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'TRANSPORT ADENOSINETRIPHOSPHATASE' IN ELECTRIC ORGAN. THE RELATION BETWEEN ION TRANSPORT AND OXIDATIVE PHOSPHORYLATION

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In 1957 Skou showed that the 'microsomal' fraction from minced crab nerves contained an adenosinetriphosphatase (ATP-ase) activated by sodium and potassium ions, and he suggested that such an enzyme might be involved in the active transport of sodium and potassium across cell membranes. Evidence for this view has been provided by experiments on red cell membranes, in which it has been shown that (i) a part of the ATP-ase activity occurs only when potassium is present outside the cells and sodium is present inside; this activity is accompanied by an influx of potassium and efflux of sodium (Glynn, 1962*a*; Whittam, 1962): and (ii) there are close resemblances between the ATP-ase and the ion transport system especially in the way they are affected by cardiac glycosides (Post, Merritt, Kinsolving & Albright, 1960; Dunham & Glynn, 1961).

Active transport in red cells is relatively sluggish. R. D. Keynes (personal communication) has evidence, from measurements of heat production, that in the electric organ of *Electrophorus electricus* ion pumping is extremely active just after a discharge; it therefore seemed worth while to examine the ATP-ase activity of this organ.

The experiments to be described fall into two groups and the paper will therefore be presented in two parts. Part I describes experiments which show that the microsomal fraction of electric organ is very rich in ATP-ase activity, and that more than 90% of this activity has properties similar to that part of the red cell ATP-ase that has been incriminated in active ion transport. Similar findings have been reported recently by Albers & Koval (1962) and Bonting & Caravaggio (1963). Part II describes experiments which were designed to show whether the ion transport system in the cell membrane and the system responsible for oxidative phosphorylation in mitochondria were similar, and, in particular, whether ion transport involved cyclical oxidation and reduction of intermediates. This problem will be discussed more fully before the relevant experiments are described.

A brief account of the experiments reported here has already been published (Glynn, 1962*b*).

PART I: 'TRANSPORT ADENOSINETRIPHOSPHATASE' IN ELECTRIC ORGAN

METHODS

Preparation of electric organ

Portions of electric organ were kindly supplied by Dr R. D. Keynes of the Institute of Animal Physiology, Babraham. The eel was decapitated and the electric organ dissected out and placed in a suitable Ringer's solution at room temperature (Keynes & Martins-Ferreira, 1953). As much fibrous tissue as could easily be removed was cut away, and a weighed portion of the organ was homogenized in about twelve volumes of an ice-cold solution containing (mM): sucrose 250; trishydroxy-methylaminomethane-hydrochloride (tris-HCl) pH 7·4, 30; and tris-ethylenediaminetetraacetate (tris-EDTA) 2. To homogenize the material it was placed first in a 'Paladin' blender, 1 min at speed 2, and then in a 'Potter' type Perspex-in-glass homogenizer, 15 sec with a motor-driven pestle. The homogenate was centrifuged for 12 min at 1200 *g* to get rid of cell debris, bits of fibrous tissue, etc., and then for 15 min at 9000 *g* to remove mitochondria. If any floating bits of fatty material were present they were removed by filtration through glass-wool. The 'microsomal' fraction was then separated by spinning for 3 hr at 35,000 *g* and rejecting the supernatant. The precipitate was resuspended in a small volume of the same solution—say twice the volume of the original electric organ—and dispersed with a small Perspex-in-glass homogenizer to give an opalescent fluid without any particles visible to the naked eye. This fluid was spun for 10 min at 9000 *g*, the precipitate rejected, and the supernatant used as the enzyme preparation. All centrifuging was done at between 0 and 10° C, and the final suspension was stored in a refrigerator at 4° C, or frozen in dry-ice and acetone and stored at −20° C. Freezing does not seem to affect the activity of the preparation, but it is not known if there is any advantage as the preparation loses very little activity in 3 weeks at 4° C, and stability has not been investigated over longer periods.

Reagents

ATP was obtained from the Sigma Chemical Co., St Louis, Missouri, as the crystalline disodium salt or as the dipotassium salt.

Tris was the 'tris 121 grade' supplied by the Sigma Chemical Co.

Salts. NaCl and KCl were obtained 'spectroscopically pure' from Johnson Matthey and Co. Ltd. $MgCl_2$ was prepared from 'spectroscopically pure' Mg rod and A.R. grade HCl. Tris-EDTA was made from the free acid that had been supplied by British Drug Houses Ltd and recrystallized from hot water.

Ouabain (strophanthin G) was obtained from British Drug Houses Ltd. The solid was dissolved in 80% (v/v) ethanol (A.R. grade) and the alcoholic solution diluted with water.

Assay of ATP-ase activity

Incubation was carried out in 5 ml. graduated tubes with glass stoppers. The reaction was started by the addition of ATP in a volume of fluid equal to one tenth of the total volume, so that the cooling effect was small. Incubation was generally carried out at 37° C. This is warmer than the eels' natural environment, but in preliminary experiments phosphate formation was linear with time over 20 min, so the enzyme was not being damaged by the high temperature. In a single experiment in which activities at 30 and 37° C were compared, total activity was only about 13% less at the lower temperature, but a smaller fraction of the activity was sensitive to ouabain. At the end of the incubation period the tubes were transferred to an ice bath and ice-cold trichloroacetic acid (55 g/100 ml.) was added to give a final concentration of 5 g/100 ml. Inorganic phosphate was estimated in this solution by the method of Fiske & Subbarow (1925), or of Weil-Malherbe & Green (1951).

RESULTS

The effects of sodium, potassium and ouabain

Characteristic features of the fraction of the red cell ATP-ase involved in ion transport are that activity requires the simultaneous presence of sodium and potassium, as well as magnesium, and that activity is completely inhibited by cardiac glycosides at a concentration of 10^{-5} g/l. The results of an experiment to determine the effect of the absence of potassium on the microsomal ATP-ase are shown in Table 1. Table 2 shows the results

TABLE 1. The effects of the absence of K, of ouabain and of sodium lauryl sulphate on microsomal ATP-ase from electric organ

Condition	Activity (m-mole orthophosphate liberated/l. original organ/ 30 min)
Control	35·9
	37·2
	33·5
K absent	3·2
	3·3
	3·5
Ouabain present (7×10^{-5} g/l.)	2·1
	2·6
	2·3
Na-lauryl sulphate present (0·5 g/100 ml.)	3·2
	3·3
Na-lauryl sulphate (0·5 g/100 ml.)	2·9
+ouabain (7×10^{-5} g/l.)	3·2
	3·3

Incubation medium (mM): Na 60; K 16; Mg 3·2; ATP 3; tris 22; EDTA 0·2; sucrose 25. Temp. 37° C; pH 7·4. The tubes were placed in the water-bath 10 min before the addition of ATP.

of a similar experiment to determine the effect of the absence of sodium. In each experiment the cardiac glycoside ouabain caused a reduction in activity similar to that produced by absence of the univalent ion. In the experiment summarized in Table 2 there was a large ATP blank, and the difference between the sodium-deficient tubes and the ouabain tubes is not significant. (The cause of the large blank is that the potassium salt of ATP is relatively unstable at 4° C, so that nearly a fifth of the ATP had broken down before the start of the experiment.) The results given in Tables 1 and 2 are from experiments on different preparations, and the activities are not directly comparable, but taken together they show that more than 90% of the total ATP-ase activity requires the simultaneous presence of sodium and potassium and is inhibited by ouabain.

TABLE 2. The effects of the absence of Na, and of ouabain on microsomal ATP-ase from electric organ

Condition	Activity (m-mole orthophosphate liberated/l. original organ/ 20 min)
Control	48
	48
	50
Na absent	1
	3
	4
Ouabain (3×10^{-5} g/l.)	5
	4
	5

Incubation medium (mM): Na 67; K 16; Mg 3·2; ATP 3; tris 20; EDTA 0·14; sucrose 16·7; ethanol 0·3% (v/v). Temp. 37° C; pH 7·4. The tubes were placed in the water-bath 10 min before the addition of ATP. This experiment and that of Table 1 were performed on different preparations and the activities are not directly comparable.

The effect of sodium lauryl sulphate

It seemed possible that the small amount of activity that remained in the presence of glycosides represented enzyme that had come 'unhitched' from the transport mechanism during the preparation. If it did, one might hope to 'unhitch' more of the enzyme by treating the preparation with a detergent. The results with sodium lauryl sulphate (0·5 g/100 ml.) presented in Table 1 show that this does not occur. The glycoside-sensitive fraction is completely abolished but the residual activity is not affected.

DISCUSSION

The experiments that have been described show that the electric organ is rich in ATP-ase activity, and that most of this activity behaves like the 'transport ATP-ase' of red cells. With a homogenized preparation it is of course impossible to observe active transport, so that direct correlation of transport and ATP-ase activity is impossible; nevertheless, the predicted association of plentiful (Na + K)-activated ATP-ase with a vigorously transporting tissue is found, and it is unlikely that this association is fortuitous. If the (Na + K)-dependent ATP-ase activity is expressed in terms of the dry weight of the microsomal preparation, the rate of splitting is about 0·33 μmole/mg min. The (Na + K)-dependent ATP-ase activity of red cell ghosts expressed in terms of the dry weight of the ghosts is about 400 times less. Since this work was done, Bonting & Caravaggio (1963) have published the results of a much more complete investigation of the distribution of (Na + K)-activated ATP-ase in different

tissues, which show a good correlation between ion transport and ATP-ase over a very wide range of activities. Red cells and electric organ come at opposite ends of the range.

PART II: THE RELATION BETWEEN ION TRANSPORT AND OXIDATIVE PHOSPHORYLATION

If the system responsible for the transport of sodium and potassium ions is associated with a magnesium-activated ATP-ase bound to the cell membrane, it bears at least a superficial resemblance to the system responsible for oxidative phosphorylation in mitochondria. For in the absence of oxidizable substrates mitochondrial particles will show Mg-activated ATP-ase activity (Cooper & Lehninger, 1957; Lardy, Johnson & McMurray, 1958), and in the presence of oxidizable substrates they appear to bind potassium ions (Gamble, 1957). The mechanism of oxidative phosphorylation is not known, but it is clear that oxidation of the substrate takes place by the passage of hydrogen atoms or electrons along a chain of intermediary carriers, each of which undergoes oxidation and reduction in a cyclical fashion, and that some of these intermediate steps are coupled to the synthesis of ATP from ADP and inorganic phosphate. In 1961 Chance, and independently Klingenberg & Schollmeyer (1961), showed that under certain conditions the oxidative phosphorylation system could be made to run backwards, so that the splitting of ATP was accompanied by the passage of electrons the 'wrong way' along the carrier chain. If the oxidation and reduction of one or more of the carriers occurred at different sites, the process of 'reductive dephosphorylation' could be responsible for the active transport of ions. It seemed worth while to seek evidence that might show whether a mechanism of this kind could be operating in sodium and potassium transport across cell membranes, and the electric organ ATP-ase provided a convenient material with which to work.

The investigation has followed three lines. First, the effects on the ATP-ase of two supposedly specific inhibitors of oxidative phosphorylation have been determined. Secondly, the ATP-ase has been treated with a number of oxidizing and reducing agents in the hope that if one or more of the hypothetical intermediates was held entirely in its oxidized or reduced form the whole reaction chain might be stopped. Thirdly, direct spectroscopy has been used in an attempt to identify intermediates known to occur in the oxidation chain in mitochondria.

METHODS

The preparation of the electric organ and the method of assaying ATP-ase activity were described in Part I.

Oligomycin, in alcoholic solution containing 1 mg/ml., was a gift from Dr J. B. Chappell of the Department of Biochemistry, Cambridge. The catalase preparation was a gift from Dr E. C. Webb of the same Department. Other reagents were obtained from British Drug Houses Ltd. or Messrs Hopkins & Williams Ltd; where necessary they were neutralized before use.

RESULTS

The effect of 2:4-*dinitrophenol*

In mitochondria dinitrophenol at concentrations of 0·2 mM uncouples oxidative phosphorylation and greatly stimulates ATP-ase activity. The results presented in Table 3 show that in the microsomal fraction of electric organ 1 mM dinitrophenol affects neither the (Na + K)-activated ATP-ase nor the small amount of ATP-ase activity that occurs in the absence of potassium.

TABLE 3. The lack of effect of 2:4-dinitrophenol (DNP) on the microsomal ATP-ase from electric organ

Condition	Activity (m-mole orthophosphate liberated/l. original organ/ 20 min)
Control	53·1
	53·0
	56·6
DNP (1 mM)	56·6
	54·6
K absent	4·0
	3·0
DNP (1 mM), K absent	3·1
	2·5

Incubation medium (mM): Na 64; K 16; Mg 3·2; ATP 3; tris 20; EDTA 0·2; sucrose 25. Temp. 37° C; pH 7·1. The tubes were placed in the water-bath 10 min before the addition of ATP.

The effects of oligomycin

The characteristic effects of oligomycin on oxidative phosphorylation and ATP-ase activity in mitochondria (see p. 462) were produced with concentrations of oligomycin of 1–3 μg/ml. Table 4 shows the results of an experiment to test the effect of oligomycin on the microsomal ATP-ase of electric organ. At a concentration of 3·3 μg oligomycin/ml. the ATP-ase activity is about 49 % inhibited; at 10 μg oligomycin/ml. inhibition is 75 %. Higher concentrations were not tried, as the alcohol in which the oligomycin was dissolved begins to inhibit at concentrations much above 1 %. The above figures refer to inhibition of the total ATP-ase activity when sodium, potassium and magnesium are all present. The results in Table 4 suggest that the small amount of activity that occurs in the absence of potassium may also be sensitive to oligomycin, though the

quantity of phosphate split in the absence of potassium is so small that the figures are not reliable.

If oligomycin at a concentration of 10 μg/ml. caused 75 % inhibition of the (Na + K)-activated ATP-ase, it seemed worth while to see if it had any effect on active transport. As it was not possible to measure transport in electric organ the experiment was performed on red cells. Three-week-old red cells from a blood bank were washed three times, suspended in suitable media containing glucose (see Table 5) and incubated at 37° C. After 315 min the cells were spun down, washed three times with about twelve times their own volume of isotonic KCl, lysed with distilled water and analysed for sodium. Under the conditions of the experiment control cells showed a net loss of sodium (Table 5), cells in which active transport had been

TABLE 4. The effect of oligomycin on the microsomal ATP-ase from electric organ

Condition	Activity (m-mole orthophosphate liberated/l. original organ/ 20 min)
Control	55·0
	56·1
	55·4
Oligomycin (3·3 μg/ml.)	28·9
	27·9
Oligomycin (10 μg/ml.)	15·7
	14·0
K absent	4·3
	3·9
K absent, oligomycin (10 μg/ml.)	2·1
	1·5

Incubation medium (mM): Na 64; K 16; Mg 3·2; ATP 3; tris 20; EDTA 0·14; sucrose 16·7; ethanol 1 % (v/v). Temp. 37° C; pH 7·4. The tubes were placed in the water-bath 10 min before the addition of ATP.

TABLE 5. The effect of oligomycin on sodium extrusion from intact red cells

	Na content of cells (m-mole/l. cells)	Change in Na content of cells (m-mole/l. cells)
Initial	18·3	—
	18·1	
Final control	16·0	−2·4
	15·5	
Final ouabain (2×10^{-5} g/l.)	21·5	+3·21
	21·2	
Final oligomycin (10 μg/ml.)	18·7	+0·45
	18·5	

Conditions of incubation. Duration 315 min; temp. 37° C; pH 7·2. Medium (mM): Na 150; K 5; Mg 1; phosphate 3; glucose 11; ethanol 1 % (v/v); haematocrit 10 %. Before being added to the suspending media the cells were washed three times with a chilled solution similar in composition to the control suspending medium.

inhibited with ouabain showed a net gain. With 10 μg oligomycin/ml. the cells showed a net gain, though it was a smaller gain than that found in the presence of ouabain. The results suggest that oligomycin was causing partial inhibition of active transport, though a conceivable alternative explanation would be that pumping was not affected but that the oligomycin made the cells very slightly more leaky to sodium.

TABLE 6. The effects of hydrogen peroxide and of reducing agents on the microsomal ATP-ase from electric organ

	Activity (m-mole orthophosphate liberated/l. original organ/ 20 min)	Activity in the presence of ouabain (7×10^{-5} g/l.)
Expt. 1		
Control	40·0	2·5
	39·2	1·9
Hydrogen peroxide (5 mM)	14·0	
	14·4	
	13·5	
Expt. 2		Activity in the absence of K
Control	53·1	4·0
	53·0	3·0
	56·6	
Sodium dithionite (1 mM)	46·4	2·0
	45·7	4·1
Ascorbic acid (10 mM)	38·9	
	47·5	
Ascorbic acid (10 mM) + *p*-phenylene-diamine (1 mM)	44·1	4·0
	44·6	3·9
Ascorbic acid (10 mM) + *p*-phenylene-diamine (1 mM) + catalase	45·4	
	45·1	
Expt. 3		Activity in the presence of ouabain (7×10^{-5} g/l.)
Control	93·4	2·0
K borohydride (1·3 mM)	63·6	3·4
	66·9	0·6

Incubation media. Expts. 1 and 3 (mM): Na 60; K 16; Mg 3·4; ATP 3; tris 22; EDTA 0·2; sucrose 25; ethanol 0·7 % (v/v). Temp. 37° C; pH 7·4. Expt. 2 was performed on the same preparation, under the same conditions and at the same time as the experiment presented in Table 3. In all three experiments the tubes were placed in the water-bath 10 min before the addition of ATP. (The absolute values of the activities in Expt. 3 may be wrong.)

The effects of oxidizing and reducing agents, and of sulphydryl reagents

Table 6 shows the effects on the microsomal ATP-ase activity of 5 mM hydrogen peroxide; 1 mM sodium dithionite; 1·3 mM potassium boro-

hydride; 10 mM ascorbic acid; 10 mM ascorbic acid together with 1 mM *p*-phenylenediamine; and 10 mM ascorbic acid plus 1 mM *p*-phenylenediamine in the presence of a trace of catalase. The *p*-phenylenediamine was used in the hope that it might be able to penetrate lipoprotein membranes where more hydrophilic reducing agents could not. The catalase was intended to remove any trace of hydrogen peroxide that might have been formed by oxidation of the ascorbic acid.

TABLE 7. The effects of 'sulphydryl reagents' on the microsomal ATP-ase from electric organ

Condition	Activity (m-mole orthophosphate liberated/l. original organ/ 20 min)
Control	52·1
	48·4
	53·3
K absent	4·6
	4·8
Iodoacetamide 10^{-3} M	51·3
	48·5
10^{-2} M	7·2
	11·7
o-Iodosobenzoate 10^{-4} M	17·1
	18·5
10^{-3} M	7·0
	3·4
p-Chlormercuribenzoate 10^{-4} M	2·4
	1·5
10^{-3} M	0·7
	0·9

Incubation medium (mM): Na 64; K 16; Mg 3·2; ATP 3; tris 20 plus amount required to neutralize *o*-iodosobenzoate or *p*-chlormercuribenzoate; EDTA 0·2; sucrose 25. Temp. 37° C; pH 7·1. The tubes were placed in the water-bath 10 min before the addition of ATP.

It is clear that the reducing agents all had much the same effect, causing 20–30 % inhibition. Hydrogen peroxide produced more severe inhibition (65 %) but this cannot be regarded as evidence that the over-all process involves cyclical oxidation and reduction, since hydrogen peroxide would oxidize sulphydryl groups. A number of further experiments (see Table 7) showed that it was possible to produce partial or complete inhibition of the microsomal ATP-ase with a variety of sulphydryl reagents, some of which were not oxidizing agents.

Direct spectroscopy

A suspension of the microsomal fraction in a solution containing (mM): sucrose 250; tris-HCl, pH 7·4, 30; and tris-EDTA 2, was diluted about tenfold with water, so that it was sufficiently translucent to allow measure-

ments of absorbancy to be made with a Beckmann recording spectrophotometer. Each millilitre of the diluted suspension contained the microsomal fraction from about 50 mg of original tissue. The suspension was divided into two parts; one served as a control and a trace of solid potassium borohydride was added to the other. A comparison of the light absorbed at wave-lengths from 270 to 650 mμ showed no significant difference. A small amount of hydrogen peroxide was added to the control portion and the spectra were again compared. The hydrogen peroxide caused a slight decrease in absorbancy below about 340 mμ, progressively more marked as the wave-length was reduced, but there were no signs of any characteristic absorption bands.

DISCUSSION

The idea that the transport of ions might be coupled directly to oxidation-reduction reactions arose from the work of Lund (1928) and Stiehler & Flexner (1938). Many variations of this idea have been suggested, perhaps the best known being the hypothesis of Conway & Brady (1948) and Crane & Davies (1948) linking gastric hydrochloric acid secretion with oxidation by the cytochrome system. In oxidation through the cytochrome chain, H atoms are oxidized to H^+ ions at the beginning and O atoms are reduced to OH^- ions at the end. If the beginning and end of the chain are at opposite sides of a membrane, then a flow of electrons along the chain will be accompanied by secretion of H^+ ions at one surface of the membrane and of OH^- ions at the other. With the aid of suitable shuttles or selectively permeable membranes the primary transport of H^+ and OH^- ions can, in theory, be made to drive uphill movements of ions of other species. The attractiveness of this hypothesis is that in many tissues ion transport occurs only in association with oxidation through the cytochrome system, and that, as far as the primary transport of H^+ and OH^- ions is concerned, there is no need to postulate the existence of unknown intermediates. Nevertheless, as a general explanation of ion transport it is not acceptable for the following reasons:

(i) Active transport of sodium and potassium ions in mammalian red cells has many features in common with the transport of these ions in nerve and muscle (Glynn, 1957) but the energy for it comes from glycolysis not respiration (Maizels, 1951).

(ii) The hypothesis predicts that only four univalent ions should be transported for each molecule of oxygen used. In frog skin (Zerahn, 1956; Leaf & Renshaw, 1957), frog muscle (Frazier & Keynes, 1959) and possibly in gastric mucosa (Crane & Davies, 1951; but cf. Conway, 1953) there is evidence that this ratio may be exceeded.

(iii) There is now strong evidence that ATP can provide the energy for active transport (see Dunham & Glynn, 1961, for summary and references).

In 1952 Davies & Krebs considered the conclusion of Crane & Davies (1951) that up to 12 H^+ ions could be secreted by the gastric mucosa for each molecule of oxygen used, and pointed out that such high ratios might occur if hydrogen ion transport was being driven by phosphate-bond energy through a reversal of the reactions normally coupling oxidation of pyridine nucleotides, flavoproteins or cytochromes with ATP synthesis. At that time it was not known if these coupling reactions were reversible, though it was known that, in the reversal of glycolysis, energy from ATP was used to reduce phosphoglycerate to phosphoglyceraldehyde. The demonstration by Chance (1961) and Klingenberg & Schollmeyer (1961) that 'reductive dephosphorylation' could occur in mitochondria obviously makes Davies & Krebs's hypothesis extremely attractive.

But however attractive this hypothesis may be as an explanation of hydrogen-ion secretion, the experiments described in the present paper provide little evidence that it can be applied to the active transport of sodium and potassium. The spectroscopic evidence is entirely negative; the inhibition by hydrogen peroxide may be discounted as a sulphydryl effect; and the effect of the reducing agents is too small to be used as an argument that redox mechanisms are involved. This leaves only the results with oligomycin.

Oligomycin is known to inhibit oxidative phosphorylation in intact mitochondria, but if the mitochondria are treated with uncoupling agents like dinitrophenol, so that respiration is unaccompanied by phosphorylation, oligomycin has no effect (Lardy *et al.* 1958). In submitochondrial particles prepared ultrasonically oligomycin inhibits phosphorylation but does not affect respiration (Lardy *et al.* 1958). Oligomycin inhibits the ATP-ase activity that can be induced in intact mitochondria by treatment with uncoupling agents; it also inhibits the ATP-ase activity shown by ultrasonic particles in the presence of magnesium ions (Lardy *et al.* 1958). Both in intact mitochondria and in submitochondrial particles the ATP–$^{32}P_i$ exchange that accompanies the splitting of ATP is inhibited (Lardy *et al.* 1958; Kulka & Cooper, 1962). All these observations suggest that the site of action of oligomycin lies beyond the stage of electron transfer.

Now it is known that arsenate stimulates mitochondrial respiration and induces ATP-ase activity in intact mitochondria (Crane & Lipmann, 1953; Azzone & Ernster, 1961), and stimulates the Mg^{2+}-activated ATP-ase in submitochondrial particles (Wadkins, 1960). These effects can be antagonized with inorganic phosphate, which acts competitively, and they can be prevented with oligomycin (Wadkins, 1960; Azzone & Ernster, 1961). If the effects of arsenate are due to 'arsenolysis'—and the competitive effect of phosphate makes this likely—then the fact that oligomycin prevents both the stimulation of respiration and the induction of ATP-ase activity is most simply explained by supposing that arsenate and oligomycin act at the same site and that this is the site at which phosphate is taken up.

If this view of oligomycin action is correct, the inhibition of electric-organ ATP-ase, or of sodium transport in red cells, by oligomycin provides no evidence that redox mechanisms are involved. It does, however,

suggest that there may be a relation between the 'transport ATP-ase' and the stage of oxidative phosphorylation at which inorganic phosphate is incorporated. The idea that two such fundamental processes as ion transport and oxidative phosphorylation might share a common step or steps is attractive, though it should be remembered that the evidence for it rests entirely on the supposed specificity of the inhibitory action of oligomycin. If the sharing of oligomycin sensitivity does denote a common step, then it is interesting that light-induced phosphorylation in *Rhodospirillum rubrum* is also inhibited by oligomycin (Baltscheffsky & Baltscheffsky, 1960).

SUMMARY

1. The 'microsomal' fraction of the electric organ of *Electrophorus electricus* has been found to be very rich in ATP-ase activity. More than 90 % of this activity requires the presence of both Na and K, as well as Mg, and is inhibited by ouabain. It therefore has the characteristic features of the 'transport ATP-ase' of red cells.

2. Sodium lauryl sulphate (0·5 g/100 ml.) completely inhibits the ouabain-sensitive ATP-ase activity but leaves the residual activity unaffected.

3. 2:4-dinitrophenol (1 mM) has no effect on the ATP-ase activity. Oligomycin at a concentration of 3 μg/ml. inhibits about half the activity, and at a concentration of 10 μg/ml. inhibits just under three quarters of the activity. Oligomycin also causes partial inhibition of the active Na extrusion from intact red cells.

4. The effects of a number of reducing and oxidizing agents on the microsomal ATP-ase have been investigated. The reducing agents cause about 20–30 % inhibition; hydrogen peroxide (5 mM) causes about 65 % inhibition.

5. Partial or complete inhibition of the ATP-ase activity can be obtained with several sulphydryl reagents.

6. Comparison of the absorption spectrum of the microsomal preparation in the oxidized and in the reduced state shows no trace of cytochromes, flavoproteins, ubiquinone or pyridine nucleotides.

7. It is concluded that

(i) the microsomal fraction is rich in 'transport ATP-ase';

(ii) there is no convincing evidence that oxidation-reduction reactions are involved, but

(iii) the sensitivity to oligomycin may imply that ion transport and oxidative phosphorylation share a common step.

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Note added in proof. Oligomycin inhibition of a (Na + K)-activated ATP-ase from electric organ has recently been reported by Jöbsis & Vreman (1963), who note that the inhibition was more marked at 25° than at 37° C. They also found that 1·18 μg oligomycin/ml. caused 50% inhibition of a partially purified (Na + K)-activated ATP-ase in the microsomal fraction from rabbit brain. Smaller effects of oligomycin on brain microsomal ATP-ase have been described by Järnefelt (1962) and Van Groningen & Slater (1963).

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