II. METABOLISM OF SULPHUR

III. THE EXCRETION OF CYSTINE BY NORMAL INDIVIDUALS

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IN a previous paper [Medes, 1936] it was shown that cystine occurs in normal urine and may be quantitatively precipitated as the cuprous salt, and subsequently determined by phospho-18-tungstic acid or by the method of Rossouw & Wilken-Jorden [1934; 1935] based on the Sullivan reaction. The slightly lower values obtained by the latter more nearly specific method leave open the possibility that some other S—S compound such as homocystine may comprise a small part of this output. However, some doubt is thrown upon this interpretation by the fact that technical difficulties in adapting the latter procedure to normal urine decrease its accuracy somewhat and by the fact that no homocystine was isolated along with cystine by cuprous chloride precipitation. In the present paper cystine was estimated by the method described by Medes & Padis [1936], and the total values were recorded as cystine.

A. The cystine content of normal urine

24-hour specimens of urine were collected from 50 normal individuals. The subjects were patients about to be discharged from the Lankenau Hospital and were for the most part mild surgical cases and all were pronounced free of any metabolic disturbance. The urine was acidified as soon as voided to minimize decomposition of the cystine and preserved in ice-boxes until the close of the 24-hour period. In addition to cystine, neutral sulphur, creatinine and total nitrogen of these specimens were determined quantitatively. The results, treated statistically, together with similar treatment of body weights are recorded in Table I.

Table I. *24-hour cystine excretion of 50 normal individuals, correlated with neutral sulphur, creatinine and total N output and with body weight*

	Mean	Standard deviation	Coefficient of correlation with cystine
Cystine (mg.)	30.4 $\pm$ 1.5	27.3 $\pm$ 1.9	—
Neutral S (mg.)	96.6 $\pm$ 4.2	42.8 $\pm$ 2.9	+ 0.46 $\pm$ 0.07
Creatinine (g.)	0.99 $\pm$ 0.03	0.3 $\pm$ 0.02	+ 0.65 $\pm$ 0.05
Total N (g.)	13.9 $\pm$ 0.7	4.9 $\pm$ 0.5	+ 0.10 $\pm$ 0.02
Body weight (kg.)	59.7 $\pm$ 1.0	10.4 $\pm$ 0.7	+ 0.15 $\pm$ 0.07

Cystine. The excretion of cystine averaged 30.4 mg. (1.27×10^{-4} M) per 24 hours. The high standard deviation, 27.3 ± 1.9 mg. shows the wide variation in the individual cases, the range being from 10 to 102 mg. The distribution of these cases, in 2 mg. classes, is shown in Fig. 1. All but two of the subjects form a fairly homogeneous group. With these two excluded, the average excretion

becomes 28.6 mg. If in addition the two cases lying between 50 and 60 mg. are excluded, the average is lowered to 26.6 mg. The wide scattering of values suggests that so-called cystinuria may represent a complete breakdown of some mechanism which may function imperfectly in a small number of other subjects. The distribution of neutral sulphur in 5 mg. classes is shown for comparison.

Fig. 1. Distribution of cystine in 2 mg. groups and of neutral sulphur in 5 mg. groups, excreted by 50 normal individuals.

Neutral sulphur. Total sulphur was determined by digesting 10 ml. samples of urine by Pirie's method [1932], transferring the digest to $5 \times \frac{1}{8}$ in. silica test-tubes. Boiling water was added to about 2 in. below the top, followed by 5 ml. of 5% BaCl_2 . The tubes were allowed to stand overnight, the contents centrifuged, the supernatant liquid decanted and the precipitate washed twice with water, centrifuging after each washing. The BaSO_4 was dried in the silica tube, ignited, cooled in a desiccator and weighed. The error of this method was found to be 1%. Total sulphates were also determined by the gravimetric method [Folin, 1905-06] the precipitates being transferred to the silica tubes, in which the washing and weighing were carried out.

The mean excretion of neutral sulphur was 96.6 ± 4.2 mg. per 24 hours. The standard deviation, 42.8 ± 2.9 mg., is relatively lower than that of the cystine, indicating more homogeneity in the group. Although the two individuals with high cystine output are among the highest with respect to excretion of neutral sulphur, their values are within the limits of the general group. Since the cystine sulphur constitutes only about 8% of the neutral sulphur in this group of subjects, it is not to be expected that variations in cystine would appreciably affect the neutral sulphur excretion. Nevertheless the coefficient of correlation between cystine excretion and neutral sulphur output was $+0.46 \pm 0.07$, suggesting some fundamental relationship between the two.

No significant relationship appeared between cystine output and excretion of total nitrogen or between urinary cystine and body weight. The latter lack of correlation was unexpected in view of the large positive correlation, 0.65 ± 0.05 , found between cystine and creatinine excretions and the positive correlation of 0.34 ± 0.08 between creatinine output and body weight. In spite of the heterogeneity of this group of subjects as to age and sex, the correlation between creatinine excretion and body weight was almost identical with that of Tracy & Clark's [1914] group of 26 women, whose coefficient of correlation between creatinine excretion and body weight was 0.34 ± 0.12 . In their group the mean body weight was 58.7 ± 1.00 kg. and the creatinine output 0.90 ± 0.03 g.

B. Relation of urinary cystine, amino-nitrogen and neutral sulphur

In Table II are presented metabolic studies of 5 hospital patients. The first 3 were about to be discharged from the hospital as recovered. The last two patients were cases of advanced and inoperable tumours. 24-hour collections of urine were made for a preliminary period of 3 or 4 days, followed by a second period during which cystine was fed daily in doses of 40 mg. per kg. body weight. In

3 cases a final period without cystine feeding was included. 2 days intervened between the second and third periods. All determinations were carried out as in the preceding section. Amino-nitrogen was estimated by Van Slyke & Kirk's [1933] modification of Northrup's method.

Table II. *Metabolic studies of 5 subjects during periods of cystine feeding, with special reference to the excretion of cystine, amino-nitrogen and neutral sulphur*

				Intake					
		Age yr.	No. days exp.	Wt. close of period	Protein g.	Cystine g.	Nitrogen		
Subject							Protein g.	Cystine g.	Total g.
1. Tuberculosis (recovered), initial weight 36.6 kg.		14	3	37.5	110.6	—	17.69	—	17.69
			4	38.1	103.6	1.5	16.60	0.17	16.77
2. Gasteropsis, initial weight 59.9 kg.		58	3	59.7	69.6	—	11.13	—	11.13
			3	59.7	66.4	2.4	10.73	0.28	11.01
			2	59.6	68.0	—	10.88	—	10.88
3. Osteoarthritis, initial weight 41.6 kg.		42	4	41.6	75.6	—	12.28	—	12.28
			4	42.3	82.6	1.6	13.11	0.19	13.30
			4	43.0	88.5	—	14.15	—	14.15
4. Sarcoma of abdomen, initial weight 63.6 kg.		51	4	64.4	86.7	—	13.88	—	13.88
			4	64.4	85.2	2.0	13.85	0.23	14.08
5. Carcinoma of breast, initial weight 60.0 kg.		—	3	60.2	76.7	—	12.27	—	12.27
			4	59.8	82.5	2.4	13.04	0.28	13.32
			5	60.2	86.4	—	13.63	—	13.63

Output													
		Nitrogen							Sulphur (as S)				
Sub- ject	Crea- tinine mg.	Cys- tine mg.	Cystine						Total SO ₄ mg.	Ether- eal SO ₄ mg.	Neutral S mg.	Cystine mg.	Cystine % neutral
			Total g.	Balance g.	Amino mg.	Cystine mg.	% amino	Total mg.					
1	691	28	13.50	+4.19	405	3.26	0.8	762	653	58	108	7.4	6.8
	671	21	11.65	+4.95	456	2.44	0.6	827	729	49	98	5.6	5.7
2	966	26	11.36	-0.23	212	3.04	1.4	710	619	36	91	6.9	7.5
	967	27	10.89	+0.16	208	3.14	1.5	1198	1078	37	120	7.1	5.9
	960	24	10.91	+0.03	205	2.80	1.4	820	708	43	112	6.3	5.6
3	804	20	12.04	+0.24	252	2.34	0.9	641	576	41	65	5.3	8.1
	827	21	11.59	+0.52	308	2.44	0.8	1019	942	43	77	5.6	7.3
	823	21	13.17	+0.98	240	2.44	1.0	688	614	49	74	5.6	6.8
4	987	37	13.80	+0.08	289	4.32	1.5	800	613	49	187	9.8	5.2
	979	40	12.23	+1.39	288	4.68	1.6	1295	1059	45	236	10.6	4.5
5	972	39	11.96	-0.23	324	4.54	1.4	709	499	52	210	10.3	4.9
	1021	49	12.29	+0.16	386	5.72	1.5	1163	937	51	226	13.0	5.8
	975	45	13.36	+0.03	340	5.24	1.5	850	617	47	233	11.9	5.1

All the subjects were in approximate nitrogen equilibrium except the first, a young boy, who was on a high calorie diet and was gaining rapidly in weight. The feeding of cystine, as shown by Table II, affected neither the excretion of creatinine nor that of cystine. The urinary amino-N also appeared unaffected except possibly in one case, subject 3, in whose urine the amino-N rose to an average value of 308 mg., with average values of 252 and 240 during the preceding and following periods respectively. Since it has been shown by Van Slyke & Kirk [1933] that amino-N in the urine rises after ingestion of protein, these findings indicate that other amino-acids are responsible for the increase.

Cystine N in percentage of amino-N varied from 0.8 to 1.6. There was no significant difference between the patients with tumours and the normal subjects. Highest values of amino-N were found in the urines of subject 1, who was on a high protein diet. In this instance the percentage of cystine N was lower than in any other group, but not significantly less than that of subject 3.

No increase in neutral sulphur was noted during the periods of cystine feeding, except in the case of subject 4. The cystine sulphur, as percentage of total neutral sulphur, varied from 4.5 to 7.5. Neutral sulphur and cystine sulphur were both elevated in the urines of the two patients with tumours, the ratios falling only slightly lower than those of the normal subjects. It is not known whether the 2 patients with tumours represent tumour patients as a group.

C. Metabolic variations in the cystine content of urine

In Table III is recorded the cystine output of a single individual on two standardized diets, one high protein, containing 120 g. protein daily (estimated), and the other, low protein, containing 8.5 g. protein daily. The urinary collections

Table III. *Cystine output on various diets. Total cystine output and output in moles per hour, as determined by the cuprous chloride precipitation method*

Date	Period	Hr.	Vol. ml.	Cystine			Diet
				Total $M \times 10^5$	per hr. $M \times 10^6$	per hr. mg.	
25 Nov.	7 p.m.-7 a.m.	12	535	6.42	5.35	1.29	Low protein
26 "	7 p.m.-7 a.m.	12	140	4.12	3.43	0.83	
27 "	7 p.m.-7 a.m.	12	370	7.03	5.86	1.42	High protein
28 "	7 p.m.-7 a.m.	12	750	8.11	6.75	1.63	
2 Dec.	9 p.m.-7 a.m.	10	285	4.10	4.10	0.99	Low protein
3 "	7 p.m.-7 a.m.	12	335	6.57	5.47	1.32	
4 "	7 p.m.-7 a.m.	12	625	4.88	4.06	0.98	High protein
5 "	7 p.m.-7 a.m.	12	530	5.94	4.95	1.20	
6 "	7 p.m.-7 a.m.	12	335	7.92	6.60	1.60	
7 "	7 a.m.-7 p.m.	12	410	6.05	5.04	1.42	
7 "	7 p.m.-7 a.m.	12	405	7.56	6.30	1.52	Low protein
10 "	7 a.m.-7 p.m.	12	250	6.12	5.10	1.23	
11 "	7 p.m.-7 p.m.	24	1520	10.94	4.56	1.10	
12 "	7 p.m.-7 p.m.	24	1190	12.50	5.04	1.22	
13 "	7 p.m.-7 p.m.	24	720	10.27	4.48	1.08	
14 "	7 p.m.-7 p.m.	24	770	10.93	4.55	1.10	
15 "	7 p.m.-7 p.m.	24	1045	9.84	4.10	0.99	
Av.						1.52	High protein
						1.11	Low protein

for the most part covered 12-hour periods, from 7 p.m. to 7 a.m. In the final group 24-hour periods were substituted, to ascertain the constancy of cystine output on a constant diet.

The average output on the low protein diet, as shown in Table III, was 1.11 mg. per hour, and 1.52 mg. per hour on the high protein diet. In spite of the small number of determinations, this difference seems significant, since the deviations in the two classes are relatively small, the values among the urines collected during the low protein diet ranging from 0.83 to 1.32 mg., and during the high protein diet from 1.42 to 1.63.

The average output in the final series of collections during the low protein diet was 1.11, a value identical with the average of the entire low protein periods. The average deviation was ± 0.07 . It is not clear how far these deviations

represent variations in the daily output, or to what extent they represent the errors in the determinations of urines of different volumes and acidity.

Since the variation in output of a single individual on varied diets is relatively slight, it may reasonably be concluded that the wide differences shown among the 50 subjects studied are fundamental differences in individual treatment of cystine. It would be of interest to determine whether these variations are metabolic or whether they are renal (leakage of cystine through the kidney). The high positive correlation between excretions of cystine and creatinine indicate that metabolic differences between individuals play the greater role.

SUMMARY

The cystine excretion of 50 normal individuals was determined as 30.4 ± 1.5 mg. per 24 hours, with a range of 0.8–84 mg. per 24 hours.

There was a positive correlation of 0.46 ± 0.07 between excretions of cystine and neutral sulphur, and a positive correlation of 0.65 ± 0.05 between urinary cystine and creatinine. No significant correlation was found between cystine excretion and output of total nitrogen or body weight.

Cystine sulphur comprised from 5 to 8% of the neutral sulphur and from 4.5 to 8% of the amino-nitrogen.

The output of cystine by a single individual ranged from an average value of 1.11 mg. per hour on a low protein diet to 1.52 on a high protein diet.

Differences between individuals are greater than between variations of a single individual on different diets.

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