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# SULFUR METABOLISM IN CYSTINURIA<sup>1</sup>

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Although the literature contains numerous studies of cystinuric cases, further data on the metabolism of various sulfur compounds in the cystinuric individual is desirable, not only for the light it may throw on an interesting anomaly but also because the cystinuric may be valuable for the investigation of the course of sulfur metabolism in the normal individual. Among all the elements concerned in animal metabolism, sulfur is unique in that it undergoes the largest change of valence, the maximum valence change of  $-2$  to  $+6$  being accomplished when hydrogen sulfide is oxidized to sulfate, and a change only slightly smaller being involved in the normal metabolism of cystine. Since the magnitude of this change is not paralleled by any other element it hardly seems surprising that some individuals fail to accomplish complete oxidation of ingested sulfur. Indeed we might reasonably expect cystinuria to be of much more common occurrence than it is. The following report deals with the results of administering certain sulfur compounds to a cystinuric.

## EXPERIMENTAL

The subject, a 14 year old boy, had a history of kidney involvement with a right nephrectomy for multiple kidney stones at age 12. The surgical aspects of the case have been commented on recently by Herman and Lee (1). The subject is Case II in their report. Cystine has been constantly present in the subject's urine, quantitative determinations showing excretion of 0.4 to 0.5 gram cystine per day. However, examination of the urines of both parents gave negative results. At the time of our experiments the subject weighed 35 kilos and was otherwise in normal physical condition.

<sup>1</sup> A preliminary abstract of this paper was presented at the Pittsburgh meeting of the American Association for the Advancement of Science, December 1934.

A preliminary abstract of the work was published in the Proceedings of the Society of Experimental Biology and Medicine 1934, 32, 455.

Total sulfur was determined by the Benedict-Denis procedure and total sulfate-sulfur after hydrolysis with HCl. In all cases the determination was made gravimetrically as BaSO<sub>4</sub>. Cystine was determined by the Sullivan colorimetric procedure as modified by Brand *et al.* (2).

Table I shows the sulfur distribution in 24-hour urine samples of the subject at various periods over several months. With the exception of the experiments of September 23 and September 24, the subject was on normal miscellaneous diet with a somewhat higher sulfur intake than would be furnished by the two quarts of milk ingested per day (and for one day previous) during the experiment of September 23 and 24. On May 24, ingestion of 10 grams sodium bicarbonate daily was begun. Although direct cystine determinations were not made during the April period, they were made for some days before May 24 (not recorded on the table) and the results indicate that the ingestion of sodium bicarbonate has no effect on the degree of sulfur oxidation nor on the cystine output. In this we are in agreement with the results of Lewis and Lough (3) and Robson (4), as opposed to those of Looney, Berglund and Graves (5). The lack of effect of either sodium bicarbonate or sodium citrate in decreasing the cystine output of the cystinuric has been confirmed by later experiments. During the latter part of May, the sodium bicarbonate was replaced by an equivalent amount of sodium citrate which was ingested daily until the September experiments. No effect on the cystine output or on the sulfur distribution was observed. We feel, therefore, that the only object to be gained by the feeding of base to the cystinuric patient is that of keeping the cystine more completely in solution and of preventing formation of calculi. In this, we are merely increasing the natural tendency of cystinuric urines to be more alkaline than those of normal subjects because of the usual restriction of protein in the diet and also because of the ex-

TABLE I  
Oxidation of sulfur by a cystinuric subject

Date	Volume	Nitrogen N	Total S	Sulfate S	Unoxidized S	Percentage oxidation	Cystine S	Cystine S per cent of unoxidized S	Remarks
	<i>cc.</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>	<i>grams</i>	<i>per cent</i>	
April 23	3400	7.58	0.632	0.357	0.275	56.5			Normal hospital diet
April 24	3700	7.30	0.589	0.370	0.219	62.8			" " "
April 25	3100	6.22	0.614	0.330	0.284	53.8			" " "
April 26	2670	6.80	0.542	0.356	0.186	65.7			" " "
April 27	1810	7.02	0.669	0.423	0.246	63.2			" " "
May 24	1320	6.82	0.604	0.398	0.206	65.9	0.099	48.0	10 grams NaHCO <sub>3</sub> per day
May 25	1760	6.86	0.566	0.332	0.234	58.7	0.134	57.2	" " " "
May 26	1300	7.06	0.551	0.314	0.237	57.0	0.107	45.1	" " " "
May 27	1280	8.02	0.583	0.368	0.215	63.1	0.112	52.1	} 5 grams glutamic acid + 2.55 grams glycine daily in addition to NaHCO <sub>3</sub>
May 28	780	5.83	0.456	0.269	0.187	59.0	0.087	46.5	
May 30	980	7.19	0.533	0.346	0.187	64.9	0.093	49.7	
May 31	1060	6.90	0.485	0.305	0.180	62.9	0.105	58.3	
June 1	950	6.69	0.496	0.302	0.194	60.9	0.108	55.7	
September 23	1245	6.77	0.434	0.225	0.209	51.8	0.100	47.8	Milk (2 quarts) + crackers and fruit
September 24	800	7.28	0.471	0.274	0.197	58.2	0.080	40.6	" " " " " "
October 27	1230	7.78	0.679	0.422	0.257	62.2	0.094	36.7	Egg diet (55 grams egg protein)
October 28	740	8.13	0.902	0.607	0.295	67.3	0.078	26.3	" " " " " "
November 5	1140	4.88	0.528	0.268	0.260	50.7	0.080	30.8	Low protein (30 grams protein)
November 6	960	4.60	0.513	0.257	0.256	50.1	0.081	31.5	" " " " " "
September 23	715	5.87	0.684	0.567	0.117	82.9			Normal subject-same age. Milk (2 quarts) + crackers and fruit
September 24	1065	9.15	0.711	0.590	0.121	83.0			

cretion of an appreciable portion of the sulfur as cystine instead of as sulfuric acid. The fact that in cystinuria one of the principal sources of urinary acidity (sulfuric acid) is diminished in quantity furnishes, to a partial degree, the alkalinity favorable to keeping the cystine in solution. If we compare the sulfur oxidation in normal with that in cystinuric subjects and calculate the effect of this deficiency in sulfuric acid in the cystinuric on the primary-secondary phosphate ratio (the chief buffer system of normal urine), we find that the cystinuric urine should average from 0.4 to 0.8 pH higher than the normal depending on variations in sulfur and phosphate output. The urinary acidity of the present cystinuric has been compared with that of a normal subject of the same age and sex, both being on identical diets for several days. Complete urine samples collected in each case over a period of two and one-half days gave average pH values of 6.5 for the normal and 7.1 for the cystinuric.

The solubility curve of cystine with change in pH demonstrates the possibility of increasing its

solubility at higher pH values and makes it difficult to evaluate the conclusions of Patch (6) as to the lack of efficacy of alkali. In the case of our present subject, x-ray examination has indicated a definite improvement resulting from administration of alkali. It is obvious that if a subject has already deposited small calculi or "gravel," administration of sufficient alkali to markedly increase the solubility of the cystine and cause it to be excreted in solution will produce a temporary rise in the total cystine excretion.

The suggestion has sometimes been advanced that the metabolism of cystine is so bound up with the formation of glutathione that administration of glycine and glutamic acid might decrease cystine excretion. While there is little to support such a view we thought it worth while to feed these two amino acids in equimolar amounts for a period of some days. As indicated in Table I, 5.0 grams glutamic acid + 2.55 grams glycine (supplying a total of 0.95 gram nitrogen) were fed daily for approximately one week. Neither during that period nor for some days following

was there any noticeable effect on the amount of cystine excreted. In this, our results are as completely negative as those of Robson (4) when glutamic acid alone was fed.

In investigating the metabolism of sulfur compounds administered by mouth we have also used a procedure in which urine collections were made by two hour periods during the day and for the following 12 hour period overnight. The subject, having been kept on a nonprotein diet the day before the experiment, voided and discarded his urine at 6 a.m. Samples were then collected at 7, 9, and 11 a.m., etc. until 7 p.m. The following 12 hour period (7 p.m. to 7 a.m.) was also collected. Since the compound to be studied was ingested at 7 a.m. on the day of the experiment, 24 hours were allowed for its elimination, a period insufficient in most cases, for complete elimination of the sulfur. On the day of the experiment no food was ingested until the evening meal and practically no protein was allowed until the following day. Constant quantities of water were taken hourly but in spite of this the bi-hourly output often varied considerably. The sulfur compound was ingested by mouth in quantity amounting to 0.40 gram sulfur.

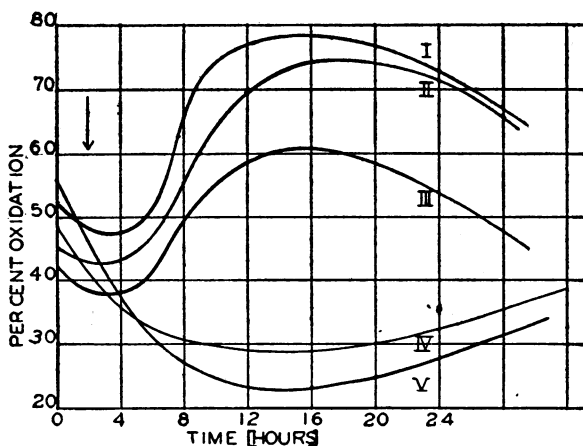


FIG. 1. PERCENTAGE OXIDATION OF SULFUR RESULTING FROM ADMINISTRATION OF VARIOUS SULFUR COMPOUNDS IN AMOUNTS EQUIVALENT TO 0.4 GRAM SULFUR.

- I = l-cystine
- II = dl-cystine
- III = dl-methionine
- IV = blank
- V = cysteic acid

Arrow indicates time of administration of compound

Since the chief variation in the effects of different sulfur compounds is to be found in the proportion of the sulfur oxidized and excreted as sulfate, the results of a number of such experiments are summarized in Figure 1 by plotting percentage oxidation against time in hours. The percentage oxidation represents the ratio of total sulfate sulfur to total sulfur in the urine specimen. This percentage is higher in the samples collected earlier in the day but as the experiment more nearly approaches a fasting basis, this figure in blank experiments becomes approximately 30 per cent. Under the subject's normal dietary conditions this percentage is roughly doubled. The later rise in the curve is the result of the resumption of food intake on the sulfur partition in the sample collected the following morning.

In agreement with the classical picture of cystinuria, the present subject is able to oxidize free cystine, administered by mouth, as well as a normal individual. This is indicated by the rise in the curves for both l- and dl-cystine during the early part of the 24 hour period.

The comparative feeding of l- and racemic cystine was prompted by the remarkable results reported by Loewy and Neuberg (7) who found a cystinuric subject capable of oxidizing "stone cystine" to sulfate but incapable of so oxidizing "protein cystine" (from hair). The suspicion that such results might be caused by difference in optical configuration (the "protein cystine" having been racemized during preparation) is increased by the findings of duVigneaud, Craft and Loring (8). These investigators obtained, with rabbits, a percentage oxidation for d-cystine of approximately half the magnitude of that for the l-isomer. While it is true that our figures show somewhat slower oxidation of racemic cystine during the first 12 hours, the differences are not great and the results, as a whole, more nearly approach those of Hele and Pirie (9) on dogs and Lawrie (10) on rats. The figures for the excess sulfate excreted as a result of cystine ingestion, when corrected for the average fasting level, indicate a somewhat higher recovery of the sulfur for l- than for racemic cystine, but here again the differences are not highly significant, considering the difficulty of assigning a definite value for the average fasting level.

In the case of the l-cystine experiment, about 75 to 80 per cent of the sulfur administered, was recovered as sulfate-sulfur in 24 hours, whereas the same calculation applied to the experiment with racemic cystine indicates a recovery of 60 to 65 per cent. In neither case was any significant rise in unoxidized sulfur noted. In studying the absorption of cystine from dogs with isolated intestinal loops, Andrews and Johnston (11) obtained results indicating slightly more rapid absorption of racemic cystine. This, however, by no means implies a more rapid or complete metabolic oxidation.

Although administration of cysteic acid to the cystinuric individual would not be expected to produce results different from those of the normal, such an experiment appears not to be recorded in the literature and we therefore included in Figure 1, the curve of one of several such experiments, conducted in the same way as described above. Since the cysteic acid is not converted to sulfate by the organism, the output of sulfate-sulfur remains relatively constant while the unoxidized portion increases moderately and the oxidation percentage drops. In another cysteic acid experiment, the percentage of sulfur oxidation reached the low level of 17.4 per cent. However, the absolute increase in sulfur elimination was in

all cysteic acid experiments so slight that the sulfur recovery amounted to only 30 to 40 per cent of that fed. This is all the more surprising in view of the very rapid absorption of cysteic acid from intestinal loops of dogs observed by Andrews and Johnston (11), but accords with the low recoveries of sulfur observed by Schmidt and Clark (12) after feeding cysteic acid to dogs. Further investigation of the cause of these low recoveries of cysteic acid, both in the normal and the cystinuric organism is in progress.

The administration of methionine to the cystinuric individual presents several features of much interest. Not the least of these is the suggestion of Brand, Cahill and Harris (13) that cystinuria may be essentially a disturbance in methionine metabolism as indicated by their finding that the output of cystine by a cystinuric individual was much increased (nearly doubled) by administration of large doses of methionine. The methionine curve in Figure 1, obtained by feeding an amount of methionine equivalent to 0.40 gram sulfur, demonstrates that this amino acid is not nearly so readily oxidized to sulfate as is cystine.

In Table II are presented the results of a longer experiment in which methionine equivalent to 0.50 gram sulfur was fed after a preliminary period, and twelve hour urine samples were collected for

TABLE II  
*Administration of dl-methionine*  
*(Urine samples collected in five 12-hour periods)*

Period	Volume cc.	Total N grams	Total S grams	Sulfate S grams	Unoxidized S grams	Percentage oxidation	Cystine S grams	Methionine S grams	$\alpha \times$ cc. (2 dm.) degrees
<i>Cystinuric subject</i>									
I	640	4.63	0.294	0.180	0.114	61.2	0.058	0.014	-115
II*	690	3.92	0.453	0.243	0.210	53.6	0.062	0.084	-138
III	350	4.23	0.364	0.249	0.115	68.4	0.052	0.020	-70
IV	490	3.72	0.276	0.152	0.124	55.1	0.042	0.018	-113
V	710	4.19	0.310	0.173	0.137	55.8	0.071	0.017	-135
<i>Normal subject</i>									
I	350	5.34	0.383	0.327	0.056	85.4		0.019	-39
II*	1160	6.83	0.772	0.633	0.139	82.0		0.069	-116
III	560	6.42	0.457	0.393	0.064	86.0		0.028	-45
IV	800	6.28	0.511	0.440	0.071	86.1		0.023	-64
V	310	4.10	0.262	0.211	0.051	80.5		0.014	-43

\* 2.33 grams. dl-methionine (=0.50 gram S) administered at the beginning of Period II.

48 hours after its administration. In this case, the cystinuric was compared with a normal subject of the same age and sex. Both subjects were kept on the standard milk-cracker diet used for the experiments of September 23 and September 24 in Table I during the day preceding the first period of urine collection as well as during the entire experiment. The methionine was administered by mouth at the beginning of Period II.

Examination of Table II shows that here again, administration of methionine has little influence on the percentage oxidation of the sulfur with either subject since it produces proportional increases in both oxidized and unoxidized fractions. The figures for cystine sulfur excreted by the cystinuric demonstrate that this increase is not to be accounted for by an increased cystine output. The sharp rise in unoxidized sulfur noted in Period II is not accompanied by a significant rise in cystine sulfur while the rise in the latter in Period V is evidently the result of an increased urine volume at that time (see below). One striking difference to be noted between the two subjects is the promptness with which the normal subject excreted the extra sulfur administered as contrasted with the slower and somewhat erratic excretion by the cystinuric patient.

The methionine administered was the dl-form whereas the natural isomer is the l form ( $[\alpha]_D = -7.2$ ). On the supposition that the organism most readily oxidizes the natural isomer and discards all or part of the d-form, we have sought to account for the fact that dl-methionine is less completely oxidized than cystine and gives rise to increases in the unoxidized S fraction. For this reason methionine was determined directly in these urines.

The figures were obtained by the method of Baernstein (14). For each determination a 50 cc. sample of the urine was evaporated to dryness below the boiling point and the determination was made on this dried residue. It was demonstrated that methionine, added to the urine before evaporation, could be 94 to 96 per cent recovered. Normal urines give a small titration by this method, which, expressed in terms of methionine sulfur, amounts to about 0.02 to 0.04 gram per 24 hours. The table shows that both normal and cystinuric subjects exhibited 4 to 6 fold increases

in this value during the period immediately following methionine ingestion. However, the absolute amount of the increase accounts for not more than 10 to 15 per cent of the methionine sulfur ingested although it does account for 60 to 70 per cent of the increase observed in the unoxidized sulfur fraction. The fact that the methionine was in the dl form lends some support to the view that in these experiments oxidation may be less complete than would have been the case had the naturally occurring isomer been administered.

The last column of the table shows the product obtained by multiplying the optical activity observed with D-light in a 2 dm. tube at 25° C. by the volume of the sample for that period. The resulting figure is therefore an arbitrary measure of the excretion of some levorotatory constituents and is obviously higher with the cystinuric because of the presence of l-cystine. With moderately constant urinary volumes, marked increases in cystine output should be evident in these figures. However, the only case in which such an increase occurs is in Period II of the normal subject when the methionine ingestion was followed by marked diuresis. That this increase in the optical figure is not due to excretion of cystine or homocystine is evidenced by the fact that all samples from the normal subject gave negative cyanide-nitroprusside tests. In this, we fail to confirm the results of Virtue and Lewis (15) and Vars (16) with rabbits and dogs respectively but it should be noted that our dose, per kilo of body weight, is far less than that used by them. The possibility of formation of homocystine from the methionine in the cystinuric is practically excluded by the fact that Folin-Marenzi determinations (which respond to both cystine and homocystine) have given values for cystine which were not appreciably higher than those obtained by the more specific Sullivan method.

In order to investigate the effect of still larger doses of methionine on the cystinuric organism, a similar experiment was run on the same subject over a period of fifteen days under carefully controlled dietary conditions. The diet used contained 300, 50 and 100 grams respectively of carbohydrate, protein, and fat daily. Twenty-four hour urine collections were made. After establishing this metabolic level for several days, the

subject was given a 5.0 gram dose of cystine in water suspension. On the 4th, 5th and 6th days after cystine ingestion large doses of dl-methionine were given. The results are summarized in Table III.<sup>2</sup>

The results of this experiment confirm our previous conclusions: that with the present cystinuric subject, no very marked increase in cystine

may therefore conclude that there is no evidence of excretion of homocystine. The figures for methionine sulfur again show an increase following methionine ingestion although the amount is small as compared with the amount of methionine ingested. The column showing optical activity (Table III) confirms the previous conclusion as to the constancy of cystine output.

TABLE III  
*Administration of di-methionine*  
(Twenty-four hour urine samples)

Day	Volume	N	Creatinine	Total S	Sulfate S	Unoxidized S	Percentage oxidation	Cystine S	Methionine S	$\alpha \times$ cc. (2 dm.)
	<i>cc.</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>	<i>grams</i>	<i>grams</i>	<i>degrees</i>
1	1840	7.27	0.78	0.712	0.236	0.476	33.6	0.147	0.038	-320
2	1520	7.52	0.67	0.685	0.320	0.365	46.7	0.126	0.041	-340
3	1580	7.31	0.75	0.756	0.297	0.459	39.3	0.124	0.039	-420
4	2000	7.71	0.82	0.616	0.362	0.254	58.8	0.135	0.028	-360
5*	1790	7.93	0.76	1.440	1.126	0.314	78.2	0.091	0.022	-300
6	1680	7.11	0.58	0.425	0.192	0.233	45.3	0.088	0.021	-320
7	1740	7.02	0.51	0.452	0.236	0.216	52.2	0.090	0.025	-320
8	1540	6.58	0.81	0.518	0.267	0.251	51.5	0.087	0.022	-400
9†	1490	7.03	0.73	0.824	0.473	0.351	57.4	0.101	0.051	-280
10‡	1620	6.92	0.68	0.788	0.461	0.327	58.5	0.102	0.044	-260
11‡	2000	7.51	0.51	0.952	0.645	0.307	67.7	0.108	0.077	-460
12	2000	7.16	0.66	0.496	0.238	0.258	48.0	0.111	0.021	-440
13	1960	7.01	0.69	0.576	0.313	0.263	54.3	0.108	0.019	-460
14	1760	6.83	0.52	0.404	0.217	0.187	53.7	0.099	0.023	-420
15	1480	6.87	0.80	0.572	0.276	0.296	48.3	0.095	0.020	-340

\* 5 grams l-cystine (=1.333 gram S) administered at the beginning of the 5th day.

† 2 grams dl-methionine (=0.43 gram S) administered at the beginning of both 9th and 10th days.

‡ 5 grams dl-methionine (=1.075 gram S) administered at the beginning of the 11th day.

excretion follows the ingestion of large amounts of methionine. It should also be noted that what increase in cystine output was observed accompanied the larger urine volumes. We have noted, on several other occasions, that diuresis is accompanied by an increased cystine output. The divergence between our results in this particular and those of Brand and coworkers (13), suggests that there exists considerable variation between cystinuric subjects, and points to the desirability of experimentation with as wide a range of subjects and conditions as possible. The figures for cystine sulfur were obtained, as before, by the Sullivan method but no appreciable increase was obtained by the use of the Folin procedure. We

<sup>2</sup> We wish to acknowledge the kindness of Dr. Erwin Brand of the New York State Psychiatric Institute in furnishing the dl-methionine used in this experiment. We wish also to acknowledge the assistance of Kathleen C. Andrews in controlling the dietary conditions of the experiment and in carrying out numerous analyses.

The percentage recovery of the sulfur is, as would be expected, somewhat low. One could hardly expect to administer doses of cystine and methionine of the size used here without having considerable loss in the feces.

In spite of the constancy of the total nitrogen there is obviously considerable variation in the creatinine figures, and it is somewhat suggestive that the most marked variations date from the day on which the large dose of cystine was given. The variability of creatinine output in cystinuric subjects has been commented on both by Alsberg and Folin (17) and by Brand, Harris and Biloan (2). These authors ascribe a rise in creatinine which they observed to an unusual amount of exercise on the part of their subjects and since our present subject was leading a normal school life during the experiment with the daily amount of exercise varying considerably this may account for our variations. The extremely cooperative attitude of the subject as well as the constancy of the

total nitrogen figures negates any assumption of incomplete collections.

TABLE IV

*Increase in free cystine content of cystinuric urines on standing*

Free cystine content of sample		
At once	After 10 days	After 100 days
grams	grams	grams
0.034	0.044	0.044
0.023	0.036	
0.050	0.072	
0.034	0.043	0.044
0.036	0.052	
0.030	0.038	0.036
0.056	0.056	0.041
0.039	0.038	0.037
0.050	0.057	0.061
0.039	0.041	

Table IV illustrates an interesting quality exhibited by some cystinuric urines. Brand, Harris and Bilon (2) have pointed out that the Sullivan colorimetric method for cystine when applied to very freshly voided cystinuric urines, sometimes gives values which are decidedly lower than those obtained when the same method is applied later to the same samples. Because of the well known specificity of the Sullivan procedure and its usual failure to respond to cystine complexes, this increase was explained by assuming the excretion of an unstable complex which quickly decomposed. The figures recorded in Table IV show that in most cases we substantiate these findings as far as our subject is concerned. It will be noted that this increase, when it is observed, is maintained on standing for over 3 months (at 0° and preserved with chloroform). We have also noted that cystinuric urines over eight months old show practically no further change in cystine concentration. The statement made by Magnus-Levy (18) that such urines lose their cystine after some months is hard to explain except on the assumption that slow racemization occurred and that the higher solubility of the racemized cystine adversely affected the precipitation method he used.

The hypothetical cystine complex is, however, far less stable than the data in Table IV imply. We have observed an increase of practically the same magnitude after keeping the fresh sample for 24 hours at 0° C. The instability of the com-

plex is further emphasized by the fact that our present subject, while giving evidence of its excretion, has also formed deposits of practically pure cystine. As far as we are aware, no cystinuric urine on record has ever given a *negative* Sullivan test when freshly voided, and certainly such a finding could hardly be expected in any subject with even small deposits of free cystine in kidney or bladder.

Further investigation of the chemistry of this complex is highly desirable since some means of increasing its stability might have practical bearing on the treatment of cystinuria; the excretion of a soluble complex might avoid the dangers of calculus formation. At present, however, we must admit the possibility that this increase in color may be merely due to the influence of some other urinary constituents on the Sullivan reaction.

#### SUMMARY

A case of cystinuria accompanied by calculus formation has been investigated with regard to the metabolism of various sulfur compounds by the subject.

The cystine output is unchanged by daily administration of alkali (sodium bicarbonate or sodium citrate) over a period of several months. Deposition of calculi has, however, evidently been prevented by this means.

Administration of equivalent amounts of glycine and glutamic acid is without effect on the rate of cystine excretion.

Administration of l-cystine by mouth results in practically complete oxidation of the latter; administration of dl-cystine is followed by slightly less efficient oxidation.

Cysteic acid, administered by mouth causes no increase in sulfate excretion.

Following administration of dl-methionine to the cystinuric subject we have observed: (1) No significant increase in cystine excretion, (2) no excretion of homocystine, (3) definite but very slight excretion of methionine.

The increase reported by Brand and coworkers (2) in the apparent cystine content of these urines on standing has also been observed by us.

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