Amperometric Determination of Sulphydryl Content of Blood and Tissues

THE total, non-protein and protein sulphydryl contents of biological materials can be determined by using two different analytical procedures: one for the determination of total sulphydryl (protein sulphydryl + non-protein sulphydryl) values and the other for the determination of non-protein sulphydryl values. The protein sulphydryl values are obtained by subtracting the non-protein sulphydryl values from the protein sulphydryl values. Most of the available methods, however, are often complicated, time-consuming and more or less non-specific. In an earlier communication[1] it was reported from this laboratory that, with a little modification, the amperometric method of Benesch, Lardy and Benesch[2] can be used for the specific determination of the total sulphydryl content of biological materials. It has since been observed that the method can be adapted for the non-protein sulphydryl determinations in the same materials. Thus it is possible to use a simple, specific and rapid method for the determination of all the three sulphydryl fractions in biological materials.

Table 1. CONCENTRATION OF TOTAL, NON-PROTEIN AND PROTEIN SULPHYDRYL IN RAT BLOOD AND TISSUES
The means of results with number of determinations in parentheses are given. The results are expressed in mgm./100 gm. for wet tissues and mgm./100 ml. for blood

Sample analysed	Total sulphydryl	Non-protein sulphydryl	Protein sulphydryl*
Whole blood	91·4 (8) 83·6–100·0	2·9 (4) 2·5–3·3	88·5
Liver	55·4 (7) 51·4–58·8	26·4 (4) 23·4–32·0	29·0
Kidney	48·3 (3) 45·0–53·0	11·3 (4) 8·7–13·1	37·0
Heart	31·2 (3) 28·0–33·0	7·2 (4) 6·8–7·9	24·0
Pancreas	25·4 (6) 20·0–30·4	6·7 (4) 6·5–6·9	18·7

* These values were obtained by subtracting the means of non-protein sulphydryl values from the means of total sulphydryl values.

The amperometric titration apparatus, with the exception of the platinum electrode, is assembled according to Benesch, Lardy and Benesch. For the construction of the platinum electrode, four strands (each 35 mm. long and 0·5 mm. diam.) of tightly twisted platinum wires are sealed in a soft-glass tubing in such a way that 15–20 mm. of the bare metal protrudes beyond the glass. The projecting wires are bent laterally into a U-shape. This electrode, in contrast to that used by others[3], greatly increases the sensitivity of the apparatus. For total sulphydryl determinations tissue homogenates and hæmolysed blood samples[1] are used. For non-protein sulphydryl determinations, sulphosalicylic acid (2·5 per cent solution) extracts of blood and tissues prepared according to the method of Benesch and Benesch[3] are used. Tissue homogenates (1–2 ml.), whole blood (0·1 ml. hæmolysed with 1 ml. of de-ionized distilled water), and sulphosalicylic acid extracts (1–5 ml.) of blood and tissues are titrated with 0·002 *M* silver nitrate in *tris* (hydroxymethyl) aminomethane buffer[2], *p*H 7·4. Each increment (0·1–0·2 ml.) of silver nitrate is added at 1-min. intervals and the microammeter reading is taken at 55 sec. after each addition. It is essential to neutralize the sulphosalicylic acid extracts before addition to the buffer. The neutralization is carried out with 0·8 *M* sodium bicarbonate, the amount being determined in a separate titration using methyl orange as an indicator. During the titration of tissue homogenates pieces of connective tissue may adhere to the platinum electrode and cause mechanical interference with current readings. This is easily eliminated by first rotating the electrode for a few minutes in the buffer containing the homogenate (before the addition of silver nitrate) and then removing the adhering pieces from the electrode. To increase the specificity of the method a 'blank' titration is carried out with each estimation, using an excess of a 0·001 *M* solution of *p*-chloromercuribenzoate in 0·05 *N* sodium hydroxide. The technique of the 'blank' titration is very similar to those used by other workers[4,5]. Where x ml. of 0·002 *M* silver nitrate is required for a titration, $2x + 0·1$ ml. of 0·001 *M* *p*-chloromercuribenzoate solution is added to block the sulphydryl groups. *p*-Chloromercuribenzoate is allowed to react for 5 min. before the titration. The final volume of the titration mixture, including the *p*-chloromercuribenzoate solution, is kept at 30 ml. Calculation: 1 ml. of 0·002 *M* silver nitrate ≡ 0·066 mgm. of sulphydryl.

The results of sulphydryl determinations, using the above method, in blood and tissues of rats (200–350 gm., both male and female) are summarized in Table 1.

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[1] Bhattacharya, S. K., *Biochem. J.*, **69**, 43p (1958).
[2] Benesch, R. E., Lardy, H. A., and Benesch, R., *J. Biol. Chem.*, **216**, 663 (1955).
[3] Benesch, R. E., and Benesch, R., *Arch. Biochem.*, **28**, 43 (1950).
[4] Benesch, R., and Benesch, R. E., *Arch. Biochem.*, **19**, 35 (1948).
[5] Goldzieher, J. W., Rawls, W. B., and Goldzieher, M. A., *J. Biol. Chem.*, **203**, 519 (1953).

Selenite as a Relatively Weak Inhibitor of some Sulphydryl Enzymes

CONTINUING our studies on the oxidation of sulphydryl compounds[1], we found that selenite was a very active catalyst for the oxidation of cysteine, glutathione, dihydrolipoic acid, and coenzyme *A*. For the oxidation of glutathione, selenite was a far better catalyst than cupric chloride, the most active catalyst known previously[2]. Selenite is poisonous to animals but it is also claimed that selenium is an essential element. Selenium poisoning is an important unsolved problem: the toxic symptoms are complex and the mechanism of the poisoning is still obscure. Some investigators[3,4] believe that selenium compounds exert their toxic effect through interference with certain enzyme systems in the living organism, particularly through mercaptide formation with sulphydryl enzymes. However, most studies on enzyme inactivation had been done with tissue homogenates or yeast cells; and further information on the reaction of selenium salts with purified enzymes seemed desirable. The present communication reports the results of a study of the comparative inhibition of six well-known sulphydryl enzymes by selenite, cupric chloride, and *p*-chloromercuribenzoate.