The Oxidative Activity of Particulate Fractions from Mosquitoes

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Watanabe & Williams (1951, 1953) were the first to perform biochemical studies on insect sarcosomes. Evidence was provided for the operation of the citric acid cycle in sarcosomes of the housefly *Musca domestica* (Sacktor, 1953, 1954, 1955; Chefurka, 1955) and of the blowfly *Phormia regina* (Lewis & Slater, 1954). It was also shown that oxidation is accompanied by the esterification of inorganic phosphate. The patterns of oxidation were further explored in the mitochondrial and supernatant fractions of the honey-bee *Apis mellifera* (Hoskins, Cheldelin & Newburgh, 1956).

As part of a study on the metabolism of insects and on the mode of action of insecticides, the respiration and oxidative phosphorylation of washed particles obtained from the mosquito *Aedes aegypti* have been investigated.

MATERIALS AND METHODS

Colony of Aedes aegypti *L.* This was maintained by a slight modification of the method of Casanges, McGovern & Chiles (1949). The mosquitoes were kept at 28° and 80% relative humidity and fed on 5% sucrose solution and water.

Materials. Nembutal [sodium ethyl-(1-methylbutyl)-barbiturate] was a product of the Abbott Laboratories. Reduced cytochrome *c* was prepared by the reduction of the commercial product with $Na_2S_2O_4$, followed by aeration. All other materials used in the present investigation were the same as described elsewhere (Avi-Dor & Mager, 1956).

Isolation of the respiratory particles. Mosquitoes (6 g.) of mixed sexes, 4–10 days old, were immobilized by cooling to 0–3° and crushed for 3 min. with a cooled pestle and mortar. The procedure was carried out in the cold room (1°). The standard extraction medium (5 ml./g. of mosquitoes) consisted of sucrose (0·33 M), human serum albumin (0·6%) and ethylenediaminetetra-acetic acid (EDTA, 10^{-4} M) adjusted to pH 7·4 with NaOH. The resulting brei was squeezed through four layers of gauze and centrifuged at 600 *g* to remove unbroken cells and cell fragments, and the particles remaining in the supernatant were sedimented at 8000 *g* for 7 min. After two washings with 70 ml. of the extraction medium the particles were suspended in the same medium so that 1 ml. contained 20–25 mg. (dry wt.) of the particles ('respiratory particles'). A turbidimetric measurement was used as a rapid check of the particle concentration. Approx. 35 mg. of particles was obtained from 1 g. of mosquitoes.

'Aged particles' were obtained by incubating the respiratory particles for various times at 30°, centrifuging at 8000 *g* for 7 min. and resuspending in the original volume of extraction medium.

'Homogenized particles' were prepared by disintegrating the standard preparation in a 'Virtis 45' homogenizer at 0°. The additional treatment was the same as that for the 'aged particles'.

Measurement of oxidative phosphorylation. The main compartment of the Warburg flasks contained all the components with the exception of glucose and hexokinase. The latter components were added from the side arm after incubation for 7 min. Initial manometer readings were taken 2 min. after the addition and the O_2 uptake was followed for 30 min. The reaction was then stopped by adding 0·3 ml. of 50% (w/v) trichloroacetic acid, and the contents of the flasks were centrifuged. Inorganic phosphate was determined in samples of the supernatant by the method of Lowry & Lopez (1946). The controls, which served as a basis for the calculation of the esterified inorganic phosphate, were deproteinized 2 min. after the addition of glucose and hexokinase. Correction was applied for the phosphate liberated from added adenosine triphosphate (ATP) by ATPase during the time of the experiment (6–7 μmoles of phosphate in 30 min.).

Analytical methods. Nitrogen was determined according to Johnson (1941). The ATPase assay was carried out by determining the phosphate liberated from ATP under the standard conditions of the experiment.

The reduced diphosphopyridine nucleotide (DPNH) was generated by a system containing 0·33 μmole of DPN, 0·1 ml. of ethanol, 0·3 ml. of yeast alcohol dehydrogenase and 90 μmoles of semicarbazide hydrochloride. The assay of the DPNase activity of the respiratory particles, and all other standard procedures used, were essentially the same as those previously described (Mager & Avi-Dor, 1956; Avi-Dor & Mager, 1956).

All other methods are referred to in the text.

RESULTS

It was shown by Watanabe & Williams (1951) that a gentle grinding of the thorax of the blowfly results in the liberation of a considerable proportion of sarcosomes from the myofibrils. The integrity and activity of the isolated respiratory granules are conditioned by the composition of the extraction medium. To avoid shrinkage, swelling and fuzzy degeneration control of the tonicity by sucrose, and addition of protective agents such as proteins and ethylenediaminetetra-acetic acid (EDTA), was advocated by various authors (Watanabe & Williams, 1951, 1953; Slater & Cleland, 1953; Sacktor, 1954; Lewis & Slater, 1954). The effect of variations in the method of preparation of the particles from mosquitoes was therefore investigated.

Effect of the composition of the extraction medium and the reaction mixture

The concentrations of sucrose or EDTA could be varied considerably in both the extraction medium and the final reaction mixture without affecting the rate of oxidation of α-oxoglutarate by the respiratory particles (Table 1). Omission of the sucrose or the chelating agent resulted, however, in a lowered respiratory rate. Inclusion of human serum albumin or other proteins also proved to be favourable.

Effect of time of grinding, number of washings, ageing and homogenization of the particles

Lewis & Slater (1954) found that blowfly sarcosomes were sensitive to variations in the time of grinding during the isolation procedure. With the mosquito, increasing the time of grinding from 1 to 3 min. had no noticeable effect, and the yield of the respiratory granules was considerably higher (Table 2). Repeated washing of the particles did not affect the respiration. Ageing of the particles at 30° lowered the rate of oxidation of α-oxoglutarate more than that of fumarate. Similarly, the oxidation of α-oxoglutarate was more sensitive to mechanical injury caused by homogenization (Table 2).

Cofactor requirements

The standard particle preparation required only the addition of ATP and Mg^{2+} ions for the rapid oxidation of α-oxoglutarate. ATP could be replaced partially by adenosine diphosphate or monophosphate and Mg^{2+} by Mn^{2+} ions. Calcium ions moderately inhibited the respiration (Table 3). Ageing of the particles increased the ATP and Mg^{2+} ion requirement and necessitated the addition of DPN (0·06 μmole/flask) to restore the full rate of respiration. Disruption of the particles in a 'Virtis 45' homogenizer produced a phosphate-dependent system. The rate of oxidation of the substrates by the particle fragments increased with the phosphate concentration. Arsenate could be substituted for phosphate in approximately equimolar concentration. In contrast with these findings, similar concentrations of arsenate slightly inhibited the respiration of the intact particles. In the damaged preparation coenzyme A stimulated the oxidation of α-oxoglutarate. Lipoic acid and cytochrome *c* had no effect.

Oxidation of various substrates and oxidative phosphorylation

The rate of oxidation of citric acid-cycle members and related compounds is shown in Table 4. All the citric acid-cycle intermediates were utilized. It is noteworthy, however, that neither β-hydroxybutyrate nor choline chloride, which are usually oxidized by mammalian mitochondria, was metabolized.

The P:O ratios obtained with the standard

Table 1. *Effect of the composition of the extraction medium and the reaction mixture on the uptake of oxygen*

Each flask contained 100 μmoles of 2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer, pH 7·4, 8 μmoles of ATP, 8 μmoles of $MgCl_2$, 0·33 μmole of DPN, 30 μmoles of α-oxoglutarate, 0·5 ml. of a suspension of the respiratory particles (approx. 4 mg. of protein) and sucrose, EDTA and protein, as indicated. Total vol. 3 ml. The centre well contained 0·2 ml. of 20% KOH. Gas phase, air. Incubation temp., 28°.

Extraction medium			Reaction mixture			
Sucrose (M)	Albumin (%)	EDTA (M)	Sucrose (M)	Albumin (%)	EDTA (M)	Uptake of O_2 (μmoles/hr.)
0	0·6	10^{-4}	0·22	0·1	10^{-4}	3·2
0·22	0·6	10^{-4}	0·22	0·1	10^{-4}	8·5
0·33	0·6	10^{-4}	0·22	0·1	10^{-4}	8·8
0·50	0·6	10^{-4}	0·22	0·1	10^{-4}	8·5
0·88	0·6	10^{-4}	0·22	0·1	10^{-4}	5·1
0·33	0·6	10^{-4}	0·06	0·1	10^{-4}	9·1
0·33	0·6	10^{-4}	0·22	0·1	10^{-4}	8·4
0·33	0·6	10^{-4}	0·33	0·1	10^{-4}	8·1
0·33	0·6	10^{-4}	0·50	0·1	10^{-4}	7·5
0·33	0·6	10^{-4}	0·88	0·1	10^{-4}	6·5
0·33	0·6	0	0·22	0·1	0	4·7
0·33	0·6	0	0·22	0·1	10^{-4}	6·1
0·33	0·6	10^{-4}	0·22	0·1	10^{-4}	8·9
0·33	0·6	10^{-3}	0·22	0·1	10^{-4}	7·0
0·33	0·6	10^{-2}	0·22	0·1	10^{-4}	4·2
0·33	0·6	10^{-4}	0·22	0·1	10^{-4}	9·0
0·33	0·6	10^{-4}	0·22	0·1	10^{-3}	6·7
0·33	0·6	10^{-4}	0·22	0·1	10^{-2}	3·5
0·33	0	10^{-4}	0·22	0	10^{-4}	4·2
0·33	0·6	10^{-4}	0·22	0·1	10^{-4}	8·5
0·33	1·2	10^{-4}	0·22	0·2	10^{-4}	8·0

particle preparation were fairly constant (Table 5). The mechanically damaged phosphate-dependent preparation did not esterify inorganic phosphorus.

Effect of inhibitors on respiration and oxidative phosphorylation

The oxidation of α-oxoglutarate, succinate and DPNH was found to be sensitive to potassium cyanide (10^{-4} M) and sodium azide (10^{-2} M). Alkylating SH reagents like iodoacetate, iodoacetamide and fluoropyruvate suppressed the oxidation of α-oxoglutarate, but did not reduce significantly the activity of the succinic oxidase or DPNH oxidase. In contrast with the selective inhibitory activity displayed by the alkylating compounds, *p*-chloromercuribenzoate, which forms mercaptides with thiol compounds, and antimycin A inhibited the oxidation of all three substrates tested (Table 6).

The classical uncoupling agent of oxidative phosphorylation, 2:4-dinitrophenol, disconnected nearly completely the respiration from phosphorylation at a concentration of 5×10^{-5} M. Nembutal was less effective in this respect, in accordance with the findings of Brody & Bain (1954) for mammalian brain mitochondria. Chloretone decreased the respiration and the phosphorylation nearly in the same proportion. Phosphorylation was more affected than respiration by the insecticide 2:2-bis-(*p*-chlorophenyl)-1:1:1-trichloroethane (DDT) (Table 7).

Table 2. *Effect of variations in the method of preparation of the respiratory particles on the rate of uptake of oxygen*

The extraction medium contained 0·33 M-sucrose, 0·6% human serum albumin and 10^{-4} M-EDTA. The composition of the reaction mixture was: 0·22 M-sucrose, 0·1% albumin, 10^{-4} M-EDTA, 100 μmoles of tris buffer, pH 7·4, 40 μmoles of phosphate buffer, pH 7·4, 8 μmoles of ATP, 8 μmoles of $MgCl_2$, 0·33 μmole of DPN and 30 μmoles of the substrate ('standard reaction mixture'). Homogenization and ageing of the particles were carried out as described in the Methods section. The amount of particles added, on the basis of dry wt., was approximately equal in each (12 mg.). Conditions of the experiments were otherwise as described in Table 1.

Treatment of the particles	Uptake of O_2 (μmoles/hr.)	
	α-Oxoglutarate	Fumarate
Grinding		
1 min.	7·7	7·8
3 min.	7·2	7·6
Homogenization*		
Low speed	5·9	7·8
Medium speed	1·6	5·8
High speed	0·6	4·1
Washing		
Once	6·9	7·9
Twice	6·7	7·6
Three times	6·5	6·2
Ageing		
None	6·3	7·6
15 min.	5·4	7·1
30 min.	0·4	5·0

* Low speed means 15 000 rev./min., medium speed 30 000 rev./min., and high speed 45 000 rev./min., in a 'Virtis 45' homogenizer.

Spectrophotometric demonstration of individual enzymes

Spectrophotometric methods were used to demonstrate in the mosquito particles the presence of some of the enzymes which form part of the citric acid-cycle system in mammalian mitochondria and also some components of the terminal respiratory chain. The presence of a very active malic dehydrogenase could be shown by following the rate of decrease of optical density at 340 mμ on the addition of oxaloacetate to a reaction mixture containing DPNH (Fig. 1). With malate as the substrate, a rapid increase of optical density at 240 mμ (see Racker, 1950) indicated a potent furmarase activity (Fig. 2).

The oxidation of DPNH by the respiratory particles could be revealed either by measuring the decrease of optical density at 340 mμ (Fig. 3) or by using cytochrome *c* (Fig. 4) or 2:6-dichlorophenol-indophenol as electron acceptors (Fig. 5).

Cytochrome oxidase activity was assayed by measuring the rate of oxidation of reduced cytochrome *c* at 550 mμ (Fig. 6).

ATPase and DPNase activity of the respiratory particles

The respiratory particles showed significant ATPase activity. Under the standard conditions of the reaction (see Table 2), 1·7 μmoles of phosphate/mg. of protein were split in 30 min. Omission of Mg^{2+} ions from the reaction mixture diminished slightly the ATPase activity (1·5 μmoles of phosphate/mg. of protein), while replacement of the Mg^{2+} ions by an equimolar amount of Ca^{2+} ions increased somewhat the rate of liberation of inorganic phosphate (2·2 μmoles of phosphate/mg. of protein).

Under the conditions of the experiment described in Table 2, approx. 50% of the added DPN disappeared (0·18 μmole) during incubation for 30 min. This activity is 30 times smaller than the value found for the rate of destruction of the pyridine nucleotide by guinea-pig-kidney mitochondria under similar conditions and is comparable with the DPNase activity of pig-heart-muscle preparations (Mager & Avi-Dor, unpublished results).

Table 3. *Effects of phosphate, arsenate, cations and cofactors on the rate of uptake of oxygen*

The concentration of some of the components in the standard reaction mixture (see Table 2) was varied as indicated, and the effect of additional cofactors was tested. For the method of preparation of the untreated respiratory particles, aged particles (15 min. at 30°) and homogenized particles, see Methods section and Table 2. The amount of the particles added, on the basis of dry wt., was approximately equal in each (12 mg.). Conditions of the experiments were otherwise as in Table 1. AMP, Adenosine monophosphate; ADP, adenosine diphosphate; CoA, coenzyme A.

		Uptake of O_2 (μmoles/hr.)					
		Untreated particles		Aged particles		Homogenized particles	
Variable component	Concn. (μmoles)	α-Oxo-glutarate	Fumarate	α-Oxo-glutarate	Fumarate	α-Oxo-glutarate	Fumarate
Phosphate	0	8·0	9·0	7·0	8·3	1·5	1·7
	5	—	—	—	—	3·7	4·1
	10	—	—	—	—	4·0	5·2
	40	8·1	9·1	7·1	8·3	5·0	6·2
	100	7·6	8·8	6·8	8·8	4·9	5·9
Phosphate replaced by arsenate	10	—	—	—	—	2·5	3·0
	40	6·0	6·8	5·8	6·2	4·1	5·1
$MgCl_2$	0	4·8	5·1	3·0	4·0	1·7	2·4
	2	6·8	7·6	5·8	7·6	4·5	4·5
	8	8·0	9·0	7·2	8·0	5·0	5·1
	20	8·0	8·8	7·0	8·2	5·0	4·8
$MgCl_2$ replaced by $CaCl_2$	8	3·1	4·2	1·8	3·3	1·8	1·8
$MgCl_2$ replaced by $MnSO_4$	8	5·4	6·2	4·1	5·1	3·4	3·2
DPN	0	7·3	6·8	4·1	3·3	2·0	1·5
	0·06	7·4	8·3	7·0	7·8	4·8	3·8
	0·33	7·8	8·3	7·2	8·2	5·0	5·0
	0·66	7·8	8·2	7·8	8·3	6·5	6·2
ATP	0	2·7	3·1	1·2	2·3	2·3	1·7
	3	6·9	8·4	5·8	6·8	2·1	3·6
	8	8·2	8·8	7·2	8·3	5·0	5·6
ATP replaced by AMP	8	5·5	4·6	5·0	4·3	4·6	4·2
ATP replaced by ADP	8	7·2	7·0	6·8	7·0	4·8	4·6
CoA	0	8·1	8·8	7·2	7·8	5·0	6·2
	500 μg.	7·9	8·6	7·4	7·6	7·6	6·3

Table 4. *Oxidation of citric acid-cycle intermediates and related compounds by respiratory particles*

The composition of the reaction mixture was as in Table 2. Conditions of the experiment were as in Table 1.

Substrate	Uptake of O_2 (μmoles/hr.)
Acetate	3·8
Pyruvate	3·8
Citrate	4·7
(±)-*iso*Citrate	4·2
α-Oxoglutarate	7·8
L-Glutamate	5·6
DPNH	8·1
Succinate	8·5
Fumarate	9·2
L-Malate	6·9
Oxaloacetate	3·7
β-Hydroxybutyrate	0·0
Choline chloride	0·0
None	0·0

Table 5. *Oxidative phosphorylation*

The main compartment of each flask contained the components listed in Table 2. Glucose (100 μmoles) and hexokinase (100 K.M. units*) were added from the side arm after incubation for 7 min. at 28°. Determination of the inorganic phosphate esterified was carried out in the way referred to under Methods. Conditions of the experiment were otherwise as in Table 1.

Substrate	Uptake of O_2 (μg.atoms/30 min.)	Inorganic phosphorus esterified (μg.atoms/30 min.)	Ratio, P:O
α-Oxoglutarate	7·8	17·9	2·3
Succinate	8·5	12·0	1·4
L-Malate	6·9	11·0	1·6

* Kunitz & McDonald (1946).

Table 6. *Effect of inhibitors on the respiration of the standard particle preparation*

Standard reaction mixture was as described in Table 2. The inhibitors were added at the concentrations indicated. Conditions of the experiment were otherwise as in Table 1.

Inhibitor	Concn. of inhibitor (M)	Concn. of inhibitor (μg./ml.)	Inhibition of the rate of uptake of O_2 (%) α-Oxoglutarate	Succinate	DPNH
KCN	10^{-4}	—	38	54	31
	10^{-3}	—	62	85	77
NaN_3	10^{-3}	—	25	15	10
	10^{-2}	—	96	78	45
Malonate	10^{-3}	—	28	38	10
	10^{-2}	—	43	83	15
Arsenite	10^{-4}	—	65	0	0
	10^{-3}	—	84	15	10
Iodoacetate	10^{-4}	—	55	0	10
	10^{-3}	—	96	10	20
Iodoacetamide	10^{-4}	—	54	0	25
Fluoropyruvate	10^{-4}	—	42	15	30
p-Chloromercuribenzoate	10^{-5}	—	42	48	68
	10^{-4}	—	96	100	88
Antimycin A	—	0·03	0	0	0
	—	0·10	62	68	78
	—	0·2	68	86	81

Table 7. *Effect of inhibitors on oxidative phosphorylation*

Substrate: 30 μmoles of α-oxoglutarate. The inhibitors were added to the standard reaction mixture (see Table 5) at the concentrations indicated. Conditions of experiment were otherwise as in Table 1.

Inhibitor	Concn. of inhibitor (M)	Uptake of O_2 (μg.atoms/30 min.)	Inorganic phosphorus esterified (μg.atoms/30 min.)	Ratio, P:O
None	—	10·7	24·2	2·2
2:4-Dinitrophenol	10^{-4}	10·0	0·0	0·0
	5×10^{-5}	10·3	1·8	0·2
	10^{-5}	10·7	12·5	1·2
	5×10^{-6}	11·2	26·0	2·3
DDT	3×10^{-4}	5·4	0·7	0·2
	10^{-4}	9·7	12·6	1·3
	10^{-5}	10·6	21·2	2·0
	10^{-6}	10·7	25·7	2·2
Chloretone	3×10^{-3}	5·2	7·8	1·5
	10^{-3}	10·6	19·7	1·8
	10^{-4}	10·6	24·4	2·3
Nembutal	10^{-3}	4·3	0·0	0·0
	10^{-4}	9·6	1·8	0·2
	5×10^{-5}	10·5	17·4	1·6
	10^{-5}	10·6	24·2	2·2

DISCUSSION

The large number of mosquitoes necessary for the preparation of the respiratory particles made the separation of the head and abdomen from the thorax impracticable. Microscopic observations showed, however, that the sarcosomes of the thorax contributed the largest fraction of the granules present. In general, the particle preparation obtained from *Aedes aegypti* resembled in its properties the respiratory particles derived from other insects (Sacktor, 1954, 1955; Lewis & Slater, 1954). All the citric acid-cycle intermediates were oxidized and the respiration was linked to phosphorylation. For obtaining maximal rates of oxidation and phosphorylation, regulation of the tonicity of the medium and addition of protective agents (EDTA, serum albumin) were found to be necessary.

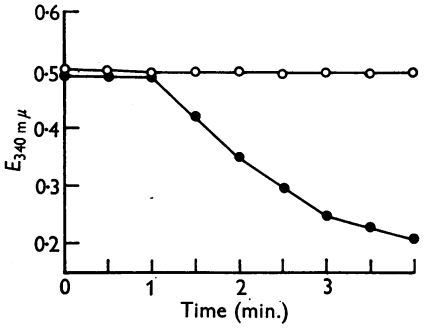

Fig. 1. Malic dehydrogenase activity. Each cell contained 30 μmoles of phosphate buffer, pH 7·4, and the particle preparation (0·02 mg. of protein). Total volume, 3 ml. At zero time 0·33 μmole of DPNH was added; ●, 5 μmoles of oxaloacetate were added at 1 min.; ○, no oxaloacetate was added. The control cell contained water.

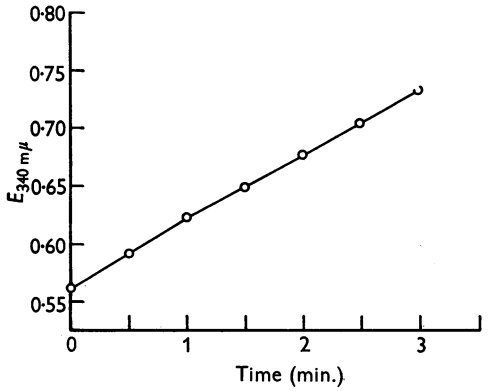

Fig. 2. Fumarase activity. Composition of the reaction mixture was as in Fig. 1. At zero time 20 μmoles of L-malate were added. The control cell contained water.

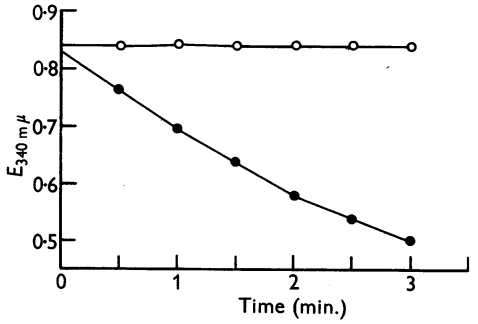

Fig. 3. DPNH oxidase activity. Each cell contained 30 μmoles of phosphate buffer, pH 7·4, 8 μmoles of ATP, 8 μmoles of $MgCl_2$, and the particle preparation (0·13 mg. of protein). Total volume, 3 ml. At zero time 0·66 μmole of DPNH was added; ○, KCN (3 μmoles) added; ●, no KCN added. The control cell contained all the components except DPNH. Disappearance of DPNH was followed at 340 mμ.

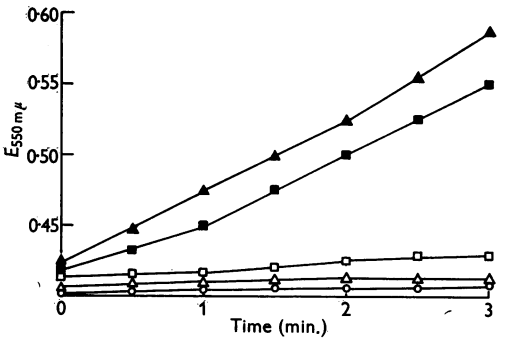

Fig. 4. DPNH–cytochrome *c* reductase activity. Each cell contained, in addition to the components indicated in Fig. 3, 0·74 μmole of oxidized cytochrome *c*. ○, No substrate added; △ and ▲, 0·66 μmole of DPNH; □ and ■, 10 μmoles of succinate; ▲ and ■, KCN (3 μmoles) added; △ and □, no KCN added. The control cell contained all the components with the exception of the substrate. Reduction of cytochrome *c* was followed at 550 mμ.

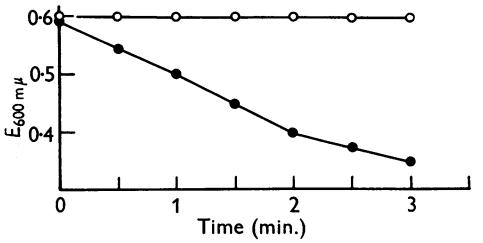

Fig. 5. Diaphorase activity. Each cell contained, in addition to the components indicated in Fig. 3, 0·15 μmole of 2:6-dichlorophenolindophenol. ●, KCN (3 μmoles) added; ○, no KCN added. The control cell contained all the components with the exception of the dye. Reduction of the dye was followed at 600 mμ.

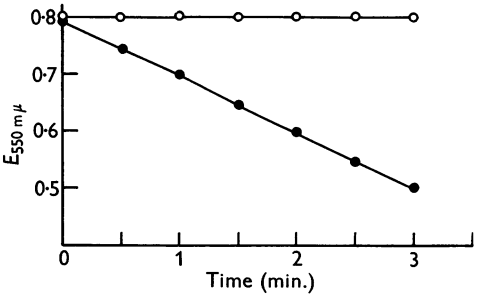

Fig. 6. Cytochrome *c* oxidase activity. Each cell contained, in addition to the components indicated in Fig. 3, 0·5 μmole of reduced cytochrome *c*. ○, KCN (3 μmoles) added; ●, no KCN added. The control cell contained all the components with the exception of reduced cytochrome *c*. Oxidation of cytochrome *c* was followed at 550 mμ.

Though the oxidation of the various substrates by the undamaged particles required only ATP and magnesium, the mechanically injured granules displayed a need for phosphate, DPN and increased concentrations of ATP and magnesium. In addition to these cofactors, supplementary coenzyme A accelerated the rate of oxidation of α-oxoglutarate in the injured granules. Arsenate in equimolar amounts could replace phosphate in the damaged preparation, but was slightly inhibitory with the intact, phosphate-independent particles. As the accelerating effect of arsenate was apparent only in the injured, non-phosphorylating preparation, it could not act as a substitute for phosphate in oxidative phosphorylation (cf. Crane & Lipmann, 1953). The requirement for relatively high phosphate concentrations seems to be a distinctive feature of damaged subcellular units, as it was also shown by pig-heart succinic oxidase preparations, obtained under rather drastic experimental conditions (Keilin & Hartree, 1949).

The effect of inhibitors on respiration and on oxidative phosphorylation was similar to that reported for mammalian mitochondria (Brody & Bain, 1954; Dianzani & Scuro, 1956; Avi-Dor & Mager, 1956); however, all the phosphorylation linked to oxidation of α-oxoglutarate was abolished by 10^{-4}M-2:4-dinitrophenol. If the higher P:O ratio obtained with this substrate, compared with that observed with malate, indicates phosphorylation at substrate level, the latter should also be sensitive to 2:4-dinitrophenol in this case. The insecticide DDT inhibited the phosphorylation at lower concentrations than those at which it inhibited the respiration. This is in agreement with the findings of Sacklin, Terriere & Remmert (1955) in housefly sarcosomes.

SUMMARY

1. Respiratory particles obtained from the mosquito *Aedes aegypti* L. oxidized all the citric acid-cycle intermediates and showed oxidative phosphorylation.

2. Particles prepared by a mild extraction procedure showed no cofactor dependence, except a requirement for adenosine triphosphate and Mg^{2+} ions. Mechanical injury produced a preparation dependent on phosphate, diphosphopyridine nucleotide and coenzyme A. Arsenate could replace phosphate in the latter preparation in equimolar amounts.

3. The effect of inhibitors on respiration and phosphorylation was investigated. All the phosphorylation linked to the oxidation of α-oxoglutarate was abolished by 10^{-4}M-2:4-dinitrophenol. The phosphorylation was more affected by 2:2-bis-(*p*-chlorophenyl)-:1:1:1-trichloroethane (DDT) than was the respiration.

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