GLUTATHIONE REDUCTASE ACTIVITY IN BLOOD AND BODY FLUIDS [1]

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Reduced glutathione (GSH) is readily oxidized by the cytochrome systems of animal tissues (1). This oxidation is reversed by the action of an enzyme, glutathione reductase (GR), which catalyzes the reduction of oxidized glutathione (GSSG) by reduced triphosphopyridine nucleotide (TPNH).

$$TPNH + GSSG + H^{+} \rightleftharpoons TPN + 2GSH$$

It has been suggested that GR is largely responsible for the ability of living tissues to keep glutathione primarily in the reduced form, a condition which may be important in the maintenance of sulfhydryl enzymes in the active state (2).

It is the purpose of this report to present the findings of the study designed to determine the presence and significance of GR in human and animal blood components and in other body fluids.

METHODS AND MATERIALS

Glutathione reductase activity may be measured by various techniques (3), including a spectrophotometric assay. Spectrophotometrically, GR activity is determined by following at 340 mμ the decrease in optical density of a reaction mixture containing TPNH. The optical density change reflects the conversion of TPNH to TPN during the course of the reduction of oxidized glutathione by GR.

Oxidized glutathione, TPNH, and bovine serum albumin were obtained commercially and were used without further purification.

Oxidized glutathione was prepared for use by dissolving 200 mg. in 5.0 ml. of distilled water; the solution was prepared daily. TPNH was prepared by dissolving 20 mg. in 3.0 ml. of buffer, and further dilution with buffer was made in order to have a final solution of optical density between 750 and 850 at 340 mμ; this solution was also prepared daily. Bovine serum albumin, free of GR activity, was used in amounts of 50 mg. dissolved in 10.0 ml. of buffer. The buffers (0.15 M at pH 7.6) were either potassium dibasic phosphate or tris (hydroxymethyl) aminomethane (Tris).

Serum, plasma with heparin, citrate or oxalate, and heparinized whole blood were obtained by venipuncture, without regard to the fasting state. Oxalated plasma showed consistently lower activities and was not used for the study. Serum and plasma were separated by centrifuging at 2,000 r.p.m. for ten minutes at room temperature. Unless otherwise stated, all blood and body fluids samples were refrigerated at 4° C. within three hours after collection and were used for GR assay within three days after collection. Control studies demonstrated that enzyme activity was not appreciably changed during these lengths of time at room temperature. Cerebrospinal fluid specimens were obtained by spinal tap between lumbar vertebrae 4 and 5. Specimens were centrifuged at 2,000 r.p.m. for 10 minutes at room temperature and the supernatant was used for GR determination. Serous effusions were obtained by the usual technique of thoracentesis or paracentesis and the specimens handled as described for cerebrospinal fluids. Lysed red cells were prepared by using 0.1 ml. of heparinized packed erythrocytes which was diluted with 5.0 ml. of distilled water and centrifuged at 2,000 r.p.m. for 10 minutes to remove ghosts.

Tissue specimens were obtained during the course of postmortem examination and within five hours of death. The tissues were used within two hours after sampling, and were prepared for GR assay by homogenizing with normal saline, at room temperature. The loss of activity after death and during the two hours after sampling was not estimated.

The patients from whom body fluid specimens were obtained were either supervised clinically and/or were evaluated by a review of the clinical records by the authors. The stated diagnoses were arrived at on the basis of the clinical and/or postmortem findings.

Glutathione reductase activity was determined spectrophotometrically. To 0.5 ml. serum or plasma were added 2.3 ml. of phosphate buffer and 0.1 ml. of TPNH. The mixture was incubated at room temperature (26° C.) for 20 minutes after which 0.1 ml. of GSSG was added. The reaction was followed in a model DU Beckman spectrophotometer using a corex cell with 1 cm. lightpath. At 15 or 30 second intervals, five consecutive readings were made of the change in optical density. From the average decrease in optical density during five 15 or 30 second intervals, the change in optical density per minute per ml. of serum or plasma was calculated.

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TABLE I

The effect of heat on glutathione reductase *

Duration of time heated (min.)	Serum GR activity (U/ml./min.)
0	160
5	80
10	60
15	40
60	12

* Serum was obtained from a patient with acute toxic hepatitis and heated in a water bath for different lengths of time at 56° C.

One unit of GR activity is defined as a change in optical density of 0.001 per ml. per minute at 340 mμ.

The same procedure was followed for the measurement of GR activity in the case of serous effusion, lysed red cells, and cerebrospinal fluid, except that 0.2 ml. of bovine albumin was added to the incubation mixture and the amount of phosphate buffer was reduced to 2.1 ml. (4).

RESULTS

Some properties of serum glutathione reductase

Sera kept frozen for one week at −10° C., or kept in the refrigerator at 4° C. for an equal length of time, showed no significant decrease in activity.

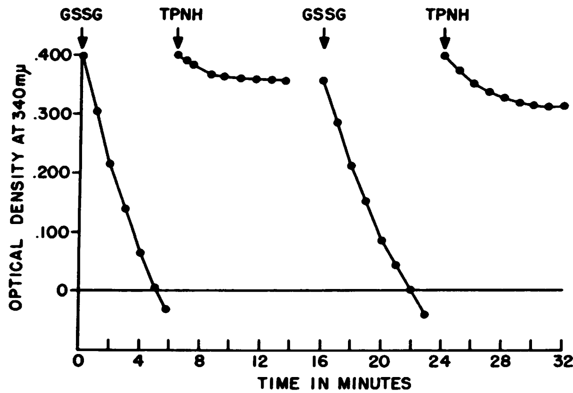

FIG. 1. THE CHANGE IN OPTICAL DENSITY WHICH RESULTS WHEN SERUM GLUTATHIONE REDUCTASE IS MEASURED AS DESCRIBED IN THE TEXT

At zero time, the reaction was begun by adding 0.1 ml. of GSSG to the reaction mixture of phosphate buffer, serum, and TPNH. The change in optical density was linear for two minutes and then slowed in rate. At six minutes, the decrease in optical density was no longer perceptible. At that time, 0.1 ml. TPNH was added and reaction resumed but proceeded slowly and terminated before the fourteenth minute. When the optical density no longer changed, 0.1 ml. GSSG was added and the reaction resumed and the change in optical density was linear until the twenty-third minute.

TABLE II

Glutathione reductase activity in human tissue homogenates *

Tissue homogenates	GR activity (U/ml./min./Gm. of wet tissue)
Liver	3,200
Kidney	3,200
Pancreas	1,300
Heart	600
Thyroid	260
Lysed erythrocytes	200
Spinal cord	160
Lung	120
Serum	40

* In each instance the values given are the average of two tissue determinations except for spinal cord and thyroid tissues, in which cases only one specimen of each was examined.

Heating sera at 56° C. for five minutes resulted in a decrease in GR activity of approximately 50 per cent, whereas heating for a longer period of time caused further decrease in activity (Table I).[3] Other investigators found a definite decrease in GR activity of plant tissue, following dialysis for a period of 17 to 72 hours and against a phosphate buffer of 0.025 M at pH 6.2 (5). In the present study no significant decrease in GR activity was noted when sera were dialyzed against 0.15 M phosphate buffer pH 7.6 at 4° C. for eight hours. Addition of manganous salts in a concentration of 0.4 times 10^{-4} M per test did not affect the activity of the serum (5).

Initial studies were done with Tris buffer; how-

TABLE III

Glutathione reductase activity of heparinized plasma and lysed erythrocytes of 10 species of animals

Animal	GR activity	
	Plasma (U/ml./min.)*	Lysed erythrocytes (U/ml. packed cells/min.)*
Hamster	70	200
Rabbit	40	600
Guinea pig	27	100
Monkey	26	300
Goat	16	60
Mouse	16	50
Chicken	8	200
Dog	6	200
Pig	4	800
Rat	4	80

* One sample of each was studied.

[3] Two experiments were done on two different sera with approximately the same results.

ever, GR activity was found to be approximately twice as high on phosphate buffer as compared to Tris buffer. Optimal phosphate buffer osmolarity for the enzyme reaction was found to be 0.15 M. No significant difference in GR activity was noted between pH 8.1 to 7.3, although a decided decrease in GR activity was observed when the pH of the reaction mixture went beyond these values.

If the enzymatic reaction was followed spectrophotometrically until no further change in optical density occurred, the reaction resumed upon the addition of oxidized glutathione attesting to the presence of excess TPNH and to the attainment of a chemical equilibrium (Figure 1).

Glutathione reductase activity of blood

Serum and heparinized plasma have similar GR activity. Slight degrees of hemolysis did not significantly influence the enzymatic activity of serum or plasma. Sera obtained from 98 normal individuals ranging in age from 10 to 60 years had a mean activity of 40 units per ml. per minute, with a standard deviation of 15 units. Using two standard deviations, the normal range of activity for human serum is 10 to 70 units. No significant difference in serum GR activity was noted in regard to the fasting state, sex, age or color. Table II compares the GR activity of human tissue homogenates, including lysed erythrocytes.

The GR activity of heparinized plasma and lysed erythrocytes from 10 species of animals is presented in Table III.

Glutathione reductase activity of body fluids obtained from patients with various disease states

Although a wide spectrum of patients with various disease states was examined, abnormally increased serum GR was noted in patients with acute hepatic disease and in patients with carcinoma. Table IV lists the serum GR activity in 104 diseased individuals studied. Figure 2 shows the serial alterations of serum GR activity in a patient with acute homologous serum hepatitis. Although only a limited number of cerebrospinal fluids and serous effusions were examined (Tables V and VI), apparently increased GR activity was

TABLE IV

Serum glutathione reductase activity in patients with various disease states

Diagnosis	No. of patients	No. of samples	GR activity		
			10–70	71–200	201–700
Viral hepatitis (1st week)	7	26	0	25	1
Hepatic coma	1	2	0	1	1
Laennec's cirrhosis	4	4	4	0	0
Lung tuberculosis	2	2	2	0	0
Systemic lupus erythematosus	2	3	1	0	2
Acute ileitis	1	1	1	0	0
Acute gout	1	5	5	0	0
Toxic hepatitis due to salicylates	4	8	5	3	0
Acute rheumatic fever	3	9	9	0	0
Acute myocardial infarct (1st week)	5	15	15	0	0
Coronary insufficiency	1	2	2	0	0
Congestive heart failure	3	3	3	0	0
Multiple sclerosis	1	1	1	0	0
Hypoparathyroidism	1	1	1	0	0
Macroglobulinemia	1	2	2	0	0
Acute glomerulonephritis	1	2	2	0	0
Pyelonephritis	1	1	1	0	0
Bilateral kidney infarcts	1	1	0	1	0
Infectious mononeucleosis	1	3	3	0	0
Diabetes	2	3	3	0	0
Gaucher disease	1	1	0	1	0
Iron deficiency anemia	2	2	2	0	0
Carcinomas	38	55	11	31	13
Sarcomas	6	7	5	2	0
Hodgkin's disease	7	13	13	0	0
Leukemia	6	7	7	0	0
Thymoma	1	1	1	0	0
Total	104	180			

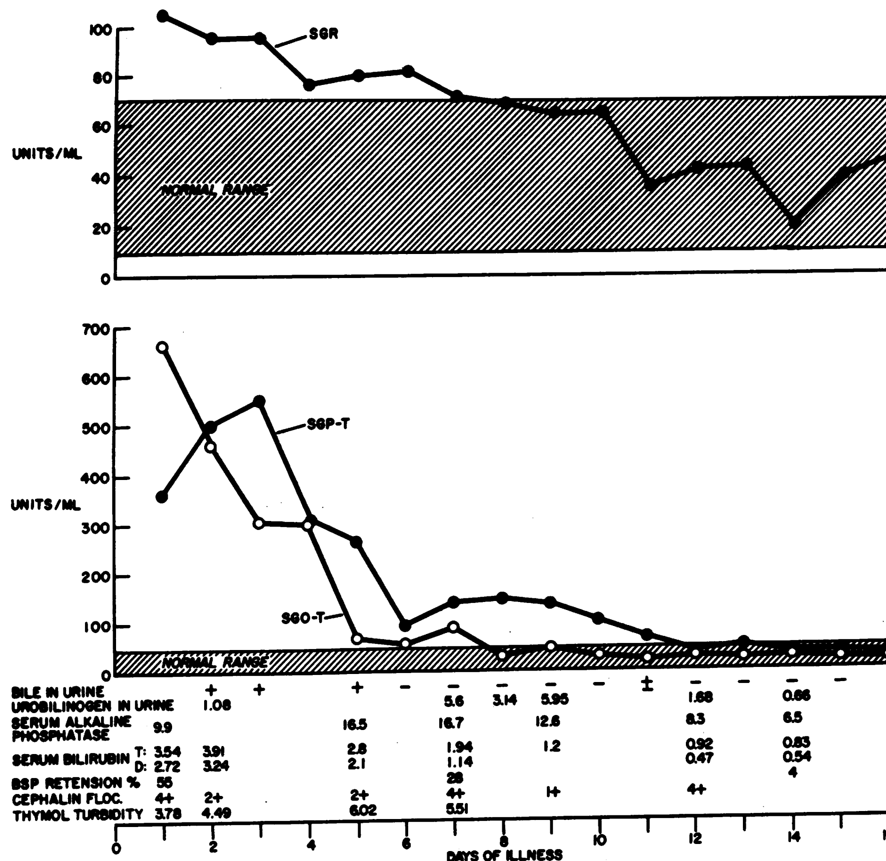

FIG. 2. SERIAL ALTERATIONS OF SERUM GLUTATHIONE REDUCTASE ACTIVITY IN A PATIENT WITH ACUTE HOMOLOGOUS SERUM HEPATITIS

Serum glutathione reductase activity, serum glutamic oxalacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) were followed for 16 days, together with liver function tests.

noted only in cerebrospinal fluid from individuals with acute bacterial meningitis and in serous effusions containing malignant cells. It is pertinent to note that patients with elevated GR activity in pleural or peritoneal fluids did not necessarily have elevated serum activity of the enzyme.

SUMMARY AND CONCLUSIONS

Body fluid and tissue homogenate GR activity can be readily measured spectrophotometrically. All human sera, erythrocytes, tissue homogenates, heparinized plasma, and ten different animal samples of plasma and erythrocytes examined had GR

TABLE V

Cerebrospinal fluid glutathione reductase activity in patients with various central nervous system disease states

Diagnosis	No. of patients	GR activity (*U/ml./min.*)			
		0–5	6–10	11–20	21–33
Ruptured lumbar disc	5	4	1	0	0
Cerebral concussion	1	1	0	0	0
Migraine	1	1	0	0	0
Possible colitis	1	1	0	0	0
Acute labyrinthitis, diffuse	1	0	1	0	0
Hydrocephalus	1	1	0	0	0
Cerebral hemorrhage	2	1	1	0	0
Meningococcic meningitis	1	0	0	1	0
Tuberculous meningitis	1	0	0	0	1

TABLE VI

Glutathione reductase activity of pericardial, pleural, and peritoneal serous effusions

Patient No.	Cavity site of effusion	Diagnosis	Effusion GR activity	Serum GR activity
Ef 1	Pericardial	Acute idiopathic pericarditis	32	60
Ef 2	Pleural	Pneumonitis	8	20
Ef 3	Pleural	Pneumonitis	16	34
Ef 4	Pleural	Hodgkin's disease	22	
Ef 5	Pleural	Hodgkin's disease	24	
Ef 6	Pleural	Lymphosarcoma	24	80
Ef 7	Pleural	Lymphosarcoma	60	
Ef 8	Pleural	Reticulum cell sarcoma	60	90
Ef 9	Pleural	Malignant lymphoma	12	24
Ef 10	Pleural	Adenocarcinoma of breast, metastatic	2,100	32
Ef 11	Pleural	Adenocarcinoma of unknown origin, malignant cells in effusion	300	32
Ef 12	Peritoneal	Laennec's cirrhosis	5	
Ef 13	Peritoneal	Laennec's cirrhosis	6	60
Ef 14	Peritoneal	Adenocarcinoma of colon, metastatic	260	132
Ef 15	Peritoneal	Adenocarcinoma of breast, metastatic and lymphosarcoma	1,760	16
Ef 16	Peritoneal	Adenocarcinoma of uterus, metastatic	56	80

activity. The mean GR activity of normal human sera is 40 ± 15 units per ml. Normal human erythrocytes have two to three times the GR activity of the respective plasma. Increased serum GR activity was noted primarily in patients with homologous serum and infectious hepatitis, toxic hepatitis due to salicylates, hepatic coma, and in patients with carcinoma usually in the disseminated phase of the disease; patients with lymphomata do not appear to have increased serum GR activity. The only individuals with appreciable cerebrospinal fluid GR activity are those with acute bacterial meningitis. Serous effusions with appreciable GR activity were noted to have malignant cellular constituents. Extension of these observations on the relationship of GR activity of serum, cerebrospinal fluid and serous effusions to various disease states will be necessary in order to evaluate the clinical significance of alterations of GR activity in body fluids.

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