GLUTATHIONE CONTENT OF NORMAL ANIMALS.

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Previous contributions from this laboratory have clearly demonstrated that glutathione is specifically concerned in the chemical defense of the body against the action of certain poisons (arsenic, copper, gold, cyanide, methylene blue, etc.). We put forward the theory that the toxic action of these substances is due to a primary disturbance of the cellular oxidation-reduction processes, resulting from a chemical interaction of the poison with the glutathione of the cells. With this knowledge as a foundation it is very desirable to explore this subject in every detail in order to throw some light on the chemical side of cellular mechanics under normal and pathological conditions. In our previous work we were hampered by a lack of knowledge of the actual quantities of glutathione available in the body. The figures given by Hopkins (1) in his orginal paper could not be relied on because of the large and variable losses involved in the chemical isolation of the substance from biological material. The need for a reliable method seemed to be satisfied with the appearance of the paper by Tunnicliffe (2). After subjecting his method to several tests with regard to reliability and accuracy we adopted it for our purpose.

Method.

Extraction of Tissues.—The extraction of the finely macerated material with 10 per cent trichloroacetic acid according to Tunnicliffe is very satisfactory, provided that the tissues are rapidly worked up immediately after the death of the animal. If this precaution is not observed losses are apt to occur.

Titration.—After many attempts to use starch as an internal

indicator in the iodine titration we arrived at the conclusion that the values thus obtained might sometimes be as much as 50 per cent higher than the figures obtained with sodium nitroprusside as an external indicator. To mention only one example: A rabbit liver was thoroughly hashed and extracted three times in the usual manner with trichloroacetic acid. The combined extracts were divided into six samples of equal volume. Three of these titrated with starch gave an average of 351 mg. of glutathione per 100 gm. of tissue, whereas the other three titrated with sodium nitroprusside gave 225 mg. per 100 gm. We therefore reached the conclusion that starch is not specific enough as an indicator and we adopted sodium nitroprusside.

Is All Glutathione in Reduced Form?—A large number of various trichloroacetic acid tissue extracts were titrated with N/100 iodine both before and after reduction with metallic magnesium (100 mg. for an average sample). Any undissolved magnesium was removed by filtration before the titration. The results so obtained clearly indicated that the increase in the titration value resulting from the reduction rarely exceeded more than 5 per cent. The conclusion was therefore justified that nearly all of the glutathione of the extract was present in the reduced (SH) form and for this reason the figures in the following tables will refer, if not stated otherwise, to the preformed SH- glutathione.

Specificity.—After completion of our work, a colorimetric method for the estimation of cystine, cysteine, and glutathione by Sullivan (3) appeared. This method is based on the fact that cysteine gives a color reaction with naphthoquinone and so does cystine after reduction, whereas glutathione reacts only after hydrolysis. Through the cooperation of Dr. Sullivan it was found that the trichloroacetic acid tissue extracts, prepared from liver, muscle, brain, and kidney of the rat, gave no test[1] for either cystine or cysteine when tested with the naphthoquinone reagent. If, however, the extracts are first subjected to acid hydrolysis, they then will yield a positive test. This furnishes experimental proof for the contention of Hopkins (4) that tissues do not contain an appreciable amount of either cystine or cysteine. Hence, the principal objection to the iodine titration method,

[1] Less than 25 parts per million.

i.e. the interfering presence of cysteine or cystine, has been removed in the case of normal tissues. The only known substances which occur under pathological conditions, and which give a nitroprusside test are acetone and acetoacetic acid in cases of disturbed fat metabolism, and cysteine and cystine in cases of cystinuria. It is obvious that the method cannot be applied under such conditions, without reservations. It is safe, however, to conclude that the method actually estimates the glutathione of normal tissues.

The naphthoquinone test is not yet adapted for the estimation of glutathione in trichloroacetic acid tissue extracts, and no direct comparison therefore can be made with the results obtained by iodine titration.

Accuracy.—If care is taken in the various manipulations the method easily yields results with an error not exceeding 5 to 10 per cent.

Most of the work was carried out on our standard inbred colony of rats, kept on a standard diet consisting of corn and wheat meals, dried milk powder, inorganic salt mixture, and cod liver oil. The food was withdrawn from the animals about 18 hours before the tissues were used and during this time they were kept in a room at 29–30°C. The animals were then weighed and bled to death by decapitation. For the analysis of the entire animal the bodies of several animals were run repeatedly through a meat grinding machine until a perfectly uniform pulp was obtained. For the analysis of the embyros a sufficient number of the proper weight was collected and this material was then minced with scissors and ground up with washed quartz sand. When sufficient material was available the composite sample was divided into several aliquot portions which were analyzed separately. The analysis of the individual organs was carried out by rapidly dissecting out the organs after death and combining a sufficient number of them to yield enough material for analysis. The blood was defibrinated and either analyzed as such or separated into cells and serum by centrifugation at high speed.

In order to eliminate as much as possible individual variations in the glutathione content, a very large number of analyses was made on the same type of material and the results were then averaged.

DISCUSSION OF RESULTS.

It will be seen from the data included in Table I that the glutathione content of the entire animal declines gradually, the decline being most pronounced between the earlier stages of the embryonic period and the age when the animals have reached a body weight of about 25 gm. From there on the drop is rather small. Part of this diminution in the glutathione content of the animal with increasing age may be due to the development of structures (bony skeleton), which do not contain this substance. However, Jackson and Lowrey (5) found for instance that the

TABLE I.

Glutathione of Entire Animal (Rat).

Range of weight of animals.	No. of animals used.	Average glutathione (mg. per 100 gm. of tissue).	Remarks.
gm.			
0.068–0.811	391	60	Embryos.
1.042–1.974	288	58	
2.321–2.948	99	54	
3.895–4.667	104	44	
4.646–4.950	29	36	Newly born.
23–26	38	32	Nursing.
30–50	20	31	Weaned and placed on standard diet plus lettuce.
137–170	20	23	

weight of the ligamentous skeleton of the albino rat never exceeds 18 per cent of the total body weight, whereas the glutathione content drops to nearly one-third of the initial value. Whatever the true explanation may be it is interesting to point out that the glutathione concentration of the body as a whole declines simultaneously with the growth rate. Whether variations in cellular glutathione concentration are directly related to the growth impulse remains to be decided by further work.

As concerns the magnitude of the glutathione content of the whole rat, it is obvious that this animal disposes of considerable quantities of the substance. If the same proportion should also

hold for the human species, an adult weighing 60 kilos would contain about 12 to 14 gm. of glutathione.

The figures in Table I are useful for expressing the relation between the mass of tissue glutathione and that of a fatal dose of a specific poison such as sodium cyanide. 1 mg. of cyanide injected subcutaneously is just sufficient to kill a rat weighing 100 gm. and containing about 25 mg. of glutathione, or, to put it differently, the fatal dose of cyanide is reached when 1 molecule of the poison is injected for each 5 molecules of tissue SH- glutathione.[2] In a recent paper (6) evidence was presented supporting the view that the reaction between cyanide and glutathione is probably best expressed by the following equation:

$$NaCN + R \cdot S - S \cdot R + H_2O = NaCNO + 2\,R \cdot SH$$

As we have shown in this paper that most of the glutathione of the tissues is present in the SH form and less than 10 per cent in the S-S form, it is obvious from the above equation that the ratio of 1 mol of NaCN to 5 mols of tissue glutathione agrees very well with a stoichiometric proportion between the two substances and adds further proof that the cyanide action is primarily due to a chemical reaction with the tissue glutathione. Similar considerations might be applied to other specific poisons of glutathione, but this will be reserved for another paper.

Tables II to V inclusive give the variations noted in the glutathione content of various organs at different age periods of the animal. Taken as a whole the liver is the richest organ, next come the kidney, brain and muscle, the latter containing relatively little glutathione. The testes of rats weighing from 142 to 178 gm. gave an average value of 149 mg. per 100 gm. of tissue. These data agree in a general way with those reported by Tunnicliffe (2), but indicate that fluctuations apparently occur in some organs as a result of age.

Finally, Table VI presents the data concerning blood. The first few analyses of rat blood indicated the presence of appreciable amounts of glutathione, which was contrary to Tunnicliffe who claimed that blood does not contain this substance. The glutathione is evidently present in the SH and S-S form in

[2] The molecular weight of SH- glutathione is approximately five times greater than that of NaCN.

TABLE II.

Glutathione of Liver (Rat).

Range of weight of animals.	No. of animals used.	Average glutathione (mg. per 100 gm. of tissue).	Remarks.
gm.			
1.02–1.83	129	151	Embryos.
20–26	108	261	
41–59	135	171	
64–77	30	154	
86–94	33	135	
142–178	45	179	
188–206	20	177	
245–276	30	204	

TABLE III.

Glutathione of Skeletal Muscle (Rat).

Range of weight of animals.	No. of animals used.	Average glutathione (mg. per 100 gm. of tissue).
gm.		
20–26	108	24
41–59	135	25
64–77	30	23
86–94	33	27
142–178	45	32
188–206	20	34
245–276	30	24

TABLE IV.

Glutathione of Brain (Rat).

Range of weight of animals.	No. of animals used.	Average glutathione (mg. per 100 gm. of tissue).
gm.		
20–26	108	102
41–59	135	99
64–77	30	74
86–94	33	78
142–178	45	132
188–206	20	112
245–276	30	40

the blood corpuscles, whereas the serum is free of the substance. These findings agree with the data recently published by Uyei (7) who also found glutathione in the blood cells of several species of animals. The data are in harmony with previous observations (8) showing that blood serum has neither reducing power when

TABLE V.

Glutathione of Kidney (Rat).

Range of weight of animals.	No. of animals used.	Average glutathione (mg. per 100 gm. of tissue).
gm.		
20–26	108	156
41–59	135	111
64–77	30	52
86–94	33	45 (?)
142–178	45	115
188–206	20	94
245–276	30	19

TABLE VI.

Glutathione of Blood of Different Species.

(Mg. per 100 Gm. Sample.)

	Defibrinated blood.		Cells.		Serum.	
	SH-glutathione.	Total glutathione.	SH-glutathione.	Total glutathione.	SH-glutathione.	Total glutathione.
Dog	20	24	55	79	0	0
Rat	22	30	46	53	0	0
Hog	31	39	70	93	0	0
Beef	35	39	73	81	0	0
Calf	36	38	66	74	0	0
Sheep	38	41	86	100	0	0
Rabbit	49	54	104	114	0	0
Guinea pig	49	58	143	151	0	0

tested with reduction indicators, nor does it yield a positive nitroprusside test. It was then pointed out that the serum (or plasma) is not concerned with oxidation-reduction phenomena, these being confined to the cells, and this view is again confirmed by the present observations.

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CONCLUSIONS.

1. The reliability of Tunnicliffe's method for the quantitative estimation of glutathione has been established as a suitable method for the analysis of tissues. The method indicates glutathione, as both cysteine and cystine were found to be absent from the tissue extracts.

2. The glutathione is largely present in the SH form, less than 10 per cent occurring in the S-S form.

3. The total glutathione content of the albino rat declines with increasing age.

4. Variations in glutathione content of individual organs occur, due apparently to age.

5. Blood serum does not contain glutathione, but the blood cells do.

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