Studies on the Adenosine Triphosphate–Phosphate Exchange and the Hydrolysis of Adenosine Triphosphate Catalysed by a Particulate Fraction from the Mosquito

By Y. AVI-DOR and O. GONDA
Israel Institute for Biological Research, Ness-Ziona, Israel

(*Received* 28 *August* 1958)

The citric acid cycle is known to be connected with the esterification of inorganic phosphate both in mammalian (Lehninger, 1954) and in insect (Sacktor, 1954; Lewis & Slater, 1954; Gonda, Traub & Avi-Dor, 1957) respiratory particles. In addition to the net synthesis of adenosine triphosphate, liver mitochondria and submitochondrial fractions also catalyse an exchange between adenosine triphosphate and labelled inorganic phosphate (^{32}P) in the absence of apparent respiration (Boyer, Luchsinger & Falcone, 1956; Swanson, 1956; Cooper & Lehninger, 1957). Strong evidence exists for a functional association of this exchange reaction with oxidative phosphorylation and 2:4-dinitrophenol-stimulated adenosine triphosphatase activity.

In a continuation of a study on the oxidative and phosphorylative metabolism of insects and on the mode of action of insecticides, the properties of the enzyme systems catalysing the adenosine triphosphate–^{32}P exchange and the hydrolysis of adenosine triphosphate have been investigated in preparations obtained from the mosquito *Aedes aegypti* L. The effect of various inhibitors and of 1:1:1-trichloro-2:2-di-(*p*-chlorophenyl)ethane on these enzymic activities has been tested.

EXPERIMENTAL

Insect colonies. *Aedes aegypti* L. were reared as described before (Gonda *et al.* 1957). Houseflies (*Musca domestica*) were cultivated by the Peet–Grady method as modified by Tahori (1955).

Materials. All nucleotides were products of Sigma Chemical Co. Fluoropyruvic acid was synthesized according to Blank, Mager & Bergmann (1955). Crystalline 1:1:1-trichloro-2:2-di-(*p*-chlorophenyl)ethane (DDT), m.p. 108°, was used.

Human serum albumin of 85% purity (containing 15% of globulin) prepared according to Nitschmann, Kistler & Lergier (1954) was obtained from Marcus Memorial Institute for Plasma Drying and Fractionation, Tel-Aviv. Solutions of the albumin were freshly prepared before use and adjusted by 0·5M-2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer to pH 7·4. Tris and phosphate buffers used were tris–HCl and NaH_2PO_4–Na_2HPO_4 mixtures respectively. They were prepared according to Colowick & Kaplan (1955).

$Na_2H^{32}PO_4$ was obtained from The Radiochemical Centre, Amersham, Bucks. The neutralized stock solution contained approx. 1·5 μc/ml. and gave $1{\cdot}5 \times 10^5$ counts/min./ml. in a thin mica-window Geiger counter tube. The radioactivity was measured by evaporating a portion (0·2 ml.) of the ten-times diluted stock solution to dryness on a planchet of 2 cm.^{2} surface under an infrared lamp.

All other chemicals were of analytical grade.

Isolation of respiratory particles. Respiratory particles from the mosquito were obtained as described by Gonda *et al.* (1957), except that albumin was omitted from the isolation medium. The standard isolation medium (5 ml./g. of mosquito) contained: sucrose 0·33M and ethylenediaminetetra-acetic acid (EDTA), 0·1 mM, adjusted to pH 7·4 with NaOH. The particles were washed twice with the isolation medium (70 ml. each time), and resuspended in 0·33M-sucrose. Respiratory particles were also prepared from whole houseflies by the same method. Guinea-pig liver mitochondria were prepared according to the method of Schneider (1948). Unless otherwise indicated, the respiratory particles obtained from the mosquito were used in the following experiments.

Methods. The exchange between ATP and ^{32}P was assayed by a method based on the procedure described by Swanson (1956). The composition of the reaction mixture (total volume 1·5 ml.) is described in Table 1. Incubation was carried out in Pyrex test tubes in a water bath at 28°. The reaction was terminated by the addition of 4·5 ml. of 5% (w/v) trichloroacetic acid and the acidified sample centrifuged to remove protein. A portion (1 ml.) of the supernatant was used for the determination of the phosphate liberated from ATP (ATPase activity). To a 3 ml. portion of the supernatant 1 ml. of an aqueous suspension of acid-washed Norit A (equivalent to approx. 25 mg. dry wt.) was added. The charcoal was separated by centrifuging, washed once with 10 ml. of trichloroacetic acid (5%, w/v), twice with 10 ml. of water and resuspended in a small volume of ethanol. The ethanol suspension of the charcoal was transferred quantitatively to planchets of 2 cm.^{2} surface and dried under an infrared lamp. The radioactivity of the nucleotides adsorbed on the charcoal was counted in a thin mica-window Geiger counter tube and was expressed as counts/min./total nucleotide fraction. The counts measured in a control, in which trichloroacetic acid was added before the particle preparation, were deducted from the experimental values (as a rule the counts in the control exceeded only slightly the background counts).

A correction for the absorption of the charcoal layer was obtained from an empirical calibration curve which was prepared by evaporating samples of ^{32}P in the presence and in the absence of 25 mg. of Norit A. The specific activity of

Table 1. *Effect of the composition of the isolation medium on adenosine triphosphate–^{32}P exchange and adenosine triphosphatase activity*

Composition of isolation medium was as indicated. The isolated particles were washed twice with 0·33 M-sucrose. Composition of the 'standard' assay medium: tris buffer (10 mM), pH 7·4, phosphate buffer (2·7 mM), pH 7·4, 0·2 ml. of the stock solution of ^{32}P, sucrose (0·25 M), ATP (7 mM), human serum albumin 1% (w/v), EDTA (mM), respiratory particles from mosquito (equivalent approx. to 0·2 mg. of N/ml. of assay medium). Total volume: 1·5 ml. Enzymic activities were measured as described under Methods. Time of incubation: 10 min. Temp. 28°. ΔP: inorganic phosphate liberated (μmoles).

Composition of isolation medium	Concn. (M)	ΔP		Total counts in nucleotides/min.	
		Albumin and EDTA omitted	'Standard' assay medium	Albumin and EDTA omitted	'Standard' assay medium
Sucrose	0·33	2·8	2·0	20	60
Sucrose EDTA	0·33 0·0001	2·2	1·8	30	3034

Table 2. *Effect of the composition of the washing medium*

Composition of isolation medium: sucrose 0·33 M, EDTA 0·1 mM. Composition of washing medium was as indicated in the Table. Other conditions of the experiment were as described in Table 1.

Composition of washing medium	Concn. (M)	ΔP		Total counts in nucleotides/min.	
		Albumin and EDTA omitted	'Standard' assay medium	Albumin and EDTA omitted	'Standard' assay medium
Sucrose	0·33	2·1	1·8	29	3100
Sucrose Albumin	0·33 1% (w/v)	1·9	1·4	70	2530
Sucrose EDTA	0·33 0·001	2·3	1·8	36	3150
Sucrose EDTA Albumin	0·33 0·001 1% (w/v)	1·2	1·4	2980	3220

Table 3. *Effect of the composition of the assay medium*

Respiratory particles were isolated and washed as described under Methods. Standard assay medium, but albumin and EDTA were omitted if not indicated. Other conditions were as described in Table 1.

Additions			
Albumin (%, w/v)	EDTA (mM)	ΔP	Total counts in nucleotides/min.
None	None	1·8	18
	0·1	1·6	20
	1·0	1·0	18
	10·0	0·4	16
0·5	None	1·8	27
1·0		1·7	26
2·0		1·7	35
1·0	0·1	1·6	208
1·0	1·0	1·0	2870
1·0	10·0	1·0	3400

^{32}P decreased during the time of incubation owing to the liberation of unlabelled phosphate from ATP. The magnitude of the change in the specific activity of ^{32}P varied under different conditions according to the ATPase activity. Therefore the experimentally found radioactivity of the nucleotide fraction was multiplied by the factor: average concentration of inorganic phosphate during the time of reaction/concentration of inorganic phosphate at zero time.

Nitrogen was determined according to Johnson (1941), and inorganic phosphate by the method of Fiske & Subbarow (1925).

RESULTS

Effect of the composition of the isolation, washing and assay media

Previous workers have shown that the addition of serum albumin and EDTA is necessary for the oxidative phosphorylation in insect-particle preparations (Sacktor, 1954; Lewis & Slater, 1954; Gonda *et al.* 1957). Therefore the effect of these substances on the two phosphorylative processes was studied. The composition of the medium was varied in: (*a*) isolation of the particles from the tissue; (*b*) washing of the particles; (*c*) assay of ATP–^{32}P exchange. The data presented in Tables 1–3 show that the presence of EDTA (0·1 mM) in the medium used for the isolation of the mosquito respiratory particles from the tissue was

required for a significant incorporation of ^{32}P into ATP under otherwise optimum conditions. Furthermore, either the assay medium or the sucrose solution used for the washing of the particles had to be supplemented with both EDTA and albumin [optimum concentrations: mM for EDTA and at least 1% (w/v) for albumin]. Neither of the two agents singly facilitated the ATP-^{32}P-exchange reaction. With albumin and EDTA in the assay medium the addition of these substances to the washing medium was unnecessary and the addition of albumin was even slightly inhibitory. Further, EDTA at high concentrations inhibits the hydrolysis of ATP, whereas albumin has no effect on ATPase activity. The requirement for albumin and EDTA seems to be characteristic of insect particles (mosquito, housefly), since guinea-pig liver mitochondria catalysed the exchange reaction in their absence (Table 4).

Effect of particle concentration

The ATP-^{32}P exchange was found to increase with the particle concentration up to a limit of a concentration equivalent to 0·3 mg. of N/ml. of assay medium. No splitting of ATP occurred at particle concentrations below 0·1 mg. of N/ml. of assay medium (Fig. 1).

Kinetics

The kinetics of the ATP-^{32}P exchange and of the hydrolysis of ATP are compared in Fig. 2. It can be seen that the splitting of ATP ceases after 10 min., whereas the exchange reaction proceeds at a gradually diminishing rate. It should be noted that after 30 min., when the exchange had practically come to an end and about 90% of the ATP was still unhydrolysed, the radioactivity of the phosphate groups in the nucleotide fraction was only approx. 10% of the value calculated for a state of equilibrium between the terminal phosphate of ATP and the added ^{32}P.

When the respiratory particles were pre-incubated for 20 min. at 28° with the assay medium, but the ATP was added only after the pre-incubation, the incorporation of ^{32}P into the nucleotide proceeded at the same rate as under the usual conditions of the reaction. Hence the cessation of the exchange was not due to an inactivation of the particles during the incubation. Accumulation of an inhibitory substance in the medium was indicated by the finding that when the respiratory particles, incubated with the complete reaction mixture, were separated after 10 min. by centrifuging and fresh particles were added to the supernatant no further exchange was observed (Fig. 2).

Table 4. *Requirement for albumin and ethylenediaminetetra-acetic acid of respiratory particles from different sources*

Respiratory particles were obtained as described under Methods, and were added in a concentration equivalent to 0·2 mg. of N/ml. of assay medium. The composition of the assay medium and other conditions of experiment were as described in Table 1, except that albumin and EDTA were added as indicated below.

Source of respiratory particles	Additions to assay medium: Albumin (%, w/v)	Additions to assay medium: EDTA (mM)	ΔP	Total counts in nucleotides/min.
Mosquito	1	1·0	1·4	3340
Housefly	0	0	1·1	120
	0	1·0	0·7	300
	1	0	1·2	200
	1	1·0	0·8	2800
Guinea-pig liver	0	0	0·0	5230
	1	1·0	0·0	5180

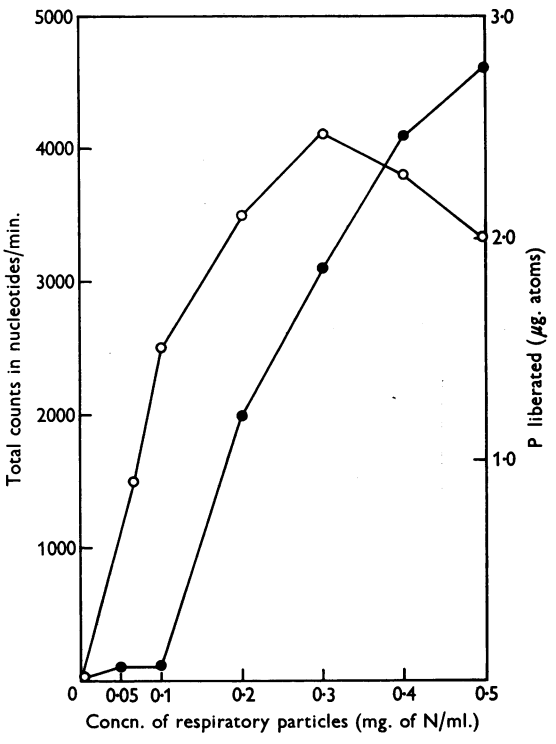

Fig. 1. Effect of the concentration of the respiratory particles on the ATP-^{32}P-exchange reaction and on the ATPase activity. ○, Total counts in nucleotides/min.; ●, μg. atoms of P liberated. Conditions of reaction were as described in Table 1. Incubation was for 10 min. at 28°.

Effect of pH

Exchange between ATP and ^{32}P was observed over a wide range of pH values with a broad peak in the range pH 7·5–8·0. No similar peak was apparent in ATPase activity. The rate of hydrolysis of ATP increased with a rise in the pH (Fig. 3).

Effect of nucleotides

Exchange between ^{32}P and nucleotides occurred at maximum velocity with ATP as the substrate. The rate of incorporation of ^{32}P into adenosine diphosphate (ADP) was approx. 30% of the maximum rate (probably as a result of myokinase activity). Inosine triphosphate (ITP) and adenosine monophosphate (AMP) did not become labelled under the conditions of the experiment. Addition of ADP or ITP inhibited significantly the incorporation of ^{32}P into ATP, whereas AMP was not inhibitory (Table 5).

The nucleotide specificity of the 'ATPase' differed from the exchange system in its effect on ITP. The rate of hydrolysis of ITP was even more rapid than that of ATP. ADP was split slowly and AMP not at all. ADP slightly decreased the rate of hydrolysis of ATP, when it was added in an equimolar amount (Table 5).

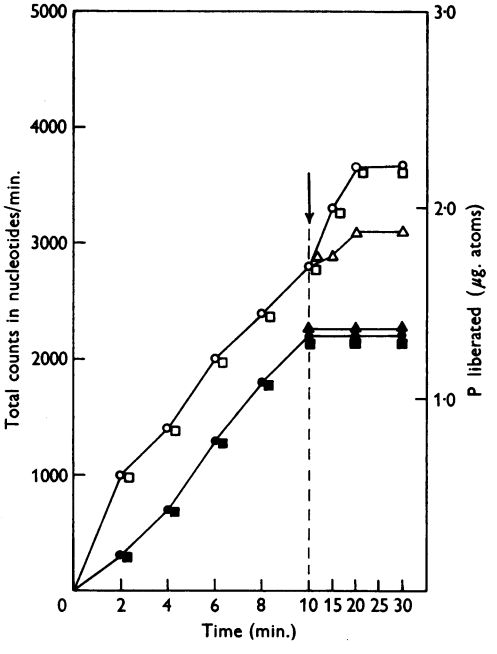

Fig. 2. Kinetics of the ATP–^{32}P exchange and the hydrolysis of ATP. ○, ●, Standard particle preparation; □, ■, particles pre-incubated with the assay medium for 20 min. at 28° in the absence of ATP; △, ▲, at the time indicated by the arrow, particles in experiment ○, ● were replaced by an equal amount of fresh respiratory particles. Other conditions of the reaction were as described in Table 1. Open points (○, □, △) total counts in nucleotides/min.; filled points (●, ■, ▲): μg. atoms of P liberated.

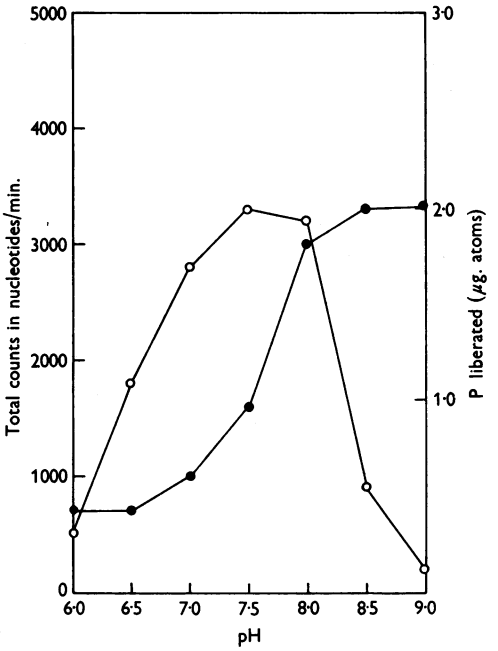

Fig. 3. Effect of pH on ATP–^{32}P exchange and on the ATPase activity. ○, Total counts in nucleotides/min.; ●, μg. atoms of P liberated. Conditions of experiment were as described in Table 1.

Table 5. *Effect of nucleotides*

Particle preparation was isolated and washed as described under Methods. Composition of assay medium, with the exception of the nucleotide, and the conditions of reaction were as described in Table 1.

Nucleotide added	Concn. (mM)	ΔP	Total counts in nucleotides/min.
None	—	0·0	15
ATP	7·0	1·2	3080
ADP	7·0	0·6	910
AMP	7·0	0·0	18
ITP	7·0	1·6	60
In the presence of 7 mM-ATP			
ADP	2·0	1·4	2710
ADP	4·0	1·0	2020
ADP	7·0	1·0	1480
AMP	7·0	1·2	2950
ITP	2·0	1·2	3110
ITP	4·0	1·8	2600
ITP	7·0	2·4	1340

Effect of respiratory inhibitors

A number of substances which inhibit the oxidation of various substrates of the citric acid cycle by mosquito respiratory particles (Gonda *et al.* 1957) were tested for their effect on the ATP–^{32}P-exchange and ATPase activity. Potassium cyanide, sodium azide and *p*-chloromercuribenzoic acid, which were found to inhibit the oxidation of both α-oxoglutarate and succinate, also inhibited the exchange reaction to a similar extent. Arsenite and fluoropyruvate, which were found to inhibit only the oxidation of α-oxoglutarate, were without effect on the ATP–^{32}P exchange (Table 6).

Effect of 2:4-dinitrophenol

2:4-Dinitrophenol (DNP), which is well known as an uncoupler of oxidative phosphorylation in mammalian as well as in insect respiratory particles, inhibited the ATP–^{32}P exchange to an extent of 60% at a concentration of 50 μM. Inhibition of the exchange was, however, not paralleled by a strong acceleration of ATPase activity as it is known to occur in liver mitochondria (Boyer *et al.* 1956). Magnesium-stimulated ATPase activity was also unaffected by DNP (Table 7).

Table 6. *Effect of respiratory inhibitors*

Respiratory particles were isolated and washed as described under Methods. The composition of the assay medium and other conditions of the reaction were as described in Table 1.

Inhibitor	Concn. (mM)	ΔP	Total counts in nucleotides/ min.
None	—	2·4	3400
KCN	0·1	2·4	2208
	1·0	2·0	1510
NaN_3	1·0	0·0	1470
	10·0	0·1	30
p-Chloromercuribenzoate	0·01	2·5	3100
	0·1	2·4	120
Arsenite	0·1	2·4	3200
	1·0	2·4	2950
Fluoropyruvate	0·1	2·3	3150
	0·6	2·4	3510

Table 7. *Effect of 2:4-dinitrophenol*

Respiratory particles were isolated and washed as described under Methods. The composition of the assay medium and the conditions of the reaction were as given in Table 1.

Concn. of DNP (mM)	Concn. of Mg^{2+} ions (mM)	ΔP	Total counts in nucleotides/ min.
None	—	1·4	2995
0·01	—	1·4	3015
0·03	—	1·4	2820
0·05	—	1·6	1250
0·10	—	2·3	210
—	1·0	3·2	2700
0·10	1·0	3·1	70

Effect of DDT

It has previously been shown that the effect of DDT on oxidative phosphorylation in mosquito particles resembles in many respects that of DNP (Gonda *et al.* 1957). It is seen from Table 8 that the two substances have a similar effect on the exchange and ATPase reactions. DDT at a concentration of 50 μM inhibited significantly the exchange reaction but had little effect on the rate of hydrolysis of ATP. In these experiments DDT was added to the reaction mixture either dissolved in a water-soluble solvent such as ethanol or propanol, or in the form of an emulsion in paraffin oil. Since the detergents tested were deleterious for the enzyme system, albumin was used as a stabilizer of the DDT suspension. The albumin added increased also the stability of the paraffin oil emulsion. A medium containing 50 μM-DDT showed no visible turbidity and became opalescent only when the concentration was increased over and above this value. DDT was completely ineffective in a paraffin oil emulsion. It should be noted that the solvents used (ethanol, propanol) had also some inhibitory effect on the exchange reaction. Guinea-pig liver mitochondria were much less sensitive to DDT than the respiratory particles obtained from the mosquito or the housefly (Table 8).

DISCUSSION

The enzyme system which catalyses the exchange between ^{32}P and ATP in respiratory particles obtained from the mosquito resembles its counterpart in the mammalian mitochondria in many respects. As in mitochondria and submitochondrial preparations from the liver (Cooper & Lehninger, 1957), exchange takes place only with ATP as the substrate, and not with ITP. ADP is inhibitory, and both preparations are sensitive to DNP. Although no endogenous respiration could be demonstrated with the conventional manometric technique, a correlation exists between the effect of respiratory inhibitors on the exchange on the one hand and on the oxidation of succinate on the other.

The reason for the decline in the rate of incorporation of ^{32}P into ATP, before the isotopic equilibrium between the terminal phosphate of the latter and the labelled inorganic phosphate was reached, is not clear. The experimental data are consistent with the view that, during the time of incubation, an inhibitor is formed in the presence of ATP. Although ADP, the product of the hydrolysis of ATP, was indeed found to inhibit the

Table 8. *Effect of* 1:1:1-*trichloro*-2:2-*di*-(p-*chlorophenyl*)*ethane*

Particles were obtained as described under Methods and were added in a concentration equivalent to 0·2 mg. of N/ml. of assay medium. Composition of the assay medium and other conditions of the experiment were as described in Table 1. DDT was added to the reaction mixture dissolved in the solvents indicated.

Source of the particles	Solvent 1% (w/v)	DDT (μM)	ΔP	Total counts in nucleotides/min.
Mosquito	None	None	1·8	3085
	Ethanol	None	1·8	2400
		50	1·8	1900
		100	1·7	1470
		200	2·3	89
	Propanol	None	1·7	1907
		50	1·8	1150
		100	1·9	820
		200	2·2	70
	Paraffin oil	None	1·8	3120
		100	1·8	3200
		400	1·9	3020
Housefly	None	None	0·7	2515
	Propanol	None	0·9	1845
		100	1·2	870
Guinea-pig liver	None	None	0·0	5850
	Propanol	None	0·2	4870
		100	0·2	4550
		200	0·4	3900

exchange, the amount of ADP which could be present after 30 min. of incubation in the reaction mixture is too small to account for the cessation of the exchange.

The addition of serum albumin to preparations of insect sarcosomes has been shown to maintain phosphorylation (Sacktor, 1954; Lewis & Slater, 1954), and to restore phosphorylative activity in mouse-liver mitochondria that were inactivated completely by ageing (Pullman & Racker, 1956). Polis & Shmukler (1954) isolated a haemoprotein which is released from aged mitochondria and which uncouples phosphorylation. This haemoprotein ('mitochrome') is strongly bound by serum albumin. The results of the present investigation can be understood on the basis of the assumption that albumin binds the substance which inhibits ATP–^{32}P exchange. This is indicated by the finding that the presence of albumin was not found to be obligatory throughout the incubation period, provided that it was added to the washing medium during the isolation procedure. Albumin exerted its effect on the exchange, however, only when EDTA was also present.

The ATPase of the mosquito respiratory particles resembles also the ATPase of liver mitochondria. The relative rates of the hydrolysis of different nucleotides (ATP $\cong$ ITP $\gg$ ADP) are similar (Kielley & Kielley, 1951). The lack of proportionality between enzymic activity and particle concentration at low tissue concentrations observed in the mosquito particles has been described also in liver mitochondria (Potter & Recknagel, 1951). The most conspicuous difference between the ATPase of liver mitochondria and that of the mosquito particles is that the acceleration of ATPase and the inhibition of the exchange reaction by DNP are not as closely related in insect sarcosomes as in liver mitochondria (Boyer *et al.* 1956).

The inhibition of the ATP–^{32}P exchange by DDT is of interest in view of the observed great quantitative difference between the insect and mammalian respiratory particles in the extent of inhibition (more than 50% inhibition in insect particles and less than 10% in mammalian liver mitochondria by 0·1 mM-DDT). It should be noted, however, that a statement about the 'concentration' of DDT is ambiguous and might be misleading. DDT is not in a true solution in the system and is divided between at least three phases: the oily suspension, an aqueous phase and the respiratory particles. An equilibrium unfavourable for the particles may explain the lack of effect of DDT in a paraffin oil emulsion. Experiments are now in progress to relate the inhibitory effect of DDT and some of its toxic and non-toxic derivatives on phosphorylative processes to the true concentration of these substances in the respiratory particles.

SUMMARY

1. Adenosine triphosphatase and the enzyme system catalysing the incorporation of ^{32}P into adenosine triphosphate have been studied in

respiratory particles prepared from the mosquito *Aedes aegypti* L.

2. Addition of ethylenediaminetetra-acetic acid to the isolation medium and of ethylenediaminetetra-acetic acid and albumin either to the washing liquid or to the assay medium has been found obligatory for the exchange reaction.

3. The effect of nucleotides, respiratory inhibitors and inhibitors of oxidative phosphorylation on the exchange reaction and on the adenosine triphosphate activity has been investigated.

4. Quantitative differences exist between the response of insect and mammalian respiratory particles to 1:1:1-trichloro-2:2-di-(*p*-chlorophenyl)-ethane (DDT). 0·1 mM-DDT inhibits the exchange reaction by more than 50% in insect sarcosomes whereas the inhibition in mammalian liver mitochondria is less than 10%.

We are indebted to Dr Rachel Galun and Dr A. S. Tahori for the cultivation of the test organisms.

REFERENCES

Blank, I., Mager, J. & Bergmann, E. D. (1955). *J. chem. Soc.*, p. 2190.
Boyer, P. D., Luchsinger, W. W. & Falcone, A. B. (1956). *J. biol. Chem.* **223**, 405.
Colowick, S. P. & Kaplan, N. O. (1955). In *Methods of Enzymology*, vol. 1, p. 143. New York: Academic Press Inc.
Cooper, C. & Lehninger, A. L. (1957). *J. biol. Chem.* **224**, 561.
Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
Gonda, O., Traub, A. & Avi-Dor, Y. (1957). *Biochem. J.* **67**, 487.
Johnson, M. J. (1941). *J. biol. Chem.* **137**, 575.
Kielley, W. W. & Kielley, R. K. (1951). *J. biol. Chem.* **191**, 485.
Lehninger, A. L. (1954). *Harvey Lect.* **49**, 176.
Lewis, S. E. & Slater, E. C. (1954). *Biochem. J.* **58**, 207.
Nitschmann, H., Kistler, P. & Lergier, W. (1954). *Helv. chim. acta*, **37**, 866.
Polis, B. D. & Shmukler, H. W. (1954). *Abstr. Pap. Amer. Chem. Soc.* 126th Meeting, p. 72C.
Potter, V. R. & Recknagel, R. G. (1951). In *Phosphorus Metabolism*, vol. 1, p. 377. Ed. by McElroy, W. D. & Glass, B. Baltimore: Johns Hopkins Press.
Pullman, M. E. & Racker, E. (1956). *Science*, **123**, 1105.
Sacktor, B. (1954). *J. gen. Physiol.* **37**, 343.
Schneider, W. C. (1948). *J. biol. Chem.* **176**, 259.
Swanson, M. A. (1956). *Biochim. biophys. Acta*, **20**, 85.
Tahori, A. S. (1955). *J. econ. Ent.* **48**, 638.

The Biosynthesis of Porphyrins from Porphobilinogen by *Rhodopseudomonas spheroides*

By H. HEATH and D. S. HOARE
Department of Chemical Pathology, University College Hospital Medical School, London, W.C. 1

(*Received* 18 *August* 1958)

In a comprehensive review (van Niel, 1944) of the non-sulphur purple and brown bacteria or Athiorhodaceae there was described the production of a diffusible red pigment from growing cells. Some properties of this pigment were described, including some of its absorption characteristics and solubility in organic solvents. In the light of subsequent studies it seems clear that the pigment described was a porphyrin derivative. Since members of the Athiorhodaceae are photosynthetic and thus contain bacteriochlorophyll and, further, since they have been shown to be rich in haem derivatives (Elsden, 1954) they possess potent enzyme systems capable of synthesizing the porphyrin nucleus. The studies of Lascelles (1955, 1956) with whole-cell suspensions of *Rhodopseudomonas spheroides*, a typical member of the group of the Athiorhodaceae, have indeed shown that these organisms can bring about a synthesis of free porphyrins and bacteriochlorophyll from glycine together with certain intermediates of the tricarboxylic acid cycle.

Lascelles (1955, 1956) found that δ-aminolaevulic acid was converted into porphyrins by cell suspensions of *R. spheroides* and during the initial stages of the reaction porphobilinogen diffused out of the cells. However, when porphobilinogen was added to washed cell suspensions of *R. spheroides* it was not converted into porphyrins, although it is a porphyrin precursor in other systems. (For a review of the interrelationships of δ-aminolaevulic acid, porphobilinogen and porphyrins see Rimington, 1957.) Bogorad & Granick (1953) have shown that porphobilinogen is converted into porphyrins by suspensions of *Chlorella vulgaris* provided that they have been previously subjected to freezing and thawing. This suggests that porphobilinogen is not permeable to intact whole cells; a similar observation was made with avian erythrocytes (Dresel & Falk, 1956*a*).

The findings of Kikuchi, Shemin & Bachmann (1958) and of Gibson (1958), establishing the biosynthesis of δ-aminolaevulic acid from glycine and