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The Oxidation of Glutathione in Human Erythrocytes— A Reversible Reaction.

R. H. TURNER. (Introduced by J. H. Musser.)

From the Department of Medicine, Tulane University School of Medicine.

The observations here reported have been made while working on an adaptation for human blood of Tunnicliffe's[1] method for the determination of a reduced glutathione. A complete report of these studies will appear later.

The general plan of the experiments was as follows: a given specimen of human blood was saturated with CO_2, a sample or samples removed for estimation of reduced glutathione; the remaining blood was saturated with air, again sampled and the remainder saturated a second time with CO_2 and a portion taken for determination of reduced glutathione. Saturation with CO_2 or with air was accomplished by shaking the blood gently in a 250 cc. Erlenmeyer flask in a specially designed motor-driven apparatus as a stream of moist CO_2 or air was passed through the flask. Samples were removed with a 10 cc. Oswald-Folin pipette and allowed to run into 10% trichloracetic acid. The samples which were saturated with CO_2 were run into the acid contained in flasks in which the air had been replaced by CO_2. The trichloracetic acid filtrates were titrated with 0.001 N iodine solution to the starch end point and with 0.001 N thiosulphate to the disappearance of color, as suggested by Perlzweig and Delrue.[2] The excess of potassium iodide they suggest was

[1] Tunnicliffe, H. E., *Biochem. J.*, 1925, ix, 777.

[2] Perlzweig, W. A., and Delrue, G., *Biochem. J.*, 1927, xxi, 1416.

omitted. The nitroprusside reaction as an outside indicator has been used as a check on the starch indicator in a large number of determinations on human blood. When the nitroprusside end point is used the figures obtained are from 85% to 95% of those obtained with filtrates containing a higher concentration of reduced glutathione and poorer agreement when the concentration is less. It appears that the same substance is being measured by either of the indicators and starch is much more accurate in my hands.

Calculations were based on 100 cc. packed erythrocytes. The volume of packed cells dealt with in a given sample was estimated from accurate hematocrit readings. The glutathione content of human serum and of a few white cells present in the bloods studied (less than 2,000 per cm.) has been shown to be so low that it may be disregarded in these experiments.

TABLE I.

Experiment No.	First CO_2	Air	Second CO_2
1	104	75	102
	100	76	101
2	114	76	116
	119	76	114
3	75	49	77
	76	49	76
4	99	62	94
5	107	78	112

The variation in reduced glutathione content of human blood, in mg. per 100 cc. packed erythrocytes, when saturated with CO_2 or with air.

In Table I the content of reduced glutathione is shown in milligrams per 100 cc. packed erythrocytes. The content of reduced glutathione in the blood when saturated with CO_2 is seen to be from 40 to 55% greater than when saturated with air. The alternate oxidation and reduction of this part of the glutathione present may be repeated a number of times without any permanent change.

It is obvious that any method for the quantitative estimation of reduced glutathione in red blood cells which does not take into account the ease with which oxidation or reduction takes place may involve an error of as much as 50%.

The conversion by CO_2 of oxyhemoglobin of the erythrocytes is apparently accompanied by a conversion of a considerable part of the oxidized glutathione into reduced glutathione. This suggests that there might be an intimate interrelationship between these 2 substances which would be of broad physiological significance.