

CXXII. THE ACTION OF IODOETHYL ALCOHOL ON THIOL COMPOUNDS AND ON PROTEINS.

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MOWAT AND STEWART [1934] have made a study of the inhibitory action of iodoethyl alcohol on the rate of disappearance of glucose from shed blood. Iodoethyl alcohol appears to be only about half as efficient as iodoacetic acid in inhibiting the glycolysis. The further observation is made that although iodoacetic acid reacts with glutathione, iodoethyl alcohol appears not to do so at all. The conclusion is drawn that although iodoacetic acid may inhibit glycolysis by interaction with thiol groups of the enzyme, the mechanism of inhibition by iodoethyl alcohol must be different.

It is the purpose of the present work to show that iodoethyl alcohol does react with thiol groups in the same way as iodoacetic acid [Dickens, 1933; Michaelis and Schubert, 1934]. In the first place the compound



has been isolated in crystalline form and identified by analysis and properties. It was prepared by the action at room temperature of iodoethyl alcohol on cysteine in aqueous solution at a p_{H} in the bicarbonate-carbonic acid range.

To show that iodoethyl alcohol also reacts with glutathione it was thought sufficient not to isolate the compound as in the case of cysteine but merely to measure the decrease with time in iodine titre of a solution containing glutathione

Table I.

Each experiment is made with 50 ml. of *M* phosphate buffer with enough added KOH solution to neutralise added cysteine hydrochloride, glutathione or iodoacetic acid. Either 0.4 g. cysteine hydrochloride or 0.7 g. glutathione is dissolved in this buffer and if an iodo-compound is to be added, 0.5 g. of either iodoacetic acid or iodoethyl alcohol. At the time intervals mentioned 10 ml. samples are withdrawn and dropped into 5 ml. of 25 % acetic acid. The samples are titrated with 0.0406 *M* alcoholic iodine, the number of ml. of this solution being recorded in the table. After the last sample has been withdrawn the p_{H} is measured with a glass electrode.

Time mins.	ml. iodine solution used.					
	Cysteine			Glutathione		
	Alone	+ CH ₂ I. CH ₂ OH	+ CH ₂ I. COOH	Alone	+ CH ₂ I. CH ₂ OH	+ CH ₂ I. COOH
0.5-1	12.9	12.9	3.3	10.7	10.7	3.1
3	—	—	0.7	—	—	0.7
6	—	—	0.2	—	—	0.3
11	—	—	0.0	—	—	0.1
75	12.9	11.0	—	10.5	9.3	—
160	12.8	9.6	—	10.2	7.9	—
280	12.0	8.0	—	9.8	5.8	—
p_{H}	7.70	7.64	7.55	7.70	7.65	7.57

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and iodoethyl alcohol at p_H 7.5. This rate of decrease is of about the same order as with cysteine in which case the compound itself had been isolated. The data are given in Table I. It is at once evident that although iodoethyl alcohol reacts with the thiol group of cysteine and glutathione it does so at a rate very much slower than iodoacetic acid. Some similar experiments were made with iodoacetamide and showed that this substance reacts as quickly with either cysteine or glutathione as does iodoacetic acid, but since the figures have no value except to show roughly the speed of the reaction they are not included here.

15 g. NaHCO_3 are placed in an Erlenmeyer flask through which a steady stream of nitrogen is passed. Through a dropping funnel is added a solution of 6.4 g. cysteine hydrochloride in 15 ml. water, and then 7.2 ml. iodoethyl alcohol are washed in with 15 ml. more water. The mixture is allowed to stand 12 hours. The solution is then acidified with HCl and neutralised to Congo red paper with NaOH. It is now evaporated to dryness *in vacuo* and the residue extracted with 75 to 100 ml. of 85 % alcohol. The mixture is filtered and to the filtrate about 500 ml. acetone are added. A white crystalline precipitate forms. This may be recrystallised several times more by precipitation from 60 to 80 % alcohol with acetone. The product is dried *in vacuo*. Found C, 36.61; H, 6.32; N, 8.27; S, 19.13 %. $\text{C}_5\text{H}_{11}\text{O}_3\text{NS}$ requires: C, 36.36; H, 6.72; N, 8.49; S, 19.39 %.

That the iodoethyl alcohol has reacted with the SH group and not the NH_2 group of the cysteine is evident from the negative nitroprusside test.

Behaviour with proteins. Mirsky and Anson [1935] have shown that iodoacetic acid reacts completely with the thiol groups of certain proteins. The data listed in Table II show that iodoethyl alcohol and iodoacetamide react similarly with a thiol-protein, but that the iodoethyl alcohol does not react as rapidly as does iodoacetic acid or iodoacetamide.

The protein used for the experiments is a protein prepared from wool by reduction with alkaline thiolacetate [Goddard and Michaelis, 1934]. This protein contains about 12 % cystine, and may easily be reduced. 1.75 g. of dry protein and 50 ml. of molar phosphate buffer (p_H 7.4), containing 1 milliequivalent of neutralised thiolacetic acid, are added to each of 4 flasks through which a steady stream of N_2 is passed. Two hours are allowed for reduction of the proteins. (The proteins are not completely dissolved, but solid proteins are reduced by thiolacetate, and react with iodo-compounds.) To three of the flasks 50 ml. of *M* phosphate buffer (p_H 7.4) containing 2 milliequivalents of either iodoethyl alcohol, iodoacetamide or neutralised iodoacetic acid, are added. The fourth flask serves as a control. After 2 hours the p_H is determined with a glass electrode, and the proteins are then brought to the turning point of methyl red with acetic acid. The proteins are then dialysed for 3 days against running water. Samples of these proteins are prepared for analysis by precipitation with trichloroacetic acid, washed with acetone and acid acetone and dried in a vacuum desiccator [see Mirsky and Anson, 1935]. The cystine analyses are made by the method of Folin and Marenzi [1929], after oxidising the acid hydrolysate with 3 % H_2O_2 . Moisture content is determined by drying to constant weight at 105°. A similar experiment was carried out at higher p_H using sodium veronal as buffer.

The reduction of the proteins by thiolacetic acid is not complete, as is shown by the analyses for residual cystine given in Table II. When the reduction is carried out at a higher p_H the reduction is more complete as is shown by the lower residual cystine values. The reaction of the iodo-compounds with the thiol groups was complete in all preparations except that with iodoethyl alcohol at p_H 7.4, this preparation alone gave a positive nitroprusside test on the protein dialysed free from thiolacetic acid.

Table II.

Figs. % based on dry weight of the proteins.
 Proteins dried at 105° to constant weight.

Protein	Cystine	Sulphur	Nitrogen
A. Wool protein (control)	12.1, 12.3	3.21	15.9
B. Prepared from A by iodoacetate at			
p_H 7.4-6.99	2.06	3.31	15.7
p_H 9.5-8.6	1.42	—	—
C. Prepared from A by iodoacetamide at			
p_H 7.4-7.33	2.25	3.25	14.8
p_H 9.5-8.8	1.7	—	—
D. Prepared from A by iodoethyl alcohol at			
p_H 7.4-7.1	6.25	3.29	14.5
p_H 9.5-9.0	1.27	—	—

DISCUSSION.

It thus appears that iodoethyl alcohol reacts with the thiol groups of cysteine and glutathione or of proteins derived from wool just as does iodoacetic acid, only the reaction is much slower. With the wool proteins this slowness is less apparent, since even at p_H about 7, half of the SH of the protein has reacted in 2 hours. With cysteine or glutathione on the other hand in 2 hours less than 25 % of the SH group has reacted. Mowat and Stewart, in testing the action of iodoethyl alcohol on glutathione, allowed only 1 hour, while in testing the action on glycolysis the reaction covered 5 to 7 hours. This coupled with the fact mentioned above that iodoethyl alcohol, in the case studied, reacts faster with protein SH than with glutathione may easily account for the apparent findings of Mowat and Stewart. Therefore we do not believe that the existing evidence requires that a new assumption be made that the mechanism of inhibition by iodoethyl alcohol is different from that by iodoacetic acid.

SUMMARY.

It has been shown that iodoethyl alcohol reacts with SH groups of cysteine, glutathione and proteins derived from wool. There is no need to assume different mechanisms of inhibition of glycolysis by iodoacetic acid and by iodoethyl alcohol.

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