The Inhibition of Thiol Enzymes by Lachrymators*

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This work was undertaken in order to throw light upon the biochemical mechanism of the lachrymatory effect of certain war gases. Owing to the relationship of one of these, ethyl iodoacetate, to the well known biochemical inhibitor, iodoacetic acid, the study was first directed towards enzymes known to be inhibited by this compound, namely, certain thiol enzymes, or enzymes whose activity appears to depend on the integrity of sulphhydryl groups. Iodoacetic acid inhibits muscle glycolysis and yeast fermentation by acting on the triose-phosphate dehydrogenase and the yeast alcohol dehydrogenase (Dixon, 1937; Rapkine, 1938; Adler, Gunther & von Euler, 1938). Dickens (1933), and Quastel (1933), showed that iodoacetic acid reacted with thiol groups in cysteine, reduced glutathione and proteins. Since then the list of thiol enzymes has been growing steadily (see Barron & Singer (1945) and Hellermann (1939) for reviews). The following work shows that the lachrymators react with simple thiol groups, that they inhibit almost all the enzymes generally considered to be of a thiol nature, that these enzymes can be protected by free sulphhydryl groups, and that most of the enzymes not affected by the lachrymators are believed on other evidence not to contain essential thiol groups. It is suggested that inhibition by the lachrymators may be used as a test for thiol enzymes in general.

METHODS

The lachrymators tested were as follows: ethyl iodoacetate, methyl iodoacetate, bromoacetophenone, chloroacetophenone, bromobenzyl cyanide and chloropicrin. They were dissolved (0.25M) in ethylene glycol monoethyl ether and in all cases the same amount of solvent was added to the control. The experiments were carried out at a temperature of 37° unless otherwise stated.

RESULTS

Reaction with enzymes

Succinic dehydrogenase

The system used for testing this enzyme was prepared from heart muscle, following the method used by Straub (1939) for the preparation of diaphorase, as far as the precipitation

at pH 4.6. This precipitate was suspended in a small amount of phosphate buffer pH 7.4, giving a highly active preparation containing all the enzymes necessary for the oxidation of succinic acid by oxygen, namely, succinic dehydrogenase, flavoprotein, cytochrome c and cytochrome oxidase. The enzyme was tested in Barcroft manometers, using about 0.1 ml. of enzyme suspension in 1 ml. phosphate buffer pH 7.6 and 1.6 ml. water. KOH papers were used to absorb any CO₂ formed. Sodium succinate (0.4 ml., 20 mg./ml.) was tipped from a Keilin tube at time 0 into the right-hand flask. The lachrymator was tipped in from another Keilin tube either with the substrate or at a given time before it.

Fig. 1 shows various results obtained. The action of the poison is progressive, so that the inhibitory effect depends on both the concentration of the

Fig. 1. The effect of lachrymators on the oxygen uptake of the succinic dehydrogenase—cytochrome system. Curve 1, control. Curve 2, m/10,000 ethyl iodoacetate. Curve 3, m/3000 ethyl iodoacetate. Curve 4, m/10,000 bromoacetophenone. Curve 5, m/15,000 chloropicrin. Curve 6, m/10,000 chloropicrin. The lachrymators were added with the substrate at time 0, and the curves show how the inhibition increases with time.

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poison and the time of incubation. The rate of inhibition falls off as the reaction between the enzyme and lachrymator proceeds, an equilibrium finally being reached if excess poison is not used.

Ethyl iodoacetate takes longer to reach equilibrium than the other lachrymators. The inhibitory reaction also depends on the presence of any protective substances, especially denatured enzyme, which inevitably occur in varying concentrations in such an enzyme preparation. Therefore any comparative work must be carried out on the same preparation and as far as possible at the same time. Table 1 shows the effects of the different lachrymators on the succinic dehydrogenase—cytochrome system.

Table 1. *Inhibition of succinic dehydrogenase—cytochrome system by lachrymators*

Poison	Conc.	In-cubation (min.)	In-hibition (%)
Bromobenzyl cyanide	M/3,000	0	70
		5	85
Ethyl iodoacetate	M/1,500	10	97
		M/3,000	90
		M/6,000	85
		M/12,000	60
Chloropierin	M/15,000	15	72
		M/50,000	68
		M/100,000	40
Chloroacetophenone	M/3,000	0	20
		5	42
		20	82

Fig. 2. The effect of lachrymators on succinic dehydrogenase. The cytochrome system has been inhibited by KCN, and methylene blue used in its place. ○—○ Control; □—□ M/10,000 ethyl iodoacetate; ×—× M/10,000 bromoacetophenone; △—△ M/10,000 chloropierin.

By adding 0.3 ml. 0.01M-neutralized KCN and 0.3 ml. of 0.5% methylene blue the cytochrome system is inhibited, and the succinic dehydrogenase remains the only active enzyme in the system,

methylene blue catalyzing the reaction with oxygen. Fig. 2 shows that the effect of the lachrymators on this system is just as great as before, thus making it clear that the lachrymators attack the dehydrogenase itself. Experiments on the oxidation of *p*-phenylenediamine by the cytochrome oxidase in the preparation show that this enzyme is not affected by M/1500 methyl iodoacetate.

Experiments on protection. It was found that addition of cysteine or reduced glutathione before adding the lachrymator protected the enzyme from the expected inhibition. Considerable excess, about nine times as much thiol as lachrymator, was necessary for complete protection. This may be due partly to the greater affinity of the lachrymator for the enzyme than for the simple thiol, as suggested below, but also it is known that the simple thiols are rapidly oxidized by the enzyme system here used.

Table 2 shows the results, the inhibition being calculated as far as possible from the initial rate over the first 5 min. The enzyme was incubated with the

Table 2. *Protection of succinic dehydrogenase by cysteine and reduced glutathione from the effects of the lachrymators*

Poison	Conc.	Conc. of GSH	Initial in-hibition (%)
Ethyl iodoacetate	M/5000	0	70
		M/2000	40
		0	85
Bromoacetophenone	M/9000	0	85
		M/3000	70
		M/2000	58
		M/1000	30
Chloropierin	M/9000	Conc. of cysteine	
		0	95
		M/1000	20
Bromoacetophenone	M/4500	0	85
		M/1000	25
Bromoacetophenone	M/9000	0	95
		M/3000	75
		M/1000	0

poison, with or without the thiol compound, for 20 min., so that the rate of increase in inhibition during the period of the experiment was usually fairly slow (cf. Fig. 3).

This protection could be used to determine accurately the inhibition produced by the lachrymator after a given time, because by tipping in excess cysteine or glutathione the poisoning could be arrested at any required point. For instance Fig. 3 shows the effect of adding excess glutathione to the succinic system after incubation for 20 min. with M/3000 ethyl iodoacetate. In the flask in which glutathione (GSH) has not been added the inhibition continues to increase, whilst in the flask in which GSH is added with the substrate, the inhibition is arrested, and remains at that level at

which it was in both experiments when the substrate was added. These results show that although GSH protects the enzyme, there is no reversal, and in fact no evidence of reversal was ever obtained in working with lachrymators on succinic dehydrogenase.

Fig. 3. Protection of succinic dehydrogenase from the effects of ethyl iodoacetate by reduced glutathione. $\times-\times$ Control; $\Delta-\Delta$ after 20 min. incubation with $m/3000$ ethyl iodoacetate, excess GSH is tipped in with the substrate at time 0; $\circ-\circ$ after incubation with ethyl iodoacetate the substrate alone is tipped in at time 0.

Since it thus appears that the poisoning is arrested immediately the thiol compound is added, it is possible to obtain figures for the relation between the time of action of the poison and the activity of the enzyme. Table 3 shows the results of adding cysteine ($m/1500$) at varying times after the addition of bromoacetophenone to succinic dehydrogenase. It appears that the rate of poisoning is not uniform, but falls off until no further poisoning can be obtained over a considerable period of time. This is the result that might be expected if the rate of inhibition depended on the concentrations of both the enzyme and the poison, and if both were destroyed during the reaction. However, destruction of the poison by something other than the enzyme would produce similar results, and it is clear from the slightly varying levels of inhibition produced by the poison on different preparations containing the same amounts of active enzyme that such destruction does occur. Only experiments with pure enzyme could clarify this point.

Table 3. Effect of terminating bromoacetophenone poisoning of succinic dehydrogenase with cysteine

Conc. of bromoacetophenone	Time of incubation before cysteine added (min.)	Activity remaining (%)
$m/82,000$: Exp. I	2	82
	5	45
	10	22
Exp. II	4	50
	8	45
	16	29
$m/37,000$	2	35
	5	17
	7	17

The most interesting protection is that by boiled enzyme. It was found that 0.2 ml. of boiled inactive enzyme would completely protect an equal amount of the active enzyme from poisoning by $m/9000$ bromoacetophenone, which without the boiled enzyme produced an inhibition of 95%.

Choline dehydrogenase

The enzyme was prepared from rat liver (Mann, Woodward & Quastel, 1938) by grinding in a mortar with phosphate buffer pH 7.4. The suspension was then homogenized in the Booth-Green bacterial mill for 15–20 min., centrifuged, and the fluid dialyzed against running tap-water for 1–2 hr. This preparation keeps for about 2 days at 0° . The oxidation of choline using 1 ml. of enzyme was followed either by oxygen uptake in Barcroft manometers or by methylene blue reduction in evacuated Thunberg tubes. The preparation contained all the factors necessary for the oxidation of choline by oxygen.

Table 4 shows the effects of the lachrymators after 20 min. incubation. As with succinic dehydrogenase, the inhibition was progressive, and the results show the initial inhibition when the substrate was first added, measured over the first 5–10 min.

Table 4. The effects of lachrymators on choline dehydrogenase

Conc. of poison	Inhibition (%)
A. In Barcroft manometers:	
Chloroacetophenone $m/2500$	65
$m/1500$	95
Bromobenzyl cyanide $m/2500$	80
Bromoacetophenone $m/2500$	90
$m/4500$	90*
Ethyl iodoacetate $m/1500$	—
(No incubation)	20
(20 min. incubation)	80
B. In Thunberg tubes:	
Bromobenzyl cyanide $m/6000$	50
$m/3000$	60
$m/2000$	75

* Obtained with a specially purified preparation.

Table 5 shows the protection afforded by cysteine against $M/1500$ chloroacetophenone, after 20 min. incubation with the poison, as measured in Barcroft manometers. In this experiment it was found that 0.2 ml. of boiled enzyme was sufficient to give complete protection.

Table 5. Protection of choline dehydrogenase by cysteine against $M/1500$ chloroacetophenone

Conc. of cysteine	Inhibition (%)
0	95
$M/500$	82
$M/250$	45

Yeast alcohol dehydrogenase

The enzyme was prepared by the first step of the method of Negelein & Wulff (1937), which gives a very active preparation. Juice prepared from dried yeast was heated, cooled and a half volume of acetone was added to the centrifugal fluid. The precipitate was discarded and a further half volume of acetone was added. This precipitate, containing the enzyme, was dried over H_2SO_4 and dissolved in water for use. It was tested in Thunberg tubes with methylene blue. In order to complete the system, cozymase and diaphorase were added and semicarbazide to remove the acetaldehyde formed. The enzyme was incubated with the lachrymators for 15 min. before the ethanol and dye were tipped from the stopper.

Table 6 shows that the lachrymators have a considerably greater effect on the enzyme than has iodoacetate.

Papain

British Drug Houses papain was used, and activated by adding 30 mg. cysteine in solution at pH 4.8 to 5 ml. of 0.5% papain solution. The substrate used was fresh milk plus 0.2 vol. of 0.2M-acetate buffer, the mixture having a pH of 4.6.

The reaction with ethyl iodoacetate is complicated by the necessity of activating papain with cysteine which would react with the iodoacetate; while if the papain and iodoacetate were incubated together before addition of cysteine, it seemed likely, from what is already known about papain, that the thiol group in the enzyme might be masked in a disulphide linkage. This proved to be the case, as the following results show. Activated papain (1 mg.) clotted the milk in 2 min., whereas 10 mg. natural papain

were necessary for this clotting time. When incubated with 0.01M-ethyl iodoacetate before activation and then treated with $M/60$ cysteine, 20 mg. of papain were necessary for the same speed of clotting, whereas if first activated with 0.02M-cysteine, incubated with 0.02M-ethyl iodoacetate and then treated with 0.04M-cysteine, no activity remained.

This showed that the papain could be regarded as less susceptible to the ethyl iodoacetate before activation, as was to be expected if in the inactive form the thiol group were combined in an S—S linkage.

Fig. 4. The effect of the lachrymators on pyruvic oxidase of *Esch. coli*. Incubation 20 min. Curve 1, control and with $M/1600$ sodium iodoacetate. Curve 2, $M/6000$ ethyl iodoacetate. Curve 3, $M/1600$ methyl iodoacetate. Curve 4, $M/3000$ chloropicrin. Curve 5, $M/3000$ bromoacetophenone.

Pyruvic oxidase

The enzyme was prepared from *Esch. coli* by the method of Still (1941) and tested in Barcroft manometers as described by him. Fig. 4 shows the results.

Table 6. The effect of lachrymators on yeast alcohol dehydrogenase.

	Time of reduction of methylene blue		Inhibition (%)
	min.	sec.	
Control	1	45	—
+ Na iodoacetate ($M/3000$)	2	35	60
+ ethyl iodoacetate ($M/3000$)	> 60	—	100
+ ethyl iodoacetate ($M/6000$)	8	—	85
+ bromoacetophenone ($M/3000$)	> 60	—	100
+ chloropicrin ($M/3000$)	> 60	—	100
+ bromobenzyl cyanide ($M/3000$)	> 60	—	100
+ chloroacetophenone ($M/3000$)	8	—	85

Triosephosphate dehydrogenase

A 5% suspension of muscle acetone powder, prepared as described by Green, Needham & Dewan (1937), was centrifuged and the soluble portion used as the source of the enzyme and of the other enzymes necessary for the testing reaction. The substrate was added in the form of hexose-diphosphate solution, which is rapidly converted into triose-

phosphate by the zymohexase present in the extract. This latter enzyme is not inhibited by the lachrymators. The reaction was carried out in Barcroft manometers in bicarbonate solution equilibrated with a gas mixture containing 5% CO₂ in N₂. Coenzyme I was added as hydrogen carrier, and pyruvate to accept the hydrogen removed from the triosephosphate. The phosphoglyceric acid formed was measured by the CO₂ evolution.

Fig. 5.

Fig. 6.

Fig. 5. The effect of lachrymators and sodium iodoacetate on triosephosphate dehydrogenase. Incubation 20 min. Curve 1, control for curves 2 and 3. Curve 2, m/3000 chloropicrin. Curve 3, m/3000 bromoacetophenone. Curve 4, control for curves 5 and 6. Curve 5, m/3000 sodium iodoacetate. Curve 6, m/3000 ethyl iodoacetate.

Fig. 6. The effect of methyl iodoacetate and sodium iodoacetate on xanthine oxidase. Incubation 15 min. Curve 1, control. Curve 2, m/660 sodium iodoacetate. Curve 3, m/660 methyl iodoacetate.

Table 7. *The effect of lachrymators on triose phosphate dehydrogenase*

Poison	Conc.	Inhibition (%)
Na iodoacetate	m/3000	90
	m/5000	60
Methyl iodoacetate	m/3000	90
	m/5000	50
Ethyl iodoacetate	m/3000	90
Chloropicrin	m/3000	20
Bromobenzyl cyanide	m/2000	45
Bromoacetophenone	m/2000	50
	m/3000	30
Chloroacetophenone	m/1000	30

Table 8. *The effect of the lachrymators on xanthine oxidase, tested in Thunberg tubes*

Poison	Time of reduction of methylene blue		Inhibition (%)
	min.	sec.	
None	—	50	—
Bromobenzyl cyanide (m/1200)	10	—	93
Bromoacetophenone (m/625) (m/2400)	8	—	85
	4	30	80
Chloropicrin (m/1200) (m/500)	1	45	66
	3	—	75

This enzyme differs from all the other thiol enzymes in being quite as sensitive to the iodoacetate ion as to the lachrymatoms. Fig. 5 shows the effects of $m/3000$ iodoacetate and some of the lachrymatoms. The two experiments recorded here have different controls owing to the varying activity of the muscle powder. Because the control reaction is not linear, the degree of inhibition is not readily determined, but Table 7 shows the initial inhibitions obtained after 20 min. incubation with the lachrymatoms.

Xanthine oxidase

The enzyme was used in the form of a whey preparation as described by Dixon & Kodama (1926) and the oxidation of hypoxanthine by oxygen followed in Barcroft manometers. Portions of 1.6 ml. of a 10% solution of the enzyme were added to both flasks and also 0.05 ml. of an active preparation of catalase, to destroy any hydrogen peroxide formed. Hypoxanthine (1 mg.) was added from a Keilin tube at time 0. The enzyme was incubated with methyl iodoacetate for 15 min. before adding the substrate.

Fig. 6 shows the effect of $m/660$ methyl iodoacetate as compared with the same concentration of sodium iodoacetate.

The other lachrymatoms were tested by the Thunberg tube method, using methylene blue. Table 8 shows the results.

Cholinesterase

The enzyme was prepared in a purified form from horse serum by the method of Stedman & Stedman (1935). The hydrolysis of acetylcholine was followed in Barcroft manometers, by measuring the amount of CO_2 set free from bicarbonate-Ringer solution by the free acid formed. After 15 min. incubation with the lachrymatoms 3 mg. of acetylcholine in neutralized solution were tipped in.

Table 9 shows the effects of the lachrymatoms on this enzyme after 30 min. incubation. It is clear that it is much less sensitive than any of the previous enzymes. A crude extract of rabbit's brain, prepared by grinding the brain with sand and pressing through muslin, was also tested, but the brain enzyme was found to be unaffected by $m/1250$ bromoacetophenone.

Table 9. The effect of lachrymatoms on cholinesterase from horse serum

Poison	Conc.	Inhibition (%)
Bromobenzyl cyanide	$m/900$	25
	$m/365$	70
Bromoacetophenone	$m/1250$	Nil
	$m/365$	60
Chloropicrin	$m/365$	Nil
Ethyl iodoacetate	$m/600$	Nil

Urease

Urease tablets (0.1 g.) were extracted with 10 ml. phosphate buffer pH 7.4 and the insoluble part filtered off. The activity was measured by the method of Sumner (1926), slightly modified. 1 ml. of a solution containing 5.4 g.

K_2HPO_4 , 4.25 g. KH_2PO_4 and 0.15 g. urea/100 ml. was added to 1 ml. of the enzyme solution, and after standing at room temperature for the required time, the ammonia content of the samples was estimated with Nessler solution.

It was found that after 15 min. incubation with $m/400$ ethyl iodoacetate 50% inhibition was produced, while $m/600$ bromoacetophenone gave 80% inhibition. The enzyme was therefore not as sensitive as might be expected from its known thiol nature, but this might have been due to impurities in the solution.

Carboxylase

The CO_2 liberated by dried yeast extract acting on pyruvate was measured in Barcroft manometers. An inhibition of about 20% was obtained by incubating the yeast with $m/1500$ ethyl iodoacetate for 15 min. before adding the substrate.

Glyoxalase

The enzyme was prepared from horse liver and tested by the method of Lohmann (1932). The methylglyoxal was placed in the flasks of Barcroft manometers with the enzyme, the glutathione being tipped in from Keilin tubes.

Fig. 7. The effect of the lachrymatoms on glyoxalase and its coenzyme, reduced glutathione. Curve 1, enzyme and 50 $\mu g.$ GSH. Curve 2, 50 $\mu g.$ GSH incubated with 300 $\mu g.$ bromoacetophenone before addition to the enzyme. Curve 3, $m/2300$ bromoacetophenone incubated with the enzyme, before 400 $\mu g.$ GSH were added. Curve 4, 50 $\mu g.$ GSH incubated with 500 $\mu g.$ ethyl iodoacetate before being added to the enzyme. Curve 5, 50 $\mu g.$ GSH incubated with 260 $\mu g.$ chloropicrin.

Fig. 7 shows the results of treating the enzyme or the coenzyme, reduced glutathione, with lachrymatoms. In curve 1, 50 $\mu g.$ of GSH were added to

the enzyme from a Keilin tube at time 0. Curve 2 shows the effect of incubating the GSH in the Keilin tubes with 300 μ g. of bromoacetophenone before tipping. The concentration of lachrymator was c. $m/70$ in the Keilin tube and, after tipping, $m/2300$ in the flask. In curve 3, $m/2300$ bromoacetophenone was incubated with the enzyme and then 400 μ g. of GSH were tipped in. While the incubation of the lachrymator with the GSH has almost completely destroyed the activity, treatment of the enzyme appears not to inhibit. This is due to the fact that the rate depends partly on the concentration of GSH. Curves 4 and 5 show the destruction of GSH by incubating 50 μ g. GSH with 500 μ g. ethyl iodoacetate and 260 μ g. chloropicrin respectively. It is clear that all three lachrymators destroy GSH.

Metabolism

Muscle respiration

Pigeon-breast muscle or rat-leg muscle was minced in a Latapie mincer with the grinding die removed, and suitable amounts (usually about

taken. The method was that of Stare & Baumann (1936). The lachrymators were tipped in during the experiment so that the rates of O_2 uptake of each portion of tissue both before and after the addition of the poison were known. Portions of chopped rat-leg muscle were similarly treated.

Fig. 8 shows the effects on the respiration of rat-leg muscle of adding 0.0005M-bromoacetophenone at the point indicated by the arrow. While the control continued at the rate already established, the muscle treated with the lachrymator immediately ceased to respire. Similar results were obtained with pigeon-breast muscle, of which the respiration was almost completely inhibited by $m/1500$ ethyl iodoacetate.

Yeast fermentation

The fermentation of glucose by baker's yeast was followed in Barcroft manometers by measuring the CO_2 produced in the absence of O_2 , the flasks being filled with N_2 freed from O_2 by passing over heated copper. The lachrymators were tipped in from Keilin tubes so that accurate measure of the time of incubation could be obtained.

All the lachrymators, except chloropicrin, inhibit glucose fermentation by yeast, the inhibition being progressive and irreversible. The inhibition produced by 0.0002M-ethyl iodoacetate, bromoacetophenone or bromobenzyl cyanide becomes almost complete in about 20 min. Chloroacetophenone produces about 60% inhibition in 40 min. The inhibition produced by 0.0002M-chloropicrin reached 30% in 5 min., but did not increase during the next 20 min. The reason for this will be explained in the next section.

Yeast respiration in glucose

The oxygen uptake of baker's yeast was measured in Barcroft manometers, KOH papers being used to absorb the CO_2 formed. 17 mg. of glucose were used as substrate although the respiration would normally continue without added glucose. In order to measure carbohydrate respiration solely the yeast was starved for 24 hr., when the residual respiration without added glucose was very small.

Table 10 shows the inhibition produced after 30 min. incubation, the initial rate of respiration at this time being determined. The inhibition of respiration was progressive, except with chloropicrin. When starved yeast was treated with $m/750$ methyl iodoacetate, it was found that the poison had no effect on the small oxygen uptake (30 μ l. in 90 min.) which occurred without the addition of a substrate, but when 17 mg. of glucose were added, the great increase in respiration (200 μ l. in 15 min.) which took place in the control was completely absent in the poisoned yeast, whose respiration remained at the same level as the control yeast without added glucose.

Fig. 8. The inhibition of rat-leg muscle respiration by bromoacetophenone. ○—○ Control; ×—× as control at the beginning, $m/2000$ bromoacetophenone being added at the arrow.

200 mg.) were weighed directly into the manometer flasks. The solution contained 1 mg. of succinate to stabilize the respiration. Any non-cyclic oxidation of succinate would be finished before readings were

Table 10. *The effect of the lachrymators on yeast (glucose) respiration*

(Conc. poison, M/6000; incubation 30 min.)

Poison	Inhibition (%)
Chloropicrin	40
Chloroacetophenone	70
Ethyl iodoacetate	82
Bromobenzyl cyanide	88
Bromoacetophenone	95
Ethyl iodoacetate, incubation 10 min.	
Conc.	Inhibition (%)
M/1,500	85
M/3,000	80
M/6,000	75
M/12,000	65
M/24,000	55
M/48,000	35

It will be noticed from this table that chloropicrin, in marked contrast to its effect on other enzymes, has only a very slight effect on yeast. The reason for this appears to be that yeast can destroy chloropicrin; when treated with 0.001M-chloropicrin, the initial inhibition of 60% is no longer shown after washing on the centrifuge, and the poisoned yeast becomes as active as the control. Similar treatment of ethyl iodoacetate-poisoned yeast produces no reactivation. Moreover, if M/750 chloropicrin is incubated with yeast for 2 hr. and then the solution is added to the succinic dehydrogenase system, no inhibition at all is obtained, showing that the chloropicrin must have been completely destroyed. Chloropicrin incubated without yeast—and bromoacetophenone incubated with yeast—both gave complete inhibition of the dehydrogenase system. This is the only case found in dealing with lachrymators in which there is evidence of reversal of the inhibition, and it seems likely that it is due to a special enzyme in the yeast, which can remove the chloropicrin from its combination with the inhibited enzyme.

Table 11 shows the enzymes which have been found to be unaffected by the lachrymators in the concentrations shown.

Table 11. *Enzymes not affected by the lachrymators*

Enzyme	Poison	Conc. tested
Cytochrome oxidase	Methyl iodoacetate	M/1500
Carbonic anhydrase	Bromoacetophenone	M/1500
Diaphorase	Methyl iodoacetate	M/300
D-Amino-acid oxidase	Methyl iodoacetate	M/750
Zymohexase	Methyl iodoacetate	M/1500
Glucose dehydrogenase	Ethyl iodoacetate	M/1500
Lactic dehydrogenase (muscle)	Methyl iodoacetate	M/1500
Malic dehydrogenase	Methyl iodoacetate	M/300

DISCUSSION

The foregoing experiments suggest that the action of the lachrymators on the body may be due to inhibition of thiol enzymes, many of which, such as succinic dehydrogenase and pyruvic oxidase, are known to play an important part in the general metabolism of the body. From an analogy with the iodoacetate ion (Dickens, 1933), it is probable that the lachrymators combine irreversibly with the enzyme, their toxicity being dependent on the halide group. They are far more toxic to almost every enzyme than iodoacetic acid, and can be regarded as reliable thiol reagents, when applied in low concentrations. Triosephosphate dehydrogenase shows a marked exception to this generalization, for it is as sensitive to ionic iodoacetate as to its esters, while the other lachrymators have a smaller effect. The only example observed of reversibility is in the inhibition of yeast by chloropicrin.

On the assumption that the lachrymators react only with the thiol group it is suggested that xanthine oxidase, an enzyme not hitherto known to belong to the thiol class, should be included in this class. It has long been known that this enzyme is very sensitive to oxidation, especially by hydrogen peroxide (Dixon & Kodama, 1926), and recently Mann & Quastel (1946) have shown that it is inhibited by oxygen at 1 atm. They suggest that this might be due to the formation of hydrogen peroxide, but in the experiments shown in this paper the peroxide was destroyed by catalase, so that it is more probable that the oxidase is a true thiol enzyme. This is the only flavoprotein which appears to be sensitive to the lachrymators.

There are many enzymes which are completely insensitive to the lachrymators, such as cytochrome oxidase and lactic dehydrogenase, and all workers are agreed that these enzymes are insensitive to all thiol reagents. There are a few enzymes which are only slightly sensitive, such as cholinesterase. Nachmansohn & Lederer (1939) suggested that this was a thiol enzyme, but they found it no more sensitive to thiol reagents than it is to the lachrymators. Finally there is the considerable class of thiol enzymes, which are very sensitive to lachrymators. Dickens (1946) has shown how these enzymes are sensitive to high oxygen pressures, and among them he includes choline oxidase, shown here to be a thiol enzyme. He finds that D-amino-acid oxidase is not sensitive to high oxygen pressure, nor is it sensitive to the lachrymators, although it is sensitive to arsenicals (Barron & Singer, 1945).

The lachrymators appear to react more readily with proteins containing thiol groups than with simple thiols, such as cysteine. While about nine molecules cysteine/molecule lachrymator are required for complete protection, the denatured

enzyme preparation will give protection in concentrations which contain no appreciable thiol groups demonstrable by the nitroprusside test. The combination with the denatured enzyme preparation must have been much faster than with the active enzyme, since if there had been any appreciable competition some of the latter must have been destroyed, and this protective action might be expected on the theory that denaturation increases the number of reactive thiol groups; denatured egg white, however, although it is known to contain numerous thiol groups, had no protective power.

The experiments with papain support the theory that this enzyme contains a thiol group which in the inactive form is probably present as S—S. When the partially inactive form was treated with ethyl iodoacetate and activated after this treatment, the inhibition was not complete. In contrast to this, when the papain was first activated with cysteine and then poisoned, complete inhibition was obtained, although excess cysteine was added after the poison.

The action of yeast on chloropicrin is of considerable interest. It appears that the yeast is capable of destroying the chloropicrin, and that washing can produce complete reactivation of the yeast. As this is the only case in which reversal has been obtained,

experiments might be carried out on other enzymes, to see whether yeast could reactivate them when they have been poisoned with chloropicrin. It suggests that reversal might be a question of finding a reactant with a sufficiently powerful affinity for the lachrymator.

SUMMARY

1. An account is given of the effects of various lachrymators on enzyme systems. It is shown that they inhibit all the enzymes which are generally accepted as thiol enzymes, the inhibition being progressive and irreversible in all cases except that of chloropicrin on yeast.

2. The enzymes can be protected against the inhibition by reduced glutathione, cysteine, or denatured enzyme. A considerable excess of the simple thiols is required for complete protection.

3. It is shown that the lachrymators inhibit the respiration of muscle. They also inhibit equally the fermentation and respiration of glucose by yeast.

4. It is suggested that the lachrymators act like iodoacetate in that they combine irreversibly with thiol groups in the enzyme, but they are much more powerful than iodoacetate.

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* Information available on application to Ministry of Supply, London.