7	11·2	865	6·71	5·80	—	86·3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	April 6th.
8	11·2	815	6·43	5·68	—	88·3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
9	8·4	1050	5·35	4·76	·29	89·0	5·5	0·988	0·549	0·438	·19	·25	55·6	44·4	19·6	24·8	7·38	4·11	3·28	1·4	1·8	
10	12·7	915	9·30	7·94	·29	85·4	3·2	1·847	1·248	0·595	·19	·40	67·6	32·4	10·4	22·0	7·94	5·37	2·57	0·8	1·8	
11	11·4	870	8·57	7·45	·34	86·9	4·0	1·736	1·095	0·641	·23	·41	63·5	36·5	13·8	22·7	8·10	5·12	2·98	1·1	1·9	
12	13·9	930	6·35	5·34	·33	84·0	5·2	2·809	2·081	0·728	·22	·51	74·1	25·9	7·7	18·2	17·69	13·11	4·58	1·4	3·2	4 gm. Cystin by mouth.
13	11·8	1145	8·46	7·34	·39	86·7	4·6	2·140	1·541	0·599	·26	·34	72·0	28·0	12·0	16·0	10·12	7·28	2·84	1·2	1·6	April 12th.
14	14·1	1180	9·88	8·79	·42	89·0	4·3	1·961	1·415	0·546	·28	·27	72·2	27·8	14·1	13·7	7·94	5·73	2·21	1·1	1·1	
15	11·0	1040	8·29	7·07	·41	85·3	4·9	1·487	0·993	0·494	·27	·22	66·6	33·3	18·0	15·3	7·17	4·80	2·38	1·3	1·1	
16	13·3	1400	8·62	7·84	·34	90·9	4·0	1·615	1·143	0·472	·23	·24	70·8	29·2	14·1	15·1	7·49	5·30	2·19	1·1	1·1	
17	14·3	1380	11·16	9·93	·47	88·9	4·2	2·104	1·548	0·556	·31	·25	73·6	26·4	14·6	11·8	7·54	5·55	1·99	1·1	·9	
18	14·5	800	8·69	7·82	—	89·9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
19	13·4	1390	9·49	8·45	—	88·9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5 gm. Arginin by mouth.
20	14·4	1200	10·11	8·63	—	85·4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	April 19th.
21	12·7	830	11·29	10·18	—	89·9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
22	13·4	1300	10·37	9·13	—	88·1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
23	13·7	1150	9·92	9·11	—	91·9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
24	11·6	1065	9·78	9·01	—	92·1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5 gm. Tyrosin by mouth.
25	12·6	1250	11·26	9·55	—	86·4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	April 24th
26	13·2	1075	11·50	10·20	—	88·6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	

* These Cystin estimations were carried out by Mr Gaskell.

TABLE II. *Case* 2.

Date Oct. 1907	Volume c.c.	Nitrogen, gm.						Per cent. of total nitrogen							Sulphur, gm. SO_3						Per cent. of total S.					
		Total	Urea	Ammonia	Uric acid	Creatinin	Residual	Urea	Ammonia	Uric acid	Creatinin	Residual	Cystin	C/N×100	Total	Total sulphate	Neutral	Ethereal SO_4	Cystin	Residual	Total sulphate	Neutral	Cystin	Residual	$\frac{\text{Ethereal } SO_4}{\text{Total } SO_4}\times 100$	$\frac{\text{Total sulphur}}{\text{Total nitrogen}}\times 100$
Day 1	1300	6·88	5·60	·24	·11	·33	·59	81·46	3·53	1·60	4·76	8·63	·38	5·5	1·511	0·799	·702	—	·25	·45	52·9	47·1	16·1	30·5	—	8·78
2	1725	9·61	8·11	·42	·15	·37	·56	84·42	4·35	1·56	3·83	5·75	·44	4·3	2·136	1·046	1·090	·163	·29	·80	49·0	51·0	13·6	37·4	15·8	8·89
3	2335	10·00	8·17	·39	·14	·40	·90	81·72	3·92	1·40	4·00	8·96	·35	3·3	2·177	1·033	1·144	·176	·23	·91	47·5	52·5	10·9	41·6	17·1	8·70
4	1860	13·64	11·51	·47	·24	·49	·94	84·36	3·44	1·77	3·57	6·86	·46	4·3	2·852	1·651	1·201	·318	·39	·81	57·9	42·1	13·7	28·4	19·3	8·36
Nov.																										
Day 1	1575	15·70	13·02	·32	·21	2·15		82·94	2·06	1·34	13·70		·43	2·7	3·037	1·940	1·097	·169	·28	·81	63·90	34·1	7·2	26·9	8·72	7·74
2	1898	18·71	15·81	—	—	—		84·96	—	—	—		·39	2·1	3·449	2·199	1·250	·194	·26	·99	63·79	36·21	7·4	28·8	8·82	7·38
3	1951	16·90	14·37	·43	·20	1·90		85·00	2·55	1·18	11·27		·40	2·4	3·053	2·004	1·049	·146	·26	·78	65·65	34·35	8·7	25·7	7·27	7·22
4	1812	14·56	12·18	·36	·19	1·83		83·62	2·51	1·33	12·54		·32	2·2	2·729	1·732	·997	·149	·21	·79	63·46	36·53	7·7	28·9	8·59	7·50

sulphur in terms of nitrogen (S/N × 100) are examined (Fig. 3). There appears a large increase in the sulphur output on the cystin day, an increase almost entirely as sulphate. There is a slight rise however in the neutral sulphur of the urine, but no change in the cystin output. The excess sulphur over the average corresponds on April 12th to 2·4 gm. cystin and on April 13th to 0·8 gm. cystin, or 3·2 gm. in all. On this single occasion therefore the ingested sulphur of the cystin was almost entirely excreted as sulphate.

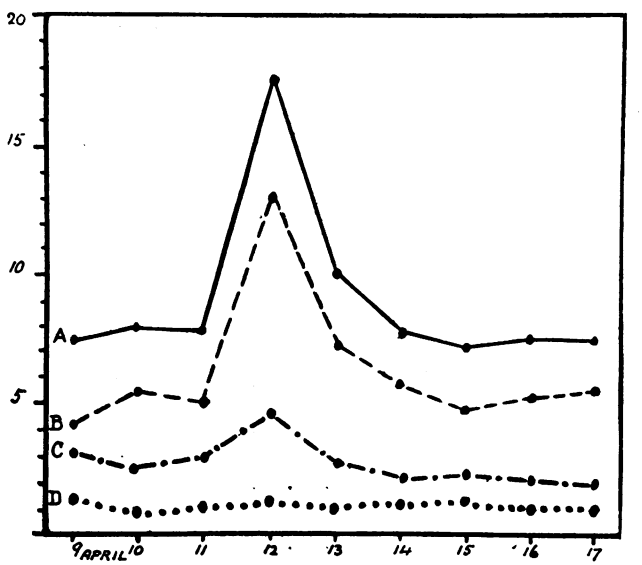

Fig. 3. Case 1. The curves represent the output of total sulphur (A), total sulphate (B), neutral sulphur (C), cystin sulphur (D), expressed as percentages of total nitrogen. On April 12th 4 gm. cystin were administered to the patient by the mouth. (See Table I.)

4. *The diurnal variation.* The recorded observations on the quantity of cystin excreted during the day and night respectively are extremely contradictory. Ebstein[22], Piccini and Conti[23] considered that the day urine contained the larger amount. Bartels[24] came to the opposite conclusion. These observers relied on precipitation tests, and their conclusions would easily be explained by differences in dilution. Wolf and Shaffer[17] give values for nitrogen, sulphur &c. in the urine over four hourly periods after the ingestion of 50 gm. casein. The outputs of neutral sulphur, total sulphur, rest nitrogen follow the output of nitrogen very closely. The maxima occur in the third period. The

maxima for ammonia and carbon are earlier in the second period. They conclude that the processes giving rise to rest nitrogen and neutral sulphur are either identical or run an absolutely parallel course. The close agreement between the neutral sulphur and total sulphur seemed

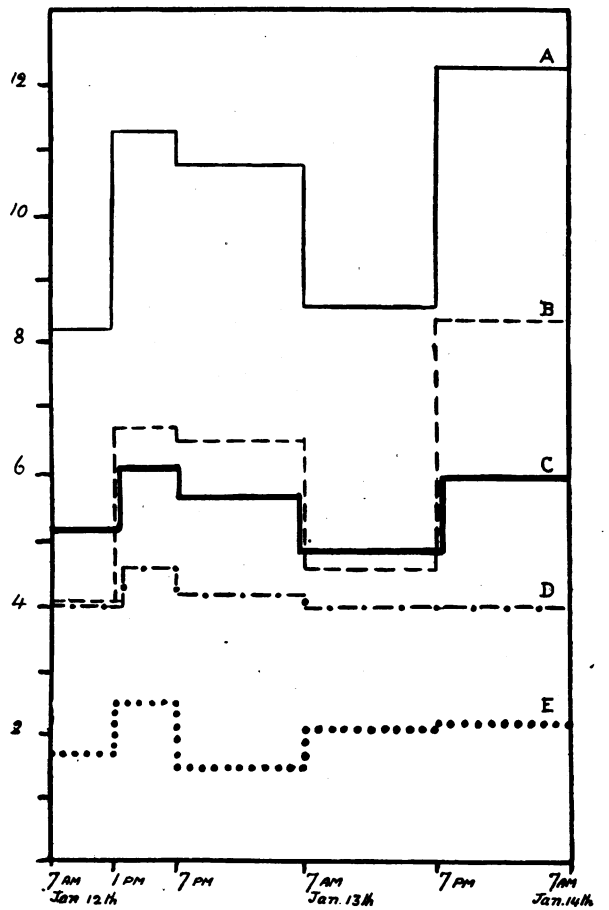

Fig. 4. Case 3. The curves show the average output per hour of total sulphur (A), total sulphate (B), neutral sulphur (D), as centigrams SO_3, of cystin (E), in centigrams, and of total nitrogen (C), in decigrams. (See Table III.)

to call for further investigation, as this agreement is not seen in the daily output with alteration of diet. I obtained therefore specimens of urine from the third case, collected over consecutive periods of the day and night. Unfortunately it was not possible to place the patient on

any standard diet. Precautions similar to those employed in the preceding instances were taken. The first experiment of this nature covered the days Jan. 12th—13th.

Food taken. Jan. 12th, 1908.		
8.30 a.m.	Breakfast.	Bacon, bread, butter, tea.
10.30 a.m.		Orange.
1.30 p.m.	Dinner.	Steak and cow-heel stew, potatoes, cabbage.
5.30 p.m.	Tea.	Bread, butter, tea, celery, rhubarb pie.
8.0 p.m.		Half pint barley water.
10.0 p.m.		Half pint barley water.
Jan. 13th.		
6.30 a.m.	Breakfast.	Bread, butter, rhubarb pie.
12 noon.		Steak and cow-heel stew, mince pie.
7.0 p.m.	Tea.	Fish, bread, butter, tea.

The urine was collected in five lots.

Estimations were made of total nitrogen, total sulphur, total sulphate and where possible cystin (Table III). When the average excretion of these bodies (Fig. 4) is considered, it is seen that whereas the total sulphur and total nitrogen are much higher at night, the neutral sulphur remains extremely constant.

This experiment was repeated on the days Feb. 9th, 10th. The food on this occasion consisted of:

Feb. 9th	9.0 a.m.	Breakfast.	Bacon, bread, tea.
	1.30 p.m.	Dinner.	Roast beef, sprouts, potatoes.
	6.0 p.m.	Tea.	Bread, butter, stewed rhubarb.
,, 10th	6.30 a.m.	Breakfast.	Tea, bread, butter, rhubarb.
	12 noon.	Dinner.	Beef, bread, tea.
	7.0 p.m.	Tea.	Fish, bread, butter.

The urine was collected in four-hourly periods (Table IV) on these days.

There was the same rise in the output of oxidised sulphur towards night, while the neutral sulphur was more constant (Fig 5).

On both occasions the cystin was rather variable, but there was no difference in the total output for day and night.

	Day	Night
Jan. 12—13th	·25	·18
,, 13—14th	·25	·26
Feb. 9—10th	—	·29 (11 hours)
,, 10—11th	·20	·20

In these experiments the increased output of total nitrogen and total sulphur towards night follows the period of increased protein intake. It is particularly interesting to notice the regular output

of neutral sulphur, an output apparently quite independent of the food taken.

Although the cystin varies from hour to hour, the difference between day and night output is very slight. Here again there is no evidence that the food has any influence on the cystin excreted.

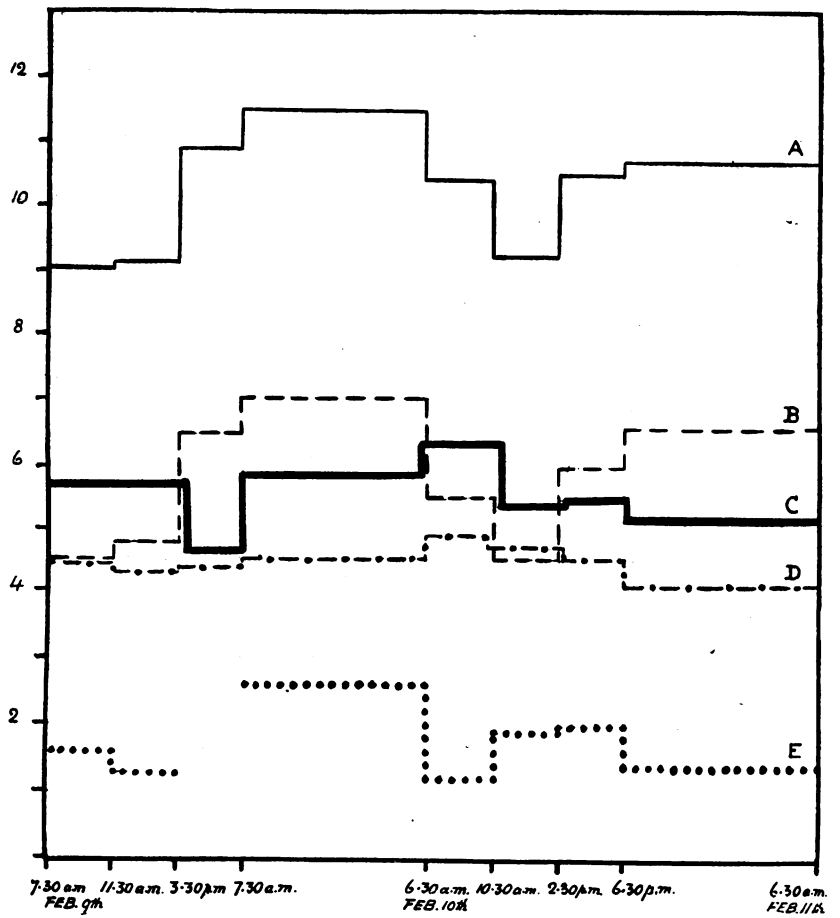

Fig. 5. Case 3. The curves represent the average output per hour of total sulphur (A), total sulphate (B), neutral sulphur (D), as centigrams SO_3, of cystin (E) in centigrams, and of total nitrogen (C) in decigrams. (See Table IV.)

THE EXCRETION OF DIAMINES.

The presence of diamines in the excreta of cystinurics has been demonstrated by a number of observers. Their occurrence in each case

TABLE III. *Case* 3.

Date		Volume c.c.	Total nitrogen, gm.	Cystin, gm.	Total sulphur as gm. SO_3	Total sulphate as gm. SO_3	Neutral sulphur as gm. SO_3	Hours of collection	Rates of excretion, gm. per hour: Total nitrogen	Total sulphur	Total sulphate	Neutral sulphur	(Cystin)	Remarks
Jan. 12th 1908	7 a.m.—1 p.m.	378	3·12	·10	0·491	0·245	0·245	6	·52	·082	·041	·041	(·017)	8.30 a.m. Breakfast.
	1 p.m.—7 p.m.	405	3·69	·15	0·677	0·403	0·274	6	·61	·113	·067	·046	(·025)	1.30 p.m. Dinner.
	7 p.m.—7 a.m.	1058	6·82	·18	1·290	0·781	0·509	12	·57	·108	·065	·042	(·015)	5.30 p.m. Tea.
Jan. 13th	7 a.m.—7 p.m.	935	5·83	·25	1·032	0·551	0·479	12	·49	·086	·046	·040	(·021)	6.30 a.m. Breakfast. 12 noon Dinner.
	7 p.m.—7 a.m.	682	7·21	·26	1·481	1·005	0·476	12	·60	·123	·084	·040	(·022)	7 p.m. Tea.

TABLE IV. *Case* 3.

Feb. 9th 1908	7.30 a.m.—11.30 a.m.	265	2·28	·064	·360	·181	·179	4	·57	·090	·045	·045	(·016)	9 a.m. Breakfast.
	11.30 a.m.— 3.30 p.m.	270	2·28	·051	·365	·193	·172	4	·57	·091	·048	·043	(·013)	1.30 p.m. Dinner.
	3.30 p.m.— 7.30 p.m.	194	1·83	—	·438	·260	·178	4	·46	·109	·065	·044	—	6 p.m. Tea.
Feb. 10th	7.30 p.m.— 6.30 a.m.	485	6·48	·286	1·261	·775	·486	11	·59	·115	·070	·045	(·026)	
	6.30 a.m.—10.30 a.m.	422	2·53	·048	·416	·220	·196	4	·63	·104	·055	·049	(·012)	6.30 a.m. Breakfast.
	10.30 a.m.— 2.30 p.m.	234	2·16	·076	·368	·181	·187	4	·54	·092	·045	·047	(·019)	12 noon Dinner.
	2.30 p.m.— 6.30 p.m.	236	2·20	·079	·422	·242	·180	4	·55	·105	·060	·045	(·020)	
Feb. 11th	6.30 p.m.— 6.30 a.m.	516	6·27	·196	1·288	·794	·494	12	·52	·107	·066	·041	(·016)	7 p.m. Tea

is very variable from day to day, but when present they are found in considerable quantity about ·3 to ·5 gm., in the 24 hours. Cadaverin is the commoner diamine in the urine, putrescin in the fæces. Cadaverin is closely related to lysin, putrescin to arginin. Loewy and Neuberg[21] found that lysin administered by the mouth to their patient was excreted as cadaverin, arginin as putrescin. Garrod and Hurtley[11] could not demonstrate any putrescin in the urine of the patient following the administration of arginin by the mouth.

1. *In the urine and in the fæces.* Although the urine was frequently examined for diamines in all three cases, and the fæces similarly examined in case 1, cadaverin was only encountered on one occasion, *i.e.* in the second sample of urine of case 2, received July 25th, 1907. On this occasion the urine was kept for three weeks under toluene before analysis. Every fraction taken yielded diamine. On benzoylation a product was obtained which, after repeated crystallisation from alcohol and water, gave a melting point of 129° C., and behaved in all respects like benzoyl-cadaverin. The yield was large, 0·5 gm. for every 1500 c.c. of urine benzoylated.

The solubility of this compound is by no means negligible. The precipitation from urine takes at least 24 hours, and if the urine is filtered immediately after benzoylation, only a small part of the benzoyl compound is present in the precipitate. On standing benzoyl-cadaverin in almost pure form is slowly deposited from the filtrate. Furthermore, the liquors from which the benzoyl-cadaverin is recrystallised is still rich in the product, so that either only a small quantity of solvent must be used, or the mother-liquors must be evaporated to small bulk on the water-bath, and the impure product so obtained again crystallised. Unfortunately benzamide is always formed together with other benzoyl compounds, and benzamide melts at the same temperature as benzoyl-cadaverin—129° C. The solubility, however, of benzamide is very much greater, so that only urines which contain a large quantity of ammonia (by decomposition of urea or otherwise) are likely to cause any trouble. No real difficulty, even in these cases, need be encountered, as the substances are very different in appearance as well as in solubility, and are readily distinguished during recrystallisation.

In this instance no benzoyl-putrescin was found but mixed with the benzoyl-cadaverin was a very small amount of impure substance melting at 196°—200° C. The quantity was too small for further purification or analysis. The product may have been identical with the compound melting at 205° C., described by Garrod and Hurtley[11] in another case.

On Dec. 8th, 1907 a third sample of urine from this patient was received. As the previous supply had been kept for some weeks before analysis, it was thought that the occurrence of cadaverin might possibly be accounted for by decomposition of diamino-acids, present in the fresh urine. Therefore, on this occasion five lots of urine, 1500 c.c. each, were taken and treated as follows:—

Lot 1. Benzoylated at once. No diamines obtained.
Lot 2. Allowed to undergo decomposition at room temperature, 6°—15° C. Benzoylated on Jan. 24th, 1908. No diamines obtained.
Lot 3. Kept under toluene in stoppered bottle at 12°—20° C. Benzoylated on Feb. 26th. No diamines obtained.
Lot 4. Kept under toluene in corked bottle at 12°—20° C. Benzoylated on Feb. 26th. No diamines obtained.
Lot 5. Kept under toluene in bottle, partly closed with slit cork, at 12°—20° C. Benzoylated on Feb. 26th. No diamines obtained.

There is no evidence that the presence of diamines in urine is connected with decomposition. Cammidge and Garrod[25] arrived at the same conclusion in 1900.

2. *The effect of diet and the administration of arginin.* Garcia[26] expressed an opinion that the quantity of diamines excreted depended on the amount of protein food. Thiele[16] concluded from single-day analyses on varied diets that the character of the food-stuffs had some effect on the excretion. The amount of benzoyl-cadaverin on a meat diet was 1·108 gm. in 24 hours, ·218 gm. in starvation, and ·366 gm. on a mixed diet. The observations are, however, too limited to carry much weight. Alsberg and Folin[15] found that on their nitrogen-rich diet their patient excreted 1·6 gm. undetermined N., as against the normal 0·6 gm. They supposed that, if this undetermined nitrogen represented amino-acids of the food that had escaped destruction, their patient on a nitrogen-free diet would excrete an amount of undetermined N., comparable with the normal figure. This they found was not so. On such a diet the undetermined nitrogen 0·8 gm. was double the normal figure of 0·4 gm. Wolf and Shaffer[17] found a similar result, 1·8 gm. on high N., ·58 on a low N. diet, the difference for the normal however in the latter instance is not so marked. There was a slight rise in undetermined nitrogen after the patient received 50 gm. casein, but no alteration in the proportion to total nitrogen. Thus there is no reason to suppose from such indirect evidence that the amount of food protein has any marked effect on the excretion of diamines. Furthermore, with the remarkable exception of the case recorded by Loewy and Neuberg[21], cystinurics have shown no intolerance to arginin or other diamino-acid.

As already stated arginin was administered to Case 1 without producing any putrescin in the urine. Nitrogen determinations were carried out on Case 2. The undetermined nitrogen was rather high, but as the diets were not properly controlled, no importance can be attached to these figures. (Table III.)

3. *Search for diamino-acids.* It was further suggested that the diamines of the urine might result from decomposition of diamino-acids excreted as such. This idea received support from the fact that in the second case the smell of cadaverin was frequently noted during evaporation of the alkaline urine before fusion, in order to make total sulphur determination, even when no diamines were detected by benzoylation. The smell became apparent after the ammonia had been driven off and before the urine began to char. Marriott and Wolf[27] in 1906 drew attention to the fact that diamino-acids had never been sought for in the urine of cystinurics. Consequently fresh urine was obtained from the patient, and examined for diamino-acids by the method employed by Wohlgemuth[28].

This may be summarised as follows :—

Four litres of urine were precipitated with basic lead acetate. The filtrate freed from lead by sulphuretted hydrogen gas, and evaporated to 1000 c.c., under reduced pressure. This was allowed to stand for seven days. No tyrosin separated.

The urine was then precipitated in the presence of sulphuric acid with phosphotungstic acid (about 1 lb. was required), and filtered—*Filtrate A*, *Precipitate A*.

Filtrate A. The phosphotungstic acid was removed by baryta, and the filtrate freed from barium by carbon dioxide followed by ammonium carbonate. The solution was then evaporated to 500 c.c. at 40° C. and 18 mm. Hg. and treated with β-naphthalene-sulphochloride for glycocoll. A small amount of impure substance was obtained, insufficient in quantity for identification.

Precipitate A. After washing, precipitate was suspended in water and decomposed by baryta. The barium phosphotungstate filtered off and the filtrate freed from barium by carbon dioxide and ammonium carbonate. The precipitation with phosphotungstic acid &c. was then repeated for further purification. After the removal of the barium, the filtrate and washings were evaporated at 40° C. and 18 mm. Hg. to 500 c.c. The liquid was treated with ammoniacal silver nitrate to remove purins, and the filtrate freed from excess of silver by sulphuretted hydrogen. After the excess of gas had been driven off, the filtrate was treated with Hopkin's mercuric sulphate reagent to remove any cystin present, and the filtrate again treated with sulphuretted hydrogen to precipitate the excess of mercury.

The liquid so obtained was then evaporated to 100 c.c. on the water-bath, and alcoholic picrolonic acid added to precipitate any arginin present. A yellow precipitate was obtained that dissolved almost at once, when washed with water, and was therefore only the precipitated reagent.

The filtrate and washings, containing excess of picrolonic acid, were evaporated to a small volume on the water-bath, shaken out several times with benzol to remove the picrolonic acid, and finally decolourised with charcoal. The colourless filtrate did not yield any permanent precipitate (lysin picrate) with picric acid.

The process was repeated on a further 6 litres of urine without producing any positive result.

Search for leucin, tyrosin, glycocoll &c.

Piccini and Conti[23] recognised tyrosin crystals in the urine of their patient, Percival[29] both tyrosin and leucin, Moreigne[30] tyrosin, although this last observer may have confused tyrosin with cystin hydrochloride. In their case Abderhalden and Schittenhelm[12] were able to identify, by full analysis, tyrosin and leucin obtained from the urine of their patient, while Fischer and Zuzuki[31] found tyrosin in a cystin calculus. These substances were sought for without success by Simon[32], Alsberg and Folin[15], Garrod and Hurtley[11]. Loewy and Neuberg[21] could only find tyrosin in the urine of their patient after the administration of tyrosin by the mouth. Simon[32], Garrod and Hurtley[11], Alsberg and Folin[15], Thiele[16] were unable to confirm this in other cases. There is no reference made in the literature to the amount of glycocoll excreted in cystinuria under ordinary circumstances. Loewy and Neuberg[33] found that their patient excreted about 20 % glycocoll unchanged after its administration by the mouth, although he could fully oxidise polypeptides containing this amino-acid.

Although tyrosin and leucin were sought for on several occasions in the three cases recorded in this paper, no trace of them could be demonstrated. The reaction of the urines with Millon's reagent did not suggest tyrosin on any occasion, save after the administration of tyrosin on both occasions to the first case C. W. On each occasion a small quantity of the "253° body" of Garrod and Hurtley[11] was obtained by benzoylation. A small quantity of glycocoll was isolated as the naphthalene-sulpho-compound from the urine of the first case, but not in excess of the normal figure.

Summary.

1. The cystin output in the three cases examined falls within the ordinary limits—·3 to ·5 gm. per diem.

2. The amount of cystin and also of neutral sulphur does alter slightly with the diet. The alteration is however slight and not in proportion to the alteration in total nitrogen and total sulphur. This is in agreement with the observations of Abderhalden[13] and of Thiele[16], but contrary to those of Alsberg and Folin[15], Shaffer

and Wolf[17]. The cases are not strictly comparable, as no wide variation in diet could be maintained in the former instances, while with Alsberg and Folin[15], and also with Shaffer and Wolf[17], the patient was placed on nitrogen-free and on nitrogen-rich diets. As a possible explanation it is suggested that the body processes, of which the excreted cystin is the last stage, require only a limited supply of food protein. This supply is early provided and only fails when the food protein is cut down to a very low figure.

3. Cystin administered by the mouth to one patient (C. W.) was excreted almost entirely as sulphate.

4. On an ordinary mixed diet the excretions of cystin and neutral sulphur are very regular through the day and night, and do not show the same marked rise towards evening as is observed in the outputs of total nitrogen and total sulphur.

5. Cadaverin was found only once in the urine of one patient (Case 2). The smell of cadaverin was frequently encountered in evaporating the urine of this patient for sulphur determination, suggesting the presence of this body in small amount. There is no evidence that decomposition of the urine has anything to do with the occurrence of diamines.

6. Arginin, given by the mouth to one patient (Case 1), was not excreted as putrescin.

7. The urine of one patient (Case 2) was searched for diamino-acids without success.

8. Glycocoll was not present in excess. Tyrosin and leucin were not found.

After giving tyrosin to one patient (Case 1) the urine gave a Millon's reaction rather more marked than the normal, and on benzoylation a small quantity of the body melting at 253° C.

This research was undertaken at the suggestion of Dr Garrod, to whom the author is much indebted for constant advice and invaluable help.

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