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# STUDIES ON THE SOLUBILITY OF CYSTINE UNDER VARIOUS CONDITIONS, AND ON A NEW METHOD OF CYSTINE PREPARATION.

By

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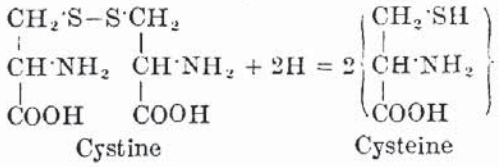
## I. Introduction.

It is a well known fact that the preparation of cystine, one of the biologically important amino acids, from hydrolysate of hair for the purpose of various biochemical studies is not easy. Its yield is very scanty in many cases and it is very difficult to eliminate the defield tyrosin completely from the cystine obtained. In order to find the method, which can increase the yield of cystine from protein-hydrolysate, I feel the necessity of knowing the solubility of cystine under various conditions. For this purpose it is necessary to know the quantitative estimation of the whole amount of cystine in the liquid and the cystine remaining in the media after crystallisation. It has been regretted, however, that there was not any method of easy and accurate quantitative estimation. Recently, Okuda reported on the iodine metho l of the quantitative determination of cystine, and I adopted his method for my studies. I shall briefly describe here Okuda's iodine method:

After the hydrolysate of proteins by hydrochloric acid is decolorized by the addition of animal charcoal, and a small amount of zinc powder is put into the liquid, the cystine is reduced to cysteine as follows:



Cystine Cysteine

Caustic soda solution is added to make the acidity of the liquid to 2% hydrochloric acid solution. Into a certain amount of that liquid (20.0 cc.) are added 5.0 cc. of a 5% potassium iodide solution and also 5.0 cc. of a 4.0% hydrochloric acid solution. When a potassium iodate solution having a certain fixed value (1/300 mol) is dropped into the liquid, iodine will be freed according to the following equation:

$$5KI + KIO_3 + 6HCl = 3H_2O + 6I + 6KCl$$

The freed iodine immediately acts upon the cysteine in the solution at room temperature, and cystine and HI are produced according to the following reaction:

$$2\left\{\begin{array}{l}CH_2\cdot SH\\ |\\ CH\cdot NH_2\\ |\\ COOH\end{array}\right\} + 2I = \begin{array}{l}CH_2\cdot S-S\cdot CH_2\\ |\qquad\quad |\\ CHNH_2\quad CH\cdot NH_2\\ |\qquad\quad |\\ COOH\quad COOH\end{array} + 2HI$$

Cysteine iodine Cysteine

When all the cysteine has been oxidised completely, the freed iodine from more than a drop of potassium iodate solution gives a yellow colour to the liquid. If this yellow colour should be left unchanged for one minute the final reaction is attained; and the amount of cystine is calculated from the amount of consumed iodate solution.

Experimentally, if 4.65 cc. of a 1/300 mol potassium iodate solution is used 0.01 gm. of cysteine (0.01 gm. cysteine = 0.0101 gm. cystine) will be completely oxidised:

$$\frac{\text{consumed cc. of } KIO_3 \times \dfrac{\text{total amount of hydrolysats}}{20{\cdot}0}}{4.65} \times \frac{100}{\text{amount of protein employed in gm.}}$$

= Cystine contents in % in the protein.

It has been generally known that for the separation of cystine, a certain H-ion concentration was necessary. I adopted the H-ion concentration test by the use of the indicator method and know that the pH optimum for the separation was pH=4.8. This fact has favoured the preparation of cystine to my satisfaction. According to the results of my long series of experiments, to make clear the necessary conditions for the separation of cystine, I have learnt that the kind of electrolyte which had been dissolved in the medium interfered to a considerable extent in the separation of cystine. Especially NaCl and $Na_2SO_4$ augmented the solubility of cystine, while $NH_4Cl$ or $CH_3COO(NH_4)$ had little effect. In the routine method of the preparation of

cystine, NaOH or $Na_2CO_3$ had been employed for the lowering of the H-ion concentration of the hydrochloric acid hydrolysate of hair. In this case, however, the NaCl, which had been produced by the neutralization process, would augment the solubility of cystine, and interfere with its crystallization. For the lowering of the acidity of the their hydrolysate, therefore ammonium water should be employed. I also studied the influence of ethyl-alcohol on the solubility of cystine. I have devised an easy and fruitful method for the preparation of cystine, as compared with the hitherto known methods.

## II. Influence of the H-ion concentration of the media on the solubility of cystine.

The crystallization optimum or solubility minimum of the hardly soluble electrolytes which having acid and alkaline reaction, amino acids etc., conforms with the isoelectric points, which fact has been pointed out by Michaelis and Davidsohn. Recently Sano carried out an investigation on the solubility of leucin, tyrosin and cystine, which are hardly soluble amino acids, at various H-ion concentrations and supported the theory of Michaelis and others. Having been assured of the fact that the solubility of cystine oscillated according to the concentration and kinds of salts dissolved in the media (as it is stated in Section III), I tested the solubility of cystine at various H-ion concentrations of media, the salt concentration of which had been put at a certain fixed value. From the results, I have learnt the fact that the solving curves of cystine at the certain fixed concentrations of salts obtained by employing divergent H-ion concentration were almost indifferent to the kinds and concentration of salts; and they were all similar. The amount of residue cystine dissolved in the medium after the separation of cystine oscillated only according to the variations of the H-ion concentration of the medium.

*A. Solubility of cystine at the various H-ion concentrations (in a 0.046 mol NaCl media.)*

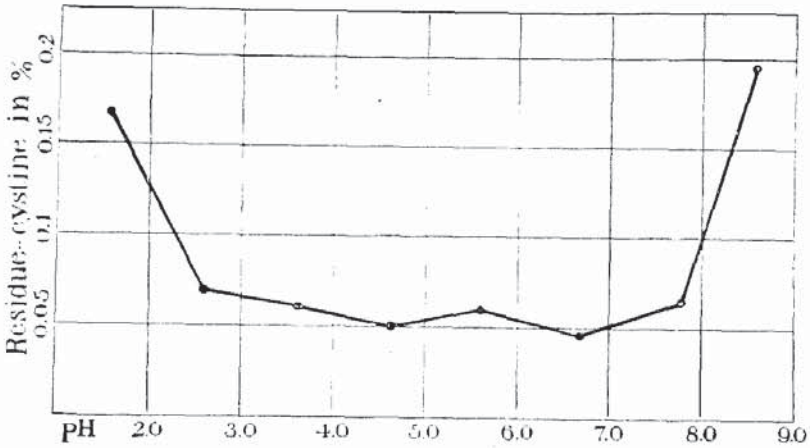
A series of test tubes are filled each with 5.0 cc. of a 1.0 % cystine hydrochloric acid solution (1.0 % HCl) and then a few drops of indicator added. They were then warmed in the water bath (which process had been applied to prevent immediate crystallization). Into the liquid is then dropped enough of a 0.1 mol caustic soda solution to attain the lowering of the H-ion concentration to the desired H-ion concentration. Then distilled water and a 0.5 mol NaCl solution are added in adequate proportions to make the NaCl concentration of each test tube to be 0.046 mol, and the total amount of each test tube to be 30.0 cc. by adding of 0.046 mol NaCl solution. The tubes are then cooled for one hour in an ice and NaCl mixture (at 0°C), and the cystine is crystallized. The contents of the tubes are then filtered through a small piece of filter paper and then a certain amount of the filtrate (viz. 10.0 cc.) is withdrawn into a test tube and the amount of cystine is estimated according to the method of Okuda, as described above.

TABLE I.

Solubility of cystine at the various H-ion concentrations (in a 0.046 mol NaCl solution).

| No. of test-tube | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| pH | 1.6 | 2.6 | 3.6 | 4.6 | 5.6 | 6.7 | 7.8 | 8.9 | 9.1 |
| Residue cystine % in the filtrate | 0.168 | 0.070 | 0.062 | 0.049 | 0.059 | 0.045 | 0.064 | 0.195 | 0.427 |

Fig. 1.
Solubility-curve of cystine at the various H-ion concentrations (in a 0.046 mol NaCl solution).




*B. Solubility of cystine at divergent pH in a 0.046 mol ammonium chloride solution.*

The following Table II shows the results of the test carried out after the same method as in Experiment A. the only difference being that a 0.046 mol of ammonium chloride solution is employed as the medium instead of the same mol solution of NaCl at the time of crystallisation.

TABLE II.

Solubility of cystine at the various H-ion concentration (in a 0.046 mol $NH_4Cl$ solution).

| No. of test-tube | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| pH | 1.6 | 2.6 | 3.6 | 4.8 | 5.6 | 6.8 | 7.6 | 8.9 |
| Residue cystine % in the filtrate | 0.138 | 0.068 | 0.057 | 0.049 | 0.056 | 0.040 | 0.051 | 0.346 |

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