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Methods

**Reactive Sulphydryl Groups present in Horse (*Equus Ferus Caballus*)
Carbonmonoxyhaemoglobin by Titrating with 5, 5'-Dithiobis-2-Nitrobenzoate (DTNB)**

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Abstract

This research was carried out to determine the number of reactive sulphydryl groups in horse (*Equus ferus caballus*) haemoglobin. Haemolysate was prepared from the horse blood. The haemoglobin was separated into major and minor fractions using carboxymethyl cellulose (CMC-52). Each haemoglobin fraction, in phosphate buffer pH 7.6, was accurately measured into several clean, dry test tubes. Increasing volumes of stock 5,5'-dithiobis-2-nitrobenzoate (DTNB) were added to the different test tubes and left to equilibrate for 3 hours. Absorbance of each solution in each test tube was read at 412 nm. A graph of change in absorbance against the volume of DTNB was plotted; maximum change in absorbance was obtained at the point where the graph levels off. The ratios of 5-thio-2-nitrobenzoate (TNB) concentration to the concentration of haemoglobin tetramer (Hb₄) were calculated and plotted against the volume of DTNB. The number of sulphydryl groups reacting with DTNB was found to be two in both the major and minor haemoglobin.

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