CVIII. THE EFFECT OF IODOACETIC ACID AND OTHER SUBSTANCES ON THE DISAPPEARANCE OF GLUCOSE FROM SHED BLOOD.

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RECENT work on the breakdown of glucose to lactic acid, while it has produced a number of new facts and hypotheses, has not by any means succeeded in producing a clear and unequivocal account of the reactions involved. Thus on the one hand we have the demonstration that iodoacetic acid, which prevents lactic acid formation in muscle, reacts with and destroys glutathione the co-enzyme of glyoxalase. On the other hand, it has been claimed that muscle, from which glutathione has been removed by dialysis, is still capable of lactic acid production provided that adenosinetriphosphate and magnesium ions are present.

The experiments described in this paper were undertaken in the hope that shed blood, in which the process of glycolysis is continuous, would prove a simpler system than muscle, the underlying assumption being that in both tissues lactic acid formation followed the same course. As will be seen, this assumption proved not to be justified, but this fact in itself seems to be one of considerable importance.

EXPERIMENTAL METHODS.

Human venous blood was used, with addition of sodium oxalate as an anti-coagulant. In some cases the blood was diluted with an equal amount of water (a procedure shown to be without appreciable influence on the rate of disappearance of sugar) and in some the p_H was adjusted to 8.5 by addition of sodium hydroxide solution. This adjustment of p_H , while it increased the rate of glycolysis in the control samples, as compared with blood at the normal p_H , did not affect the inhibition of glycolysis by iodoacetic acid *etc.* Measured amounts of this blood mixture were transferred to separate test-tubes and to them the various substances to be studied were added in $N/10$ solution. Acids were used in the form of sodium salts (at p_H 7.5) and $N/10$ NaCl was used as a control, and, where necessary, for adjustment of volume. Incubation was at 37°. Sugar estimations were made by the method of Hagedorn and Jensen.

Iodoacetic acid was prepared by the action of potassium iodide on chloroacetic acid and purified by repeated precipitation from benzene solution with light petroleum. Iodoethyl alcohol was synthesised by the method of Hendry [1889], the final product being decolorised by sodium thiosulphate and distilled *in vacuo*. *l*-Bromopropionic acid was synthesised by the method of Volhard [1887] and from it a specimen of *l*-iodopropionic acid was prepared by the method of Abderhalden and Guggenheim [1908]. The β -iodopropionic acid was a Kahlbaum preparation, kindly presented to us by Dr H. S. Rule.

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RESULTS AND DISCUSSION.

Iodoacetic acid proved to be the most efficient of the substances tested in preventing the disappearance of glucose. Nevertheless it was without appreciable effect in concentrations of the order capable, in cardiac muscle, of completely inhibiting anaerobic carbohydrate breakdown. Bromoacetic acid was almost as active, chloroacetic acid less so; whilst iodoethyl alcohol and α -iodopropionic acid displayed smaller though very definite powers of inhibition of glycolysis. β -Iodopropionic acid however was quite inactive (Table I).

In a number of experiments it was noticeable that iodoacetic acid, while completely inhibiting glycolysis from the end of the first hour of incubation to the seventh hour, allowed some apparent decrease of sugar during the first hour. In part this may be due to the time necessary for the iodoacetic acid to exert its effect—*i.e.* to react with glutathione. Since, however, the method of protein precipitation used in the Hagedorn and Jensen method does not completely remove non-carbohydrate reducing substances, it seems likely that this apparent decrease in the blood-sugar may be due to the destruction by the iodoacetic acid of glutathione and possibly other substances such as ergothioneine.

The experiment quoted in Table I (a) shows the inhibition well maintained at 24 hours. This was not always the case, though usually the control, even then, showed the lowest sugar content. Most probably this was due to the fact that no special precautions were taken to exclude bacterial contamination.

Table I. *Effect of iodoacetic acid, iodoethyl alcohol, α -iodopropionic acid, bromoacetic acid and chloroacetic acid on the rate of disappearance of sugar from blood.*

(a) 0.7 ml. *N*/10 NaOH added to each 2.0 ml. blood. 0.25 ml. *N*/10 solution of reagent (neutral) or NaCl added per 5.0 ml. of blood mixture. Incubation at 37°.

mg. sugar per 100 ml. blood.

Time hours	Control	Iodoacetic acid	Iodoethyl alcohol	α -Iodopropionic acid
0	87	87	87	87
1	79	84	82	77
3	55	84	81	69
5	55	84	75	59
7	37	85	61	57
24	35	80	59	57

(b) As above, but 0.4 ml. *N*/10 chloroacetic acid, 0.2 ml. *N*/10 bromoacetic acid or *N*/10 iodoacetic acid (neutralised) and volumes adjusted with *N*/10 NaCl.

mg. sugar per 100 ml. blood.

Time hours	Control	Iodoacetic acid	Bromoacetic acid	Chloroacetic acid
0	225	225	225	225
3	201	224	215	209
5	179	209	211	91

Blood from a case of diabetes.

Chloro-, bromo- and iodo-acetic acids are all known [Dickens, 1933] to react with glutathione *in vitro*, and the velocity constants of the reactions have been determined. The action between glutathione and chloroacetic acid proceeds very much more slowly than those between glutathione and bromo- and iodo-acetic acid. In accordance with this, chloroacetic acid is much less efficient than the

other two substances in preventing glycolysis, but it is rather surprising to find it as efficient as it is—roughly half as efficient as bromoacetic acid. This is particularly so when one remembers that even in relatively greater concentrations it is without inhibitory action on the lactic acid production of cardiac muscle [Gaddie and Stewart, 1934]. These facts immediately suggest that the mechanism of sugar breakdown in blood differs from that in muscle and that the action of chloroacetic acid is not confined to the destruction of glutathione.

It is obviously desirable to test the effect of glutathione on the other two substances capable of inhibiting glycolysis (iodoethyl alcohol and α -iodopropionic acid) as well as the related compound, β -iodopropionic acid, which had no such effect. This became especially important when it was shown [Gaddie and Stewart, 1934] that none of these substances had an inhibitory effect on the frog's heart comparable with that exerted by iodo- and bromo-acetic acids. The method of testing was as follows: Solutions of the substance to be tested ($M/10$) and glutathione ($M/100$) were prepared at p_H 7.5, and 10 ml. of each solution were mixed with 10 ml. of a phosphate buffer at the same p_H . From this mixture, 5 ml. were at once removed and examined immediately for hydriodic acid. Further samples were removed at intervals of 15 minutes (the mixture meanwhile standing at room temperature) and tested in the same way. Control experiments were made without glutathione. Estimation of hydriodic acid was made by adding to the sample 2 ml. of $N/10$ potassium ferricyanide containing zinc sulphate, and 2 ml. of 3 % acetic acid, extracting the liberated iodine with chloroform (three extractions using 2 ml. of chloroform each time) and titrating the chloroform extracts with $N/200$ sodium thiosulphate. This method was tested on standard solutions of potassium iodide and was found to give satisfactory results.

When β -iodopropionic acid was tested in this way, the reason of its inactivity in preventing glycolysis became apparent at once—even in the absence of glutathione it lost hydriodic acid with much greater rapidity than did iodoacetic acid in presence of glutathione.

The α -iodopropionic acid, however, was stable alone and reacted with glutathione much more slowly than did iodoacetic acid (Table II), a finding in keeping with its smaller power of preventing the disappearance of sugar.

Table II. *Effect of iodoacetic acid, iodoethyl alcohol and α -iodopropionic acid on glutathione in vitro.*

ml. $N/100$ thiosulphate used in titrating iodine liberated per 5 ml. of solution.

Time min.	Iodoacetic acid	Iodoethyl alcohol	α -Iodopropionic acid
0	0.540	0.03	0.170
20	1.055	0.02	0.425
40	1.100	0.02	0.575
60	1.150	0.025	0.660

Iodoethyl alcohol, on the other hand, failed to react with glutathione, or rather, failed to liberate hydriodic acid in the presence of glutathione, although it was perfectly capable of inhibiting glucose breakdown. This can only mean that iodoethyl alcohol reacts with glutathione in some other way, or that its effect is exercised entirely on some other part of the system responsible for the conversion of glucose into lactic acid. The first of these alternatives seems to be excluded by the finding that the sulphydryl group (probably the essential part of the molecule for co-enzyme activity, and the most likely part to undergo

change) is unaffected by incubation with iodoethyl alcohol at p_H 7.5. This is shown by the fact that after incubation of the mixture for $1\frac{1}{2}$ hours the amount of sulphhydryl present is undiminished when tested both colorimetrically with sodium nitroprusside and titrimetrically with iodine. Still further evidence of the independence of glutathione and iodoethyl alcohol is afforded by the fact that added glutathione does not abolish the inhibitory action of iodoethyl alcohol on glycolysis, whereas it does abolish the action of iodoacetic acid (Table III).

Table III. *Effect of added glutathione on the inhibition of glycolysis in presence of iodoacetic acid and iodoethyl alcohol. Experiment as in Table I but 0.5 ml. reagent per 5 ml. blood mixture, and 20 mg. glutathione (in neutral solution) volumes adjusted with N/10 NaCl.*

mg. sugar per 100 ml. blood.					
Time hours	Control	Iodoacetic acid	Iodoacetic acid and glutathione	Iodoethyl alcohol	Iodoethyl alcohol and glutathione
0	103	103	105	104	103
1	91	93	95	90	91
3	55	93	56	83	85
5	49	91	54	75	74

We are therefore driven to the conclusion that the actions of iodoacetic acid and iodoethyl alcohol, though leading to the same end-result, are fundamentally different at least in part. Moreover, since iodoethyl alcohol inhibits glycolysis in blood with an activity roughly half that of iodoacetic acid, but is totally ineffective in cardiac muscle at a concentration tenfold that of iodoacetic acid capable of complete inhibition of glycolysis, it follows that some part of the glycolytic mechanism present and of importance in blood is absent from cardiac muscle, or at least is not of critical importance in the latter.

The only alternative to this hypothesis appears to be the idea that iodoethyl alcohol is acting merely as a general enzyme poison. Indeed, when one considers the quantity involved, such an idea might be entertained with regard not only to iodoethyl alcohol, but also with regard to iodoacetic acid itself. For there is no proof that the whole action of this latter substance consists in its destruction of the co-enzyme of glyoxalase, and one might consider the restorative effect of added glutathione (which seems to be complete) as being merely an *in vitro* reaction resulting in the destruction of the enzyme poison and the replacement of the co-enzyme previously lost.

We have not tested this alternative hypothesis completely, and, indeed, there are not many enzyme systems in blood which have been thoroughly studied. We have, however, tested the effect of both iodoacetic acid and iodoethyl alcohol on the blood-phosphatase. The blood, made alkaline to p_H about 8.5 by means of sodium hydroxide was incubated with excess of sodium glycerophosphate solution and addition of sodium iodoacetate or iodoethyl alcohol in the concentrations known to inhibit glycolysis. In both cases samples withdrawn at 24 and 48 hours showed decreased hydrolysis of the organic phosphate as compared with the controls (Table IV). Since however the phosphatase may form part of the system responsible for the breakdown of glucose to lactic acid, this cannot be taken as evidence that the two iodo-compounds act as general enzyme poisons. It is perhaps noteworthy that they are without any inhibitory effect on the xanthine oxidase of milk.

Table IV. *Effect of iodoacetic acid and iodoethyl alcohol on blood-phosphatase.*

	mg. P per 100 ml. blood.		
Time (hours)	0	24	48
Control	7.44	27.18	39.17
Iodoacetic acid	7.18	21.44	31.63
Control	6.40	38.30	47.11
Iodoethyl alcohol	6.23	28.30	30.45

SUMMARY.

Iodoacetic acid inhibits the disappearance of glucose from shed blood, but only in concentrations much higher than those required to prevent lactic acid formation in cardiac muscle. Bromoacetic acid has an almost equal effect. Chloroacetic acid, α -iodopropionic acid and iodoethyl alcohol have similar, though weaker actions. β -Iodopropionic acid is without effect probably on account of the rapidity with which it loses iodine.

The effect of iodoacetic acid, but not that of iodoethyl alcohol, is counteracted by addition of glutathione. Iodoethyl alcohol is also peculiar in not reacting with glutathione *in vitro* to produce hydriodic acid. Both iodoacetic acid and iodoethyl alcohol, in concentrations capable of preventing sugar disappearance, inhibit the action of blood-phosphatase.

The significance of these findings is discussed in relation to the mechanism of carbohydrate breakdown.

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