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# DETERMINATION OF GLUTATHIONE AND GLUTATHIONE PEROXIDASE ACTIVITY AFTER DEATH



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## Introduction

Free radicals (atoms or molecules containing unpaired electrons, viz. superoxide anion radicals, hydrogen peroxide and hydroxyl radicals) are normal products of several essential cellular processes. Such radicals, especially those derived from oxygen, can potentially injure cells and tissues. Aerobic organisms have evolved elaborate mechanisms for protecting themselves from the toxic consequences of oxygen metabolism. There is now evidence that several insults (viz. tissue irradiation, hyper and hypo-oxygenation syndromes, cancer, aging and inflamatory states) (1-6) which result in tissue injury do so by generating toxic metabolites of oxygen in amounts which exceed the capacity of tissues to protect themselves.

In mammals, glutathione (GSH) and glutathione peroxidase (GSH-Px; EC 1.11.1.9) are primarily involved in protecting cellular structures against peroxides and free radicals (7). Using GSH as the reducing equivalent, GSH-Px reduces hydrogen peroxide to yield water and glutathione disulfide (GSSG). This is a major pathway of $H_20_2$ metabolism in many cells, and one which also catalyzes reduction of other peroxides, making it important for the protection of membrane lipids against oxidation.

To our knowledge there was none dealing with the post mortem evolution of such mechanisms. In this study, total glutathione and selenium-dependent glutathione peroxidase were measured in blood-"seroplasma" from corpses following death from a variety of causes. It is hypothesized that the cellular oxidant stress generated in some cases could be manifested as a change in glutathione status or glutathione peroxidase activity.

## Materials and methods

Studies were performed on 41 cadavers autopsied in the Institute of Forensic Pathology of the University of Copenhagen (Denmark) within 72 h. after death. Corpses were maintained at 4°C. until the autopsies were performed. The population studied comprised 26 men and 15 women (aged 20 to 93 years) grouped according to cause of death based on physiopathological similarities as follows : group I : respiratory failure (19 cases); groupe II : myocardial infarction (4 cases); group III : intoxication (8 cases); group IV : brain injury (6 cases); group V : multiple traumatism (4 cases).

Blood samples were collected from the femoral vein in polyethylene tubes. After centrifugation, the supernatant (or "seroplasma") was removed and stored at -20°C. until analysis for glutathione and glutathione peroxidase. Since post mortem "seroplasma" could be contaminated with blood from autolysis, haemoglobin was also quantified in the supernatant in order to determine whether erythrocyte contamination could affect the other analytical parameters.

Total glutathione (GSH + GSSG) was measured according to the method of Griffith (8). Aliquots of "seroplasma" were mixed with 10% 5-sulphosalicylic acid and after centrifugation the supernatant solution was assayed. The rate of formation of 2-nitro-5-thiobenzoic acid was recorded at 412 nm (30°C.). Selenium-dependent glutathione peroxidase activity was measured according to Paglia and Valentine (9) using t-butyl hydroperoxide as the substrate. Linear rates of NADPH oxidation were recorded spectrophotometrically (340 nm) at 37°C.

## Results and discussion

Table I shows GSH and GSSG concentration and glutathione peroxidase activity in post mortem "seroplasma" from cadavers after different causes of death.

Total glutathione concentration in post mortem "seroplasma" did not differ significantly between the various groups based on the cause of death. For plasma glutathione the reference interval *in vivo* is about 1 to 3 µmol (8). The total glutaghione contents found in the present study in post mortem

"seroplasma" are higher than expected (Table I), due possibly to the inevitable contamination of plasma by erythrocytes as a result of post mortem haemolysis.

As mentioned above, reduced glutathione (GSH) is the predominant non-protein thiol in mammalian tissues, but under conditions of marked toxicity or oxidative stress, intracellular oxidized glutathione (GSSG) substantially increases. After death, total glutathione is not a good parameter to detect cellular oxidative stress. It could well be desirable to determine intracellular oxidized glutathione (GSSG) in order to determine the potential usefulness of the GSH/GSSG ratio in assessing the degree of cellular oxidant stress.

**Table I.**

| | *Group* | *n* | *GSH + GSSG (µmol/l)* | *GSH-Px (mU/mg protein)* |
|---|---|---|---|---|
| I. | Respiratory failure | 19 | 3.671 ±0.416 | 1.580 ±0.158 |
| II. | Myocardial infarct. | 4 | 3.922 ±0.778 | 0.767 ±0.287 |
| III. | Intoxication | 8 | 3.765 ±0.865 | 1.457 ±0.113 |
| IV. | Brain damage | 6 | 3.480 ±0.335 | 1.103 ±0.151 |
| V. | Multiple traumat. | 4 | 2.437 ±0.509 | 0.936 ±0.223 |

*Each value represents mean +/- SEM.*

Glutathione peroxidase activity shows a small decrease in myocardial infarction, multiple traumatism and brain injury as compared with other causes of death. Several authors have noted that cellular levels of enzymes, protective against peroxidative stress (viz. glutathione peroxidase), are found to be increased after ischemic injury and reperfusion of tissue.

It seems difficult to explain our findings but they could be related to the duration and intensity of agonal suffering. When death occurs suddenly or very rapidly (as in death by myocardial infarction, multiple traumatism or brain injury) there is not enough time for an increase of cellular levels of enzymes protective against oxidative tissue injury to rise significantly.

It could be hypothesized that given enough time, these enzyme activities would increase, thus protecting the tissue from the toxic consequences of oxygen

metabolism. In any case, our findings regarding glutathione peroxidase activity should be interpreted with caution in view of the low number of cases studied.

**Table II.**

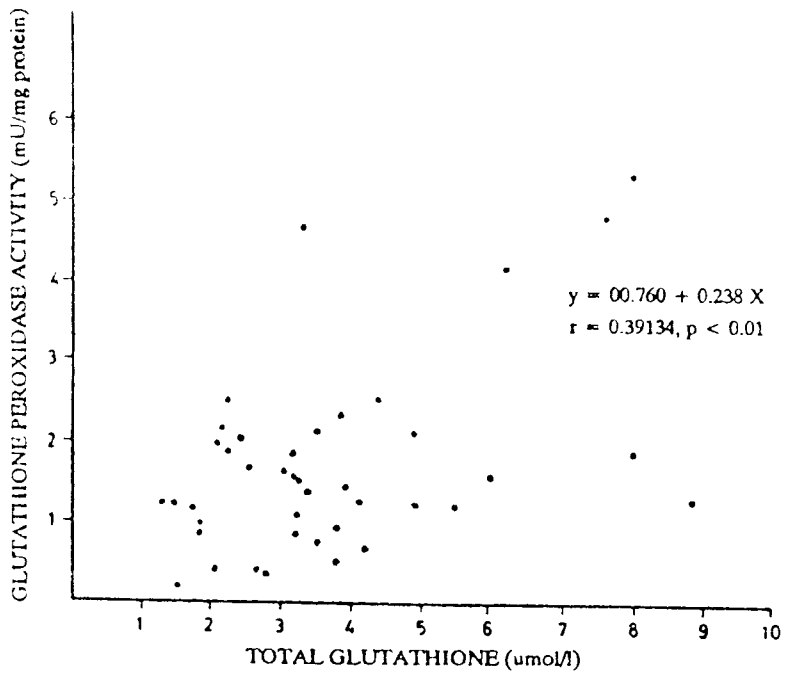
| | | *GSH + GSSG (mol/l)* | *GSH-Px (mu/mg protein)* |
|---|---|---|---|
| AGE | < 50 years (n = 17) | 3.520 ± 0.426 | 1.326 ± 0.131 |
| | > 50 years (n = 24) | 3.595 ± 0.360 | 1.342 ± 0.140 |
| SEX | Male (n = 26) | 3.309 ± 0.303 | 1.369 ± 0.135 |
| | Female (n = 15) | 4.010 ± 0.517 | 1.269 ± 0.119 |

*Each value represents mean +/- SEM.*

Table II presents the values of these parameters in relation to age and sex. No significant differences were noted in any of the cases.

On the other hand, a significant positive correlation ($r = 0.39134$, $p < 0.01$, 39 d.f.) was found between glutathione peroxidase activity and total glutathione concentration (Figure 1) with lowest values noted in the myocardial infarction and multiple traumatism groups, followed by the brain injury group. Higher levels were noted in the respiratory failure and intoxication groups.

Fig. 1



It will be important to quantify these studied parameters in other body fluids known to be well protected against post mortem blood contamination and more resistant to putrefaction than blood. Further investigation will be needed to provide more information on these interesting observations.

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## References

1. FREEMAN B.A. & CRAPO J.D., *Biology of disease, Free radicals and tissue injury*, 1982, Lab. Invest., 47, pp 412-426.

2. SIES H. & CADENAS E., *Biological basis of detoxication of oxygen free radicals*, in *Biological basis of detoxication,* Caldwell J. & Jakoby W.B. (Eds), Academic Press, San Diego, 1983, pp. 181-211.

3. HALLIWEL B. & GUTTERIDGE J.M.C., *Oxygen toxicity, oxygen radicals, transition metals and disease,* 1984, Biochem. J., 219, pp. 1-14.

4. DEL MAESTRO R.F., *An approach to free radicals in medicine and biology*, 1980, Acta Physiol. Scand., 492, pp. 153-168.

5. MASON R.P. & CHIGNELL C.F., *Free radicals in pharmacology and toxicology. Selected topics*, 1982, Pharmacol. Rev., 33, pp. 189-211.

6. McCORD J.M., *Oxygen-derived free radicals in post-ischemic tissue injury*, 1985, N.Engl.J.Med., 312, pp. 159-163.

7. TAPPEL A.L., *Measurement of and protection from in vivo lipid peroxidation*, in *Free radicals in biology,* vol.4., Pryor W.A. (Ed.), Academic Press, New York, 1981, pp. 1-47.

8. GRIFFITH O.W., *Glutathione and glutathione disulphide*, in *Methods of enzymatic analysis,* vol.8, 3th ed., Bergmeyer H.U. (Ed.) Verlag Chemie, Weinheim, 1985, pp. 521-529.

9. PAGLIA D.E. & VALENTINE W.N., *Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase,* 1967, J. Lab. Clin. Med., 70, pp. 158-169.