CLXXIV. NOTE ON THE REDUCTION OF THE DISULPHIDE GROUP BY ENZYME SYSTEMS.

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AT the end of a paper by Wieland and Bergel [1924] it was claimed that dithiodiglycollic acid was reduced to thioglycollic acid by succinic acid with the succinoxidase of washed liver mince.

Hopkins and Dixon [1922] had found that the succinoxidase system was quite unable to reduce oxidised glutathione. There were two points of difference between their experiments and those of Wieland and Bergel.

(*a*) Wieland and Bergel used dithiodiglycollic acid, whereas Hopkins and Dixon used glutathione.

(*b*) Wieland and Bergel used washed liver mince, whereas Hopkins and Dixon used washed muscle.

The experiments here described were begun with the object of determining which of these differences was responsible for the discrepancy in the results, but it was found that Wieland and Bergel's results could not be confirmed. Repeating their method with liver mince, and also using active enzyme extracts prepared from liver according to Ohlsson's method [1920], no increased reduction either of dithiodiglycollic acid or of glutathione was obtained in the presence of succinate.

Prof. Wieland has since kindly written to say that the work has been repeated but not confirmed in his laboratory.

It seemed possible that the disulphide-containing molecule might in some way become activated at a surface in the tissue, and in this state be liable to reduction not only by the tissue itself but by active hydrogen from other sources. As, however, enzyme systems producing active hydrogen are heterogeneous, the active hydrogen may not be available at the surface activating the disulphide which would also be a heterogeneous system. Methylene blue might, however, act as a homogeneous intermediary between the donator and acceptor. This possibility has been tested.

Xanthine oxidase and hypoxanthine were used as the primary source of active hydrogen, which kept the methylene blue reduced to the leuco-form, the experiment being carried out anaerobically. Muscle residue and oxidised glutathione were present, but again no more —SH was formed than in a control without hypoxanthine. Neither leucomethylene blue, therefore, nor hypoxanthine with xanthine oxidase, will reduce —SS— even in the presence of muscle residue.

Thus though attempts by several workers have been made to link up the disulphide group with hydrogen transportase systems, the thermostable tissue substance remains the only biological mechanism proved to reduce the disulphide group.

Experimental.

Dithiodiglycollic acid with succinoxidase preparations and succinate.

Exp. 1. Into a flask (*A*) 50 g. of liver mince thoroughly washed with distilled water were introduced, then 20 cc. of 2 % dithiodiglycollic acid solution at p_H 8 in phosphate buffer, and 0·5 g. sodium succinate in 10 cc. buffer, and a few drops of chloroform. A control (*B*) containing exactly the same materials without succinate was also set up. The two flasks were filled with oxygen-free nitrogen and shaken for 7 hours at 37°. Then each was filtered and titrated with 0·1 *N* iodine in an atmosphere of nitrogen.

6·2 cc. of filtrate from (*A*) required 0·8 cc. 0·1 *N* iodine,
11·0 cc. of filtrate from (*B*) required 1·5 cc. 0·1 *N* iodine,

which is equivalent to 0·8 cc. iodine for 6·2 cc. of the liquid.

The above procedure follows the method of Wieland and Bergel, except that they make no mention of the p_H of the liquids, and less succinate and dithiodiglycollic acid were used than in their experiments. Also, instead of marking the filter flask so as to measure 10 cc. liquid for titration and finally squeezing out the tissue to estimate the total volume, the liquid filtered from each flask was measured after titration, and this volume and titre for the two flasks were compared.

Exp. 2. A similar experiment at the same initial p_H was carried out using water instead of buffer solution as medium.

24 cc. of filtrate (*A*) required 3·0 cc. 0·1 *N* iodine solution,
24·5 cc. of filtrate (*B*) required 3·1 cc. 0·1 *N* iodine solution.

Exp. 3. With the succinoxidase preparation, the flasks contained 25 cc. of the active alkaline phosphate extract from sheep's liver, 10 cc. of 2 % dithiodiglycollic acid in *M*/15 sodium phosphate brought to p_H 8 with *N* NaOH solution, and in (*A*) 0·5 g. of sodium succinate. The two flasks were filled with oxygen-free nitrogen and shaken gently for 7 hours at 37°. Before filtering, 10 cc. of 10 % trichloroacetic acid were introduced, which by precipitating proteins gave easy filtration and a clear filtrate in which the —SH is not easily autoxidisable.

30·9 cc. filtrate (*A*) required 1·1 cc. 0·1 *N* iodine solution,
30·3 cc. filtrate (*B*) required 1·1 cc. 0·1 *N* iodine solution.

Thus, in no experiment has any extra reduction of —SS— occurred in the presence of succinate.

A similar experiment with glutathione and enzyme extract also showed no extra —SH formed in the presence of succinate.

Glutathione and muscle residue with leucomethylene blue.

Exp. 4. Two flasks were set up each containing 3 g. of thermostable residue, prepared according to Hopkins and Dixon's [1922] standard method from rabbit muscle, 25 cc. of a 10 % solution in phosphate buffer of xanthine oxidase-caseinogen preparation, prepared according to Dixon and Thurlow [1924], 10 cc. of a buffer solution containing 5 mg. oxidised glutathione per cc., and 2·5 cc. of 0·02 % methylene blue solution. The p_H of each was adjusted to 7·5, and 2·5 cc. of a solution containing 2 mg. hypoxanthine per cc. were added to (*A*), 2·5 cc. of water being added to (*B*).

The two flasks were filled with oxygen-free nitrogen and shaken for 7 hours at 37°. Then 15 cc. of 20 % trichloroacetic acid solution were run into each, the liquid was filtered off and titrated with 0·01 *N* iodine solution using the nitroprusside external indicator.

35 cc. of the liquid from both (*A*) and (*B*) required about 7 cc. of 0·01 *N* iodine.

Thus no extra —SH had been formed by the hypoxanthine in flask *A* donating hydrogen to the —SS— in presence of the muscle system, even with methylene blue as intermediary.

The enzyme preparations used in all cases were shown to be active by the reduction of methylene blue in the presence of their respective substrates.

Summary.

1. Neither dithiodiglycollic acid nor glutathione is reduced by succinoxidase and succinic acid.

2. No extra reduction of —SS— in presence of muscle residue is caused by the sources of active hydrogen tried.

REFERENCES.

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