A Method of Estimating Cysteine, Cystine, and Their Derivatives in Tissues and Biological Fluids, and The Application of The Method

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A METHOD OF ESTIMATING CYSTEINE, CYSTINE, AND THEIR DERIVATIVES IN TISSUES AND BIOLOGICAL FLUIDS, AND THE APPLICATION OF THE METHOD[1]

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Several years ago the writer established a method (4), the so called Iodine Method, for the determination of cysteine in the cleavage products of proteins. The basis of this method depends upon the fact that among amino-acids cysteine alone reacts very actively with iodine, in an acid solution of a certain concentration, and at room temperature. The principle of the method is to titrate cysteine in a hydrochloric acid solution, in the presence of iodide, with a standard iodate solution. This method is also used for the determination of cystine in proteins, after reducing it into cysteine.

Some SH compounds such as glutathione of HOPKINS (2), thiasine of BENEDICT, NEWTON, and BEIRE (1) and synpectothion of HUNTER and EAGLES (3) were found in tissues.

TUNNICLIFFE (5) has published a method determining reduced glutathione, extracting it from tissues with trichloracetic acid and titrating with iodine solution. But we found that the titration of SH group, with iodine solution, in trichloracetic acid, gives no reliable results. Furthermore cysteine, thiasine, and synpectothion will interfere with the TUNNICLIFFE'S method.

With respect to the estimation of cystine, oxidized glutathione, or R_2S_2 compounds in tissues, as far as we know, no work has been done.

1 The method was published in Japanese in the Journal of the Agricultural Chemical Society of Japan, 3, 1097, 1927.

The present communication deals with a method for the estimation of cysteine, cystine and allied compounds in tissues and biological fluids. The general plan of this method is analogous to that just described in the case of their determination in proteins. The only remarkable difference are the removal of proteins from the sample to be examined by means of sulphosalicylic acid, and the estimation of RSH and R_2S_2 compounds in the solution of this acid instead of hydrochloric acid.

For the sake of convenience, some preliminary experiments, which were performed for the purpose of the establishment of the present method, will be first described here, and then the method and its application.

I. PRELIMINARY EXPERIMENTS

A. Removal of Proteins

First of all, proteins should be removed from the tissues, which are subjected to the estimation of cysteine and cystine compounds. After some experiments, sulphosalicylic acid and trichloracetic acid were found to be the most convenient protein-precipitants. The precipitation-capacity of these two acids was compared against dilute milk and egg white solutions. The experimental results are shown, in the following table, in the quantity of nitrogen in coagulum obtained by the addition of the acids.

Protein solutions	Concentration of acids	Sulphosalicylic	Trichloracetic
Egg white	2 %	10.9 mg. N	10.2 mg. N
Egg white	3 %	11.0 mg. N	11.2 mg. N
Egg white	8 %	11.1 mg. N	11.1 mg. N
Egg white	12 %	11.1 mg. N	11.0 mg. N
Milk	2.5 %	20.4 mg. N	20.5 mg. N
Milk	10 %	20.9 mg. N	20.8 mg. N

From these results we see, that the precipitation-capacity of these acids in the concentration of 2-12 per cent is practically equal.

B. Comparison of Acid Solutions

Availability of these two acids in the case of determining cysteine was compared. Different quantities of cysteine were dissolved in the acid solutions of the same acidity and volume, and then titration was made with an iodate solution in the presence of iodide.

Quantity of cysteine applied	In the case of sulphosalicylic acid solution		In the case of trichloracetic acid solution	
	KIO_3 required	Ratio	KIO_3 required	Ratio
10	12.0	9.3	13.3	7.0
5	5.6	4.5	6.3	3.8
1	1.3	1.0	1.7	1.0

In both cases, the quantity of KIO_3 required is not strictly parallel to that of the cysteine applied. But sulphosalicylic acid is superior to trichloracetic acid, as it give much better results. Even in the case of sulphosalicylic acid, of course, the nearer the concentration of cysteine in sample and control (standard), the better results are obtained.

C. Comparison of Titrating Solutions

The availability of iodine solution (in KI)[2], and of iodate solution (in presence of KI), for titration of cysteine in sulphosalicylic acid solution was compared with the following results.

Quantity of cysteine applied	M/600 KIO_3 required	Ratio	N/100 I required	Ratio
10	12.0	9.3	8.8	5.6
5	5.6	4.5	4.8	3.3
1	1.3	1.0	1.5	1.0

From this table we see that the iodate solution gives much better results than the iodine solution. We have shown by experiment also that this fact holds good in the case of trichloracetic acid solution. Consequently iodate solution, as a rule, is preferable to iodine solution, furthermore the former is more stable than the latter.

D. Concentration of Acid and Temperature of Sample Solution

As already reported (4) the temperature, acidity, and volume of cysteine solution to be examined have some influence upon the requirement of iodate solution. Hence the estimations of cysteine have to be made under strictly comparable conditions of acid-concentration and volume of the solution, and also the effect of temperature should be taken into account.

The stronger the acidity, the more distinct is the end point of titration, but the less the amount of iodate solution required. The titratable acidity equivalent to about 2 per cent hydrochloric acid was

[2] Iodine-alcohol solution gave no reliable results.

found to be advantageous. Consequently both the cysteine solution to be examined and the iodate solution for titration have to be made to correspond to n/2 sulphosalicylic acid solution.

E. Filtration

For the filtration of the protein-coagulum obtained by the addition of sulphosalicylic acid to protein solutions, three methods were tried. 1) Filtration by the use of a Buchner funnel, suction and washing. 2) Separation by means of centrifugation. 3) Filtration by the dried filter paper and no washing.

Among these the first method required much time, the second method was inconvenient, the third method was found to be favourable.

a) The first and third methods were compared, with the sample solutions containing egg white or milk and a definite quantity of cysteine, estimating cysteine in an aliquot portion of the filtrate. The results were as follows:—

	Egg white solution	Milk solution
The first method	9.8 mg. cysteine	4.9 mg. cysteine
The third method	9.7 mg. cysteine	4.8 mg. cysteine

b) To make another kind of expepiment, a definite quantity of cysteine was added to milk and to "protein free milk," which was prepared by the removal of protein from the original milk by means of sulphosalicylic acid. To both of these solutions some sulphosalicylic acid was added. Precipitation took place only in the original milk, which was filtered by the third method

	Cysteine found
Protein free milk	4.70 mg.
Original milk...	4.75 mg.

From these experimental results we see, that the adsorption of cysteine by protein-coagulum is so slight as to fall within the limits of experimental error. Consequently the third method of filtration is advantageous over the others.

F. Different Acid Solutions

a) To the equal volume of different acid solutions, such as hydrochloric, sulphuric, trichloracetic, and sulphosalicylic acids, which were made to have the same titratable acidity corresponding to 2 per cent hydrochloric acid, a definite quantity of cysteine was added and these

solutions were subjected to the iodine method at the same temperature. The results are as follows :—

	Hydrochloric	Sulphosalicylic	Trichloracetic	Sulphuric
KIO_3 required...	4.80	4.86	4.72	4.97

b) Similar experiments were carried out with hydrochloric acid, with a mixture of hydrochloric and trichloracetic acids, and with a mixture of hydrochloric and sulphosalicylic acids.

	Hydrochloric	Trichloracetic mixture	Sulphosalicylic mixture
Cysteine found	25.0	24.5	25.2

c) Similar experiments were repeated with hydrochloric acid, sulphosalicylic acid, and the mixtures of these two acids. The only difference in these cases was the use of protein free milk solution of cysteine instead of the aqueous solution.

	Hydrochloric acid	Sulphosalicyclic acid	Mixtures
Cysteine found	10.0	10.0	9.9-10.0

From these results we see, that when titratable acidity, volume, and cysteine content of sample solutions are equal, cysteine is determined with the same accuracy even in different acid or mixed acid solutions.

G. Reduction

To determine cystine by the iodine method, it should be first reduced into cysteine by means of zinc powder and acid, and the reducing power, of course, is not the same in different acid solutions.

a) The reduction of cystine by zinc and trichloracetic acid is insufficient either at room temperature or at the boiling point. This acid, therefore, can not be used for the determination.

b) The reduction, at room temperature, in the mixture of hydrochloric acid and trichloracetic acid is nearly complete, if a great deal of the former is present, while the reduction is quite readily completed in sulphosalicylic acid or in the mixture of this acid with hydrochloric acid. An experimental result is as follows :

	Reduction of cystine	
	Found	Theoretical
Trichlor. acid + much HCl	98.2	100.0
Trichlor. acid + little HCl	14.2	100.0
Sulpho. acid + little HCl...	100.0	100.0

c) To know the effect of concentrntion of acid and length of

time of reduction at room temperature, some cystine was dessolved in 10, 5, and 2.5 per cent of sulphosalicylic acid and allowed to stand for 10, 30, and 60 minutes. The reduction was nearly the same in each case.

d) As above mentioned the reduction of pure cystine by means of zinc and sulphosalicylic acid is perfect even at a room temperature. But when some inpurities are present, the reduction is not so readily as pure solution. In the case of milk solution of cystine, for instance, the reduction is not completed at room temperature. For complete reduction it is necessary to boil for ten minutes more or less.

An example.—A solution of cystine in diluted milk was precipitated with sulphosalicylic acid, and the filtrate from the protein coagulum was divided into three equal portions. The reduction was made by the addition of some zinc. 1) At room temperature. 2) At room temperature after decolorization with charcoal. 3) By boiling for 10 minutes. The results were as follows:

	Found	Theoretical
1	2.4	4.0
2	3.9	4.0
3	4.0	4.0

That is to say, the decolorization brought about a favorable condition for reduction[3], but much time was required to wash out a little cystine, which was adsorbed by charcoal. Consequently the third manipulation, that is boiling for ten minutes seems to be most convenient.

e) To know the effect of concentration of sulphosalicylic acid and the length of time of boiling upon reduction, 10 mg. of cystine was dissolved in protein free milk solutions, in which the concentration of acid were n/4, n/2, and n respectively. In each case reduction was carried out for 10 and 60 minutes, and cystine was determined. In every case, nearly the same quantity of cystine was recovered, varing from 9.9 to 10.1 mg., or averaging to 10 mg.

From these results we see that trichloracetic acid can not be used, but sulphosalicylic acid is available, reduction of 10 minutes-boiling is sufficient, and 10 mg. of cystine give rise to about 10 mg. of cysteine.

3 We called attention in the proceeding paper (OKUDA: J. Department of Agr. Kyushu Imp. Univ. 1. 190, 1925.) to the fact that the treatment with charcoal is necessary for complete reduction of cystine in urine. In the case of protein hydrolysat, the treatment also creates a favorable condition for the reduction.

H. Stability of Cysteine Solution

In the present method of determination a definite quantity, say 5 mg., of cysteine is always added to sample solutions before titration. Cysteine, however, is unstable being gradually oxidized even in acid solution. Consequently to avoid the trouble of preparing afresh cysteine solution in each case of determination, some experiments were carried out to discover the stability of cysteine solution under different conditions

N/2 sulphosalicylic acid solution containing 4.8 mg. of cysteine per 5 c.c. was prepared and placed in glass bottles, brown and colorless, provided with ground glass stroppers. These bottles were allowed to stand at room temperature (15°—20°), in a incubator at 37°, or in an ice box at 4° respectively. 5 c.c. each of the solutions was pipetted out from time to time, and cysteine was determined with the following results ;—

Time after preparation	Room temp.		Incubator		Ice box	
	Colorless	Brown	Colorless	Brown	Colorless	Brown
1st day	4.8	4.8	4.8	4.8	4.8	4.8
7th day	4.8	4.8	4.3	4.3	4.8	4.8
15th day	4.5	4.5	3.2	3.4	4.6	4.6
30th day...	4.2	4.2	1.9	2.0	4.6	4.6

That is no distinct difference were observed between brown and colorless bottles, but remarkable differences resulted at different temperatures, the cooler being the more stable.

From these results we know that the cysteine solution is usable for about 7 days after preparation when it is left at room temperature.

II. THE METHOD ESTIMATING CYSTEINE- AND CYSTINE-COMPOUNDS

This method is an application of the iodine method. Tissues are rid of proteins by means of sulphosalicylic acid, and the filtrate from the coagulum is divided into two portions (a) and (b). In (a), cysteine and SH compounds are determined and calculated as cysteine. In (b), cystine and S.S compounds are determined and calculated as cystine.

As the quantities of these compounds in tissues are usually very small, a definite amount of cysteine, say 5 mg., is added before titration,

to make a sample solution in which the concentration of cysteine is near to that of the standard cysteine solution.

Solutions required for the determination :—

I About 5 per cent KI aqueous solution.

II Normal sulphosalicylic acid solution.

III N/2 sulphosalicylic acid solution (1 c.c. of this solution corresponds to 5 c.c. of n/10 NaOH).

IV N/10 NaOH solution.

V Cysteine solution, containing 5 mg. of cysteine in the form of cysteine hydrochloride in 5 c.c. of n/2 sulphosalicylic acid. This solution should be freshly prepared each 7 days, if it left at room temperature. (Refere to I, H.)

VI M/500 KIO_3, which is prepared by dissolving 0.214 g. of pure KIO_3 in 500 c.c. of n/2 sulphosalicylic acid.

Standardization of the Iodate Solution :—The iodate solution should be standardized for the standard cysteine solution very carefully. The standard cysteine solution is prepared from the solutions III and V, and contains 2 mg. of cysteine in 20 c.c. of n/2 sulphosalicylic acid.

For the purpose of the standardization, the standard cysteine solution is mixed with 2 c.c. each of the solutions I and II, and titrated with the iodate solution until a yellow color (or a violet color when starch is used as an indicator) is produced. A thermometer is immediately inserted in the resultant solution to read the temperature at which the titration has been finished. The volume (c.c.) of the iodate solution required corresponds to 2 mg. of cysteine.

As the volume varies somewhat with the temperature of the experiment, it is convenient to place the standard cysteine solution in several flasks or test tubes, and to repeat the same experiment several times at different temperatures, so as to get a table or a curve showing the relation between the temperature and the required volume of the iodate solution. The curve obtained in such a manner is named here "Temperature Curve" and is shown in the figure I.

In the temperature curve, the temperature at which the titration is finished is taken as the ordinate, and c.c. of the iodate solution corresponding to the cysteine solution as the abscissa. The cysteine solution contains 2 mg. of cysteine, 20 c.c. of n/2 sulphosalicylic acid, and 2 c.c. each of 5 per cent potassium iodide and normal sulphosalicylic acid.

Procedure :—Take about 10 g. of tissue paste in a small mortar, add

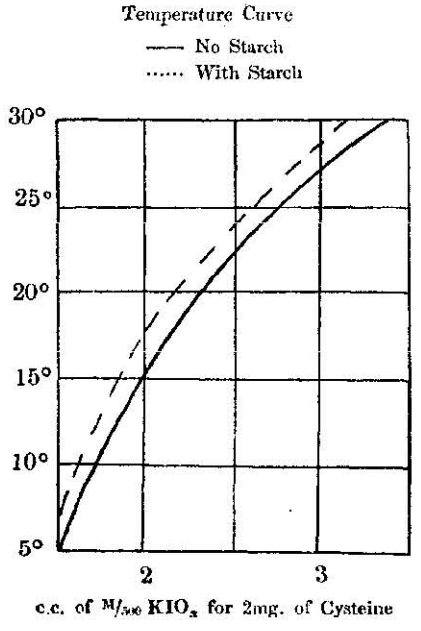

Fig. 1.

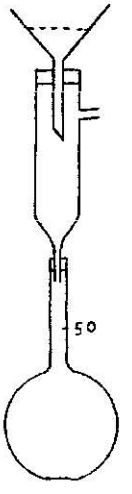

Fig. 2.

immediately about 20 c c. of normal sulphosalicylic acid, extract the soluble matters by grinding and stiring. Decant the liquid in a 100 c c. volumetric cylinder, and repeat the extraction with n/2 sulphosalicylic acid and finally transfer both liquid and coagulum in the cylinder and make up to the volume with n/2 sulphosalicylic acid. Shake from time to time and leave it for about half an hour at room temperature, and then filter through a dry filter paper in a dried flask. The filtrate is divided into two portions and serves for the estimation of cysteine and cystine.

(a) *Cysteine*:—To ascertain the concentration of the acid in the filtrate, take 1 c.c. of the filtrate and titrate it with n/10 NaOH, using azolithmin as an indicator. Take again 40 c.c. of the filtrate in another flask and add the calculated quantity of water to make a solution which has the same titratable acidity as n/2 sulphosalicylic acid. To the solution add the solution V or 5 mg. of cysteine in 5 c.c. of n/2 sulphosalicylic acid. In such a manner we obtain usually more or less 50 c.c. of a solution which contains over 5 mg. of cysteine and has the titratable acidity corresponding to n/2 sulphosalicylic acid. Take

20 c.c. of this resultant solution, add 2 c.c. each of the solutions I and II, and then titrate with M/500 KIO_3. Read the temperature of the solution and calculate the quantity of cysteine by the use of the temperature curve and according to the following formula:

$$\frac{2 \times \text{Required c.c. of } KIO_3}{\text{c.c. of } KIO_3 \text{ corresp. the Temp.}} = \text{Cysteine (mg.) in 20 c.c.}$$

(b) *Cystine*:—Take 40 c.c. of the original filtrate, add about 0.5 g. of zinc dust, boil for ten minutes for the complete reduction of cystine into cysteine, filter through a small Buchner's funnel into a special flask (Fig. 2) of 50 c.c. capacity, wash several times with a few c.c. of n/2 sulphosalicylic acid run from a burette and make up to the volume with the solution. After shaking, take 1 c.c. of the filtrate and titrate with n/10 NaOH to ascertain the acidity. Add the calculate quantity of water or normal sulphosalicylic acid to 40 or 45 c.c. of the remaining filtrate to make a solution in which the concentration of acid corresponds to n/2 sulphosalicylic acid. To this solution add the solution V, take 20 c.c. of the resultant solution and determine cysteine as mentioned above.

Calculation:—From the result obtained by (a), after the subtraction of the added quantity of cysteine, we get the quantity of cysteine and its derivatives in the sample, represented as cysteine.

And also, from the result obtained by (b), after the subtraction of the added cysteine, we get the quantity of cysteine, cystine, and their darivatives represented as cysteine.

Multiplying the coefficient 1.0, therefore, by the quantity obtained by the subtraction of the former (a) from the latter (b), we can calculate cystine and its derivatives as cystine.

Remarks.—

1) The coefficient 1.0 was established from the results of the preliminary experiments G.

2) We prescribed the use of 10 g. of tissue as a sample for a single determination, but that is not an inflexible condition, for instance, 50 c.c. of biological fluid may sometimes be used as a sample. In this case, the addition of 50 c.c. of n-sulphosalicylic acid is convenient.

Most muscles give a very hard coagulum, when concentrated acid as described is added directly, which disturbs the further extraction. In the case of muscles, therefore, it is recommended to use at first a faintly acid solution for extraction, and then concentrated acid to make the final concentration to about n/2 sulphosalicylic acid.

3) By this method, no trace or only minute traces of cysteine (or SH compounds) and cystine (or S.S compounds) were obtained from serum and boiled milk, and when pure cysteine and cystine were added to these samples, they were recovered nearly quantitatively. Consequently the constituents of serum and milk have probably no influence upon this method. Lecithin reacts with iodine, but it is precipitated by sulphosalicylic acid and the filtrate does not absorbe any iodine. Therefore its presence is of no influence. The presence of fats and sugars also has no influence. By far the most of the starch remains on the filter paper, but some kinds of starch, such as rice starch, come to the filtrate. In this case the temperature curve with starch should be used.

4) As the contents of SH and S.S compounds in tissues and biological solutions varies with the elapse of time, the sample to be examined should be acidified immediately to prevent oxidation, and a single determination should be finished within a day.

5) If the sample solution is colorless no starch is used, but if it is colored, starch is used as an indicator, although the end point of titration in this case is not so clear as in the former case. To make the starch solution, dissolve 1 g. of starch in 100 c.c. of boiling water and add 100 c.c. of 5 per cent potassium iodide aqueous solution. A certain number of drops of this solution should be used for every detemination.

6) As the standard in this titration, we applied the solution containing 2 mg. of cysteine in 20 c.c. of n/2 sulphosalicylic acid, if a sample solution was more concentric, take less than 20 c.c. and make up the volume with n/2 sulphosalicylic acid, and then make titration.

7) It is convenient, for titration to use a burette which gives readings of 1/100 c c., and also to know the volume of one drop run from the burette.

III. ESTIMATION IN ANIMAL TISSUES AND FLUIDS

The method described was applied to animal tissues and fluids. As the sample, 10 g. or 10 c.c. (or sometimes 50 c c) was used. The contents of cysteine (or SH compounds) and cystine (or S.S compounds) are indicated, unless otherwise stated, in mg. in 100 g. or in 100 c.c. of samples.

(1) Cow's milk and serum contain only minute traces or no trace of the compounds.

a)

	Cysteine[4] (SH compounds)	Cystine (S.S compounds)
Milk, 100 c.c.	0.8	1.4
Boiled Milk 100 c.c.	0.0	1.0
Blood serum 100 c.c.	0.0	1.1

b) 4.71 mg. of cysteine and 5.0 mg. of cystine were added to 10 c.c. of milk, and by the determination 4.68 mg. of cysteine and 4.97 mg. of cystine were recovered.

(2) Eggs, blood, urine, and meat extract for bacterial culture contain only a little of the compounds.

	Cysteine	Cystine	Remarks
Egg (hen), 100 c.c.	4.1	0.7	A few hours after layed.
Egg (hen), 100 c.c.	0.0	3.4	48 hours after layed.
Blood (cow), 100 c.c.	0.2	1.2	Oxalated blood, somewhat hemolized.
Blood (cow), 100 c.c.	1.2	0.4	Hemolized by saponin.
Blood (rabbit) 100 c.c.	2.1	10.6	Hemolized by saponin.
Red corpuscles (rabbit) 100 c.c.	8.2	12.2	Hemolized by saponin.
Urine 100 c.c.	1.9	3.5	—
Meat extract 100 c.c.	0.2	1.3	—

(3) Tissues

a)

	Cysteine	Cystine	Remarks
Muscle (tanny fish), 100 g.	1.4	3.7	A specimen from a market.
Liver (tanny fish), 100 g.	25.3	11.1	A specimen from a market.
Muscle (albino rat), 100 g.	9.9	6.1	Freshly killed.
Liver (albino rat), 100 g.	43.3	24.9	Freshly killed.

b). Hen's tissues. The estimation was performed with the tissues obtained from a hen which was freshly killed, and with the tissues after storage for a few days in an ice box at about 4° The quantity of SH compounds decreases gradually, and that of S.S compounds seems to increase once and then decreases.

Date after death	Muscle		Liver		Testes	
	Cysteine	Cystine	Cysteine	Cystine	Cysteine	Cystine
1	8.5	3.1	52.7	6.4	52.8	4.0
3	1.9	2.1	—	—	—	—
4	—	—	—	—	20.3	5.9
5	—0.3[5]	—1.0	—	—	—	—

Another experiment. Tissues were mixed with ten times their weight of n/2 sulphosalicylic acid solution, preserved in an ice box and

4 In the following pages, the phrase "SH compounds etc." is omitted, for the sake of brevity, and it is indicated simply as "cysteine" or "cystine."

5 This figure is due to experimental error, it should be zero.

then estimation was carried out. Even in the acid solution, the diminution was observed although the change is slower, as the following results show.

Date after death.	Muscle		Liver	
	Cysteine	Cystine	Cysteine	Cystine
3	4.2	6.0	38.9	11.3
5	—0.4	1.8	28.6	11.2

c) Ox liver. As above mentioned, even in low temperature the quantities of the sulphur compounds decrease, it is obvious that at higher temperature they decrease more quickly. To ascertain this fact, 10 g. each of ox liver paste was placed in several Erlenmyer flasks, and one of which was estimated immediately. The remaining samples were left for 20 or 68 hours at 37°, with chloroform water. The experimental results are shown in (A) in the following table. To the other samples, 20 c.c. of cystine solution (8.3 mg. cystine, PH 7.4) was added. The results were calculated to the quantity (mg.) in 100 g. of samples.

	A		B	
	Cysteine	Cystine	Cysteine	Cystine
Fresh	64.9	7.3	64.9	90.3
20 hours	32.5	25.8	33.6	92.9
68 hours	5.7	50.2	5.4	96.4

In these cases the quantity of cysteine decreased but that of cystine increased. It seems from another experimental result, that the latter would also probably have decreased, if we had left the samples a longer time.

d) To know whether the oxidation of cysteine compounds is due to an enzyme action, some experiments were performed, but we failed to secure clear results. At least, the oxidation takes place without enzyme.

e) When liver paste was placed in several small flasks, and preserved under freezing conditions in a refrigerator, the variation of the quantities of the sulphur compounds was very small. It is shown in the following table:

	Cysteine	Cystine
Fresh	73.5	4.1
42 hours	74.8	1.1
90 hours	61.3	13.6

From the results obtained as above we deduce the following facts: —Cysteine, cystine, and their derivatives are present in minute traces or sometimes absent in the biological solutions such as milk or blood serum. They are present in red corpuscles. All the animal tissues examined contained them, namely a little in muscles, comparatively much in livers and testes. In these tissues cysteine or SH compounds are always predominated. The quantities of these compounds decrease rapidly at room temperature or higher temperature, but slowly at frozen state, or in an acid solution. In these cases temporary increase of cystine or S.S compounds is observed, but they also decrease gradually. The sum of SH and S.S compounds is always decreasing. The content of these compounds seems to be much greater in the tissues of warm blooded animals than in those of cold blooded.

IV. ESTIMATION IN PLANTS

As the sample of a single determination, 20-30 g. of plant tissues were applied, and the contents are shown as follows in mg. in 100 g. of samples.

1)

	Cysteine	Cystine
Japanese medlar	0.0	4.3
Apple	0.0	3.5
Cucumber	3.2	2.5
Radish	23.5	0.0
Turnip	10.1	6.1
Germinated "Azuki" bean	2.4	2.5
"Koji" extract	0.4	0.9

2)

Young buds of a pine tree	Cysteine	Cystine
Under 3 cm. long	32.5	2.0
Over 10 cm. long	19.5	0.5

3)

Green pea	Cysteine	Cystine
Very young (a pod)	41.6	0.0
Half matured, seed.	6.1	20.7
Half matured, pod.	0.0	12.1

4) Seeds of soy beans and the plants after 4 days of Germination.

	Cysteine	Cystine
Seeds	3.8	17.6
Germinated in the light	5.4	21.9
Germinated in the dark	2.1	20.1

5) The cucumber at different stages of maturation.

	Date after the fall of flowers	Cysteine	Cystine
A species	1-2	4·9	7·7
A species	5-6	4·7	3·2
B species	1-2	7·3	6.1
B species	3-4	2·5	5·3
B species	5-6	2·5	4·5
B species	7-8	2·5	4·5

From these results, we see that the fruits, such as apples and Japanese medler, contain no cysteine or SH compounds, the roots, such as radish and turnip, contain them in comparatively large quantities. During the maturation of peas the quantity of cysteine compounds decreases, but, on the contrary, that of cystine compounds increases. During the growth of cucumber both the sulphur compounds decrease.

V. SUMMARY AND CONCLUSION

1) A method of estimating cysteine (or SH compounds) and cystine (or S.S compounds) in tissues and biological fluids was described. In this method, first of all, proteins are removed by means of sulphosalicylic acid, and then the filtrate of the protein coagulum is made to hold a definite concentration of the acid. Cysteine compounds are estimated in this solution by titration with a standard iodate solution in the presence of a certain quantity of iodide. Cystine compounds, after being reduced into cysteine compounds, are estimated in the same way.

2) Trichloracetic acid is as effective as sulphosalicylic acid as a protein-precipitant. But as the solvent for cysteine the latter is superior to the former, giving much more accurate results. Furthermore, in trichloracetic acid solution, the reduction of cystine to cysteine is incomplete, which results in inaccurate estimation. Consequently sulphosalicylic acid is preferable in either the estimation of cysteine compounds or that of cystine compounds.

3) As a titrating solution for cysteine, an iodate solution in presence of some iodide is superior to an iodine solution, giving much more accurate results. Furthermore iodate solution, as a rule, is stable for several months or a year.

4) By this method, only minute traces or no traces of cysteine, cystine and their derivatives were obtained from blood serum and boiled milk, and when pure cysteine and cystine were added to these samples, they were recovered quantitatively. Consequently, the constituents of the serum and the boiled milk have probably no influence upon this method.

5) All the extracts from fresh animal tissues examined contain cysteine and cystine compounds, the former predominating. The quantity of these compounds decreases rapidly at room temperature, but slowly in a frozen state or in a strongly acid solution.

6) Some fruits examined contain no cysteine compounds, but roots and seeds contain both cysteine and cystine compounds. During the maturation of peas, the quantity of these compounds varies.

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