BBA 45977

CONVERSION OF BIOMEMBRANE-PRODUCED ENERGY INTO ELECTRIC FORM

I. SUBMITOCHONDRIAL PARTICLES

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(Received January 19th, 1970)

SUMMARY

The hypothesis of an electric membrane potential generated by respiration or ATP hydrolysis in submitochondrial particles has been verified. To this end a number of synthetic ions penetrating lipid membranes were used.

Penetrating anions of phenyl dicarbaundecaborane (PCB^-), tetraphenyl boron and picrate were shown to accumulate in sonicated submitochondrial particles in an energy-dependent manner. The process was inhibited by rotenone, antimycin and cyanide if supported by respiration, and by oligomycin, if ATP was used as the energy source. Uncouplers were inhibitory in both cases. The following oxidation reactions were found to support the energy-dependent accumulation of PCB^-: oxidation of NADH by oxygen or fumarate; oxidation of succinate or ascorbate by oxygen; oxidation of NADPH by NAD^+. In the latter case, which is the reverse of the energy-requiring transhydrogenase reaction, ion transport was inhibited by NADH and $NADP^+$ as well as by uncouplers. Oxidation of NADH by $NADP^+$ in the energy-requiring transhydrogenase reaction was accompanied by an efflux of PCB^- anions which had accumulated during succinate oxidation. The redox 'succinate–ferricyanide' couple could not be used as a supply of energy for the accumulation of PCB^-.

Particles deprived of the coupling factor F_1 showed a decreased ability for respiration-dependent anion uptake, the process being stimulated by oligomycin. ATP-driven PCB^- accumulation was completely absent in F_1-deprived particles but could be reconstituted after preincubation with F_1.

The active accumulation of anions penetrating into particles was readily distinguished from passive anion absorption, since the latter did not require energy and could be demonstrated both in native particles and in those deprived of F_1, as well as in phospholipid micelles. The energy-dependent accumulation of anions

Abbreviations: PCB^-, phenyl dicarbaundecaborane anion; DDA^+, *N,N*-dibenzyl *N,N*-dimethyl ammonium cation; FCCP, *p*-trifluoromethoxycarbonyl cyanide phenylhydrazone; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; TTFB, tetrachlorotrifluoromethyl benzimidazole.

penetrating into submitochondrial particles was accompanied by alkalinization of the incubation medium. The efflux of ions upon the cessation of the energy supply induced acidification.

Anion accumulation was followed by the suppression of other energy-linked functions of submitochondrial particles. Under the same conditions the penetrating cations, dibenzyl dimethyl ammonium, tetrabutyl ammonium and triphenyl methyl phosphonium, did not affect either the pH of the medium or energy-linked functions.

It was concluded that a mechanism for ion accumulation in submitochondrial particles is specific for the sign of the charge but not for other features of the penetrating compounds. This mechanism operates in such a way that anions, but not cations, are pumped into the particle as if the process were supported by an electric field, orientated across the membrane, being positive inside the particles.

INTRODUCTION

The possibility of transforming the chemical energy of oxidizable substrates or ATP, into the electric energy of the membrane potential has been widely discussed since 1961, when MITCHELL[1] put forward his chemiosmotic hypothesis of oxidative phosphorylation. A number of observations have recently been published supporting the idea of the membrane potential. The majority of these findings deal with the mechanism of the active transport of metallic cations into mitochondria[2–7]. According to the membrane potential hypothesis, accumulation of Ca^{2+}, Sr^{2+}, Mn^{2+} or K^{+} in the presence of valinomycin in mitochondria is due to the movement of these cations in the electric field produced by respiration or ATP hydrolysis. This seemed an attractive interpretation, but no unequivocal proof was furnished. An alternative explanation was proposed assuming the active cation transport in mitochondria to be carried out due to conformational changes in the hypothetical 'ion translocase'[8].

If the membrane potential hypothesis is true, and cations move into mitochondria in an electric field, the mechanism of cation accumulation should be rather nonspecific. It must be operative with any ion which is able to go through the membrane, the direction of cation and anion flows being opposite.

This paper, and the three following ones, summarize the results of experiments with some artificial ions of different structure readily penetrating across the lipid membranes. Such ions were used as a means of detecting the electric potential difference across the membranes of mitochondria and photosynthetic bacteria. As a part of the study, direct evidence for the conversion of biomembrane-produced energy into the form of electric potential was obtained (preliminary note, see ref. 9).

EXPERIMENTAL

Biomolecular phospholipid membranes were prepared according to MUELLER *et al.*[10]. A solution of the total phospholipid fraction from beef heart mitochondria in heptane (20 mg phospholipids/ml) was used. The membrane resistance was about $1.10^{9}\,\Omega\cdot cm^{2}$ (for the method of measuring membrane resistance, see ref. 11). Phospholipid micelles were prepared after BANGHAM *et al.*[12] using the same heptane solution of mitochondrial phospholipids.

Phosphorylating submitochondrial particles were prepared from the 'heavy' fraction of beef heart mitochondria[13] essentially according to HANSEN AND SMITH[14]. 'Heavy' mitochondria were suspended in a solution containing 0.25 M sucrose, 0.01 M Tris–HCl, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 1 mM ATP and 1 mM succinate (pH 8.2). A suspension containing 20–30 mg of protein/ml was twice sonicated under cooling in an MSE 500-W ultrasonic disintegrator at a frequency of 20 kcycles, 0.5 A and at maximal resonance, for 1.5 min at intervals of 3–5 min. After sonication the pH of the mixture was once more adjusted to 8.2 and the mixture centrifuged at 10000 × *g* for 10 min. The sediment was removed and the supernatant was centrifuged at 105000 × *g* for 30 min. The sedimented submitochondrial particles were suspended and diluted to a concentration of 50–80 mg of protein/ml in the buffer solution of the composition mentioned above at pH 7.5.

The particles deprived of the coupling factor F_1 were prepared by the method of RACKER AND HORSTMAN[15]. Purification of F_1 was carried out according to the procedure of PULLMAN *et al.*[16] except that the heat treatment was omitted.

Measurements

The respiration of submitochondrial particles was measured polarographically using a stationary platinum electrode. The kinetics of the oxidative phosphorylation were followed by measuring the fluorescence of NADPH in a mixture supplemented with glucose, hexokinase, $NADP^+$ and glucose-6-phosphate dehydrogenase. Reversed electron transfer and the transhydrogenase reaction were measured fluorimetrically by the increase in NAD(P)H fluorescence. Fluorescence was excited with light at 360 nm and measured at 460 nm. ATPase activity (and in some experiments, oxidative phosphorylation as well) was measured according to the method of CHANCE[17] using pH meter techniques. The kinetics of the pH changes were followed with an LPU-01 meter; glass electrodes were used. (For details of the above methods, see ref. 18.)

The uptake of ions penetrating into the submitochondrail particles was measured by the method described earlier[19]. This method is based on the use of the phospholipid membrane as the selective electrode for the penetrating ion.

The experimental cell (Fig. 1) consisted of two vessels, the larger made of glass into which was inserted a smaller one made of Teflon. In the wall of the Teflon vessel a hole of 1-mm diameter was made. The hole was covered with a phospholipid membrane. Before the beginning of the experiment, both vessels were filled with the same solution containing an ionized compound that could easily penetrate through

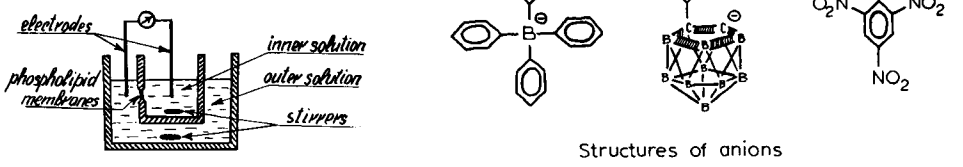

Fig. 1. The chamber for measuring the concentration of penetrating anions.

the phospholipid membrane. The volume of the solution in the internal vessel was about 2.5 ml. In the majority of experiments, phenyl dicarbaundecaborane anion (PCB^-) was used as the penetrating ion. Absorption of the anion by submitochondrial particles added to the solution in the internal vessel caused a decrease in the anion concentration inside the Teflon vessel and the appearance of a potential difference across the phospholipid membrane. In the range of PCB^- concentrations above $1 \cdot 10^{-7}$ M, a 10-fold concentration gradient of PCB^- across the phospholipid membrane gave a potential difference of 58 mV, which is in agreement with the value calculated from the Nernst equation. Thus, measurement of the potential difference across the membrane allows the kinetics of the concentration changes of penetrating anions in the incubation mixture to be followed.

RESULTS AND DISCUSSION

Selection of penetrating ions

Anions and cations capable of penetrating across the mitochondrial membrane were selected for experiments in which bimolecular phospholipid membranes were used as the model system.

Amongst the ionized compounds of greatest interest for this study would be a range of substances whose structures differ from each other and from those of the natural penetrating ions. In this case, any interaction of a penetrating ion with specific translocases in the mitochondrial membrane must be extremely unlikely.

As was shown in experiments by LEHNINGER's group and ours[9,20–24], there is a correlation between the ability of different compounds to uncouple oxidative phosphorylation in mitochondria and to increase proton conductance in phospholipid membranes. The use of the phsopholipid membrane model allowed us to find some new and very effective uncouplers of oxidative phosphorylation. This observation suggests that phospholipid membranes could also be used in the search for penetrating ions.

A number of synthetic ions were found to penetrate across the phospholipid membrane. These ions gave rise to an increase in the electric conductance of the system due to their diffusion across the membrane. When the concentration of penetrating ions on both sides of the membrane was unequal, a potential difference arose across the membrane. The negative charge was found to be on the side of lower concentration in the case of penetrating anions and on the side of higher concentration in the case of penetrating cations. Penetrating ions chosen for the subsequent experiments did not induce the conductance of the phospholipid membranes for other ions usually present in the incubation mixture with mitochondria or submitochondrial particles.

The highest penetrating ability was shown by those compounds whose ionized atom was screened by hydrophobic substituents and whose charge was strongly delocalized. Anions of phenyl dicarbaundecaborane (PCB^-) and tetraphenyl boron (TPB^-) which seemed to meet these requirements in the best way, appreciably increased the electric conductance of the membrane beginning from a concentration of $1 \cdot 10^{-9}$ M. The electric conductance had a linear dependence on the concentrations of PCB^- or tetraphenyl boron in water. A $1 \cdot 10^5$ increase in electric conductance was observed when the anion concentration was increased from $1 \cdot 10^{-9}$ to $1 \cdot 10^{-4}$ M

(Fig. 2). The picrate anion (trinitrophenol, TNP^-) also proved capable of penetrating across the membranes. These three anions (PCB^-, tetraphenyl boron and picrate) were used in the later experiments with mitochondria, submitochondrial particles and bacterial chromatophores.

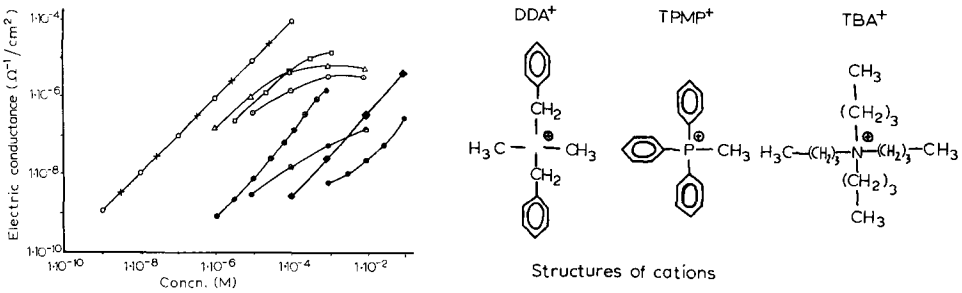

Fig. 2. The effect of synthetic ions on the electric conductance of phospholipid membranes. All probes contained 0.03 M Tris–HCl buffer (pH 7.5). ○—○, PCB^-; +—+, tetraphenyl boron; ⊕—⊕, trinitrophenol; ●—●, DDA^+; ■—■, triphenyl methyl phosphonium; ⓘ—ⓘ, tetrabutyl ammonium; □—□, DDA^+ in the presence of $3 \cdot 10^{-7}$ M tetraphenyl boron; ⊙—⊙, triphenyl methyl phosphonium in the presence of $3 \cdot 10^{-7}$ M of PCB^-; △—△, tetrabutyl ammonium in the presence of $3 \cdot 10^{-7}$ M PCB^-. This experiment as well as the following ones was carried out at room temperature.

From among the penetrating cations, three were selected: dibenzyl dimethyl ammonium (DDA^+), tetrabutyl ammonium (TBA^+), and triphenyl methyl phosphonium ($TPMP^+$). In order to obtain a measurable effect on the electric conductance, these compounds had to be added in greater amounts than the anions mentioned above. The permeability of the membrane for cations could be appreciably increased by the addition of low concentrations of penetrating anions (Fig. 2).

Anion accumulation in phosphorylating submitochondrial particles

While testing the penetrating anions in experiments with submitochondrial particles (or intact mitochondria and bacterial chromatophores as well), it was noticed that PCB^-, tetraphenyl boron and picrate were readily absorbed from solution.

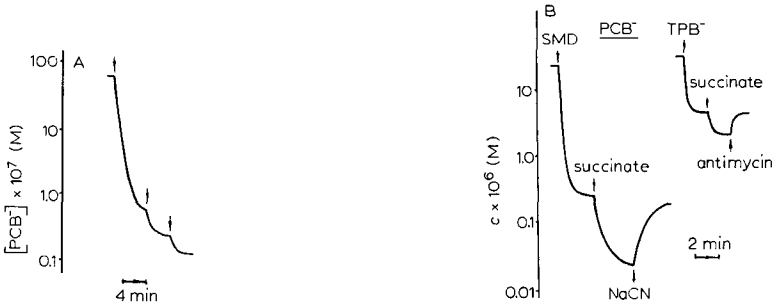

Fig. 3. The uptake of the penetrating anoins, phenyl dicarbaundecaborane (PCB^-) and tetraphenyl boron (TPB^-), by phospholipid micelles (A) and submitochondrial particles (B). A. Additions of 1.3 mg of phospholipid micelles to the water solution of PCB^- are indicated by arrows. B. Incubation mixture: 0.25 M sucrose, 0.03 M Tris buffer (pH 7.5), $5 \cdot 10^{-3}$ M $MgSO_4$. Additions: $5 \cdot 10^{-3}$ M succinate; submitochondrial particles (SMP) (0.6 mg of protein/ml); $2 \cdot 10^{-3}$ M NaCN; $1 \cdot 10^{-5}$ M antimycin A.

The simplest system tested possessing the ability to absorb the anions proved to be phospholipid micelles. Fig. 3A shows the absorption of PCB^- by micelles prepared from mitochondrial phospholipids. It is seen that the addition of micelles to the PCB^- solution results in a strong decrease in the concentration of free PCB^-. Further lowering of the anion concentration in the solution could be obtained only on the addition of another portion of micelles.

If submitochondrial particles were used instead of micelles, additional PCB^- uptake could be obtained upon the transition of particles to the energized state. Fig. 3B shows that addition of oxidizable substrate (succinate) to the particles after the termination of passive absorption of anions (PCB^- or tetraphenyl boron) induces a further lowering of the anion concentration in the solution. Cessation of the energy supply by a respiratory inhibitor leads to the efflux of anions that had accumulated during the energized state.

The extra uptake of PCB^- anions by particles previously equilibrated with PCB^- took place whenever an energized state was induced regardless of the type of energy source. Fig. 4 shows the energy-dependent PCB^- uptake by submitochondrial particles preincubated with PCB^- without an energy source. It is seen that particles take up PCB^- in response to the appearance of ATP in the system. Addition of oligomycin causes the efflux of the anions that are accumulated in the presence

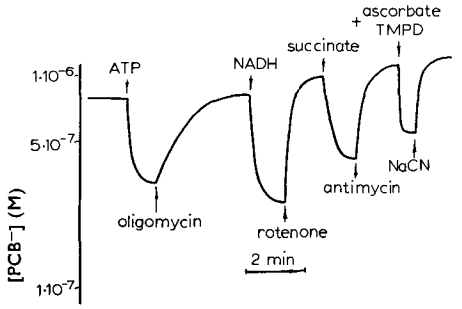

Fig. 4. The utilization of different energy sources for the energy-dependent uptake of PCB^- anions by particles. Incubation mixture: 0.25 M sucrose, 0.05 M Tris buffer (pH 7.5), $6 \cdot 10^{-3}$ M $MgSO_4$, particles (0.6 mg of protein/ml). Additions: $2 \cdot 10^{-3}$ M ATP, 2 μg/ml oligomycin, $1 \cdot 10^{-3}$ M NADH, $3 \cdot 10^{-5}$ M rotenone, 0.01 M succinate, $1.4 \cdot 10^{-6}$ M antimycin A, $2 \cdot 10^{-4}$ M TMPD, $8 \cdot 10^{-3}$ M ascorbate, $2 \cdot 10^{-3}$ M NaCN.

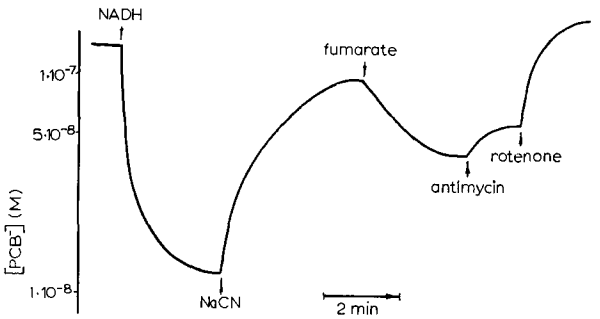

Fig. 5. PCB^- accumulation in particles supported by the operation of three and only one (first) coupling sites. Incubation mixture as in Fig. 4 with the addition of 0.13 mg/ml of alcohol dehydrogenase and 0.95% ethanol. Particles, 0.9 mg of protein/ml. Additions: $1 \cdot 10^{-3}$ M NADH, $2 \cdot 10^{-3}$ M NaCN, 0.01 M fumarate, $8 \cdot 10^{-7}$ M antimycin A, $3 \cdot 10^{-5}$ M rotenone.

of ATP. Addition of NADH results in the influx; rotenone, efflux; succinate, influx; antimycin, efflux; ascorbate and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD), influx; and cyanide, efflux of PCB^-. In Fig. 5 the energy-dependent PCB^- influxes supported by operation of the first coupling site and the total respiratory chain are compared. It is seen that oxidation of NADH by oxygen results in a more pronounced decrease in the concentration of external PCB^- than oxidation of NADH by fumarate (the oxidation rate in the latter case was much lower).

All attempts failed to demonstrate PCB^- accumulation on oxidation of succinate by ferricyanide in the presence of cyanide. It could have been a consequence of the inaccessibility for ferricyanide of internal surface of the sonicated particle membrane in whose proximity cytochromes c_1 and c should be localized[2,25].

Reversal of the energy-requiring transhydrogenase reaction as an energy source for anion accumulation

The use of anion accumulation as a probe for an energized state allows the demonstration of energy conservation during reversal of the energy-requiring transhydrogenase reaction described by ERNSTER[26,27]. Fig. 6 shows the results of these experiments. One can see (Fig. 6A) that addition of both NADPH and NAD^+ to

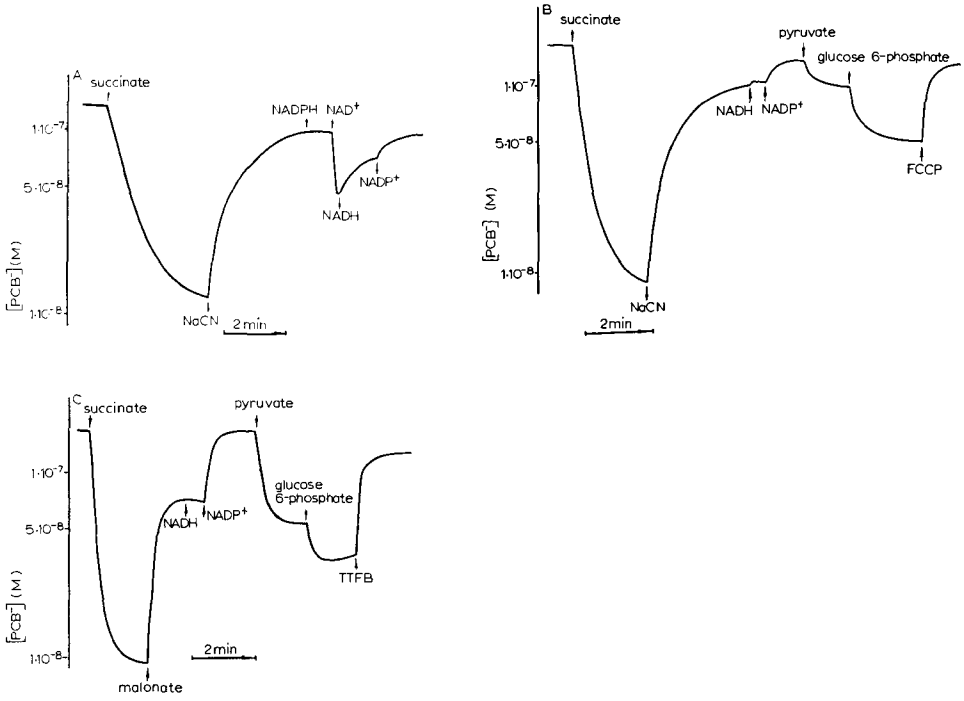

Fig. 6. Accumulation of PCB^- anions at the expense of reversal of the energy-requiring transhydrogenase reaction. In Expts. B and C the incubation mixture (see Fig. 4) was supplemented with glucose-6-phosphate dehydrogenase, 0.32 mg/ml, and lactate dehydrogenase, 0.02 mg/ml; in Expt. C with $3 \cdot 10^{-5}$ M rotenone. Additions: 0.01 M succinate, $3 \cdot 10^{-3}$ M (A) and $2 \cdot 10^{-3}$ M (B) NaCN, $1 \cdot 10^{-3}$ M NADH, $1 \cdot 10^{-3}$ M NAD^+, $1 \cdot 10^{-3}$ M NADPH, $1 \cdot 10^{-3}$ M $NADP^+$, 5 mM malonate, 0.01 M pyruvate, $2.4 \cdot 10^{-3}$ M (B) and $8 \cdot 10^{-3}$ M (C) glucose 6-phosphate, $2 \cdot 10^{-7}$ M *p*-trifluoromethoxycarbonyl cyanide phenylhydrazone (FCCP), $2 \cdot 10^{-5}$ M tetrachlorotrifluoromethyl benzimidazole (TTFB).

particles treated with rotenone, and equilibrated with PCB^- in the absence of available energy sources, induces influx of PCB^-. Subsequent addition of substrates of the energy-requiring transhydrogenase reaction, $NADP^+$ and NADH, results in the efflux of previously accumulated PCB^-.

It is remarkable that for complete reversal of the effect of NADPH and NAD^+ it is enough to equalize the concentrations of NADPH and $NADP^+$, NAD^+ and NADH. For comparison, the PCB^- uptake during succinate oxidation is shown in the same figure. Figs. 6B and 6C demonstrate the results of experiments in which different ratios of reduced and oxidized nicotinamide nucleotides were attained without any change in the size of the whole pool of $NAD(P)H + NAD(P)^+$. Submitochondrial particles were incubated with PCB^-, rotenone, skeletal muscle lactate dehydrogenase and glucose-6-phosphate dehydrogenase. It is seen that addition of succinate induces PCB^- uptake, the process being almost completely reversed by cyanide. Addition of NADH and $NADP^+$ (substrates of the energy-requiring transhydrogenase reaction) results in some increase in the PCB^- concentration which reaches the level maintained in the deenergized state. Oxidation of NADH by pyruvate induces the influx of the portion of PCB^- released during incubation with NADH and $NADP^+$. Reduction of $NADP^+$ by glucose 6-phosphate results in further uptake of PCB^- from the solution. Addition of the uncoupler, FCCP, causes the efflux of PCB^- that had accumulated after treatment with glucose 6-phosphate.

In the experiment presented in Fig. 6C, the effects of nicotinamide nucleotides were tested under somewhat other conditions. 5 mM malonate was added to particles, treated with 10 mM succinate, to decrease the rate of respiration. As is seen in the figure, addition of malonate was followed by the efflux of about half the PCB^- that had accumulated in the particles during succinate oxidation. Subsequent additions of NADH, $NADP^+$ and pyruvate resulted in PCB^- concentration changes in the same direction as in Fig. 6B, but with greater amplitude. The effect of glucose 6-phosphate was not so pronounced as in Fig. 6B. The quantitative differences in the responses between Fig. 6B and Fig. 6C seem to be due to a greater inhibition of respiration by cyanide (Fig. 6B) than by malonate (Fig. 6C).

The above results show that hydrogen transfer between two nicotinamide nucleotides is able to support PCB^- accumulation if the direction of the process is $NADPH \rightarrow NAD^+$. A decrease of $NADPH/NADP^+$ and $NAD^+/NADH$ ratios, as well as the addition of an uncoupler, results in the loss of the accumulated anions. The transhydrogenase reaction in the energy-requiring direction ($NADH \rightarrow NADP^+$) inhibits active transport of PCB^-.

Accumulation of penetrating ions in the F_1-deprived particles

An attempt was made to fractionate submitochondrial particles in order to determine the lowest level of organization allowing the phenomenon of energy-dependent anion accumulation to be observed. For this purpose the procedure of RACKER AND HORSTMAN[15] was employed. F_1-deprived particles obtained by this method lose both the knob-like subunits attached to the outer surface of the membrane and their ATP-synthetase activity, but the enzymes of the respiratory chain are retained. The fractionated particles were found to be competent in passive absorption and, to some degree, in the respiration-driven accumulation of penetrating anions. ATP did not support the anion uptake. Addition of oligomycin stimulated the process

of respiration-driven anion accumulation. Incubation of fractionated particles with the coupling factor F_1, resulted in the reconstitution of the initial system competent in both respiration- and ATP-driven anion transport (Fig. 7). These results are in agreement with the previous observations of other laboratories concerning the 'coupling' activity of oligomycin and F_1 in F_1-deprived submitochondrial particles[28,29].

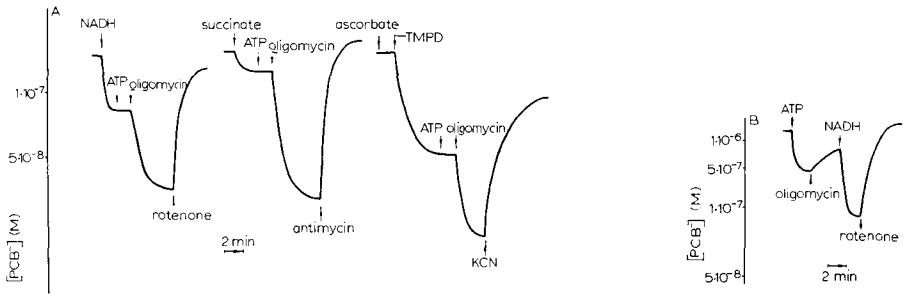

Fig. 7. Accumulation of PCB^- anions in submitochondrial particles deprived of the coupling factor F_1. Incubation mixture contained 1.7 mg protein of the F_1-deprived particles per ml, 0.25 M sucrose, 0.03 M Tris buffer (pH 7.5), $5 \cdot 10^{-3}$ M $MgSO_4$. Additions: $3.5 \cdot 10^{-3}$ M NADH, $1 \cdot 10^{-3}$ M ATP, 2 μg/ml of oligomycin, $2.6 \cdot 10^{-5}$ M rotenone, $3.5 \cdot 10^{-3}$ M succinate, $6 \cdot 10^{-6}$ M antimycin A, $2 \cdot 10^{-4}$ M TMPD, $2 \cdot 10^{-4}$ M ascorbate and $7 \cdot 10^{-3}$ M KCN. In Expt. B depleted particles were incubated for 10 min at room temperature in the presence of 0.12 mg/ml of coupling factor F_1.

pH responses of particles on the addition of penetrating ions

As can be seen from Fig. 8, addition of the penetrating anions of PCB^-, tetraphenyl boron and picrate to particles oxidizing succinate causes an increase in the pH of the incubation mixture. At the same time, the penetrating cations DDA^+, triphenyl methyl phosphonium and terrabutyl ammonium do not affect the pH. Various factors that destroy the energy supply for active anion transport reverse the pH changes completely or partially. Figs. 8A and B show the acidification of the medium after the addition of an uncoupler (TTFB), or a respiratory chain inhibitor (antimycin), to the particles that are loaded with penetrating anions during succinate oxidation.

Fig. 8C demonstrates the pH responses of F_1-deprived particles. In this case, addition of a penetrating anion results in a very small pH change. Treatment with oligomycin restores the pH responses to a level similar to that in the nonfractionated particles (alkalinization after addition of PCB^- reversed by TTFB). This effect resembles that observed by SCHOLES *et al.*[30], who showed that oligomycin stimulated the light-induced pH responses on K^+ extrusion from bacterial chromatophores (see also ref. 31). The effect of oligomycin on the pH responses of phosphorylating submitochondrial particles was mentioned by MITCHELL AND MOYLE[32].

Effect of anion accumulation process on other energy-linked functions of particles

It was found that accumulation of penetrating ions is acccompanied by the inhibition of such energy-linked functions as reversed electron transfer, reduction of $NADP^+$ by NADH, and oxidative phosphorylation. Fig. 9 shows the effect of PCB^- on the rate of NAD^+ reduction by succinate. It is seen that addition of PCB^-, up to a total concentration of $1 \cdot 10^{-6}$ M, causes a steady inhibition of reversed electron

transfer. This effect is apparently due to some uncoupling which accompanies the accumulation of PCB^- in particles. Polarographic measurements showed that PCB^- has little (if any) effect on the oxidation of NADH by oxygen within the range of the anion concentrations used in the above experiments. Relatively low concentrations of two other penetrating anions, tetraphenyl boron and picrate, also inhibited energy-linked functions in the particles. Penetrating cations under the same conditions do not affect the functions of particles up to concentrations as high as $1 \cdot 10^{-2}$ M.

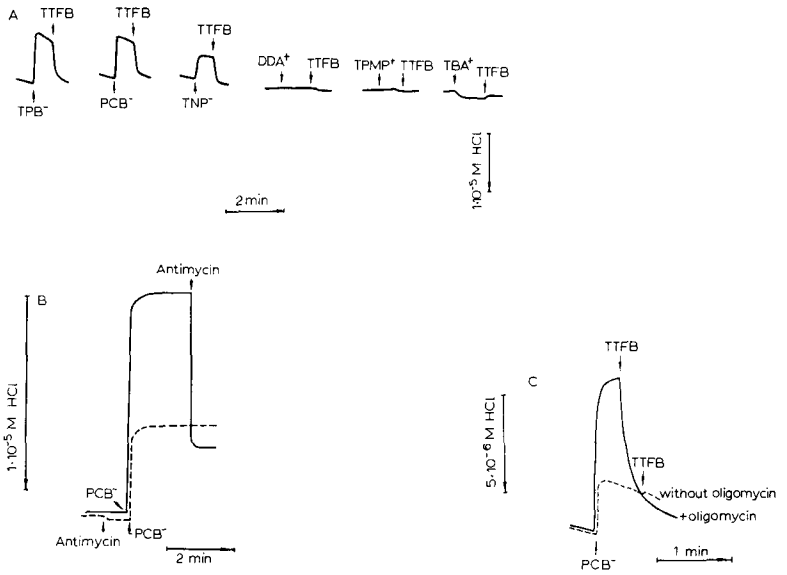

Fig. 8. pH changes on the addition of penetrating ions to a suspension of submitochondrial particles oxidizing succinate. Incubation mixture in Expts. A and B contained 0.25 M sucrose, $5 \cdot 10^{-3}$ M $MgCl_2$, $5 \cdot 10^{-4}$ M sodium succinate, $1 \cdot 10^{-3}$ M EDTA, $1 \cdot 10^{-3}$ M Tris buffer (pH 7.5), 2 g of protein/l of submitochondrial particles. Expt. C, 0.25 M sucrose, $4.7 \cdot 10^{-4}$ M succinate, $9 \cdot 10^{-4}$ M Tris buffer (pH 7.5), submitochondrial particles deprived of factor F_1 (2.1 g of protein/l). Concentrations of the additions: Expt. A, $5 \cdot 10^{-5}$ M tetraphenyl boron (TPB^-), PCB^-, trinitrophenol (TNP^-), DDA^+, triphenyl methyl phosphonium ($TPMP^+$), tetrabutyl ammonium (TBA^+), $9 \cdot 10^{-6}$ M TTFB. To the samples with DDA^+, triphenyl methyl phosphonium and tetrabutyl ammonium $3 \cdot 10^{-7}$ M tetraphenyl boron was added. Expt. B, $4 \cdot 10^{-5}$ M PCB^-, $1 \cdot 5 \cdot 10^{-6}$ M antimycin A. Expt. C, $4 \cdot 10^{-5}$ M PCB^-, $9 \cdot 10^{-6}$ M TTFB, 0.8 mg/l oligomycin. Here and below the pH scale is graduated by the response to the addition of HCl assuming the volume of the sample to be 1 l.

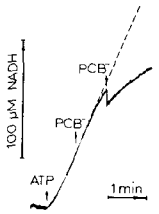

Fig. 9. The action of PCB^- on reversed electron transport in submitochondrial particles. The incubation mixture contained: 0.25 M sucrose, 0.01 M Tris buffer (pH 7.5), 0.025 M succinate, $5 \cdot 10^{-3}$ M NaCN, $2.5 \cdot 10^{-4}$ M NAD^+, 0.0125 M $MgSO_4$ and particles (1.1 mg of protein/ml). Concentrations of the additions: $1 \cdot 10^{-3}$ M ATP, $1 \cdot 10^{-7}$ M PCB^- (first addition) and $1 \cdot 10^{-6}$ M PCB^- (second addition).

The data obtained seem to be sufficient to allow the conclusion that sonicated submitochondrial particles contain an energy-dependent mechanism for ion transport. Using this mechanism, submitochondrial particles accumulate penetrating anions of different structure such as phenyl dicarbaundecaborane, tetraphenyl boron and picrate, but not the penetrating cations dibenzyl dimethyl ammonium, tetrabutyl ammonium and triphenyl methyl phosphonium.

These properties (specificity for the sign of the charge but not for other structural details of the accumulated ions) can be easily explained if one assumes that the electric field is the motive force for the active transport of ions into the particles. As far as one can judge from the direction of anion movement, the electric field is orientated across the particle membrane in a manner such that the '*plus*' is inside and the '*minus*' outside.

ACKNOWLEDGMENTS

The authors are greatly indebted to Dr. O. Yu. Okhlobistin and Dr. Yu. V. Zeifman for providing some of the substances, and to Miss T. I. Kheifets and Mr. D. O. Levitsky for translating the paper into English.

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