GLUTATHIONE IN THE BLOOD IN SENILE CATARACT AND OTHER OCULAR CONDITIONS

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Introduction

It is well known that the human lens is rich in glutathione and that this substance disappears totally from the lens in senile cataract. Glutathione is remarkable in being the most labile sulphur-containing substance in the body. It is, as we know, closely associated with tissue respiration, which assumes special importance in a non-vascular tissue such as the lens. The relation of glutathione to the general sulphur metabolism of the body has never been determined (Lewis), either in the normal subject or in disease. As a first step towards this investigation it was thought worth while to estimate the blood glutathione in cases of senile cataract and other ocular disease, the range for blood glutathione in the normal being already well known. In addition, in some concurrent investigations (Bourne and Campbell), a study was made of the sulphur metabolism in the same types of patients, by giving diets of known sulphur value and estimating the corresponding sulphur excretion in the urine. The figures obtained from the two series of experiments provide an interesting first record of the relation of blood glutathione to sulphur metabolism, particularly in senile cataract as compared with other ocular disease. In actual fact, the sulphur metabolism in these cases was not found to be abnormal, as might have been expected.

Historical

The bulk of the glutathione present in the blood is in the reduced form, which varies from 25-41 mgm. per 100 c.c. (average 34), while the oxidised form varies from 3-11 mgm. (Woodward and Fry). This is in agreement with the earlier observations for reduced glutathione in the normal subject—Benedict and Gottschall 30-48, Mason 36-58, Achard, Levy and Guthmann 36-58 and Groen and Meyer 40 mgm. It is wholly contained in the corpuscles, and if considered in relation to the blood count, the factor, reduced glutathione, red cell count, ranges from 4.9-9.1 (Woodward and Fry).

Little is known as to the variation of glutathione in disease. Achard, Levy and Guthmann (1932) found a slight decrease in anaemia, diabetes, neoplasm and tuberculosis, while Giordano and

Battistini (1933) found no variation in a number of diseases. Senturia (1934) states that there is no variation from the normal in cases of arthritis, while Loomis and Bogen (1934) found a slight increase in the reduced form, and a decrease in the oxidised, in tubercle, which they ascribed to defective aeration of the blood. Oberst and Woods (1935) recently estimated the blood glutathione in 66 women.

The only figures available for patients with ocular disease, are those of Vele (1933), who employed an older and rather less accurate method of estimation. For normal patients, she found a range of only 13.5-24.5 mgm. per 100 c.c. (average 18.25), whereas in a large variety of ocular disease she found an increase in every type of case, ranging from 15-60 mgm. She states that there was an accompanying rise in the red cell count, and whereas the factor GSH/r.b.c. was 4 in the normal, it was 6-8 in ocular disease, and even higher in keratitis.

Experimental

The usual methods for the estimation of glutathione were based on the reduction of iodine, but in the last few years, reducing substances other than glutathione have been shown to be present in the tissues, *e.g.*, thionine and ascorbic acid (Vitamin C), which interfere with the estimation. Thus in the ox lens, 1/5 of the total reduction of iodine by a trichloroacetic acid filtrate, formerly ascribed wholly to glutathione, is due to Vitamin C, and in the case of a human lens from a 30-year-old subject, 28 per cent. of the reduction was due to Vitamin C. (Euler and Martius.)

For the present series of estimations, therefore, the method of Woodward and Fry (1932) was used, in which the interference from other substances is very small. Thus the amount of thionine in the blood could cause at the most an error of 3 mgm. per cent., while Vitamin C is present in the reduced form to the extent of only 0.5 mgm. per 100 c.c. blood. One slight variation was made in the method, *viz.*, in order to obtain the figure for total glutathione, reduction of the solution with zinc was carried out at 37°C. instead of at room temperature.

In every case, samples of venous blood were analysed immediately after withdrawal, the blood being taken not less than two hours and a half after a meal. As the range of glutathione has been adequately demonstrated for the normal, the estimations on patients with senile cataract were compared with those from cases of other ocular disease, exclusive of any condition affecting the lens. In cataract patients, the blood was taken at a time when there was no active absorption of lens matter in progress, *i.e.*, after a preliminary iridectomy when the lens had not been

disturbed, or some time after an extraction when no residual cortex was present.

With very few exceptions the patients were under observation in hospital, and it was possible to study a number on a strictly balanced diet of known calorie value, for three successive days. A comparison of the blood glutathione was made in the two classes of patients (a) senile cataract, (b) ocular disease exclusive of cataract, as follows:—

- (1) On ordinary hospital diet, uncontrolled.
- (2) On controlled normal diet of known calorie value and sulphur intake.
- (3) In relation to the red cell count, on ordinary diet.
- (4) In relation to the red cell count, on controlled high protein, low protein and normal diets of the same calorie values.

An example is given of the controlled normal diet of 2,347 calories which was most used, and details of the high and low protein (*i.e.*, high and low sulphur) diets can be found elsewhere, in a full description of the investigation of the sulphur metabolism in these patients (Bourne and Campbell). The results are summarised in the following tables. The abbreviations "GSH" and "GSSG" are used to signify reduced and oxidised glutathione respectively. The sulphur and protein values of foodstuffs were taken from Sherman's "Chemistry of Food and Nutrition," 1933.

TABLE I
BLOOD GLUTATHIONE, REDUCED AND OXIDISED, IN PATIENTS
ON ORDINARY HOSPITAL DIET

Patient	Sex	Age	GSH mgm. per 100	GSSG c.c. blood	Clinical details
C. C. ...	M.	56	50·15	2·40	Senile cataract
W. S. ...	M.	71	38·95	3·58	
M. T. ...	F.	71	35·12	2·29	
L. S. ...	F.	75	53·37	1·83	
H. A. ...	M.	75	51·21	2·16	
			45·76	2·45	Average
C. B. ...	F.	9	39·87	0·00	Strabismus
G. C. ...	M.	9	32·82	3·06	Strabismus
P. ...	F.	33	36·19	4·91	Perforating injury, L.E.
H. B. ...	M.	43	41·10	0·91	Specific iritis, R. and L.
L. F. ...	F.	52	38·95	6·14	Lacrimal abscess
A. C. ...	M.	58	45·70	1·23	L. excision, choroidal sarcoma
J. L. ...	M.	62	46·01	0·91	Glaucoma, R. and L.
E. J. ...	F.	71	36·80	3·99	Secondary glaucoma, L.E.
			39·68	2·84	Average
Range—			35·12–53·37	1·83–3·58	Senile cataract
			32·82–46·01	0·00–6·14	Non-cataract cases.

TABLE II
BLOOD GLUTATHIONE IN PATIENTS ON CONTROLLED NORMAL DIET

Patient	Sex	Age	Diet Calories	GSH mgm. per 100 c.c. blood	GSSG	Clinical details
H. S. ...	F.	43	2495	39.79	6.22	Senile cataract
A. McV. ...	F.	51	2347	36.80	—	
T. H. ...	M.	54	2347	36.96	0.46	
G. W. ...	M.	55	2347	38.34	1.53	
C. B. ...	F.	57	2347	37.42	1.84	
M. C. ...	F.	57	2178	46.01	6.41	
J. B. ...	M.	65	2347	41.01	3.05	
H. S. ...	M.	67	2347	41.71	4.30	
G. S. ...	M.	68	2347	49.69	3.99	
J. W. ...	M.	69	2347	46.01	0.89	
G. L. ...	F.	79	2393	44.01	—	
				41.61	3.19	Average
M. P. ...	F.	29	2495	45.39	—	Detachment L. retina
E. O'D. ...	F.	31	2347	41.71	12.89	Keratitis R.E.
L. H. ...	M.	33	2347	42.94	9.59	Strabismus
M. P. ...	F.	47	2347	40.82	2.12	L. excision for injury
N. ...	M.	50	2347	42.01	2.78	Detachment R. retina
J. J. ...	F.	59	2347	45.39	2.46	R. corneal ulcer
M. H. ...	F.	59	2147	45.09	1.23	Iritis L.E.
				43.34	5.18	Average

Range—36.80–49.69 0.46–6.41 Cataract
40.82–45.39 1.23–12.89 Non-cataract

N.B.—The same diet was continued in each patient for three successive days for the metabolism experiments, the blood sample for glutathione being taken on the second day.

The diet of 2347 calories contained 66.94 gm. protein, 1.935 gm. sulphur, the other diets differed from this only very slightly.

TABLE III
BLOOD GLUTATHIONE IN RELATION TO THE RED CELL COUNT
IN PATIENTS ON ORDINARY HOSPITAL DIET.

Patient	Sex	Age	Red cell count	GSH	GSH r.b.c.	Clinical details
H. ...	M.	—	4,450,000	32.80	7.37	Senile cataract
W. H. ...	M.	66	5,500,000	36.80	6.60	
S. K. ...	M.	67	4,600,000	42.33	9.20	
J. L. ...	F.	68	5,050,000	47.23	9.35	
W. R. ...	M.	70	5,550,000	53.98	9.72	
A. C. ...	F.	74	5,200,000	47.85	9.19	
E. B. ...	F.	77	5,150,000	39.57	7.66	
				42.98	8.44	Average
M. F. ...	M.	10	4,100,000	33.74	8.02	Interstitial keratitis
M. V. ...	M.	10	3,750,000	37.11	9.89	" " W.R. + ve
W. ...	M.	20	4,750,000	36.19	7.61	" " W.R. + ve
F. F. ...	M.	32	5,600,000	54.29	9.69	Scleral wound, R.E.
E. W. ...	F.	40	4,700,000	35.12	7.40	Detachment of retina
H. M. ...	F.	43	5,600,000	46.93	8.38	" " "
M. W. W.	F.	62	4,300,000	46.93	10.93	Secondary glaucoma, L.E.
A. M. ...	M.	69	5,600,000	58.29	6.68	Chronic glaucoma, R. and L.
				42.07	8.56	Average

Range—32.80–53.98 6.60–9.72 Cataract
33.74–54.29 6.66–10.93 Non-cataract

TABLE IV
BLOOD GLUTATHIONE IN PATIENTS ON GRADED AND
CONTROLLED DIETS

Patient	Sex	Age	Diet	Red cell count	GSH	GSSG	GSH r.b.c.	Clinical details
H. T....	M.	22	H.P.	5,600,000	35.12	5.98	6.27	Perforating injury R.E.
			L.P.	—	35.27	2.46	6.42	
			N.	5,350,000	34.04	—	6.36	
G. G....	M.	50	L.P.	4,450,000	37.11	0.31	8.33	Glaucoma, R. and L.
			N.	—	36.50	—	8.20	
G. S....	M.	68	L.P.	5,650,000	42.33	2.66	7.49	Senile cataract, R. and L.
			N.	—	38.95	3.07	6.89	
G.W....	M.	72	H.P.	5,100,000	29.44	8.90	5.77	Senile cataract, R. and L.
			L.P.	—	37.41	3.69	7.07	
			N.	5,200,000	38.34	4.60	7.03	
D. T....	M.	73	H.P.	3,250,000	26.68	4.00	8.21	Senile cataract, R. and L.
			L.P.	—	26.07	1.61	8.02	

N.B.—Each diet was continued for three successive days, one day's interval being allowed between the change from one diet to the next. Blood was taken on the second day for estimation and cell count.

Daily diet:	Protein	Sulphur	Calories
High protein (H.P.) ...	107.42 gm.	3.987 gm.	2325
Low protein (L.P.) ...	33.23	0.931	2328
Normal (N.) ...	67.87	1.863	2261

TABLE V
EXAMPLE OF CONTROLLED NORMAL DIET OF 2347 CALORIES
PER DIEM

Food	Ounces	Gm. Protein	Gm. Sulphur
Bread	8	20.61	0.22
Butter	2½	0.63	0.006
Egg	2	7.50	0.109
Milk	10	9.24	0.094
Sugar	2	—	—
Beef	4	21.17	1.283
Potato	6	3.36	0.050
Cabbage	6	2.68	0.099
Apples	8	0.89	0.013
Cream	1	0.70	0.008
Marmalade	1	0.16	—
Total	...	66.94	1.935

distributed as follows:—

Breakfast—Bread 2 oz., butter ½ oz., 1 egg boiled.

Lunch—Bread 1 oz., butter ½ oz.

Dinner—Beef 4 oz. (sirloin steak, minced raw, weighed and cooked with water), potato 6 oz. (weighed raw after peeling, steamed), cabbage 6 oz. (weighed raw, and steamed), apples 8 oz. (weighed after peeling, stewed with 1 oz. sugar), cream 1 oz.

Tea—Bread 3 oz., butter ¾ oz., marmalade 1 oz.

Supper—Bread 2 oz., butter ½ oz., milk 5 oz.

Fluid not less than 2½ pints as tea or coffee allowed for the day, together with sugar 1 oz., and milk 5 oz.

Summary of Results

(1) There is no marked variation in the reduced glutathione of the blood, with age or diet, or in senile cataract as compared with other ocular conditions. The range for all cases of ocular disease is within the normal.

(2) In comparison with reduced glutathione, the oxidised glutathione shows considerable variation, especially in non-cataract cases, but no significant change.

(3) The factor GSH/r.b.c. is not raised above the normal value in ocular disease, and shows the same range in senile cataract as in other ocular conditions, including active keratitis (*cf.* Vele).

Comment

Although these results are negative, it is particularly interesting to find that the blood glutathione, like the blood calcium, remains constant over a certain range in a variety of conditions of age and disease. Moreover, in ocular disease, it appears to be unaffected by the sulphur metabolism of the body, even when there is a large variation in the amount of sulphur which is being ingested. This is probably true also for normal subjects.

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