

EXPERIMENTAL STUDIES ON THE COMPOUND:  
CYSTINE MERCURIC SULFATE

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Pauline D. Wyman

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## I Introduction

## II Experimental

Preparation of the Compound

Methods of Analysis

Nitrogen

Mercury

Sulfur

Analytical Data

Hygroscopicity

Potentiometric Titration

Optical Activity

## III Discussion

Composition

Gray Compound

## IV Summary and Conclusion

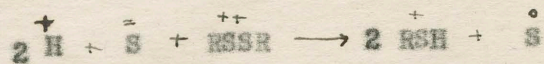


## I INTRODUCTION

Harris (1) has suggested a method for the determination of cystine in serum and egg albumin through the preparation and reduction to cysteine of the double mercuric sulfate salt of cystine. His method involves a reduction of the mercuric sulfate derivative of cystine with hydrogen sulfide with a final reoxidation of the cysteine thus formed to cystine. He has found that mercuric sulfate completely removes cystine in 5 - 7% sulfuric acid solution but that loss occurs in the recovery of cystine from the mercury precipitate. Investigators in the Cambridge Laboratory claim that a very pure form of cysteine is obtained by this method, while Andrews (2) found that the cysteine prepared by this method has an  $[\alpha]_D$  value of  $-20^\circ$  to  $-80^\circ$  as compared with that of pure electrolytically reduced cysteine of  $+9.7^\circ$ . He has found that when hydrogen sulfide is passed into a suspension of the  $\text{HgSO}_4$  salt of cystine the result is a partial reduction to cysteine and  $\text{HgS}$  according to the reactions:



3.



However, attempts to find in the HgS precipitate the free sulfur expected from this reaction have failed.

It would seem necessary that the composition of this intermediate  $\text{HgSO}_4$  derivative of cystine be ascertained before any explanation for the above discrepancies can be given.

The following account describes an attempt to determine the composition of the mercuric sulfate derivative of cystine, and to investigate some of its properties:



## II EXPERIMENTAL

Preparation of the Compound

The samples for analysis were prepared by dissolving 10 gms. of cystine in 2500 cc. of 5%  $\text{H}_2\text{SO}_4$ . In an attempt to determine the quantitative relations of  $\text{HgSO}_4$  to the cystine molecule, and assuming one  $\text{HgSO}_4$  molecule to be attached to each amino group in the "double mercuric sulfate salt" of cystine, the stoichiometrical quantity of  $\text{HgSO}_4$  reagent (10%  $\text{HgSO}_4$  in 5%  $\text{H}_2\text{SO}_4$ ) was added. A flocculent white precipitate immediately appeared. Two such preparations were made and in both cases, upon standing, the upper layers turned gray (See later discussion). One preparation was allowed to stand at room temperature while the second preparation was placed in the cold room at  $0^\circ\text{C}$  for four days, but no change was apparent in the gray compound at the end of that time. An amount of  $\text{HgSO}_4$  reagent equal to the quantity previously used, was added to both preparations thus doubling



the stoichiometrical ratio. More of the white compound precipitated.

After the addition of the excess  $\text{HgSO}_4$  and upon standing the gray compound apparently disappeared.

Both preparations were carefully washed. The details of the washing are given below as this process evidently has an important bearing upon the results obtained. The washing was difficult due to the fact that the compound was cohesive and packed down tightly on the filter~~s~~. Therefore to facilitate matters, the precipitate was transferred from the filter to a large beaker and there carefully washed with distilled water. The suspension was then filtered in successive portions, each portion being again washed when on the filter. When the entire suspension had been so treated, the precipitate was again transferred to a beaker and the process was repeated. This method of washing was continued until the test for sulfate was almost negative. The washings no longer gave a positive test for <sup>++</sup>Hg after 2.5 liters of distilled water had been used. Sample I



was washed with 61 liters of water; sample II with 55 liters. In both cases the final washings contained sulfate and were almost, though not entirely, neutral to litmus. After the first fifteen or twenty liters of washing the amount of  $\text{SO}_4$  present in the filtrate was markedly decreased, but after that it did not decrease to any marked degree.

The samples were difficult to dry, calcium chloride and sulfuric acid proving ineffective. A vacuum <sup>desiccator</sup> dessicator containing phosphorous pentoxide was resorted to and was kept at  $37^\circ\text{C}$ . In about a week the weight was nearly constant. Due to the hygroscopicity of the compound it was difficult to obtain a constant weight. (See below). Sample II was first placed in the warm room and several weeks later in a vacuum desiccator.

The use of higher temperatures for drying was precluded by the instability of the compound. At temperatures of  $250^\circ$  to  $300^\circ\text{C}$  the white compound blackens instantly with formation of metallic



mercury. This decomposition is also initiated at a temperature of 120° C and even below.

### Methods of Analysis

#### Nitrogen

Total nitrogen was determined by the Kjeldahl method, ten percent sodium sulfide being used to precipitate the mercury as HgS after digestion was complete. Blanks were run on all reagents used.

#### Mercury

An attempt was made to determine the percentage mercury present by filtering through a Gooch crucible, the HgS precipitate obtained from the Kjeldahl determination. The method was unsuccessful because of difficulties in filtering. Some HgS was apparently in colloidal form, and the whole procedure appeared to be of doubtful reliability.

Therefore the following procedure was adopted: A 0.5 gm sample was oxidized with  $\text{H}_2\text{SO}_4$  and  $\text{K}_2\text{SO}_4$  as in the nitrogen determination.



After digestion was complete, the solution was cooled (on cooling beautiful needle like crystals of  $\text{HgSO}_4$  appeared) and diluted to 200 cc. It was then heated to boiling and hydrogen sulfide passed in for three-fourths to one hour, while the solution was gently boiling on a hot plate; allowed to stand 48 hours and then filtered through a Gooch and washed free of  $\text{SO}_4$ . Very little free sulfur was present and this was extracted with carbon disulfide. The Gooch containing the  $\text{HgS}$  precipitate was dried to constant weight at  $110^\circ \text{C}$ . The filtrate was tested for complete precipitation of mercury. In most cases it had been complete. In one or two instances a maximum of two milligrams of  $\text{HgS}$  was obtained from the filtrate.

#### Sulfur

The percentage sulfur was determined by the gravimetric method with the precipitation of barium sulfate. A 0.5 - 1.0 gm sample was treated with fuming nitric acid and evaporated to dryness on a water bath. Then concentrated  $\text{HCl}$  was added and the solution again evaporated to dryness



(since nitrates are occluded by  $\text{BaSO}_4$ ). The residue was taken up with water, diluted to 300 cc. and the solution neutralized with ammonia, methyl red being used as indicator. The precipitate of  $\text{Hg}(\text{OH})_2$  which appeared dissolved on the addition of 3 cc conc  $\text{HCl}$ . The solution was heated to boiling and excess hot  $\text{Ba Cl}_2$  added drop by drop while the solution was kept gently boiling on a hot plate. The solution was digested on a water bath for two hours, allowed to stand overnight, filtered through a Gooch, washed free of  $\text{Cl}$ , ignited for half an hour to remove any organic matter and dried to constant weight at  $110^\circ$ .

The filtrate when tested showed complete precipitation of the  $\text{BaSO}_4$ .

The figures for percentage nitrogen, mercury and sulfur are given in Table I.

#### Analytical Data

Sample I	Table I		
	N%	Hg%	S%
	2.82	61.78	7.66
	2.82	61.94	7.53
	2.85	61.81	7.45
	2.91	61.90	
	2.86		
	2.85		
	2.91		
Average	2.86%	61.86%	7.55%



Sample II	N%	Hg%	S%
	3.04	60.34	7.14
	3.04	60.35	7.18
	3.05	60.36	7.34
	3.05	60.30	7.22
Average	3.05%	60.34%	7.22%

### Hygroscopicity

Mention has already been made of the difficulty in obtaining accurate weights of the samples analyzed. This was due to the slowness in drying and also to the gain in weight during the weighing of the samples. In order to determine the hygroscopicity of the compound a sample was removed from the desiccator and placed on a weighed watch glass. The percentage gain or loss in weight over a period of two weeks is given in Table II. The sample was allowed to remain in the balance case. It was found that the gain in weight was at first so rapid that no accurate weighing could be made for some time. It will be noted that the gain or loss in weight was influenced to a large extent by the condition of humidity. (On a rainy day there was a .37% gain as compared with .01% gain on the day previous)



Temperature and pressure greatly affect the rate of drying of the compound. One preparation when kept at  $37^{\circ}$  dried very much more rapidly than one placed in an ordinary calcium chloride desiccator at room temperature. A sudden gain in weight was noticed when the compound in the vacuum desiccator containing  $P_2O_5$  was removed to a room at  $23^{\circ}$  from one at  $37^{\circ}$ . A gain in weight was also noticed when air was admitted to the vacuum desiccator in the warm room.



Table II

Hygroscopicity of the Mercuric Sulfate-Cystine Compound

Date	Weight of Sample	%Gain or loss over previous weighing
12/31/28		
11.55	2.753 gas	.....
12.05	2.755	.07
12.15	2.758	.11
12.25	2.760	.07
1.10	2.761	.04
2.10	2.762	.04
3.10	2.765	.11
4.10	2.767	.07
5.10	2.768	.04
1/2/29		
9.30	2.784	.58
4.00	2.782	.07 loss
1/3/29		
10.00	2.782	0.0
5.30	2.783	.36
1/8/29	2.785	.07
1/9/29	2.7853	.01
† 1/10/29	2.7957	.37
1/11/29	2.7934	.08 loss
† 1/12/29	2.7880	.20 loss
1/14/29	2.7885	.02
1/15/29	2.7915	.11

N.B. Fourth decimal place approximate

† Rainy day

† Clear - warm

Maximum gain in weight = 1.55% on 1/10/29.



### Potentiometric Titration

To obtain a check on the analytical results, which showed a one to three ratio of cystine to mercury (see below), several potentiometric titrations were made. 0.5 gm l cystine were dissolved in 50 cc. of 5%  $\text{H}_2\text{SO}_4$  and the 10%  $\text{HgSO}_4$  reagent was added one cc. at a time, from a burette. The voltage was measured by a Type K potentiometer using a Standard Weston cell and calomel half-cell. Both bright and platinized platinum electrodes were used, but the most satisfactory results were obtained with platinum black. An agar-KCl bridge between the calomel cell and the cystine solution was necessitated by the fact that the presence of halides greatly increased the solubility of the mercury compound and gave erratic curves. For example when 25 cc. KCl solution were added a marked alteration in the titration curve resulted. Further experiments with bromide and iodide gave parallel results; the mercury-cystine compound quickly dissolved leaving only, in the case of the iodide, a slight precipitate of mercuric iodide.

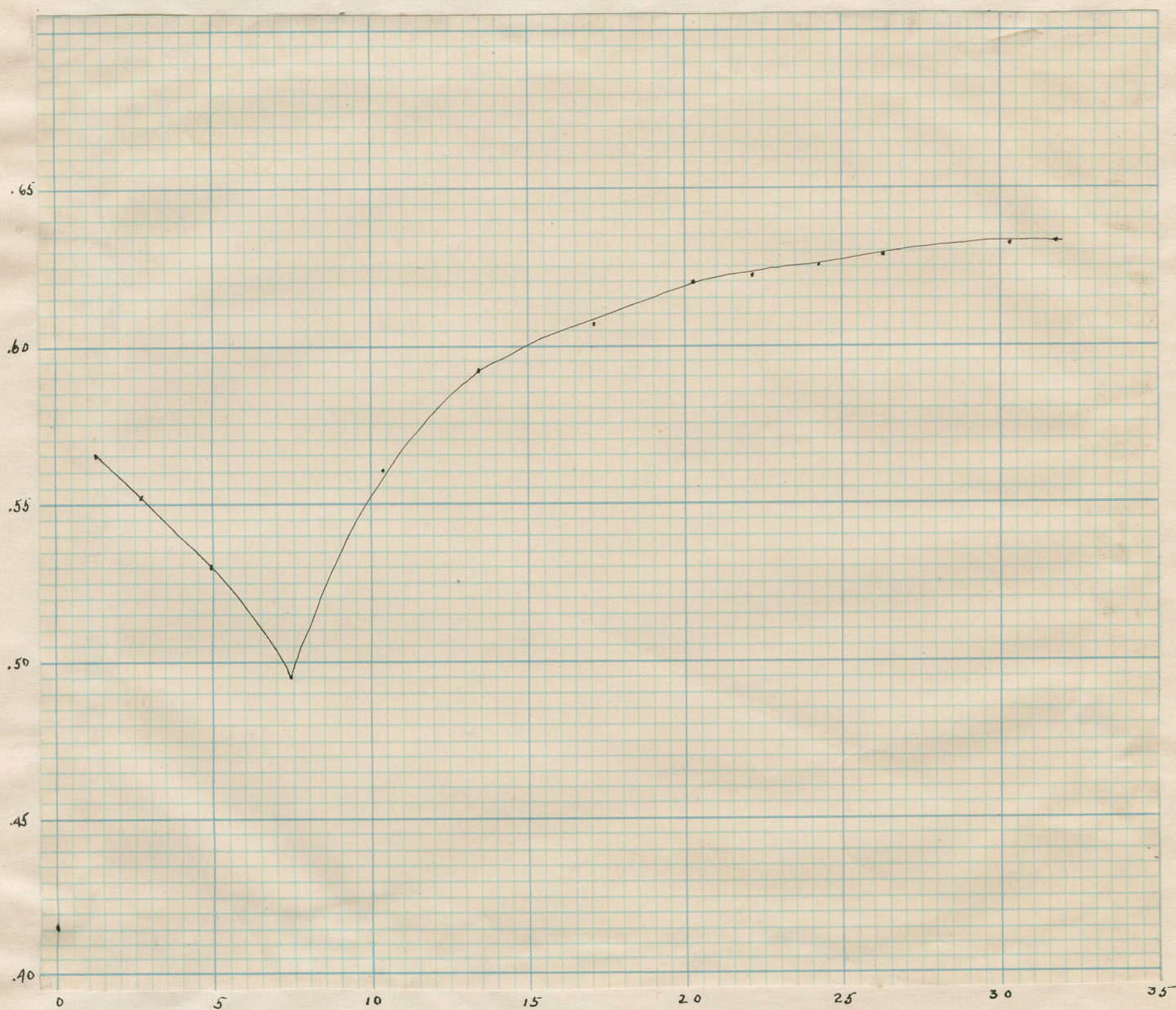
In the absence of any halide, a Curve was obtained (Fig. 1) with a



definite maximum at about 7 cc. of 10%  $\text{HgSO}_4$  reagent, with no indication of any other maximum or minimum. This would seem to show a 1 : 1 ratio of cystine to mercuric sulfate (0.5 gm. cystine reacting with 0.62 gm.  $\text{HgSO}_4$ ), with no indication of such a compound with a 1 : 3 ratio as shown by analysis. There was no evidence of any adjustment in composition of the compound.

It would seem from these results that there is a possibility, depending on the method of preparation, of the formation of more than one compound of  $\text{HgSO}_4$  with cystine.





.5 gm cystine in 50 cc 5%  $\text{HgSO}_4$  titrated with 10%  $\text{HgSO}_4$  reagent  
Ordinate - voltage      Abcissa - cc  $\text{HgSO}_4$  reagent

Fig. 1.



Optical Activity

The filtrate obtained from the precipitation of the  $\text{HgSO}_4$  - cystine was tested for the presence of an optically active substance to check Harris' statement as to the completeness of precipitation. A rotation of  $+ .15^\circ$  was observed. The initial washing gave a rotation of  $+ .13^\circ$ . These dextro-rotatory results are similar to those obtained by Andrews (unpublished results) who observed rotations of  $+ .11^\circ$ ,  $+ .05^\circ$ ,  $+ .10^\circ$ ,  $+ .07^\circ$  in such solutions. The asymmetry present in this active substance must come from the cystine and precipitation is not quantitatively complete. This constitutes all the information we have at present on this subject which needs further investigation.



### III DISCUSSION

#### Composition

The cystine molecule contains two basic and two acidic groups.

It is possible, then, that mercury may replace the hydrogen of a carboxyl group or that the  $\text{HgSO}_4$  molecule may attach itself to one or both of the amino groups. The salts thus formed may hydrolyze with the formation of a basic mercuric salt or hydroxide.

Considering the nitrogen and mercury percentages obtained in Sample I, we find a definite ratio of nitrogen to mercury of 2 : 3 or one cystine molecule to three mercury.

$$\text{N } \frac{2.86}{14.01} = .2042 \text{ (- .1021 cystine)}$$

$$\text{Hg } \frac{61.86}{200.6} = .3084$$

Assuming then that three  $\text{HgSO}_4$  molecules are attached to the cystine molecule, a compound with a molecular weight of 1130.24 would result, giving percentages of nitrogen and mercury lower than those actually found, as indicated in Table III. In other words



the molecular weight of such a compound as is indicated by a ratio of 1 cystine : 3  $\text{HgSO}_4$  is too high to agree with the experimental findings.

Table III

Possible Compositions	N%	Hg %	S%	Mol. Wt.
(A) $\text{C}_6\text{H}_{12}\text{O}_4\text{N}_2\text{S}_2 \cdot 3 \text{HgSO}_4$ <i>no space</i>	2.48	53.22	14.18	1130.24
(B) $\text{C}_6\text{H}_{10}\text{O}_4\text{N}_2\text{S}_2 \cdot 2 \text{HgSO}_4$	2.72	58.30	12.43	1032.16
(C) $\text{C}_6\text{H}_{10}\text{O}_4\text{N}_2\text{S}_2 \cdot \text{HgSO}_4 \cdot \text{Hg}(\text{OH})_2$	2.89	62.03	9.92	970.18
(D) $\text{C}_6\text{H}_{12}\text{O}_4\text{N}_2\text{S}_2 \cdot \text{HgSO}_4 \cdot 2 \text{HgO}$	2.89	62.03	9.92	970.18
(E) $\text{C}_6\text{H}_{10}\text{O}_4\text{N}_2\text{S}_2 \cdot \text{HgO} \cdot \text{HgSO}_4$	2.94	63.21	10.10	952.14
(F) $\text{C}_6\text{H}_{10}\text{O}_4\text{N}_2\text{S}_2 \cdot 2 \text{Hg}(\text{OH})_2$	3.09	66.27	7.06	908.09
(G) $\text{C}_6\text{H}_{10}\text{O}_4\text{N}_2\text{S}_2 \cdot 2 \text{Hg}(\text{OH})_2 \cdot 4 \text{H}_2\text{O}$	2.86	61.40	6.54	980.11
(H) $\text{C}_6\text{H}_{10}\text{O}_4\text{N}_2\text{S}_2 \cdot 2 \text{Hg}(\text{OH})_2 \cdot 3 \text{H}_2\text{O}$	2.91	62.55	6.66	962.11
(I) $\text{C}_6\text{H}_{10}\text{O}_4\text{N}_2\text{S}_2 \cdot 2 \text{Hg}(\text{OH})_2 \cdot 2 \text{H}_2\text{O}$	2.97	63.74	6.79	944.1

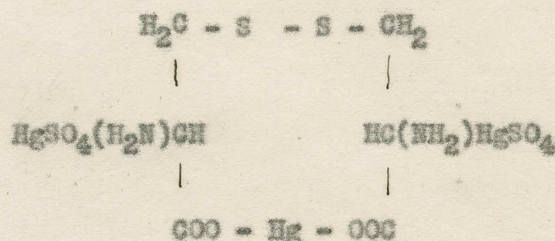
Experimental Data

Sample I	2.86	61.86	7.55	....
Sample II	3.05	60.34	7.22	....



Again if we assume two  $\text{HgSO}_4$  molecules and another mercury molecule to be present in such a compound as the following:

(B)

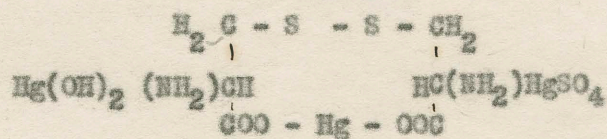


we obtain a molecular weight of 1032.16 and nitrogen and mercury percentages of 2.72 and 58.30 respectively, the mercury percentage being too low to agree with the experimental findings even for sample I.

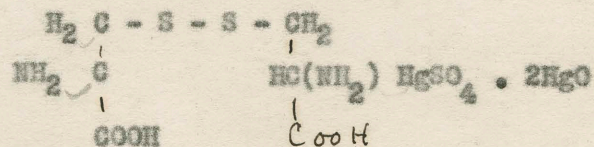
It is quite possible, however, that due to repeated washing one or both the  $\text{HgSO}_4$  molecules might have become hydrolyzed. (It is worth noting in this connection that when the samples were prepared and washed the washings were never completely free of  $\text{SO}_4$ .) If one  $\text{HgSO}_4$  had hydrolyzed the following compound (C) would result:



(c)



(D)



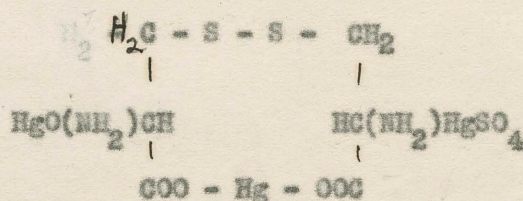
(D) represents another possible compound of the same molecular weight as (C), but with a different molecular arrangement. Both have a molecular weight of 970.17 and percentages of N and Hg equal to 2.89 and 62.03 respectively which do not differ radically from those actually obtained on analysis, i.e., N = 2.86%, Hg = 61.86%.

If both  $\text{HgSO}_4$  molecules had hydrolyzed to  $\text{Hg}(\text{OH})_2$  molecules a compound with a molecular weight of 908.09 would result which is far too low to agree with the analytical data. If three  $\text{Hg}(\text{OH})_2$  molecules were present the molecular weight would still be too low = 944.13. If three  $\text{HgO}$  molecules or two  $\text{HgO}$  and one  $\text{Hg}$  were



present compounds of even lower molecular weight would result. A compound such as the following gives a somewhat higher, but not high enough molecular weight (see table III) for the calculated percentages to agree with the experimental findings.

(E)



Compounds (C) and (D), then, are the most plausible as far as the nitrogen and mercury percentages are concerned.

But the theoretical value for sulfur for either one, 9.92%, is much higher than the analytical value obtained, i.e., 7.55%. A possible source of error in the method used for the determination



2 carbons

22.

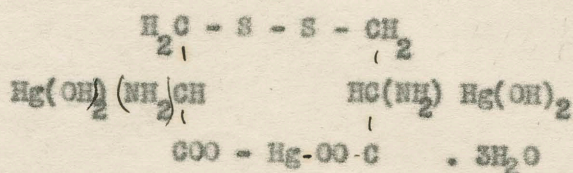
of sulfur would be in the incomplete oxidation of the sulfur with subsequent loss due to the evolution of  $\text{SO}_2$ .

Osborne<sup>(2)</sup> suggests that the fusion method for the determination of sulfur is ~~much~~ to be preferred ~~because of the possible evolution of  $\text{SO}_2$  in the nitric acid oxidation method, etc.~~ Several fusions were tried, but the method was less satisfactory due to the tendency of the melt to attack the walls of the crucible.

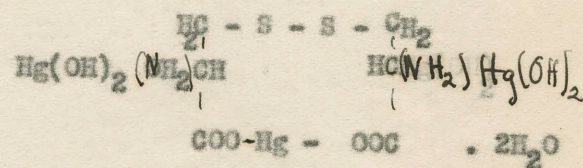
Also several oxidations with bromine in potassium bromide solution, followed by nitric acid, were tried with no better result.

Assuming the sulfur determinations to be correct <sup>and</sup> in order to account for the low value obtained without materially changing the molecular weight from 970.16, several hydrated forms were postulated containing one less sulfur atom.

(H)



(I)

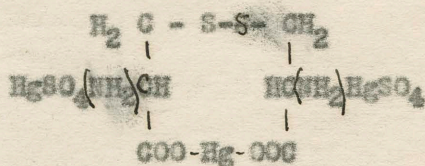




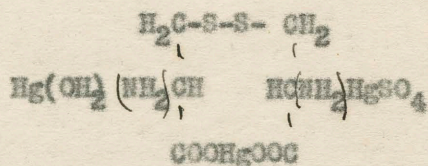
By referring again to Table III, it is readily seen that in the above hydrated forms the theoretical nitrogen<sup>and</sup> mercury percentages are raised considerably above the analytical ones, while the sulfur falls below that value found on analysis. If four molecules of water are assumed the mercury as well as the sulfur percentage falls below the analytical value. (Table III)

Thus a comparison of the sulfur, nitrogen and mercury ratios and a study of the percentage composition of the possible compounds, compared with analytical data, would indicate that we are dealing with a mixture in Sample I. It is quite possible that the compound originally precipitated may have contained two (or even three)  $\text{HgSO}_4$  molecules which on prolonged washing were either partially or wholly hydrolyzed as shown by the following examples:

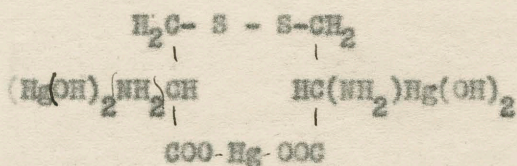




(I)



(II)



(III)

The sample analyzed may be a mixture of I, II, III representing various degrees of hydrolysis.

Similar difficulties were encountered in reconciling the analytical data obtained for Sample II with any plausible composition. Its analysis points to the same conclusion:- that a mixture is present, that such factors as temperature, amount of washing, etc., will



materially affect the composition.

#### Gray Compound

The above conclusion is further borne out by the appearance of the gray compound, previously mentioned, when the  $\text{HgSO}_4$  derivative of cystine was uncompletely precipitated. In the preparation of both samples for analysis the stoichiometrical quantity of  $\text{HgSO}_4$  reagent was added (assuming two  $\text{HgSO}_4$  molecules to be attached to one cystine molecule) and in a short time the upper layers had a grayish appearance, the rest resembling the usual flocculent white precipitate. Samples of the gray precipitate when examined under a microscope exhibited a mass of definite pin-like "crystals", (Fig. 2) some of the pins being bent but not broken.

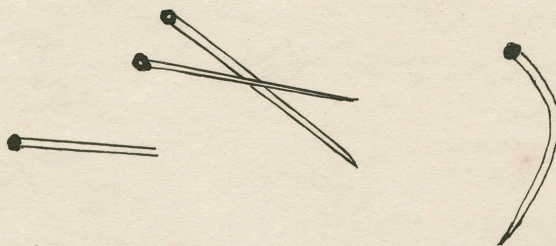


Fig. 2



Their appearance suggested the possibility of a mold, but on testing the solution for mercuric ion a positive test was obtained. It seems improbable (though not impossible), that a mold would exist in an acid solution of a mercuric salt. A temperature of  $0^{\circ}$  for four days did not affect the appearance of the gray precipitate. Samples of both gray and white precipitate were removed and placed in flasks for future reference.

To the main portion of the precipitate was added a quantity of  $\text{HgSO}_4$  reagent equal to that used in the initial precipitation. More of the usual white variety of precipitate then appeared.

In order to minimize the possibility of mold formation a one gram sample of cystine was dissolved in 250 cc. 5%  $\text{H}_2\text{SO}_4$  and the solution boiled for fifteen minutes. The stoichiometrical quantity of reagent (assuming two  $\text{HgSO}_4$  molecules) was added and the compound precipitated from the hot solution. The Erlenmeyer flask containing the solution was immediately covered with a sterilized beaker. Within an hour the



gray compound made its appearance, and later practically the entire precipitate was of this character. After cooling, a specimen was examined microscopically. Masses of short pins were observed - some arranged as in burrs. Later examination of the same slide showed the presence of long needle-like crystalline forms while the pins were fewer in number. In one instance the needles split across the longer axis. In a previous precipitation when excess  $\text{HgSO}_4$  had been added and the material stirred up, a few needles and many irregular, broken crystals were observed.

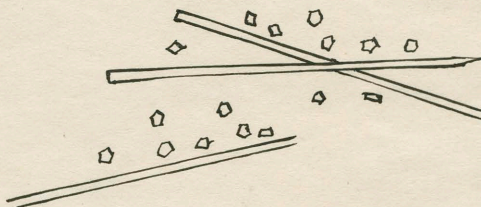


Fig. 3.



A plausible mode of formation of these fragments from the "pins" is indicated in Figure 4, which depicts various observed forms.

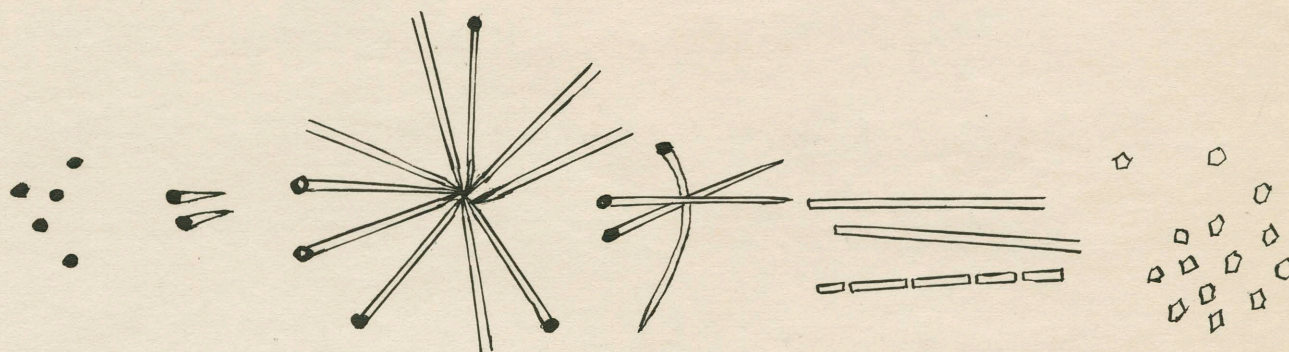


Fig. 4.

A sample of preparation II examined just before filtering (after excess  $\text{HgSO}_4$  had been added) appeared to have the final form in Figure 4. Perhaps an explanation of previous inability to obtain a crystalline form of the  $\text{HgSO}_4$  derivative of cystine is the fact that on standing the crystals become packed down into a cohesive amorphous looking-mass, which under the microscope appears to consist of masses of irregular plates.



## IV SUMMARY AND CONCLUSION

An analysis of two preparations of the  $\text{HgSO}_4$  derivative of cystine described by Harris, was made and the percentages of nitrogen, mercury and sulfur determined. The results indicated a probable hydrolysis by repeated washings, of the original compound precipitated, the sample analyzed probably being a mixture of several compounds representing various degrees of hydrolysis.

The results of the analysis showing a 1 : 3 ratio of cystine to mercury and those of the titration curves showing a 1 : 1 ratio, and the formation of the gray compound as an intermediate step, indicate the probability of formation of a series of compounds of  $\text{HgSO}_4$  with cystine with a composition depending on the method of preparation.

The possibility of formation of more than one compound with  $\text{HgSO}_4$  and cystine may account for the discrepancies already noted between Andrews and different workers in the Cambridge Laboratories, in the preparation of a pure form of cysteine by the reduction of the



$\text{HgSO}_4$  derivative of cystine.

The filtrate from the precipitation gave a slight optical rotation of  $+.15^\circ$ , and the initial washings  $+.13^\circ$ .

The compound formed is extremely hygroscopic.

It is very unstable at temperatures of  $200^\circ \text{C}$  and above.

A gray compound having an unstable pin-like structure was obtained as an intermediate step in the preparation of the sample for analysis.

It suggests a crystalline form of some  $\text{HgSO}_4$  salt of cystine, although the idea of possible fold formation is not altogether precluded.

Further standardization of the conditions of preparation of this compound is required before consistent data as to composition and properties can be expected.



## BIBLIOGRAPHY

- (1) Harris, L. J. Proc. Roy. Soc. London Series B 1913 XCIV 441
- (2) Andrews, J. C. Jour. Biol. Chem. 1926 - LVIX - 209
- (3) Osborne T. B. Jour. Am. Chem. Soc. 1902 - 24 - 140