

groups present in the membranes at the ganglion synapses and neuromuscular junctions might be obtained. These results could conceivably be used to show that solid-state forces can be applied in the adsorption state⁴.

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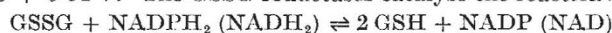
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BIOCHEMISTRY

Reduction-potential of Glutathione

THE values of the reduction-potential of GSH (E_{m7} at pH 7.0) measured with different methods (indicator dyes¹, electric measurements²⁻⁴, equilibrium with the NADPH₂/NADP-system^{5,6}, mixed disulphides^{4,7}) range from -0.35 V to $+0.04$ V. The GSSG-reductases catalyse the reaction:



Earlier attempts to demonstrate the back-reaction by spectrophotometric estimation of the NADPH₂-formation were unsuccessful^{8,9}. Rall and Lehninger⁵ calculated that the E_{m7} GSH cannot be more negative than -0.13 V (with the new value of E_{m7} NADPH₂ (ref. 10) -0.17 V); Mapson and Isherwood⁶ found with the very sensitive fluorometric method the formation of very small amounts of NADPH₂ (0.9×10^{-2} $\mu\text{mol./ml.}$).

From their equilibrium constant which is $1/K$ in our notation and the value of -0.315 V for E_{m7} NADPH₂ (ref. 10) one can calculate an E_{m7} GSH of -0.16 V. The data of Mapson and Isherwood and their calculations as well as those of Rall and Lehninger are based on the assumption that the GSH used is free of GSSG (more precisely, contains less than 10^{-6} parts GSSG). If the GSH were to contain GSSG in appreciable amounts, less NADPH₂ would be formed than corresponds to the true equilibrium; consequently the concentrations of NADPH₂ found would not be equal to those of GSSG. In fact, we have found in all GSH preparations investigated 0.5–1.0 per cent GSSG.

Therefore, we proceeded in the following fashion to determine the true equilibrium constant. First the amount of GSSG contaminating the GSH was determined. A calculated amount of NADPH₂ was then added in the cuvette of the spectrophotometer in the presence of NADH₂-GSSG reductase¹¹ in order to reduce the GSSG. After completion of this reaction a measured large excess of NAD was added and the ensuing formation of NADPH₂ was determined. The experiments were carried out under anaerobic conditions at 40° C, the temperature optimum of the enzyme, with a recording spectrophotometer.

At the beginning the solution (0.125 M phosphate-buffer pH 7.0, contained 20 $\mu\text{mol./ml.}$ GSH and as found by determination 0.16 $\mu\text{mol./ml.}$ GSSG. After addition of 0.16 $\mu\text{mol./ml.}$ enzymatically oxidizable NADH₂ 80 min elapsed until equilibrium was reached. At this time 97 per cent of the added NADH₂ and, therefore, of the GSSG had reacted. After the addition of 20 $\mu\text{mol./ml.}$ NAD the optical density showed an increase of 0.260 E within 10 min, corresponding to the formation of 84×10^{-3} $\mu\text{mol./ml.}$ NADH₂. The enzyme was found still to be active when tested by addition of 2 $\mu\text{mol.}$ GSSG.

The equilibrium concentrations were then as follows:

$$\begin{array}{r} \text{NADH}_2 \text{ formation} \quad 8.4 \times 10^{-5} \text{ M} \\ + 3 \text{ per cent 'remain'} \quad 0.5 \times 10^{-5} \text{ M} \\ \hline \Sigma = 9.0 \times 10^{-5} \text{ M} \end{array}$$

Therefore $[\text{NADH}_2]$, $[\text{GSSG}] = 9 \times 10^{-5}$ M

and $[\text{GSH}]$, $[\text{NAD}] = 2 \times 10^{-2}$ M (neglecting the minor changes caused by the reactions described).

The equilibrium constant is:

$$\frac{[\text{GSH}]^2 [\text{NAD}]}{[\text{GSSG}] [\text{NADH}_2]} = K' \approx 10^8$$

With the equation:

$$E_{m7} \text{ GSH} = E_{m7} \text{ NADH}_2 + 0.03 \log K'$$

and $E_{m7} \text{ NADH}_2 = -0.33$ V (at $+40^\circ$ C) (ref. 10) the reduction potential of glutathione is calculated to be -0.24 V (E_{m7} at $+40^\circ$ C). It may be assumed that the error of this estimate does not exceed ± 0.01 V.

Since the reduction potentials of NADPH₂ and NADH₂ are identical, the results apply also to a system mediated by the NADPH₂-GSSG reductase. The different values of Mapson and Isherwood are presumably due to a small GSSG-content of their GSH.

In investigations of the formation of mixed disulphides^{4,7} it has been shown that the reduction potentials of cystein and GSH differ only by about 0.01 V. Therefore, it may be assumed that the reduction potentials, at least of some of the SH-groups of proteins, are also in the same range.

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Locus of Biochemical Change in Mice dying from Staphylococcal Infection

RECENT advances in investigating the mechanism of staphylococcal infection in mice indicate that host carbohydrate metabolism is profoundly involved. When measurements are made in the blood of mice close to death from an intraperitoneal challenge with staphylococci there is a rise in inorganic phosphorus, alkaline phosphatase and transaminase and a fall in sodium. Less definite falls occur in sugar, cholesterol and serum carbon dioxide¹. The validity of these findings was further tested by comparing the chemical composition of the carcass at death from intraperitoneal staphylococcal infection with normal controls. A profound fall in glucose with a rise in inorganic phosphorus was found. There was also a fall in total protein and cholesterol and a rise in transaminase².

In order to examine the mechanism of these changes it was decided to determine the fate of glucose, total protein, cholesterol, inorganic phosphorus, alkaline phosphatase and transaminase in different organs of the mouse carcass. These determinations were selected as they were the most deranged values in our previous blood and carcass surveys. Values were determined for normal and intraperitoneally infected animals at death after challenge with 10^{10} *Staphylococcus aureus* (Smith). They are shown and compared statistically in Fig. 1. Glucose was only found in the liver, where it fell significantly at death. Significant changes related to carbohydrate metabolism were a fall in liver glucose, a rise in inorganic phosphorus in the liver and adrenal and a rise in alkaline phosphatase in the