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Effects of Methyl Bromide on Phosphorus Metabolism in the Adult Housefly, *Musca domestica* L.

By F. P. W. WINTERINGHAM, G. C. HELLYER AND M. A. MCKAY
*Department of Scientific and Industrial Research, Pest Infestation Laboratory,
 London Road, Slough, Bucks*

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The distribution and biochemical significance of the acid-soluble phosphorus compounds of insect tissues closely resemble those of higher animals. For example, the principal intermediates which accumulate in rat muscle (LePage, 1948) are also those found in the thoracic muscle of the housefly (Winteringham, Bridges & Hellyer, 1955*a*). Chefurka (1954) has demonstrated in homogenates of housefly tissue many of the glycolytic enzymes of the Embden-Meyerhof scheme. Oxidative phosphorylation has been demonstrated with sarcomeres isolated from blowfly muscle by Lewis & Slater (1954). Adenosine triphosphate (ATP) recovered from various insect species appears to be identical with that recovered from vertebrates (Albaum & Kletzkina, 1948; Calaby, 1951). It also appears to play a comparable role in muscle contraction (Gilmour & Calaby, 1953*a*; Maruyama, 1954*a, b*) and in the synthesis of acetylcholine (Smallman, 1956). There are, however, some differences between the phosphorus metabolism of insects and higher animals which might be significant in the mechanisms of toxic action in the intact animal. For example, Sacktor (1955) and Zebe & McShan (1957) have observed a remarkably low lactic acid dehydrogenase activity in some insect-muscle preparations and have suggested that lactic acid may not accumulate as an end product of glycolysis as it does in vertebrate skeletal muscle. An earlier intermediate such as a triose phosphate may enter the Krebs cycle more directly. Arginine phosphoric acid (Arg-P) plays the part of the phosphagen of insects, in common

with other invertebrates (Baldwin & Needham, 1934), instead of the creatine phosphoric acid of vertebrates. There is evidence that certain of the glycolytic enzymes of insect and vertebrate tissues may differ in their sensitivity to inhibitors. For example, Chefurka (1954) was unable to find any inhibition of housefly triose phosphate dehydrogenase by 0.1 mM-iodoacetate, which inhibits the mammalian enzyme (Cori, Slein & Cori, 1948). This paper describes a quantitative study, by means of the labelled-pool technique (Winteringham, 1956), of the effects of methyl bromide on the phosphorus metabolism of the adult housefly, *Musca domestica* L., *in vivo*. For a preliminary communication see Winteringham & Hellyer (1954).

METHODS AND MATERIALS

Labelling and extraction. Techniques (Winteringham *et al.* 1955*a*) for labelling with ³²P the soluble intermediates of adult houseflies *in vivo*, and for their extraction, have been used with minor modifications. Entire thoraces (minus wing and leg appendages) or abdomens have been homogenized and extracted three times with 1.05 ml. portions of ethanolic formic acid instead of 1.8 ml. portions as originally described, and concentration of the extracts *in vacuo* has been omitted. This modification was without significant effect on either recovery or distribution of the labelled compounds. Entire heads have similarly been extracted three times with 0.55 ml. portions of ethanolic formic acid.

Paper-radiochromatographic techniques. The techniques described earlier (Winteringham *et al.* 1955*a*) have been used with slight modification. Resolution of the ATP-adenosine diphosphate (ADP) fraction in the aq. acetic acid-acetone solvent originally described has been omitted in the

more recent work. It was found that when ADP occurred in significant proportions it was sufficiently well separated in the initial run in the aq. formic acid-acetone solvent, with a mean R_f value of 0.24 compared with the mean value of 0.13 for ATP. In cases of doubt unlabelled ADP was added as carrier before chromatography and located either chemically or in ultraviolet light on the final paper chromatograms. By a determination of the proportion of the total ^{32}P activity within the located boundaries of the added carrier it was possible to set an upper limit for the corresponding labelled compound recovered from the insect. This has been indicated in the results below by $\times$. A similar procedure has been adopted for other compounds in cases of incomplete resolution. In some experiments no attempt was made to resolve a composite fraction and the principal constituents of such fractions have been bracketed together in the tabulated results (see below).

Chemical identity of labelled fractions. Further hydrolysis and cochromatographic experiments with authentic compounds have confirmed the identities of the principal fractions reported earlier (Winteringham *et al.* 1955*a*), but the description of a fraction as a particular compound must not be taken to exclude the presence of other unidentified compounds in relatively small but possibly variable proportions. For example, in the ATP fraction there was an unidentified compound (UC. 1) which on hydrolysis for 10 min. in N-HCl liberated less of its ^{32}P activity as inorganic phosphate (PO_4^{3-}) than did ATP under the same conditions. In the ammoniacal methanol solvent used for resolving the composite fraction containing arginine phosphoric acid (Arg-P), adenylic acid (AMP) and glucose 6-phosphate (G 6-P), a second fraction (UC. 2) ran with AMP. This was not inosinic acid (IMP). A third compound (UC. 3) occurring chiefly in abdominal extracts, ran faster than G 6-P in ammoniacal methanol but was not fructose 6-phosphate. A fourth fraction (UC. 4) ran faster than the PO_4^{3-} fraction in the aq. formic acid-acetone solvent or was absent. In a few cases there was evidence that UC. 4 was present in a relatively small proportion and incompletely separated from the PO_4^{3-} fraction. This has been indicated by brackets in Table 3. The recent application of these techniques to the study of the distribution of phosphorus in sarcosomes isolated from the adult blowfly, *Calliphora erythrocephala*, suggests that UC. 4 is a phospholipid associated with the sarcosomes of the thoracic tissues and liberates glycerophosphate on hydrolysis. UC. 1 may similarly be coenzyme I, which also appears to be associated with the sarcosomes and represents less than 2% of the total soluble phosphorus of the blowfly thorax (S. E. Lewis & G. M. Price, personal communication).

Insects and their treatment. Female adult houseflies (*Musca domestica* L.), 3-8 days old at the time of extraction, were used throughout. The flies were drawn from the Laboratory stock, which has been maintained by continuous breeding over a period of years. All experiments were conducted at 30°. For studying the effects of toxic vapours groups of 10 flies, which had been fed with $^{32}\text{PO}_4^{3-}$, were anaesthetized for 2 min. with cyclopropane, transferred rapidly to a closed stainless-steel-gauze cage, allowed to recover completely and then immersed for the required time in a jar containing air saturated with the vapour. The exposed flies were then observed in fresh air or were immediately plunged into liquid nitrogen for dissection and extraction.

RESULTS

Mean distributions of phosphorus in normal housefly tissues. These have been determined in 49 groups of 10 adult female houseflies representing 30 different generations. The distribution of phosphorus in any extract has been taken as the mean of at least two paper-radio-chromatographic analyses (see below). The mean distributions found in the thoracic extracts (representing flight muscle), with the ranges and standard deviations, are summarized in Table 1. The mean distributions found in the head and abdominal extracts are based on 11 and 7 groups respectively. It is assumed on the basis of uniformity of labelling (see Winteringham *et al.* 1955*a*) that the distribution of ^{32}P will be the same as that of the natural phosphorus. In all cases the insects were 'labelled' and 'metabolized' for 24 hr. periods at 30° and, where necessary, brought to a state of rest by cyclopropane anaesthesia for 2 min. The mean distribution of total soluble phosphorus between the different body regions is also shown in Table 1.

Variation in replicate analyses. In order to test the reproducibility of the technique for the analysis of the labelled fractions 20 radio-chromatographic analyses was made on the same extract under routine conditions. In practice at least two analyses are made on each extract, so that the calculated standard error of the means of all possible pairs of results for a particular fraction will indicate the order of variation to be expected as a result of the analytical technique itself. The results are summarized in Table 2. The extract used in this test had been prepared from a group of flies which had been exposed to continuous cyclopropane anaesthesia for some hours, and accounts for the low ATP value.

Significance of results. The distributions found in the tissues of the treated insects have been compared with those found in normal insects (Table 1). Significance has been assessed by the *t* test and the corresponding level of probability indicated. Two groups of insects were used in experiments to study the effects of a toxic substance: a control group and a treated group whose treatments were identical except for exposure to the toxic substance. The control group provided a check on the condition of the insects used for the experiment and on the possible effects of the additional manipulation associated with exposure to the toxic substance. The results for a control group might therefore differ significantly from those of Table 1. For example, if an indirect result of exposure to the toxic substance was the inability of the insect to feed then the control insects would also be starved so that indirect effects of starvation might be assessed. In four instances the control-group

Table 1. *Distribution of soluble phosphorus in tissues of the adult housefly*

Fraction	³² P of fraction as % of the total soluble ³² P ± s.d.	Range
Head		
ATP + UC. 1	35.9 ± 6.8	25.5–45.8
ADP	4.9 ± 2.2	0 – 7.6
AMP + UC. 2	4.3 ± 1.4	1.9– 6.6
G 6-P	10.6 ± 2.8	6.7–15.2
UC. 3	0.8	0 – 3.1
Arg-P	6.2 ± 1.7	3.4– 8.5
PGA	13.0 ± 3.8	8.8–18.5
PO ₄ ³⁻	20.9 ± 4.2	13.4–28.7
UC. 4	3.4	0 – 9.5
Thorax		
ATP + UC. 1	34.7 ± 4.8	25.4–44.9
ADP	3.7 ± 2.4	0 –11.4
AMP + UC. 2	3.6 ± 1.9	0 –11.0
G 6-P	9.1 ± 3.2	3.6–17.9
UC. 3	Trace	—
Arg-P	10.5 ± 3.4	2.8–19.0
PGA	20.9 ± 4.6	11.2–33.1
PO ₄ ³⁻	16.8 ± 4.7	6.7–27.8
UC. 4	0.7	0 – 7.0
Abdomen		
ATP + UC. 1 } ADP } AMP + UC. 2 } + G 6-P + UC. 3 } + Arg-P }	21.6 ± 3.1	18.1–27.4
PGA } PO ₄ ³⁻ } + UC. 4 }	4.9 ± 1.4	3.0– 7.2
	56.5 ± 5.0	45.8–59.9
Mean ³² P in body regions as % of the total soluble ³² P	{ Head 4.7 { Thorax 40.4 { Abdomen 54.9	

Table 2. *Variation in replicate analyses of the same extract*

Fraction	³² P of fraction as % of total soluble ³² P ± s.e. of means of pairs
ATP + ADP + UC. 1	26.0 ± 1.1
AMP + Arg-P + G 6-P + UC. 2 + UC. 3	24.1 ± 1.7
PGA	24.9 ± 1.5
PO ₄ ³⁻ + UC. 4	25.0 ± 0.9

distributions of soluble phosphorus differed significantly from those shown in Table 1 and in such a way as to complicate the interpretation of differences found in the treated groups (see below). The thoracic ATP and ADP fractions of the control group in Expt. 4, Table 3, were significantly low and high respectively ($P < 0.01$) and the thoracic G 6-P was significantly high ($P < 0.02$) in the control groups of Expt. 7, Table 3. The levels of thoracic Arg-P were unusually high in Expts. 4 and 5, Table 3.

Effects of methyl bromide

The results are shown in Table 3. Two or more fractions have been bracketed when their resolution

was inadequate or not attempted. When the insects were immobilized and not allowed to recover (Expts. 1–10) there were significant and sometimes severe depletions of head and thoracic ATP fractions ($P < 0.05$). In Expt. 4 the thoracic ATP of the control group was also significantly low (see above), but methyl bromide had nevertheless caused a highly significant ($P < 0.01$) depletion of the combined ATP + ADP fraction; the corresponding fraction of the control group did not differ significantly from the normal mean of Table 1. In Expts. 2, 3, 5, 6, 7 and 10 there were significant ($P < 0.05$) increases in thoracic AMP and corresponding increases in the PO₄³⁻ fractions. In Expt. 8 there were also significant ($P < 0.01$) increases in the head AMP and PO₄³⁻ fractions.

These results strongly suggest that immobilization of the insect was associated with a breakdown of ATP according to the equation:

This is shown by the data of Table 4, which are derived from the treated groups of Expts. 1–10, Table 3, and the normal groups of Table 1. It will be seen that there is reasonable agreement between

the average changes in the AMP fraction and those calculated on the basis of the above equation and on the assumption that ADP changes according to the equation $\text{ADP} \rightleftharpoons \text{AMP} + \text{PO}_4^{3-}$. The possible effects of changes in UC. 1 and UC. 2 on the results of Table 4 have been neglected. In Expt. 6 of Table 3, however, the increase in the AMP + UC. 2 fraction was shown by cochromatography with the authentic compound and by acid hydrolysis to be due entirely to AMP.

Significantly high values for thoracic G 6-P were observed in the heavily poisoned insects of Expts. 6 and 7 ($P < 0.01$) and in Expt. 9 ($P < 0.05$). There was evidence therefore that excessive doses of methyl bromide may cause an increase in the level of thoracic G 6-P but this effect was not observed consistently (cf. Expt. 10, Table 3).

Except in Expts. 4 and 5 the level of thoracic Arg-P was reduced by exposure to methyl bromide when the insects were not allowed to recover (Expts. 1-10) and was apparently associated with the fall in ATP, the largest effect on Arg-P being observed in Expt. 7, which showed the largest depletion of ATP. If, on the other hand, the unusually high levels of thoracic Arg-P in the control groups of Expts. 4 and 5 are accepted the results of these two experiments also fall into line. Significant ($P < 0.05$) and highly significant ($P < 0.01$) falls in the levels of thoracic phosphoglycerate (PGA) were observed only after excessive exposures to methyl bromide of 10 min. or longer (Expts. 6, 8, 9 and 10). The reduction of the level of PGA clearly proceeded more slowly than that of the high-energy phosphates ATP and Arg-P. The free inorganic PO_4^{3-} fraction of head, thoracic or abdominal tissues was always increased as the result of the breakdown of ATP and Arg-P.

Effects of ethylene dichloride and iodoacetic acid

The results of some experiments with ethylene dichloride (1:2-dichloroethane) vapour and iodoacetic acid are shown in Table 5. It will be seen that narcosis with ethylene dichloride had little effect on the distribution of thoracic phosphorus except for an increase in the free inorganic phosphate fraction. Iodoacetate, on the other hand, caused marked and significant depletions of ATP and PGA in thoracic tissues, the depletion of PGA being consistent with iodoacetate acting as an inhibitor of triose phosphate dehydrogenase. There was a consequent accumulation of free inorganic phosphate. Significant ($P < 0.02$) accumulations of thoracic G 6-P were consistent with the early observation of Lundsgaard (1930) that a hexose phosphate accumulated in the iodoacetate-poisoned muscle of the frog. The breakdown of thoracic ATP in the iodoacetate-poisoned fly was accompanied by the formation of AMP. ATP was

significantly reduced in the head tissues ($P < 0.05$). The treated head and control thoracic Arg-P were significantly high ($P < 0.02$) in Expt. 3. This may have been connected with the fact that both groups in this experiment had also received relatively massive injections of fluid, the effect being masked in the treated thorax by the local action of the iodoacetate.

DISCUSSION

Methyl bromide readily and irreversibly methylates certain free and protein amino acids, especially the SH groups of cysteine and reduced glutathione, the $\text{S}\cdot\text{CH}_3$ group of methionine, and the imidazole group of histidine (Blackburn, Consden & Phillips, 1944; Winteringham & Harrison, 1946; Lewis & Eccleston, 1946; Lewis, 1948; Winteringham, Harrison, Bridges & Bridges, 1955*b*; Bridges, 1955).

The rapid blocking of the free SH groups of the tissues of *Calliphora erythrocephala* larvae *in vivo* by high concentrations of methyl bromide vapour has been demonstrated (Loveday & Winteringham, 1951). Consistent with these findings is the fact that methyl bromide has been shown to inhibit several SH enzymes *in vitro*, including yeast hexokinase (Dixon & Needham, 1946), urease and mammalian succinic dehydrogenase (Lewis, 1948). The wide dependence of glycolysis and pyruvate oxidation on SH enzymes and the SH groups of cofactors like coenzyme A and the reduced form of lipoic acid suggest that methyl bromide might be expected profoundly to disturb basal metabolism *in vivo* and to resemble in its action other SH reagents such as iodoacetate. It remains to discuss the present results against this background.

Typically, when adult houseflies are exposed to a lethal but not excessive dose of methyl bromide vapour in air, as in Expts. 1-3 and 11-13 of Table 3, they immediately collapse and appear to be deeply anaesthetized. On removal to fresh air they recover within minutes and behave normally for 1-3 hr. when, invariably, they collapse again and fail to recover. After the initial exposure to methyl bromide the flies respire at a normal or slightly supranormal rate for several hours. Respiration then gradually falls off. When the flies are exposed to an excessive lethal dose (as in Expts. 6-10, Table 3) there is no temporary recovery in fresh air and respiration falls off immediately. This is illustrated by the results shown in Fig. 1 (F. P. W. Winteringham & A. Weatherley, unpublished work). Similar observations have been made by Bond (1956), who found that the oxygen uptake of larvae or adults of *Tenebrio mauritanicus* was unaffected during exposure to LD_{50} doses of methyl bromide vapour, despite a considerable

Table 3. *Effects of methyl bromide on the distribution of the soluble phosphorus in the tissues of the adult housefly in vivo*

³² P of fraction as % of total soluble ³² P recovered								
Expt. no.	Treatment and signs of poisoning at the time of freezing and extraction	Fraction	Head		Thorax		Abdomen	
			Control	Test	Control	Test	Control	Test
1*	1 min. exposure to methyl bromide; complete collapse; immediate extraction. Similar unextracted group recovered within minutes but majority collapsed again some hours later and never recovered	ATP + UC. 1			36.3	23.0		
		ADP			0	0		
		AMP + UC. 2				6.6		
		G 6-P			25.6	14.9		
		UC. 3				0		
		Arg-P				5.7		
		PGA			21.1	19.3		
		PO ₄ ³⁻			17.0	30.5		
		UC. 4						
		2	As in Expt. 1	ATP + UC. 1			40.0	20.8
ADP					0	0		
AMP + UC. 2					5.0	9.7		
G 6-P						10.0		
UC. 3					0	0		
Arg-P					18.5	7.4		
PGA					17.6	18.7		
PO ₄ ³⁻					18.9	33.4		
UC. 4								
3*	As in Expt. 1			ATP + UC. 1			27.0	11.4
		ADP			3.2	3.1		
		AMP + UC. 2			4.1	7.5		
		G 6-P			9.7	8.4		
		UC. 3			0	0		
		Arg-P			9.2	2.8		
		PGA			16.0	15.8		
		PO ₄ ³⁻			25.0	49.7		
		UC. 4			5.8	1.3		
		4	2 min. exposure to methyl bromide; complete collapse; immediate extraction. Similar unextracted group recovered within minutes but collapsed again some hours later and never recovered	ATP + UC. 1	33.9	19.4	21.1	18.0
ADP	4.3			8.0	10.7	4.4	3.7	2.1
AMP + UC. 2	3.0			4.7	2.8	3.7	1.6	1.8
G 6-P	6.2			13.0	7.3	9.2	7.1	4.2
UC. 3	4.0			2.8	4.6 (?)	0	7.4	4.6
Arg-P	5.7			8.9	14.6	14.1	2.5	3.7
PGA	10.6			7.7	19.9	11.9	3.7	3.6
PO ₄ ³⁻	27.6			28.6	16.2	32.0	56.8	67.8
UC. 4	4.7			6.9	2.8	6.7	2.8	1.8
5	5 min. exposure to methyl bromide; complete collapse; immediate extraction. Similar unextracted group failed to recover in fresh air			ATP + UC. 1			31.3	10.0
		ADP			0	0		
		AMP + UC. 2			8.9	15.2		
		G 6-P			7.8	8.2		
		UC. 3			0	0		
		Arg-P			20.8	10.5		
		PGA			18.8	17.5		
		PO ₄ ³⁻			12.4	38.6		
		UC. 4			Trace	Trace		
		6	10 min. exposure to methyl bromide; complete collapse; immediate extraction. Similar unextracted group failed to recover in fresh air	ATP + UC. 1			39.0	13.2
ADP					0	2.1		
AMP + UC. 2					4.3	21.2		
G 6-P					8.8	17.9		
UC. 3					0	0		
Arg-P					10.1	3.1		
PGA					16.0	8.5		
PO ₄ ³⁻					21.8	34.0		
UC. 4					0	0		
7	As in Expt. 6			ATP + UC. 1			38.6	4.9
		ADP			0	3.4		
		AMP + UC. 2			1.1	10.7		
		G 6-P			17.9	24.2		
		UC. 3			Trace	0		
		Arg-P			10.4	0		
		PGA			18.7	19.9		
		PO ₄ ³⁻			13.3	36.9		
		UC. 4			0	0		

Table 3 (cont.)

³² P of fraction as % of total soluble ³² P recovered								
Expt. no.	Treatment and signs of poisoning at the time of freezing and extraction	Fraction	Head		Thorax		Abdomen	
			Control	Test	Control	Test	Control	Test
8	As in Expt. 6	ATP + UC. 1	45.2	8.3	31.0	7.7		
		ADP	0	5.0	6.4	2.4		
		AMP + UC. 2	3.2	12.6	3.2	5.4		
		G 6-P	10.9	6.4	6.7	5.3		
		UC. 3	Trace	Trace	0	0		
		Arg-P	3.4	3.2	10.9	3.2		
		PGA	13.2	14.2	23.6	10.7		
		PO ₄ ³⁻						
		UC. 4	24.1	50.3	18.2	65.3		
9	60 min. exposure to methyl bromide; immediate extraction. Similar unextracted group failed to recover	ATP + UC. 1			36.3	14.0		
		ADP			0	0		
		AMP + UC. 2				6.5		
		G 6-P			25.6	16.2		
		UC. 3				0		
		Arg-P				4.3		
		PGA			21.1	5.4		
		PO ₄ ³⁻			17.0	53.6		
		UC. 4						
10	120 min. exposure to methyl bromide; immediate extraction. Similar unextracted group failed to recover	ATP + UC. 1			27.0	6.6		
		ADP			3.2	0		
		AMP + UC. 2			4.1	11.3		
		G 6-P			9.7	6.5		
		UC. 3			0	0		
		Arg-P			9.2	7.2		
		PGA			16.0	7.0		
		PO ₄ ³⁻			25.0	58.8		
		UC. 4			5.8	2.6		
11*	1 min. exposure to methyl bromide; followed by 60 min. aeration before extraction. All flies had recovered from initial collapse. Similar unextracted group finally collapsed some hours later	ATP + UC. 1			40.0	35.2		
		ADP			0	0		
		AMP + UC. 2			5.0	6.8		
		G 6-P				6.5		
		UC. 3			0	0		
		Arg-P			18.5	11.9		
		PGA			17.6	8.5		
		PO ₄ ³⁻			18.9	31.1		
		UC. 4						
12	2 min. exposure to methyl bromide; followed by aeration. All flies recovered but collapsed 2 hr. or more later. Each fly was frozen and extracted on final collapse and an extract of 10 thoraces was pooled	ATP + UC. 1			27.0	29.9		
		ADP			3.2	2.7		
		AMP + UC. 2			4.1	4.7		
		G 6-P			9.7	4.9		
		UC. 3			0	0		
		Arg-P			9.2	12.2		
		PGA			16.0	13.9		
		PO ₄ ³⁻			25.0	26.8		
		UC. 4			5.8	4.9		
13*	1 min. exposure to methyl bromide; followed by aeration. All flies recovered but collapsed 1 hr. or more later. Each fly was frozen and extracted 3 hr. after final collapse	ATP + UC. 1	32.8	18.7	31.3	17.8	24.2	15.1
		ADP	3.8	5.9	3.5	3.8	3.2	3.9
		AMP + UC. 2	1.9	3.8	3.7	4.7	0.8	1.9
		G 6-P	13.3	8.2	7.8	10.4	7.3	5.3
		UC. 3	0	3.4	0	0	5.2	4.3
		Arg-P	8.1	10.6	16.3	9.5	6.3	6.1
		PGA	18.5	11.3	19.6	9.7	7.2	6.6
		PO ₄ ³⁻	20.0	38.1	17.8	43.4	45.5	56.8
		UC. 4	1.6	0	0	0.7	0.3	0

* A 1 min. exposure to methyl bromide was not invariably lethal. One or two insects of an exposed group failed to undergo the delayed collapse and survived. These insects were rejected.

reduction in muscular activity, and that respiration fell off only gradually thereafter.

It will be seen from Table 3 that immediately after exposing adult houseflies to methyl bromide for 1 min. there was a fall in the thoracic ATP level. This would account for the observed prostration of the insect at this stage, and differs from anaesthetic action because houseflies may be deeply anaesthetized with cyclopropane without significantly affecting the level of thoracic ATP (Winteringham *et al.* 1955a). The ATP level of flies completely immobilized by exposure to the vapour of ethylene dichloride for 6 min. was also unaffected, as shown in Table 5. Another difference between anaesthetized flies and those immobilized with methyl bromide was that the legs and wings of the former would still respond to a single direct electrical

Table 4. *Effect of methyl bromide on tissue nucleotides of the adult housefly in vivo*

Changes are expressed as percentage of total soluble ^{32}P .

	Thorax	Head
Average observed change in ATP	-21.7	-22.0
ADP	-2.2	+1.6
AMP	+6.2	+4.4
Calculated change in AMP	+8.3	+6.5

Fig. 1. Effects of just-lethal and excessive doses of methyl bromide vapour on the oxygen uptake of the adult housefly. ○, Control; ●, 1 min. exposure; ■, 15 min. exposure.

Table 5. *Effects of ethylene dichloride vapour and injected iodoacetic acid on the distribution of the soluble phosphorus in the tissues of the adult housefly in vivo*

		^{32}P of fraction as % of total soluble ^{32}P recovered from body region							
Expt. no.	Treatment and signs of poisoning at the time of freezing and extraction	Fraction	Head		Thorax		Abdomen		
			Control	Test	Control	Test	Control	Test	
1	6 min. exposure in air saturated with ethylene dichloride vapour. All flies were motionless after 5 min.; immediate extraction. Similar unextracted group recovered completely in fresh air and showed no delayed effects	ATP + UC. 1			38.4	35.1			
		ADP			0	0			
		AMP + UC. 2			4.7	4.0			
		G 6-P			17.1	9.0			
		UC. 3			0	0			
		Arg-P			9.1	5.6			
		PGA			17.9	15.9			
		PO_4^{3-}			12.8	30.4			
		UC. 4							
2	Injected intrathoracically with 5 μg . of iodoacetic acid in 1 μl . of acetone. Flies prostrated after about 2 hr. were immediately extracted. Remaining flies survived and were rejected	ATP + UC. 1			36.7	17.0			
		ADP			0	0			
		AMP + UC. 2			0	6.5			
		G 6-P			11.6	16.6			
		UC. 3			0	0			
		Arg-P			13.5	6.3			
		PGA			15.1	5.8			
		PO_4^{3-}			23.1	47.8			
		UC. 4							
3	Injected intrathoracically with 10 μg . of iodoacetic acid in 2 μl . of water. All flies were motionless after 1 hr.; extracted 2 hr. after injection	ATP + UC. 1			30.5	11.8	22.5	25.1	
		ADP	31.9	20.8	3.7	0	0	2.2	
		AMP + UC. 2		7.3	1.8	10.0			
		G 6-P		3.7	6.5	18.9			
		UC. 3	22.9	7.4	0	0	13.8	21.0	
		Arg-P		—	0	0			
		PGA		21.6	19.0	8.4			
		PO_4^{3-}	17.6	9.1	15.1	6.8	4.6	7.2	
		UC. 4	19.4	26.7	20.4	42.6	59.1	44.5	
			8.2	3.4	0	1.5	0	0	

stimulus, whereas those of the latter would not, presumably because of the depletion of muscle ATP (Winteringham & Bridges, 1955). A possible explanation of the initial fall in ATP was acute anoxia in the presence of a high concentration of methyl bromide vapour. Anoxia caused a severe depletion of ATP and phosphagen in rabbits (Marcotte-Boy & Cheymol, 1953). On the other hand, the level of thoracic ATP was little affected in flies which were drowned at room temperature by submerging them in water containing a wetting agent until all struggles and movement ceased. The fall in ATP as a result of exposure to methyl bromide thus suggests an inhibition of phosphorylation. The corresponding formation of thoracic AMP and PO_4^{3-} but not ADP (see Table 4) would be explained by the combined action of adenosine triphosphatase and myokinase (Sacktor, 1953; Gilmour & Calaby, 1953*b*; Maruyama, 1954*b*). The absence of chromatographic evidence of formation of IMP may be due to the absence of an insect adenylic acid deaminase (Gilmour & Calaby, 1953*a*). The parallel recovery of ATP with the activity of the insect (Expt. 11, Table 3) on removal to fresh air is not so readily explained in view of the irreversible nature of alkylation and enzyme inhibition by methyl bromide.

Considering the delayed collapse following the 1 or 2 min. exposures, it will be seen that when the flies finally collapsed (Expt. 12, Table 3) the distribution of thoracic phosphorus was not markedly affected except for an increase in PO_4^{3-} of borderline significance ($P=0.05$). Respiration was also normal at this stage (Fig. 1). The delayed collapse cannot then be explained on the basis of the inhibition *in vivo* of SH enzymes of glycolysis and respiration or the blocking of coenzyme A or lipoic acid function. This is also consistent with the fact that the proportion of $^{14}\text{CO}_2$ recovered in 24 hr. (45 %) from intact flies which had been intrathoracically injected with $[2-^{14}\text{C}]\text{acetate}$ at the time of the final collapse was actually higher than that (38 %) recovered from flies which had not been exposed to methyl bromide (F. P. W. Winteringham, unpublished work). The possibility of there being a local inhibition of glycolysis at the triose phosphate level in nerve tissue is not supported by the fact that in Expt. 13, Table 3 there was no significant effect on the PGA of the head, which is relatively rich in nerve tissue and poor in muscle.

The action of the brief (1–2 min.) but lethal exposures of the insects to methyl bromide does not resemble that of iodoacetate, which inhibits glycolysis at the triose phosphate dehydrogenase level (Cori *et al.* 1948). When adult houseflies were injected intrathoracically with a lethal but not excessive dose of iodoacetic acid in acetone or

water (Table 5) the majority of flies collapsed within about 2 hr. and failed to recover. Respiration similarly fell off rapidly.

Iodoacetate caused significant ($P<0.01$) reductions in thoracic ATP and PGA in immobilized insects, the latter reduction being an expected consequence of triose phosphate dehydrogenase inhibition (Expts. 2 and 3, Table 5). This is in agreement with Bettini & Boccacci (1954, 1955, 1956*a, b*). They were able closely to relate insect mortality due to iodoacetate with the inhibition of triose phosphate dehydrogenase *in vivo* and *in vitro* but not with the inhibition of succinic dehydrogenase or with the blocking of coenzyme A function, and this despite the apparent insensitivity of insect triose phosphate dehydrogenase to iodoacetate reported by Chefurka (1954). An effective reduction in insects of carbohydrate and pyruvate oxidation by iodoacetate would be expected from the classical work with vertebrate tissues by Lunds-gaard (1931) and by Peters and his colleagues (see Peters & Wakelin, 1946). The expected reduction in oxidative phosphorylation combined with the insensitivity of insect actinomyosin adenosine triphosphatase and soluble adenosine triphosphatase to iodoacetate (Maruyama, 1954*b*) would then account for the fall in ATP shown in Table 5.

Excessive doses of methyl bromide, from which the insects never recovered (Expts. 6–10, Table 3), usually caused a reduction in thoracic ATP, Arg-P and PGA with corresponding increases in PO_4^{3-} , but the pattern of changes in the head tissues of the same insects was not necessarily similar (Expt. 8, Table 3). Respiration also ceased (Fig. 1). The action of excessive doses of methyl bromide may therefore be to block SH enzymes and contingent respiration and so resemble the action of iodoacetate.

The results of Expt. 13 (Table 3) show that 3 hr. after the final collapse after just-lethal exposure to methyl bromide there were delayed but significant depletions of ATP ($P<0.01$) and of PGA ($P<0.05$), but these appeared to be too delayed to account for the final collapse of the insect (cf. Expt. 12, Table 3). Such delayed falls in ATP, PGA and other phosphorylated intermediates have been observed, moreover, in flies exposed to lethal doses of non-SH-reactive substances, and to extreme starvation, and may be non-specific and indirect consequences of the dying cell.

This study provides no evidence that the immediate and delayed signs of poisoning in the adult housefly briefly exposed to a lethal but not excessive dose of methyl bromide vapour can be explained in terms of SH enzyme inhibition or the blocking of the SH groups of coenzyme A or reduced lipoic acid. The action of lethal but not excessive doses of methyl bromide in the adult

housefly apparently differs therefore from that of iodoacetate. There is evidence, however, that excessive doses of methyl bromide resemble iodoacetate in their action as a triose phosphate dehydrogenase inhibitor and cause a marked and irreversible depletion of phosphoglycerate and muscle ATP *in vivo*.

SUMMARY

1. Adult female houseflies were exposed to a saturation concentration of methyl bromide vapour for 1 min. (lethal but not excessive exposures) or for longer periods (excessive exposures) at 30°.

2. Insects exposed for 1 or 2 min. were immobilized but recovered temporarily in fresh air. These insects finally collapsed again 1-3 hr. later and failed to recover. Respiration continued at a near normal rate for several hours after the delayed collapse.

3. Initial immobilization of the insect by brief exposure to methyl bromide was apparently due to a reversible breakdown of adenosine triphosphate (ATP) according to the overall equation: $\text{ATP} \rightleftharpoons \text{adenosine 5'-phosphate} + 2\text{PO}_4^{3-}$.

4. Excessive exposures to methyl bromide immediately and irreversibly immobilized the insect. Such exposures were associated with depletions of ATP, arginine phosphoric acid and phosphoglyceric acid.

5. Temporary recovery of the insects after brief exposures was associated with a return towards normal levels of thoracic ATP.

6. The reversible effects of brief exposures on the level of ATP, the delayed collapse of the insect without marked changes in distribution of soluble phosphorus and the sustained respiration were not consistent with the action of methyl bromide as an SH-enzyme inhibitor.

7. The effects of excessive exposures to methyl bromide resembled those of lethal but not excessive doses of iodoacetate characterized by depletion of ATP and phosphoglycerate in the thoracic tissues.

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