CLII. INTERACTION OF HALOGENACETATES AND SH COMPOUNDS.

THE REACTION OF HALOGENACETIC ACIDS WITH GLUTATHIONE AND CYSTEINE. THE MECHANISM OF IODOACETATE POISONING OF GLYOXALASE[1].

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Since Lundsgaard [1930] drew attention to the remarkably toxic nature of certain of the halogenacetic acids and their rôle as powerful and selective inhibitors of lactic acid formation and of alcoholic fermentation, these compounds have assumed great importance in physiology, and the numerous investigations of fundamental value to the theories of muscular contraction, tissue glycolysis and fermentation which have resulted show the usefulness of these labile halogen compounds as reagents in the study of intermediary tissue metabolism. But in spite of the many investigations, in no one case has the mechanism of their action been explicable in any clearly defined way, though various suggestions, such as those of Waldschmidt-Leitz and Schäffner [1932] and of Bersin [1932], have been advanced from time to time.

In view of the importance of the iodoacetate reaction, it was decided to investigate what appeared to be one of the simplest physiological transformations known at the time to be inhibited by iodoacetic acid, namely the conversion of methylglyoxal into lactic acid: a type of oxido-reduction of great interest and perhaps one directly concerned in glycolysis. Indeed Dudley [1931], who discovered the inhibitory action of iodoacetic acid on this keto-aldehyde mutase system, considered that this might well be the mode of action of the halogenacetic acids on the glycolytic process. It is true that Lohmann [1931], shortly after, found that in his glyoxalase preparations a much higher concentration of iodoacetate was needed to stop glyoxalase action than was required to check the formation of lactic acid by the Meyerhof muscle extract; he therefore considered that it was unlikely that the inhibition of muscle glycolysis could be due to poisoning of the glyoxalase system.

Nevertheless this point could not be said to be definitely cleared up, and during an attempt to investigate it further the work to be described in the present paper, which sheds some light on the mechanism of iodoacetate poisoning of glyoxalase, arose.

In view of the possibility that the method of preparation of the extracts might affect the concentration of iodoacetate necessary, the behaviour of

[1] A preliminary account of part of the work presented here appeared in a letter to *Nature* in January last [Dickens, 1933]. Owing to the author's change of laboratory, the completion of this work and its publication have been somewhat delayed.

dialysed and undialysed extracts was being investigated when a further publication from Lohmann appeared, containing the important discovery that reduced glutathione could act as co-enzyme to glyoxalase in converting synthetic methylglyoxal into lactic acid. Liver extract, from which the bulk of the proteins had been precipitated by an acetate buffer, lost nearly all its glyoxalase activity on dialysis, and this was found to be completely restored by addition of minimum quantities of reduced glutathione to the dialysed extract [Lohmann, 1932]. This discovery of Lohmann suggested as an obvious possibility that the action of iodoacetate might be upon the sulphydryl compounds in the extract, leading to destruction of glyoxalase activity by destruction of the essential co-enzyme. From his earlier paper it is evident that Lohmann [1931] did not favour the view that iodoacetate acts upon the co-enzyme systems, a suggestion put forward, certainly with little direct evidence, by Barrenscheen and Braun [1931]: on the contrary Lohmann believed that the action of iodoacetic acid was to destroy the enzyme itself.

In the present paper it is shown that (I) under physiological conditions of p_H and temperature, the halogenacetic acids readily react with sulphydryl compounds, in particular with glutathione and cysteine, forming the corresponding thio-ethers and hydrogen halide, and (II) that this action provides an adequate explanation for the destruction of glyoxalase activity by halogenacetic acids.

I. *The interaction of glutathione and cysteine with halogenacetic acids.*

Evidence of the reaction between iodoacetic acid and glutathione was first obtained manometrically. Glutathione, prepared by Pirie's [1930] method and purified through the cadmium salt [Voegtlin, Johnson and Rosenthal, 1931] was dissolved in glass-distilled water containing 0·031 M $NaHCO_3$, the solutions

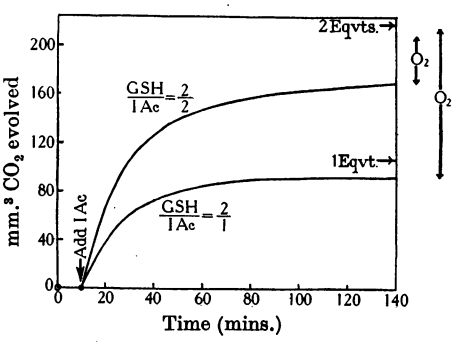


Fig. 1.

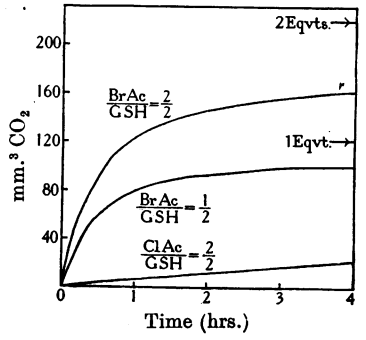


Fig. 2.

Fig. 1. *Reaction of iodoacetate with glutathione.* The oxygen uptake after 140 mins. (vessels filled with O_2 + 5 % CO_2 and trace $CuSO_4$ added) is shown by the heavy lines on the right: the units are the same as for the CO_2-liberation, and the sum of the latter with the O_2-uptake of the unchanged GSH is near the theoretical for 2 equivalents GSH.

Fig. 2. *Reaction of bromoacetate and chloroacetate with glutathione.*

having been previously saturated with a gas mixture of nitrogen with 5 % CO_2. Suitable quantities (3 cc.) of the approximately $M/300$ solution of glutathione were measured into the Warburg manometer vessels, which were provided with side-bulbs containing the $N/20$ solution of the sodium salts of the halogenacetic acids (0·1 or 0·2 cc.): the latter solutions also contained a similar excess of

$NaHCO_3$ to that present in the glutathione solution in the main part of the vessel. After filling with nitrogen containing 5 % CO_2, the vessels, attached to their manometers, were put into the thermostat at 37·5° and shaking was begun. When temperature equilibrium was attained the contents were mixed by tipping the vessel and then replacing in the thermostat. CO_2 evolution immediately began and its course was followed by the pressure readings (Figs. 1 and 2).

Table I.

37·5°; $[Na^+]=0{\cdot}032$; N_2+5 % CO_2; p_H approx. 7·4.

Iodoacetate and glutathione:

$a=3{\cdot}15\times10^{-3}$, $b=1{\cdot}61\times10^{-3}$, vol. = 3·1 cc.

t mins.	x (mm.³ CO_2)	$\frac{x}{M}\times10^{-3}$	k
10	38·5	0·55	14·5
20	61	0·88	15·1
30	72·5	1·05	14·1
40	81	1·17	13·6
		Mean	14·3

$a=3{\cdot}05\times10^{-3}$, $b=3{\cdot}12\times10^{-3}$, vol. = 3·2 cc.

10	69·5	0·97	14·5
20	108	1·50	17·5
30	126	1·76	15·5
40	140	1·95	14·6
		Mean	15·5

Bromoacetate and glutathione:

$a=b=3{\cdot}13\times10^{-3}$, vol. = 3·2 cc.

10	48·5	0·67	8·7
20	75·5	1·05	8·1
30	95	1·32	7·8
40	107	1·49	7·3
		Mean	8·0

$a=3{\cdot}24\times10^{-3}$, $b=1{\cdot}61\times10^{-3}$, vol. = 3·1 cc.

30	58·5	0·84	8·9
40	70·5	1·02	9·5
50	76	1·10	9·0
60	81	1·17	8·7
		Mean	9·0

Chloroacetate and glutathione:

$a=b=3{\cdot}13\times10^{-3}$, vol. = 3·2 cc.

120	12·5	0·175	0·16
180	17·5	0·244	0·15
240	21·5	0·300	0·14
270	23	0·320	0·14
		Mean	0·15

Table I shows that the reaction follows the bimolecular law and may be represented as follows:

$$\underset{a-x}{G.S'}+\underset{b-x}{ICH_2COO'}=\underset{x}{G.S.CH_2COO'}+\underset{x}{I'}$$

or similarly for the other monohalogenacetic acids. Similar reactions, *e.g.* of thiolacetic acid and halogenacetic acids, are well known [Klason, 1877; Klason and Carlson, 1906], but they have been surprisingly little studied by physical chemists. Quite recently, however, Hellström [1931] has studied the example (thiolacetic acid) mentioned from the point of view of Brönsted's theory and finds that the reaction follows the bimolecular law, though the velocity constant depends on the

nature and concentration of the cations present, being, when $[Na^+] = 0{\cdot}0375$ and at 25°, of the order: 150, 70, 0·70 (uncorr.) for iodo-, bromo-, chloro-acetic acids respectively. This order is very similar to that shown in the present experiments, the constants with thiolacetic acid being however about 10 times as large, despite the lower temperature used by Hellström. This series is also in agreement with the physiological action, that is it corresponds with the order of activity of the three halogen-acids in checking glycolysis or fermentation [Lundsgaard, 1930; Lohmann, 1931; Cayrol, 1931].

Whilst the above course follows the bimolecular type sufficiently accurately, it was thought necessary to isolate the products of the reaction. With glutathione the purification of the condensation compound presented difficulties, and the reaction was worked out with cysteine, where the greater stability makes the separation easier.

For this purpose cysteine hydrochloride (0·16 g. = 1 milli-equiv.) was neutralised with $N/5$ $Ba(OH)_2$ (5·5 cc.) and a neutral solution of iodoacetic acid (0·19 g. = 1 milli-equiv., dissolved in 5·3 cc. $N/5$ $Ba(OH)_2$) was added while N_2 was passed through the solutions. The reaction mixture at once became acid, and $N/5$ $Ba(OH)_2$, in all a further 5·0 cc. (1 milli-equiv.), was added to keep the reaction mixture neutral; after addition of alcohol (15 cc.) the mixture was allowed to stand overnight. In the morning a further 30 cc. absolute alcohol was added and the white precipitate of Ba salts, including some $BaCl_2$, was collected, after half an hour, in the centrifuge: the alcoholic supernatant liquid contained barium iodide. The precipitate of Ba salts was dissolved in water (3 cc.), and H_2SO_4 (*ca.* 10 cc. $N/10$) was cautiously added to give complete precipitation of the Ba as $BaSO_4$; the latter was removed and the clear colourless solution evaporated *in vacuo* over P_2O_5. A white crystalline residue began to separate when the volume was reduced to a few cc., and the whole contents of the dish crystallised overnight: traces of a yellow impurity and of HCl were removed by washing with a little ice-cold alcohol. Yield 0·15 g. = 85 % of the theoretical; colourless clusters of fine needles, M.P. 84° decomp. (uncorr.). For micro-analysis (Schoeller) the crystals were dried in the air without further purification. Found: C, 33·14; H, 5·16; N, 7·10; S, 17·69 %. $C_5H_9O_4NS$ requires: C, 33·51; H, 5·06; N, 7·81; S, 17·69 %. The course of the reaction is therefore as follows:

$$HOOC.CH(NH_2).CH_2.SH + I.CH_2COOH = HOOC.CH(NH_2).CH_2.S.CH_2.COOH + HI.$$

The thio-ether is only slightly soluble in cold water or alcohol, more soluble in hot water and freely soluble in dilute aqueous ammonia. The aqueous solution is acid in reaction.

When the same method of isolation was tried with glutathione the recovery and purity of the product were not so satisfactory. Neutral solutions ($\frac{1}{3}$ milli-equiv.) of Ba salts of glutathione and iodoacetic acid were mixed, and in all 1·55 cc. $N/5$ $Ba(OH)_2$ (calc. 1·66 cc.) were required to keep the mixture neutral. The alcoholic precipitate of Ba salts was freed from Ba by H_2SO_4 (6 cc. $N/10$). Evaporation of the filtrate *in vacuo* over P_2O_5 gave a clear sticky residue which changed to a feathery precipitate when rubbed with alcohol. The precipitate was collected and dried in a vacuum desiccator overnight. Micro-analysis (Schoeller) showed that the substance is probably the not quite pure thio-ether containing 1 mol. H_2O. (Found: C, 38·4; H, 6·0; N, 9·4; S, 7·9 %. $C_{12}H_{19}O_8N_3S$, H_2O requires: C, 37·6; H, 5·5; N, 10·9; S, 8·3 %.) Further purification of the thio-ether was not carried out: it was freely soluble in cold water, giving a solution with an acid reaction from which the substance was re-precipitated by addition of cold alcohol: the nitroprusside reaction was negative.

ERRATUM

Vol. XXVII, page 1144, line 22 from bottom

for **M.P. 84°** ***read*** **M.P. 184°.**

These experiments leave little doubt that the reaction is perfectly analogous to that with cysteine, where the condensation product is more readily isolated in the pure state.

Immediately following the publication of the preliminary report of these experiments [Dickens, 1933] the author learned in a personal communication from Dr Rapkine that he had, quite independently, studied the reaction of cysteine and iodoacetic acid: these results have now been published [Rapkine, 1933] and agree in all respects with those set out in the author's preliminary account. In addition the rate of reaction was shown by Rapkine to increase with increasing temperature and alkalinity. The reaction products (thio-ethers) were not isolated in Rapkine's experiments. Quastel [1933] has also produced interesting evidence of physiological interaction of sulphydryl compounds and iodoacetate, and in fact the paper by Quastel and Wheatley [1932] was, unknown to the author, submitted for publication before the appearance of his preliminary account. It may be mentioned here that one of the earliest recorded examples of the biological action of halogen compounds on thiol compounds appears to be that of Thunberg [1911].

II. *The inhibition of glyoxalase by iodoacetate.*

The source of glyoxalase in all experiments was rat-liver. For the preparation of the extracts the freshly removed liver was minced in a micro-mincer, ground with sand and extracted twice by thoroughly grinding with its own weight of water and centrifuging: the two extracts were then combined. For removal of protein, acetate buffer ($\frac{1}{10}$ vol. $M/3$, p_H 4) was used as in Lohmann's [1932] extracts, followed by centrifuging. $NaHCO_3$ (usually $\frac{1}{10}$ vol. 1·3 %) was added and the solution was dialysed in collodion sacs against distilled water. The water in the 1st and 2nd dialysates invariably showed a positive nitroprusside reaction: sometimes on dialysis a further slight precipitate formed, and was removed by centrifuging. In some extracts KCl (0·9 %) was used instead of water for the preliminary extraction, without any noticeable difference in the extract.

For the estimation of glyoxalase activity, chemically controlled manometric experiments were used, in which 1–2 cc. liver extract, usually diluted with an equal volume of water, were measured into the Warburg manometer vessels together with a volume of 1·3 % $NaHCO_3$ usually $\frac{1}{3}$ to $\frac{1}{2}$ the volume of the original extract, and the vessel was then filled with a gas mixture of 5 % CO_2 in N_2. The temperature for the measurements with extracts was always 30°. The p_H was controlled by bicarbonate estimations, and the CO_2-retention by acidification: with crude extracts the latter was about 20 %, but with deproteinised and dialysed extracts on the other hand the retention correction was small (about 1·5 %). Lohmann has shown that the evolution of CO_2 is an accurate measure of the lactic acid formed when suitable conditions are observed. In view of the possibility that this equivalence might be upset by the presence of iodoacetic acid, this point was controlled in selected experiments by determination of the lactic acid formed, by precipitation of proteins with the Schenk reagent and analysis of the filtrate, freed from mercury by H_2S, by Clausen's method, using Friedemann and Kendall's [1929] modification. Blanks on the extracts and methylglyoxal solutions used were invariably made, and the amounts of lactic acid found were corrected for these blank values. On the whole the agreement of manometric and chemical estimations was satisfactory, and usually 90–98 % of the manometric readings were given by the Clausen analysis. Only when very high concentrations ($M/100$) of iodoacetate were

used did there appear to be some tendency for the chemical values to be lower compared with the manometric ones, but these experiments were not important for the present purpose, as such high concentrations of iodoacetate were seldom used.

The methylglyoxal solutions used were obtained by the method of Neuberg *et al.* [1917] from the distillation of dihydroxyacetone with dilute sulphuric acid. The distillate (50 cc. from 1 g. dihydroxyacetone) was neutralised to phenolphthalein with $N/10$ NaOH before use and $NaHCO_3$ (1·3 %) to give a similar concentration to that in the extract was then added: 0·1 to 0·3 cc. of the distillate so treated was used for each experiment, being usually measured into the side bulb of the manometer vessels.

Glutathione was made by Pirie's [1930] method from yeast, was crystallised from water and then purified through the cadmium salt [Voegtlin, Johnson and Rosenthal, 1931], afterwards being again crystallised from water by evaporation over H_2SO_4 at room temperature. Dissolved in glass-distilled water it was sufficiently stable (5·0 mg. in 3 cc. $M/75$ phosphate or in bicarbonate buffer at p_H 7·4 took up about 4 mm.^{3} O_2 at 38° in 60 mins.), but on the addition of $CuSO_4$ (5×10^{-5} M) the oxygen uptake was 98·5 % of the theoretical and was complete in 60 minutes. (In confirmation of Voegtlin, Johnson and Rosenthal's finding it may be mentioned that 6 times this amount of Fe, in the form of thrice recrystallised ferric ammonium sulphate, was almost without effect on the O_2 uptake in phosphate buffer.)

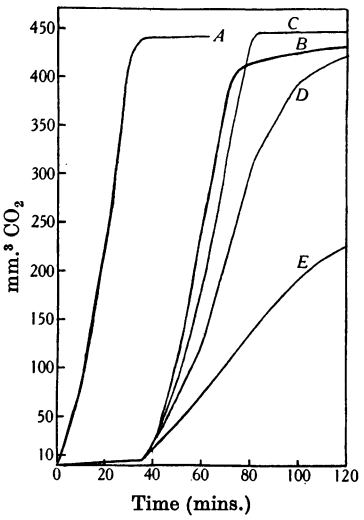


Fig. 3. *Action of iodoacetate on undialysed liver extract.*

A. Control, methylglyoxal added at 0 mins.
B. Control, methylglyoxal added at 35 mins.
C, *D* and *E*; Iodoacetate added at 0 mins. and methylglyoxal at 35 mins. Concentration of ICH_2COOH: *C*, 5×10^{-4} M; *D*, $2{\cdot}4 \times 10^{-3}$ M; *E*, 1×10^{-2} M. Chemical estimation of lactic acid formed: *A*, 1·72; *D*, 1·53; *E*, 0·70 mg. (96, 90 and 80 % respectively of that calculated from pressure changes).

The iodoacetic acid was a pure specimen kindly given me by Dr Lundsgaard. Bromoacetic acid was purified by recrystallising from ligroin, and chloroacetic acid by redistillation *in vacuo*. These acids were dissolved in glass-distilled water and neutralised with $NaHCO_3$. All glass-ware was cleaned with chromic acid

and well rinsed with glass-distilled water before drying. Naturally, the liver extracts were not metal-free, but the use of these precautions prevented loss of part of the glutathione by oxidation before the experiment was begun.

Crude liver extract. A portion of the acetate-precipitated liver extract was taken before dialysis, or in another experiment after only half an hour's dialysis to remove the greater part of the acetate. The CO_2-retention correction of the former was 20 % and of the latter only 6 %. Bicarbonate was added and $N_2 + 5$ % CO_2 passed, when the p_H was 7·4. These extracts were strongly active without added GSH; relatively high concentrations of iodoacetic acid were needed to inactivate them. From Fig. 3 it will be seen that the activity of the crude extract is not impaired by incubation. Iodoacetate in $N/2000$ concentration had no action; $N/420$ caused a perceptible though slight retardation of lactic acid formation whilst the $N/100$ solution inhibited to about 50 %. The chemical estimations showed that the manometric estimations fairly represented the lactic acid formed with the two lower concentrations (96 and 90 % of the manometric readings corresponded to the amounts of lactic acid found by Clausen determination); with the concentrated solution the discrepancy was greater. The experiment shows that a high concentration of iodoacetate is necessary to inactivate the undialysed extract.

Dialysed liver extracts. Fig. 4 shows the reactivation of an extract dialysed for 3 hours by the addition of glutathione after the extract had been inactivated by incubating for 70 mins. with $N/100$ and $N/420$ iodoacetate. Recovery of activity was complete and instantaneous on the addition of GSH equivalent only to $\frac{1}{20}$ of the amount of iodoacetate added. This effect is still clearly shown by the extract after prolonged dialysis has reduced almost to zero its power to form lactic acid without any addition other than methylglyoxal. Fig. 5 shows the behaviour of an extract dialysed for 12 hours and the amounts of glutathione necessary to restore its activity. This extract was then shaken for 40 mins. in the thermostat with the addition of methylglyoxal to all vessels, and to some of iodoacetate also, in suitable concentrations. Glutathione (0·01 mg.) was then added from the side-bulbs attached to the vessels, when as Fig. 6 shows, reactivation was complete with those vessels which had 10^{-4} and 10^{-3} M iodoacetate (the latter is a 30-fold excess over the amount of glutathione used). On the other hand in the 300-fold excess present in the case of $M/100$ iodoacetate, this small amount of glutathione was unable to bring about complete reactivation, and the lactic acid formation as shown both by manometric and chemical estimation fell to about 60 % of the control without iodoacetate. The explanation of the phenomenon of reactivation in the presence of an excess of the inhibitor, which has been repeatedly confirmed, might lie either in a destruction of iodoacetate by incubation with liver extract, for which there is no direct evidence, or it is more probably due to a much more rapid reaction of GSH with the glyoxalase system than the reaction of GSH with iodoacetic acid. The reaction with the enzyme system is certainly rapid, in view of the immediate recovery of activity resulting in an instantaneous and vigorous formation of lactic acid on adding GSH.

The behaviour of the dialysed extract is very different from that of the cruder, undialysed preparations. Fig. 7 shows an experiment with the same extract as that used for the experiment shown in Fig. 4, but after a further 5 hours' dialysis (8 hours in all) the glyoxalase action, without addition other than methylglyoxal, is now reduced to a low level, though the spontaneous increase of activity with time [*cf.* Lohmann, 1932] was rather unusually pronounced with this extract, as is shown by the upward curvature of the controls.

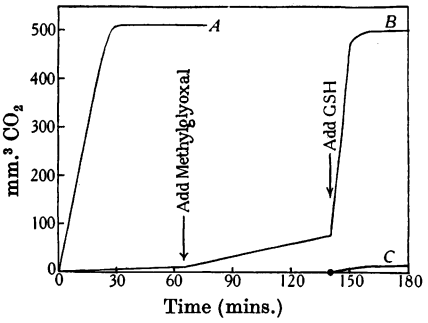
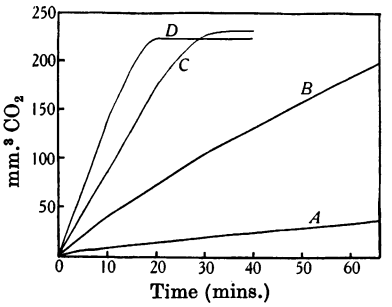


Fig. 4.



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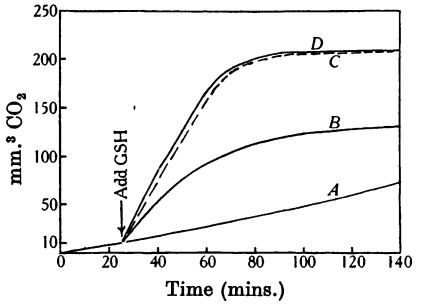

Fig. 6.

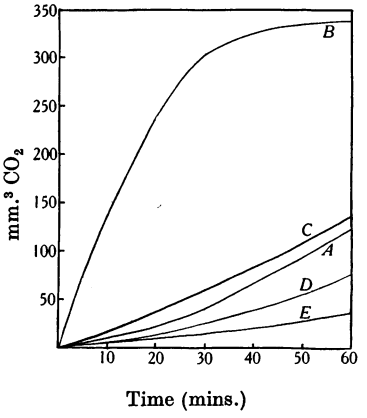

Fig. 7.

Fig. 4. *Liver extract dialysed for three hours. Reactivation by GSH after inactivation by ICH_2COOH.*

A. Control, methylglyoxal added at 0 mins.
B. (*a*) 2·4 mg. and (*b*) 0·47 mg. ICH_2COOH added at 0 mins. Methylglyoxal added at 65 mins., and 0·2 mg. GSH added at 140 mins.
C. Addition of 0·2 mg. GSH to 2·4 mg. ICH_2COOH.

Fig. 5. *Amount of GSH needed to activate extract dialysed for 12 hours.*

A. Control without added GSH. *B*, 0·01 mg.; *C*, 0·025 mg.; *D*, 0·05 mg. GSH.

Fig. 6. *Reactivation of dialysed extract by GSH after ICH_2COOH.*

The same extract as in Fig. 5 was put in the bath at 30° 15 mins. before the experiment began, with the addition of methylglyoxal and iodoacetate.
A. Control without added GSH.
B. ICH_2COOH, 10^{-2} *M*. GSH (0·01 mg.) added after 40 mins. treatment at 30° with ICH_2COOH.
C. ICH_2COOH, 10^{-3} *M*. Otherwise as *B*.
D. ICH_2COOH, 10^{-4} *M*. Otherwise as *B*.
Chemical controls. *B*, 0·48 mg. (91 %); *C*, 0·79 mg. (95 %); *D*, 0·785 mg. (95 % of manometric).

Fig. 7. *Extract dialysed for eight hours.*

A. Control without added GSH or ICH_2COOH.
To *B*, *C*, *D* and *E* was added 0·05 mg. GSH ($1·2 \times 10^{-4}$ *M*), and to *C*, *D* and *E* iodoacetate also, 20 mins. before experiment began. Methylglyoxal was added at 0 mins.
B. Control without ICH_2COOH; *C*, ICH_2COOH, $1·2 \times 10^{-4}$ *M*; *D*, 3×10^{-4} *M*; *E*, $2·5 \times 10^{-3}$ *M* ICH_2COOH.

This extract was strongly activated by addition of glutathione in $1{\cdot}2 \times 10^{-4}$ *M* concentration, and an exactly equivalent concentration of iodoacetic acid reduced the lactic acid formation to the level of the control without added GSH. The dialysed extract is therefore more than one hundred times as sensitive to iodoacetate as the cruder extracts, and the amount of iodoacetate required to bring about inactivation is almost exactly equivalent to the amount of glutathione added.

Intact tissues. The behaviour of slices of intact liver, studied by Warburg's method, by suspending in 2 cc. bicarbonate-Ringer solution at 38° and p_H 7·4, in an atmosphere of N_2 + 5 % CO_2, much more closely resembles that of the highly purified extract than it does that of the crude glyoxalase preparations. Two experiments are reproduced in Figs. 8 and 9. In the former equal weighed

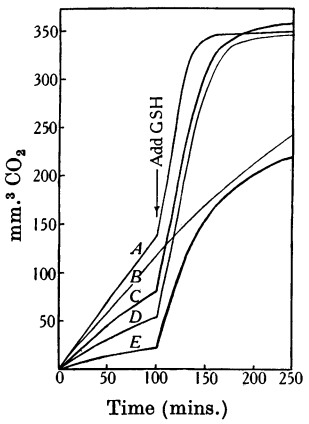

Fig. 8.

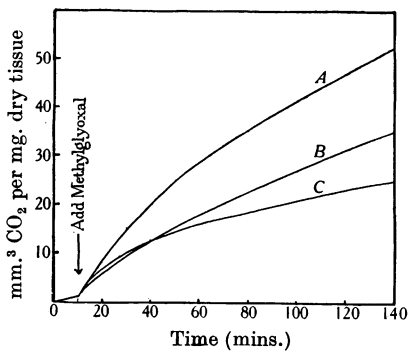

Fig. 9.

Fig. 8. *Rat-liver slices.*

Each vessel contained 50 mg. moist weight; all were put into the bath 60 mins. before first reading at 0 mins., when methylglyoxal was added.

A and *B*. Controls without ICH_2COOH; *C*, $ICH_2COOH = 1{\cdot}5 \times 10^{-4}$ *M*; *D*, $4{\cdot}8 \times 10^{-4}$ *M*; *E*, $2{\cdot}4 \times 10^{-3}$ *M* ICH_2COOH. 0·1 mg. GSH added to all except *B* at 100 mins.

Fig. 9. *Rat-liver slices.*

A. Control without ICH_2COOH.
B. Time incubated 20 mins. with $1{\cdot}5 \times 10^{-4}$ *M* ICH_2COOH before adding methylglyoxal.
C. As *B* but with 5×10^{-4} *M* ICH_2COOH.

portions (50 mg.) of the slightly rinsed slices were placed in the vessels and to some of these iodoacetic acid in concentration varying from $1{\cdot}5 \times 10^{-4}$ to $2{\cdot}4 \times 10^{-3}$ *M* was added. The slices were shaken in the manometer vessels in the thermostat for 70 mins. before adding the methylglyoxal solution (0·2 cc.) from the side-bulbs attached to the vessels. Definite inhibition of glyoxalase action was shown in the presence of even $1{\cdot}5 \times 10^{-4}$ *M* iodoacetate, whilst with 3 times this concentration the activity was only about 40 % of the controls. The activation on adding glutathione (0·1 mg.) to the contents of the vessels was very marked, though with the highest concentration of iodoacetate used ($2{\cdot}4 \times 10^{-3}$ *M*) the conversion of the methylglyoxal was only partially complete (Fig. 8).

It was found that this long preliminary treatment of the liver slices with iodoacetate is unnecessary. In the experiment represented in Fig. 9 the conditions were the same, but the methylglyoxal (not the same solution as in Fig. 6) was added to the tissues 10 minutes after the first reading (in all 20 minutes after adding the iodoacetate and placing in the bath). The curves show that there is a definite inhibition of glyoxalase activity in the intact tissue slices by $1{\cdot}5 \times 10^{-4}$ *M* iodoacetate, even after this short incubation time.

SUMMARY.

1. When neutral solutions of sodium iodoacetate and glutathione are mixed a reaction occurs which follows the bimolecular law and results in the elimination of iodide and the formation of a thio-ether. The behaviour of bromoacetate is similar but less vigorous, whilst that of chloroacetate is much less so; the respective velocity constants for the reaction at 38° are of the relative order, I:Br:Cl=15:9:0·15. With cysteine a similar reaction occurs, and the pure reaction product has been prepared in good yield under conditions of reaction and temperature not very different from the physiological.

2. The iodoacetate-inhibition of glyoxalase activity is reversed completely by addition of glutathione to the inactivated extract. It is therefore the coenzyme of glyoxalase that is inactivated by iodoacetate, the enzyme itself remaining undamaged. The concentration of iodoacetate necessary to inhibit glyoxalase preparations depends on the method of preparation of the extracts. With crude undialysed extracts high concentrations of iodoacetate (*N*/100) are necessary, whilst with the preparations subjected to thorough dialysis the amount of iodoacetate is nearly equivalent to the content of added glutathione, thus iodoacetate in about 10^{-4} *M* concentration or less may be sufficient to stop lactic acid formation from synthetic methylglyoxal in the dialysed extracts. This is of the same order of concentration as that found to inhibit the glyoxalase action of tissue slices, prepared and studied by Warburg's method. Hence it is once more shown that quantitative conclusions drawn from the behaviour of extracts may be misleading when applied to the intact tissues. The concentration of iodoacetate needed to check the conversion of synthetic methylglyoxal into lactic acid by intact tissues is of the same order as that [Krebs, 1931] required to stop the conversion of glucose into lactic acid by tissues.

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