XXVII. GLUTATHIONE.

THE OCCURRENCE AND QUANTITATIVE ESTIMATION OF GLUTATHIONE IN TISSUES.

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THE sole information available as to the glutathione content of various tissues is based upon the amounts actually isolated as described by Hopkins [1921] in his original paper.

The figures are as follows:

Yeast. 0·1–0·15 g. per kg.; 0·01–0·015 %.

Muscle. Approximately the same.

Liver. Undoubtedly richer, but yield not recorded.

Professor Hopkins informs me that from yeast later yields of the dipeptide have been greater. In one case 14 g. were isolated from 50 kg. of yeast, a yield of 0·03 % approximately.

As the method of isolation is such that fairly considerable losses are bound to occur, it is obvious that the above figures are of the right order only.

An attempt was therefore made to estimate, more directly and quantitatively, the amount of glutathione in various animal tissues.

EXPERIMENTAL.

The method adopted was as follows. A known weight of the tissue is ground in a mortar with sand and 10 % trichloroacetic acid. The mass so obtained is filtered on a small Buchner funnel and re-extracted twice with further amounts of 10 % trichloroacetic acid.

It was found by the application of the nitroprusside test that this was sufficient to extract all the soluble —SH groups of the tissues.

The use of trichloroacetic acid has the following advantages:

(i) A clear protein-free filtrate is obtained.

(ii) It does not interfere with the subsequent iodine titration.

(iii) Oxidation of the —SH groups during the extraction is prevented owing to the acidity of the solution.

(iv) Glutathione is of course not precipitated by this reagent.

The clear extracts so obtained are titrated with $N/100$ iodine solution, using sodium nitroprusside as an external indicator. From the equation

$$2R.SH + I_2 = R.S.S.R + 2HI$$

1 cc. $N/100$ $I_2 \equiv 2{\cdot}5$ mg. reduced glutathione.

The method has been controlled as far as possible in the following ways. The following substances, which might be present in an extract of tissue prepared as described and which might also react with iodine under such conditions, have been found not to interfere with the estimation:

Urea, Uric Acid, Creatinine.
Glucose. Fructose.

Known amounts of reduced glutathione added to the tissue pulp were satisfactorily estimated.

Estimations have also been made of the sulphate sulphur and total sulphur contained in tissue extracts. The difference gives the soluble organic sulphur. This amount has been found to correspond well with the value obtained for the sulphur present as —SH groups by the iodine titration method described. In no case has the sulphur calculated from the iodine titration been greater than the amount of total organic sulphur.

The following table gives some typical results.

Extract	Iodine titration; cc. $N/100$ I_2	Sulphur calculated from iodine titration as mg. $BaSO_4$	Total sulphur mg. $BaSO_4$	Sulphate sulphur mg. $BaSO_4$	Organic sulphur mg. $BaSO_4$
Liver (rabbit)	15·3	35·6	37·3	0·7	36·6
Liver (rabbit)	10·3	24·1	32	5	27
Yeast	9·6	22·4	30	3	27

It follows, therefore, that by the iodine titration of the trichloroacetic acid extract of the tissue, the —SH groups and the —SH groups alone are being estimated. The results obtained by the method are given in the table following. In calculating, the assumption has been made that all the —SH groups present are those of glutathione. This assumption is considered further in a later section.

The following procedure was adopted with animals. They were chloroformed or stunned, and bled to death from the jugular vein. The tissue required was removed as rapidly as possible and weighed. In the case of muscle, preliminary chopping with scissors before grinding with sand, was found advantageous. Cellular organs (liver and kidney) were ground directly with sand.

In the case of yeast the cells were ground with acid, transferred to a beaker and warmed to 60°, and allowed to cool before filtering. Kieselguhr was used if difficulty was experienced in obtaining a clear filtrate.

Tissue	Weight (g.)	cc. $N/100$ I_2	mg. reduced glutathione	%	Remarks
Yeast. A (1)	20	13·6	34·0	0·17	Fresh baker's yeast.
(2)	10	7·2	18·0	0·18	
(3)	5	4·2	10·5	0·21	
(4)	5	3·8	9·5	0·19	
B (5)	5	6·0	15·0	0·30	Samples had dried through keeping.
(6)	5	5·6	14·0	0·28	
Rat. Liver. A (1) ...	8·0	6·08	15·2	0·19	Animals starved for 8 hrs. to free liver from glycogen.
(2) ...	7·8	5·6	14·0	0·18	
B (3) ...	7·5	4·8	12·0	0·16	
(4) ...	9·0	6·10	15·3	0·17	
Rat. Muscle (skeletal) A	6·1	0·86	2·14	0·035	
B	7·0	1·0	2·5	0·035	
C	7·0	0·92	2·3	0·033	
Blood (whole)	—	—	—	—	Completely absent.
Rabbit. Liver A (1) ...	20	19·2	48·0	0·24	Animal starved 16 hrs. to free liver from glycogen.
(2) ...	20	20·0	50·0	0·25	
B (1) ...	20	21·6	54·0	0·27	
(2) ...	20	22·4	56·0	0·28	
Rabbit. Muscle (skeletal)	15	2·4	6·0	0·04	
	15	2·4	6·0	0·04	
	14·8	2·6	6·5	0·044	
Kidney	8·0	5·6	14·0	0·17	Mean value.
Blood (whole)	20 cc.	—	—	—	Complete absence.
Human blood	20 „	—	—	—	Complete absence.
Egg (hen)	—	—	—	—	Absent.

The mean values obtained are summarised below:

		%	Range %
Skeletal muscle	Rat	0·034	
	Rabbit	0·04–0·045	
Liver	Rat	0·18	0·16–0·21
	Rabbit	0·24	0·22–0·35
Yeast (fresh)		0·18	0·15–0·22

The possibility that an equilibrium between the —SH and the —SS— forms of the dipeptide was disturbed during the excision of the tissue and subsequent manipulation (as in the case of lactic acid) was checked as far as possible by adopting a technique similar to that used by Hopkins and Fletcher [1907] in determining the resting minimum for lactic acid in muscle.

The tissue was removed as rapidly as possible after the death of the animal into a tared, ice-cold beaker, weighed and ground under ice-cold trichloroacetic acid in a mortar surrounded by ice. The whole procedure was carried out as far as possible at 0°. No significant differences were observed between the results obtained at 0° and those at ordinary temperatures.

Discussion of Results.

The assumption has been made that the whole of the soluble sulphydryl groups in the tissues are those of glutathione. At the present moment, there is no evidence that cysteïne exists as such free in normal tissues. Extracts made from large quantities of yeast have been concentrated but in no case was cysteine (or cystine) isolated.

There remains the possibility of course that other peptides of a nature similar to glutathione exist, but until such have been definitely isolated it seems justifiable and convenient to express the results as percentage of reduced glutathione.

The close approximation of the values for the organic sulphur and the sulphur calculated from the iodine titration (which estimates —SH groups only) suggests that by far the greater part of the glutathione exists in the tissues in the reduced form. This deduction has been confirmed experimentally as follows. Two equal portions of the same sample of yeast were taken. In one the reduced glutathione was estimated as described. The other was suspended in a phosphate buffer solution at p_H 7·6 in an evacuated tube and incubated at 37° for one hour. The estimation was then carried out in the same manner. No increase in the amount of reduced glutathione was found. The same was the case with liver and muscle.

If, however, oxidised glutathione was added to the sample, a considerable increase in the amount of reduced glutathione was found. The lack of any increase in the experiment was thus due not to the inability of the tissue to reduce the —S—S— to the —SH group, but to the absence of the —S—S— group.

This result is of interest since evidence is accumulating that the —SH group is the active group in the catalytic oxidation processes in which glutathione plays a part.

The liver is undoubtedly the richest organ, and it will be noticed that the organs of the rabbit have a somewhat larger content than the corresponding organs of the rat.

The figures obtained for skeletal muscle (both in the rat and the rabbit) have been exceedingly consistent; those for the liver were variable. The variations in the case of the liver may in part be due to differences in the amount of blood remaining in the organ after bleeding the animal.

In cases where bleeding was poor, and the organ distended with blood, the percentage was always rather lower than usual. The effect of loss of moisture is well shown in the case of yeast; consequently fresh samples were obtained for each determination.

The values obtained by this method of estimation are from 3–5 times greater than the amounts actually isolated.

When the possibilities of loss in the various stages of the separation are considered, the difference may be well accounted for. At one stage alone—precipitation with mercuric sulphate reagent—losses of the order of 40 % have been recorded with cystine [Hopkins, 1921] and a similar loss is probable in the case of glutathione.

Abderhalden and Wertheimer [1923] have estimated colorimetrically the substance reacting with nitroprusside in horse muscle. This, calculated as cysteine, was present to the extent of 100 mg./kg. As glutathione, this on recalculation gives 0·02 %.

The method used by these authors depends on the matching of the nitroprusside colour with a standard mixture of dye-stuffs (Bordeaux red and methylene blue). The nitroprusside colour fades with exceptional rapidity and although, as stated by Abderhalden and Wertheimer, the colour may be made to persist to some extent by the addition of potassium cyanide, the sources of error would appear to be considerable.

It has already been pointed out that no evidence exists for the presence of free cysteine in the tissues of normal animals; and only in cases of cystinuria has the existence of cystine in the tissues been shown.

SUMMARY.

(1) Glutathione in the tissues of normal animals is chiefly in the reduced form (—SH).

(2) The sulphur equivalent to the —SH groups present accounts for the greater part of the soluble "neutral" sulphur of tissue extracts.

(3) The glutathione content of certain tissues has been estimated and values are given.

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REFERENCES.

Abderhalden and Wertheimer (1923). *Pflüger's Arch.* **198**, 122.
Hopkins (1921). *Biochem. J.* **15**, 286.
—— and Fletcher (1907). *J. Physiol.* **35**, 247.