Book-Chapter 6: **Transport and ATP synthesis in mitochondria** (1978-1984)

IV. K^+-transport: Evidence for mitochondrial F_0F_1-ATPase being a K^+-pump.

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Abbreviations: NSPM, N'-[N"-n-nonyl-4-sulfamoylphenyl]-maleimide; TTFB, 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole; ACMA, 9-amino-6-chloro-2-methoxyacridine; SMP, submitochondrial particles; o = outside mitochondria

Summary: Our experiments demonstrate that mitochondria contain an energy driven K^+/H^+-antiporter. The energy is derived from substrate oxidation by the respiratory chain. This antiporter is Mg_o^{2+}-sensitively stimulated by $NSPM_o$, Cd_o^{2+}, $dicyclohexylcarbodiimide_o$ and Ca_o^{2+}. Quinine prevents the Mg_o^{2+}-sensitivity. Ruthenium red prevents Cd_o^{2+} and Ca_o^{2+} sensitivity.
The experiments prove further that mitochondria contain an oligomycin-sensitive, ATP- driven K^+-pump, which is identical to the oligomycin-sensitive F_1F_0-ATPase. This K^+-pump is stimulated by NSPM, picrylacetate, Cd^{2+}(Mg^{2+},Ca^{2+}) and inhibited by dicyclohexylcarbodiimide. The synthesis of ATP on the P_i/H^+-symporter, as proposed, is then highly probable, or even proven. K^+/H^+-antiporter, K^+-pump and P_i/H^+-symporter are linked together in versatile energy-driven K^+/H^+-cycling and oscillations. This system is controlled by O_2, and the free Mg^{2+} and Ca^{2+} concentrations in the cytosol of the cells and most likely also able to handle "abnormal" Ca^{2+} concentrations. A high physiological K^+-gradient between cytosol (K^+=high, ~175 mM_o) and matrix (K^+=low,~1mM_i)is established and maintained by the system. Under normal physiological conditions, no protons are detectable in the bulk phase; they are therefore either moving along the membranes via hydrogen bridges, or the proton-pumping systems are directly linked. The results imply a direct connection (and regulatory function) between nervous system (brain) and mitochondria (body and brain): for thermoregulation, substrate oxidation, as well as O_2-uptake/-reduction (anorexia/dystrophia), which can lead to arteriosclerosis, heart attack, and cancer on the one hand, and edema during anoxia/hypoxia, or shock and dilusion with coma (death) on the other hand are examples. **Key words:** Energy driven K^+/H^+-antiporter, oligomycin-sensitive ATP-driven K^+ -pump, K^+/H^+-cycling, oscillations, hydrogen bridges, connection: brain-mitochondria.

Zusammenfassung: Unsere Ergebnisse zeigen, daß die Mitochondrien einen Energie-getriebenen K^+/H^+-Antiporter enthalten. Die Energie wird durch Substrat-Oxidation in der Atmungskette geliefert. Dieser Antiporter wird Mg_a^{2+}-sensitiv stimuliert durch $NSPM_a$, Cd_a^{2+}, $dicyclohexylcarbodiimid_a$ und Ca_a^{2+}. Quinine verhindert die Mg_a^{2+}-Sensivität, Ruthenium Rot verhindert die Cd_a^{2+}- und Ca_a^{2+}-Sensivität. Die Experimente beweisen weiterhin, daß die Mitochondrien eine Oligomycin-sensitive ATP-getriebene K^+-Pumpe, identisch mit der Oligomycin-sensitiven F_1F_0-ATPase, besitzen. Diese K^+-Pumpe wird stimuliert durch NSPM, Picrylacetat, Cd^{2+} (Mg^{2+}, Ca^{2+}) und inhibiert durch Dicyclohexylcarbodiimid. Die Synthese von ATP an dem P_i/H^+-Symporter - wie vorgeschlagen - ist damit sehr wahrscheinlich, wenn nicht bewiesen. K^+/H^+-Antiporter, K^+-Pumpe und P_i/H^+-Symporter sind miteinander durch Energie-

getriebene zyklische K^+/H^+-Bewegungen und Oszillationen verbunden. Dieses System wird durch O_2 und die freien Mg^{2+}-und Ca^{2+}-Konzentrationen im Cytosol der Zellen kontrolliert und ist höchstwahrscheinlich auch in der Lage, "abnormale" Ca^{2+}-Konzentrationen zu beseitigen. Ein großer physiologischer K^+-Gradient zwischen Cytosol (K^+ = hoch, ~ 175 mM_a) und Matrix (K^+ = tief, ~ 1 mM_i) wird durch das System erstellt und erhalten. Da unter normalen physiologischen Bedingungen keine Protonen in der wässrigen Phase zu sehen sind, bewegen sie sich entweder entlang der Membran über Wasserstoffbrücken oder aber die H^+-Pumpen sind direkt miteinander verbunden. Die Resultate implizieren eine direkte Verbindung (und Regulation) zwischen Nervensystem (Gehirn) und Mitochondrien (Körper und Gehirn): Thermoregulation, Substrat-Oxidation wie auch O_2-Aufnahme/-Reduktion (Anorexia/Dystrophia) mit Arteriosklerose, Herzinfarkt, Krebs auf der einen Seite und Bildung von Ödemen während Anoxia/Hypoxia oder Schock, Wahnvorstellungen mit Koma (Tod) auf der anderen Seite sind Beispiele. **Schlüsselwörter:** Energie-getriebener K^+/H^+-Antiport, Oligomycin-sensitive ATP-getriebene K^+-Pumpe, zyklische K^+/H^+-Bewegungen, Oszillationen, Wasserstoffbrücken, Verbindung: Gehirn-Mitochondrien.

Introduction

NSPM, picrylacetate and Cd^{2+} have been shown to be very potent and specific reagents for inhibition or stimulation of various membrane-associated functions, e.g. electron transfer and transport activities [1-8].

Our hypothesis for mitochondrial ATP synthesis and the consequences of this hypothesis [4] implicate the F_0F_1-ATPase as a K-pump. Past literature includes numerous reports in support of this supposition, including our own results [4, 9]. The present manuscript focuses on K^+ (Ca^{2+})-transport across the mitochondrial and submitochondrial membranes and its relationship to the energy-linked functions performed by these particles; especially electron-transfer and K^+- or proton-pumping activities. This relationship was investigated by using various functional group-trapping reagents in addition to the compounds NSPM, picrylacetate, and Cd^{2+} as mentioned above. Preliminary results were presented [10, 11].

Materials and Methods

Rat liver mitochondria were prepared according to Kaschnitz et al [12] and suspended in 0.22 M mannitol, 70 mM sucrose, 2 mM K-hepes, pH 7.4. Oxygen uptake of mitochondria was measured with an oxygen electrode. All mitochondrial preparations were checked for structural integrity using the criterion of respiratory control [13]. Assay of endogenous calcium and phosphate concentrations, as well as swelling experiments were performed as in [2, 3]. K^+-transport rates were monitored continously at 30^0C by means of a Beckman K^+-electrode. Calibration of the K^+-electrode in each experiment was done by adding a known amount of a KCl solution [2, 3, 14]. The experiments were only possible at low K^+-concentrations. pH-jump experiments using a pH-electrode were performed as in [15-17].

$\Delta\varphi$ of mitochondria was followed using DiS-C_3-(5) fluorescence changes [18]. The fluorescence emission of DiS-C_3-(5) was measured at 667 nm at an excitation wavelength of 622 nm.

Mitochondria (1.2 mg in 40 µl 0.22 M mannitol, 70 mM sucrose, 2 mM K-hepes, pH 7.4) in 2.5 ml of a solution containing 4 µM DiS-C_3-(5), 0.22 M mannitol, 70 mM sucrose, 2 mM K-hepes, pH 7.4, 20 mM KCl, 5 mM $MgCl_2$, 10 mM K-phosphate, pH 7.4, 25 µM Rotenone, were energized with 8 mM K-succinate, pH 7.4 at 30^0C.

4-Methylumbelliferon (4-MU) fluorescence was used as one more indicator of Δ pH in mitochondria [19]. The fluorescence emission of 4-MU was measured at 450 nm at an excitation wavelength of 356 nm. Mitochondria (1.2 mg in 40 µl 0.22 M mannitol, 70 mM sucrose, 2 mM K-hepes, pH 7.4) in 2.5 ml of a solution containing 0.2 µM 4-MU, 0.22 M mannitol, 70 mM sucrose, 2 mM K-hepes, pH 7.4, 20 mM KCl, 5 mM $MgCl_2$, 10 mM K-phosphate, pH 7.4, 25 µM Rotenone, were energized with 8 mM K-succinate, pH 7.4 at 30^0C.

Phosphorylating submitochondrial particles and submitochondrial particles were prepared according to published procedures [8, 20, 21].

ATP/O-ratio, ATP-P_i-exchange (without BSA and added phospholipids), ATPase activities in the presence and absence of KCl and either in an ATP regenerating system or by the amount of anorganic phosphate released, ATP-driven reduction of NAD by K-succinate, transhydrogenation from NADH to AcPyNADP and proton permeability in 150 mM KCl by the ATP-jump method were performed as described [8, 17, 22-26].

Succinate- and ATP-dependent spectral response of oxonol VI and ACMA-fluorescence in submitochondrial particles and complex V [8] were followed as described in [8].

The effect of Cd^{2+} on the respiratory chain was evaluated on phosphorylating submitochondrial particles using a Shimadzu ultraviolet 300 dual-wavelength scanning spectrophotometer [27, 28].

O-acetyl-picric acid was prepared according to [29]. ^{3}H-acetyl-picric acid was synthesized from ^{3}H-acetic anhydride (Amersham, 500 mCi/ mmol): 64.6 mg picric acid (0.282 mmol) were warmed to 56^oC, then a mixture of 25 µl acetic anhydride and 40 µl ^{3}H-acetic anhydride was added and stirred with a glass rod. After further addition of 1 µl 70 % perchloric acid, the mixture was kept for 5 minutes at 56^oC, then cooled to 0^oC and the product purified by being washed three times with 1 ml of 4 % ice-cold acetic acid and dried over anhydrous calcium chloride. The yield of ^{3}H-picrylacetate was 55 mg and the specific radioactivity was 22900 cpm/0,1 nmol.

The ^{3}H-acetyl labeling experiments were done with 10 mM in anhydrous acetone freshly dissolved ^{3}H-acetyl-picric acid. ^{3}H-acetylation studies of various particles, complex V, and F_1 were performed identically to the picrylacetate inhibition studies on their different activities.

NSPM, DCCD and the sources of the other materials used were as described [2, 3, 8, 23], or otherwise commercially available and analytically graded. 4-Methylumbelliferon and DiS-C_3-(5) were gifts from Dr. H. Kiefer, Basel Institute of Immunology.

Results

Fig. 1 shows the effects of various uncouplers and ionophores on the coupled respiration of rat

liver mitochondria. 21 nmoles/mg of the thiol reagent NSPM (A) and 4 nA/mg of the dithiol reagent Cd^{2+} (B) stimulate Mg^{2+}-dependent respiration rate [2, 30]. Inhibition of the increased respiratory rates is almost complete by addition of 5.7 mM Mg^{2+} to the assay medium directly after adding the thiol reagents, or complete inhibition results after several minutes on addition of Mg^{2+} before the compounds. 1.1 mM quinine prevents the Mg^{2+} inhibition of the increased respiratory rates (tested at NSPM-stimulated respiration). The low concentrations of the thiol compounds used completely inhibit the uncoupling normally seen with 2.4-dinitrophenol by inhibition of the phosphate-proton-symporter as discussed [2]. 1 μg of the ionophore nigericin (catalyzing K^+/H^+-exchange) (C) stimulate – similar to NSPM or Cd^{2+} - in a high K^+-concentration medium Mg^{2+}-sensitive respiration. 2.4-dinitrophenol-uncoupling is not inhibited from this type of compound as expected. 1 μg of the K^+-ionophore, valinomycin (D), stimulates respiration as nigericin does, but in contrast to the experiments with the K^+/H^+-exchanger, Mg^{2+} further stimulates the respiration rate. A similar behavior, although not as pronounced, has been obtained using 114 μM 2.4-dinitrophenol (E).

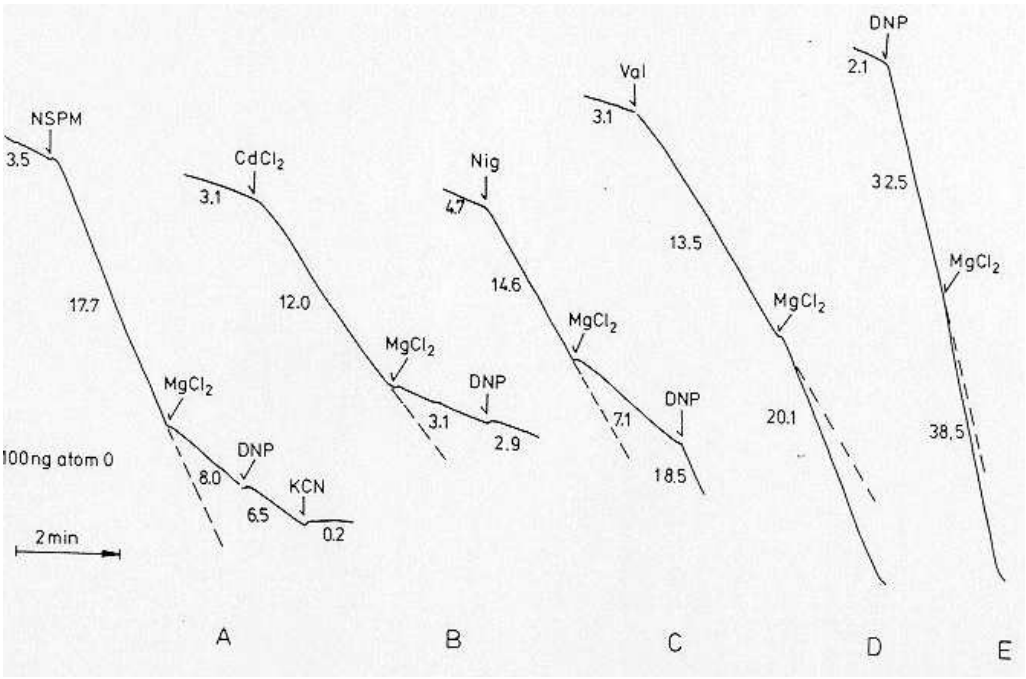

Fig.1. Effects of various uncouplers on the coupled respiration of rat liver mitochondria. The lines represent the output from the oxygen electrode. The numbers on the lines are respiration rates; nanogram atoms of oxygen per min./ per mg protein. Reaction medium contained 0.22 M mannitol, 70 mM sucrose, 2 mM K-hepes, pH 7.4, 1.2 mg mitochondria, 5 μM rotenone, 10 mM Na-succinate (A, B, E) or 10 mM K-succinate (C, D) and 30 mM KCl (C). Additions: 21 nmoles NSPM/mg protein, 5.7 mM $MgCl_2$, 114 μM DNP, 1.14 mM KCN, 5 nA $CdCl_2$/mg protein, 1 μg nigericin (Nig), 1 μg valinomycin (Val).

Fig. 2 shows active swelling of rat liver mitochondria in the presence of NSPM. 21 nmoles NSPM/mg lead to a large amplitude swelling with almost identical Mg^{2+} sensitivity under essentially the same conditions as described for the experiments depicted in Fig. 1. Cd^{2+}-induced active swelling is about half that induced by NSPM, and is sensitive to 2 nmol ruthenium red/mg mitochondria, similarly to the increased respiratory rate caused by Cd^{2+}, which is also inhibited on addition of oligomycin (+ADP). The same holds for the respiratory rate increased by Ca^{2+}/P_i. The Ca^{2+}/P_i-induced active swelling and the increased respiratory rate are strongly inhibited by 2 nmol ruthenium red/mg at concentrations also inhibiting Ca^{2+}/P_i uptake by about 50 % [2, 3, 6]. Ca^{2+}-uptake or release results in a P_i-drain in the same direction, most probably via the P_i/H^+-

symport system and the dicarboxylate carrier.

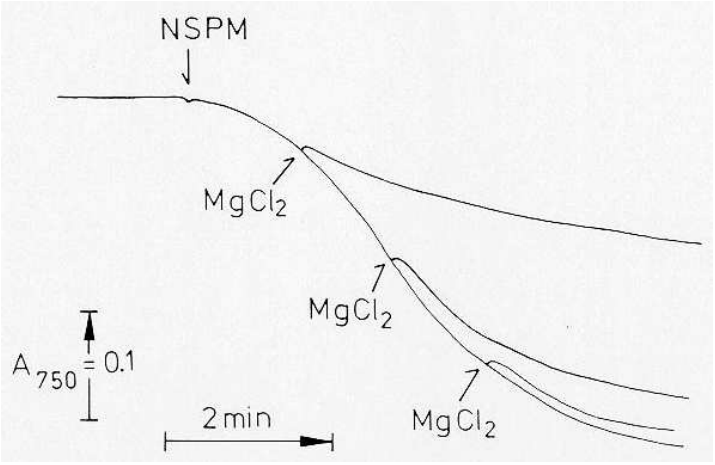

Fig. 2. Swelling of rat liver mitochondria in the presence of NSPM. Reaction medium contained 0.22 M mannitol, 70 mM sucrose, 2 mM K-hepes, pH 7.4, 1.2 mg mitochondria, 5 μM rotenone, 5 mM Na-succinate. Additions: 21 nmoles NSPM/mg protein, 10 mM $MgCl_2$.

The results presented above refer to the experiments of Connelly and Lardy [31] and should, of course, be viewed with respect to the work of Li et al [32]. A rough summation of our results makes a further exploration of the K^+-transport symport system – which is without any doubt the common denominator of our results – necessary. We therefore decided to investigate the K^+-transport activities of mitochondria.

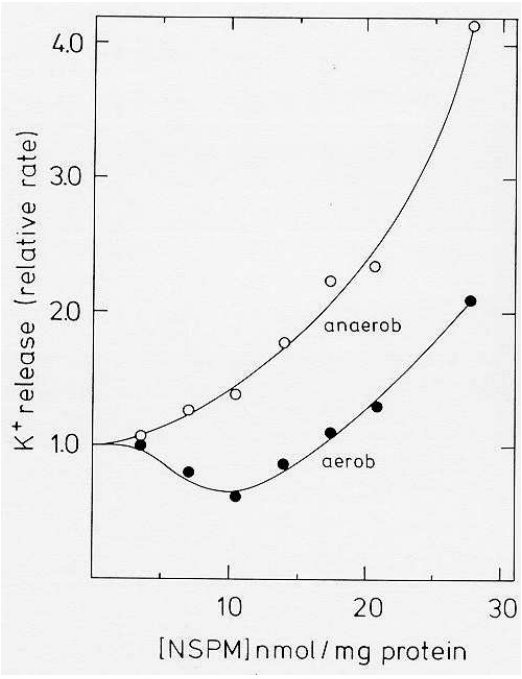

Fig. 3. K^+ release of rat liver mitochondria in the presence of NSPM. Reaction medium contained 0.22 M mannitol, 70 mM sucrose, 2 mM K-hepes, pH 7.4, 14.4 mg mitochondria, 10 μM rotenone, energization with 2 mM tris-succinate. Additions: indicated amounts of NSPM. Anaerobiosis at the break in the release curves, as calculated from oxygen-uptake measurements.

A summary of the results is presented in Fig. 3. The relative rates of K^+-release by K^+-loaded rat liver mitochondria in the presence of increasing concentrations of NSPM can be seen in the aerobe/energized state, as well as the anaerobe/deenergized state. A typical assay may be described as follows: addition of 10 μM rotenone to the mitochondria in the normal O_2-saturated assay medium leads to the release of accumulated K^+ as the endogenous substrate oxidation is blocked. Upon energization with succinate, a rapid reuptake of K^+ follows to an internal average concentration of 175 mM_i, with a remaining external concentration of 1,4 mM_o. This difference may be calculated to a K^+-concentration gradient of roughly 130 mV. After about four minutes,

there is a break in the gradually declining K_i^+ concentrations at anaerobiosis of the assay medium (as calculated from oxygen uptake measurements) to higher K^+-release values. The difference in the K^+ concentrations between the energized ($+O_2$) and deenergized ($-O_2$) state could best be resolved by using indicated concentrations of NSPM. Fig. 3 demonstrates clearly that K^+-pumping activity is linked to the substrate oxidizing respiratory chain. The results described in Figs. 1 to 3 indicate that NSPM has at least two effects on the K^+-transport system of mitochondria: at low concentrations of NSPM (below 10 nmol/mg), stimulation of K^+-uptake is visible, while at higher concentrations (above 10 nmol/mg), stimulation is counteracted by enhanced K^+-release. Taken together, both effect by NSPM add up in K^+-cycling, an enhanced Mg^{2+}-sensitive respiration-rate and swelling.

Former results imply the question about accompanying pH-changes. The aerobe pH-jump experiments, performed as described in [15], show essentially nigericin-sensitive, opposite movements of protons to K^+ (and an H^+-gradient of ~ 130 mV? which would be - together with the K^+-gradient - ~ 260 mV), with essentially the same sensitivity to NSPM. As expected, the obtained pH is much lower in the presence of phosphate than in its absence. Most interestingly, under the conditions used pH-oscillations were evident which are normally seen allusively in the other measurements. The K^+-release curve obtained under aerobic conditions shown in Fig. 3

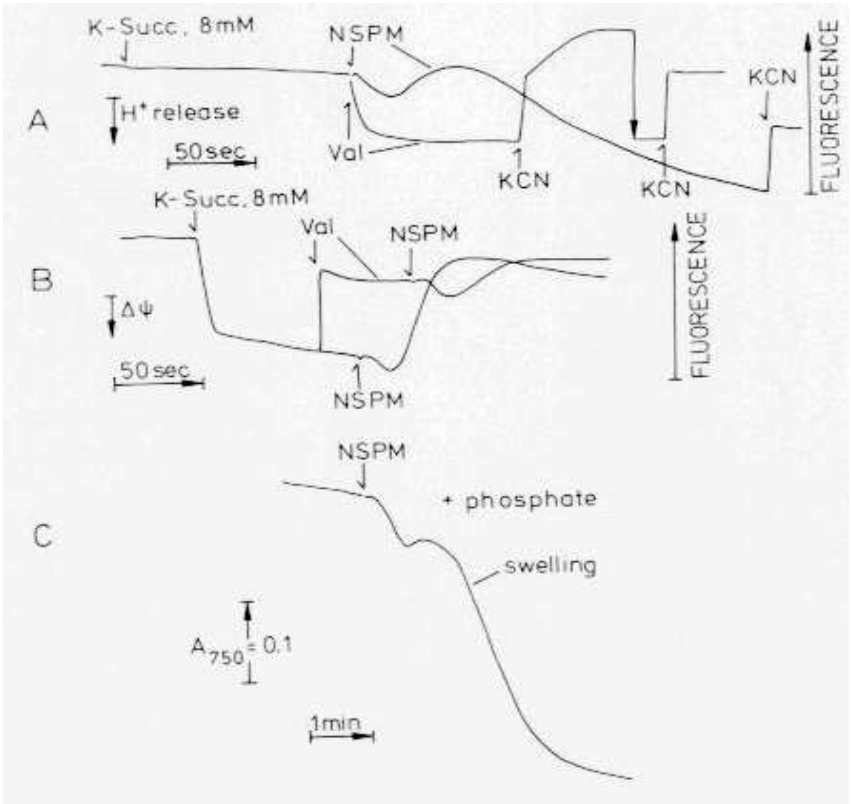

Fig. 4. pH-gradient (4-MU), membrane potential (DiS-C_3-5)) and swelling (in the presence of 10 mM K-phosphate) of rat liver mitochondria in the presence of NSPM. Reaction medium as given in Methods and Materials. Additions: 21 nmoles NSPM/mg protein, 1 µg valinomycin (Val), 0.8 mM KCN.

could be explained by the counter movement of K^+-ions. The same explanation holds for the H^+-release curve, although in this case for protons. The opposite K^+- and proton-movements are oscillating in phase.

Fig. 4 demonstrates impressively the oscillatory phenomena on proton release, membrane potential (K^+-movements), and swelling as induced by 21 nmoles NSPM/mg mitochondria. These

three reactions are almost parallel and oscillating in phase. There is no ΔpH visible under our applied conditions without the use of valinomycin or NSPM (A), but as expected, succinate induces a rapid uptake of K^+, which is sensitive to these reagents (B). The obvious result: valinomycin and NSPM change the succinate supported K^+-gradients into pH-gradients, presumably due to the K^+-cycling caused by the compounds.

Table I. The effect of NSPM on the activities of submitochondrial particles (SMP)

SMP-activity	2 min. incubation with 20 nmol/mg %
Δ pH (ACMA), succinate	400-600 a)
Δ pH (ACMA), ATP	140
H^+-release, ATP b) d)	$t_{1/2}$ ~ times 2 c)
ΔΨ (OX-VI), succinate	68 e)
ΔΨ (OX-VI), ATP	65 e)
ATP/O, succinate d)	80
ATP-P_i-exchange	70
ATPase	107
succinate to NAD, ATP-driven	7
NADH to O_2	40
NADH to Q_1	20
NADH to $K_3Fe(CN)_6$	100
ß-hydroxybutyrate to NAD	25
succinate to O_2	138
succinate-driven transhydrogenase (rot)	48
ATP-driven transhydrogenase (rot/KCN)	53/20

a) the absolute value is similar to the value obtained with ATP and is maximal after ~ 4 minutes

b) the heighth of the ATP-induced jump is at 150 mM KCl similar to the heighth of the NADH-induced jump, and $t_{1/2}$ of the H^+-release induced by ATP is ~ 5 x $t_{1/2}$ of the NADH-induced level.

c) $t_{1/2}$ increases only in the beginning seconds, then declines to $t_{1/2}$ of the control level

d) Hansen and Smith phosphorylating particles were used for these measurements, while the others were done with Löw and Vallin particles

e) abolished after ~ 4 minutes

The results described up to now demonstrate: there is a respiratory chain linked energy dependent K^+/H^+-antiport system, responsible for transformation of the energy obtained from substrate oxidation into the energy of an H^+- and K^+-gradient across the mitochondrial membrane. There is a natural limitation for the uptake of osmotically active K^+-ions: the totally extended

mitochondrial membranes! Therefore, K^+ is released (H^+ taken up) if the osmotic pressure of H_2O (as supported by the K^+-ions) becomes too high. The oscillations under our applied conditions are best described by this explanation.

NSPM, Cd^{2+}, valinomycin, and 2.4-dinitrophenol stimulate the mitochondrial ATPase activity at the applied concentrations [23] by 15 to 20 fold, with the exception of 2.4-dinitrophenol, 95 to 97 % oligomycin-sensitive [6, 8, 23, 33]. The Ca^{2+} stimulation of mitochondrial ATPase activity is sensitive not only to oligomycin, but also to ruthenium red, as expected from the other measurements.

The ^{14}C-NSPM labeling patterns of mitochondria at 20 nmol ^{14}C-NSPM/mg have been described in [3]. A ^{14}C-NSPM labeled ~80 kDa polypeptide becomes visible in SDS-polyacrylamide gels with higher concentrations of ^{14}C-NSPM [34]. This polypeptide may be identical to the K^+/H^+-antiporter described by Li et al [32].

Results from the picrylacetate experiments on mitochondria are described in [3, 5, 7] and ^{3}H-picrylacetate labeling patterns in [3, 5]. ^{3}H-Picrylacetate acetylates ~30 kDa large polypeptides as well as the chloroform/methanol extractable DCCD-binding peptides at ~12 kDa up to 2.2 nmoles/mg each. The label incorporation into the 30 kDa polypeptides is dependent on RCR [3, 5]. Labeling behavior of mitochondria towards ^{3}H-picrylacetate at the 30 kDa polypeptides resembles the incorporation of the ^{14}C-NSPM label into the 33 kDa polypeptides [3].

Table II. The effects of picrylacetate (PA), picrate, and Cd^{2+} on the activities of submitochondrial particles (SMP)

SMP-activity after 2 min. incubation		PA, 40 µM %	PA, 40 µM + BSA/%	picrate 40 µM/%	Cd^{2+} 25 µM/%
Δ pH (ACMA)	Succ	500-1200 a)	-	no effect	10
	ATP	150	-	no effect	150
ΔΨ (OX-VI)	Succ	10	-	50	50
	ATP	15	-	60	100
ATP-P_i-exchange		10	35	35	100
ATPase		70 b)	82	120 c)	100
rev. e^- (± DTE)		5	22	82	100
Succ to O_2		146	100	146	15
NADH to O_2		50	35	120	100
Succ-Transh. (rot)		71	87	68	15
ATP-Transh. (rot)		63	72	87	100

a) the absolute value is similar to the value obtained with ATP

b) 50 % ATPase at 160 µM PA

c) max. ATPase (155 %) at 160 µM picrate

Tables 1 and 2 [5] show the effects of 20 nmoles NSPM/mg submitochondria, 40 μM picrylacetate and 25 μM Cd^{2+} with 2 minutes incubation time. The results may be roughly summarized as follows:

1. Succinate- or ATP-supported ACMA response increases on NSPM or picrylacetate addition, picrate is without effect, and Cd^{2+} has the opposite effect: the succinate response is abolished, the ATP response raises to the NSPM and picrylacetate values, while $t_{1/2}$ of H^{+}-release is not affected by NSPM.

2. Succinate- and ATP-supported oxonol VI-response is lowered by NSPM and picrylacetate or picrate; Cd^{2+} lowers the succinate-supported response, but has little effect on the ATP-induced one.

3. ATP/O, ATP-P_i-exchange and ATPase are only slightly, or not at all influenced by NSPM or Cd^{2+}. In contrast, picrylacetate almost abolishes ATP-Pi-exchange and lowers ATPase, while picrate reduces ATP-P_i-exchange and stimulates ATPase.

4. ATP-driven reverse electron transfer from succinate to NAD is abolished by NSPM, picrylacetate, lowered by picrate and not much affected by Cd^{2+}. Succinate oxidation is stimulated by NSPM and picrate, but not affected by picrylacetate, and almost abolished by Cd^{2+}.

5. NSPM and picrylacetate decrease the NADH-oxidation rate, picrate stimulates this activity and Cd^{2+} shows no effect.

6. NSPM, picrylacetate and picrate lower the succinate- and ATP-driven transhydrogenase activities, but Cd^{2+} effects only the succinate-driven activity. The ATP-driven transhydrogenase is much more sensitive to NSPM in the presence of KCN instead of rotenone.

Most effects by Cd^{2+} can be easily explained. Measurements on submitochondrial particles [27, 28] indicate Cd^{2+}-induced changes in the cytochromes: The high potential cytochrome b_{562} of complex III disappears almost totally on Cd^{2+} addition. Comparing experiments with antimycin A $\pm$ Cd^{2+} on aerobe/anaerobe-working particles, indicates inhibition by Cd^{2+} of energy transfer from the high potential cyt b to the low potential cyt b .

The results described demonstrate that proton (cation) movement (ACMA response), and membrane potential (oxonol VI response) are not correlated with the ATP/O-ratio or the ATP-P_i-exchange activities performed by the particles. These results, therefore, encouraged us to more explicitly explore the energy-dependent ACMA quench and oxonolVI responses [Fig.5 to Fig.9].

The succinate dependent ACMA fluorescence quenching in the applied assay mixture is very small. But this quench can be increased to about 600 % by addition of 24 nmoles NSPM/mg particles, and can be abolished by addition of 0.2 mM $CdCl_2$. 130 nmoles N-ethyl-maleimide/mg are only marginally effective. The response remains sensitive to 2 μM of the uncoupler TTFB and to anaerobiosis under all conditions [Fig. 5]. The ATP-dependent ACMA fluorescence quenching in the same assay mixture is much larger than the succinate-dependent level, and can be increased further to about 140 % by addition of 24 nmoles NSPM/mg or 0.2 mM $CdCl_2$. The

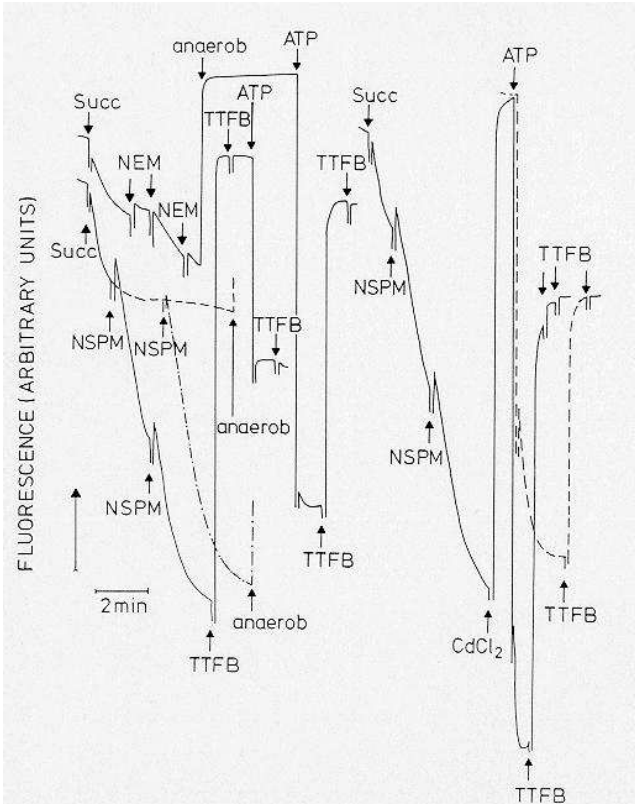

Fig. 5. Energy-dependent ACMA fluorescence quenching in the presence of NSPM, NEM and $CdCl_2$. Reaction medium contained 0.25 M sucrose, 50 mM Tris-sulfate, 10 mM $MgSO_4$, pH 7.5, 0.8 mg submitochondrial particles, 0.5 μM ACMA, energization with 5 mM Na-succinate or 5 mM Na-ATP, respectively. Additions (each): 24 nmol/mg NSPM, 43 nmol/mg NEM, 0.2 mM $CdCl_2$, 2 μM TTFB.

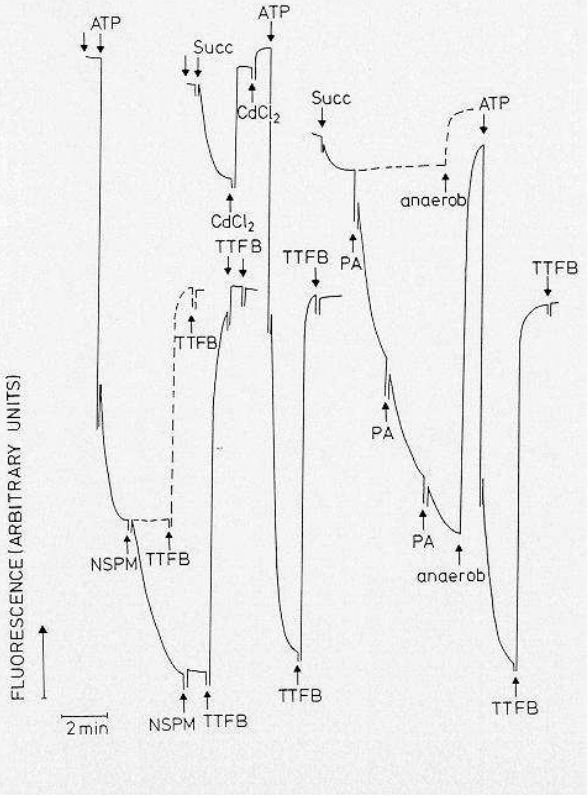

Fig. 6. Energy-dependent ACMA fluorescence quenching in the presence of NSPM, $CdCl_2$ and PA. Conditions as described in Fig. 4. Additions (each): 24 nmol/mg NSPM, 0.2 mM $CdCl_2$, 11 μM PA, 2 μM TTFB.

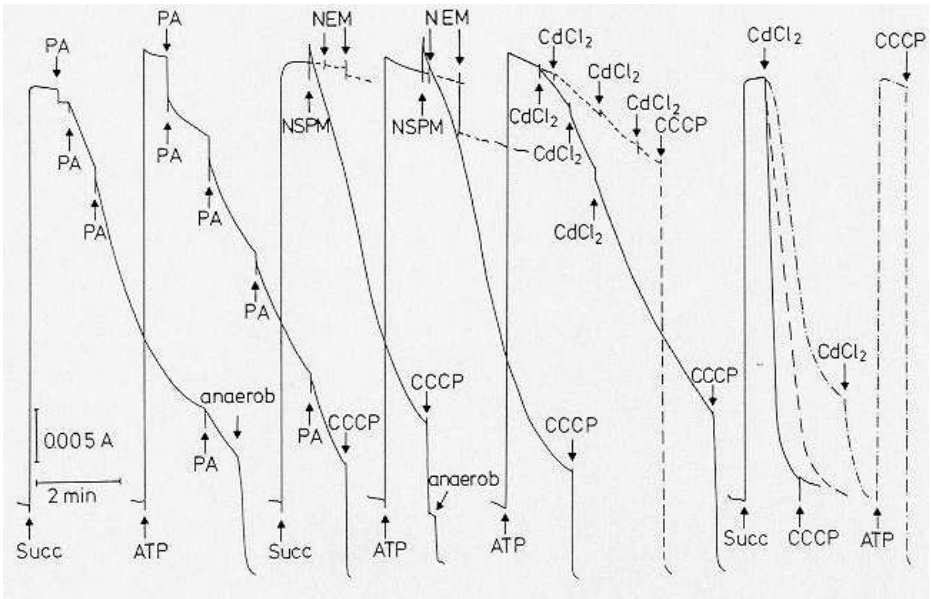

Fig.9. Energy-dependent response of oxonol VI in submitochondrial particles (SMP). Reaction medium containing 0.25 M sucrose, 50 mM Tris-sulfate, 10 mM $MgSO_4$, pH 7.5, 0.4 mg SMP, 3.8 μM oxonol VI, energization with 5 mM Na-succinate respectively 5 mM Na-ATP. Additions (each): 20 μM PA, 20 nmol/mg NSPM, 250 nmol/mgNEM; 0.4 (---) and 4 mM $CdCl_2$ (—) at ATP-, 0.4 mM (-.-), 1 mM (---) and 4 mM $CdCl_2$ (—) at succinate energization; 2 μM CCCP.

response is fully sensitive to TTFB. The increased ATP- or succinate-dependent ACMA fluorescence quench becomes equal. 33 µM picrylacetate behave similarly to 24 nmoles NSPM/mg [Fig. 6]. 36 (48) mM KCl result in nigericin-sensitive, but valinomycin-insensitive full responses of either ATP- or succinate-supported ACMA fluorescence quench. The quench induced by 18 mM $CaCl_2$ reaches a level of about 60 to 70 % of the KCl values [Fig. 7].

10 nmol dicyclohexylcarbodiimide/mg have almost no effect on the succinate/KCl-supported quench, but are highly effective in abolishing or preventing the ATP-(ATP/KCl) supported quench [Fig. 8]. All fluorescence quenches described remain fully sensitive to O_2 depletion and/or TTFB additions.

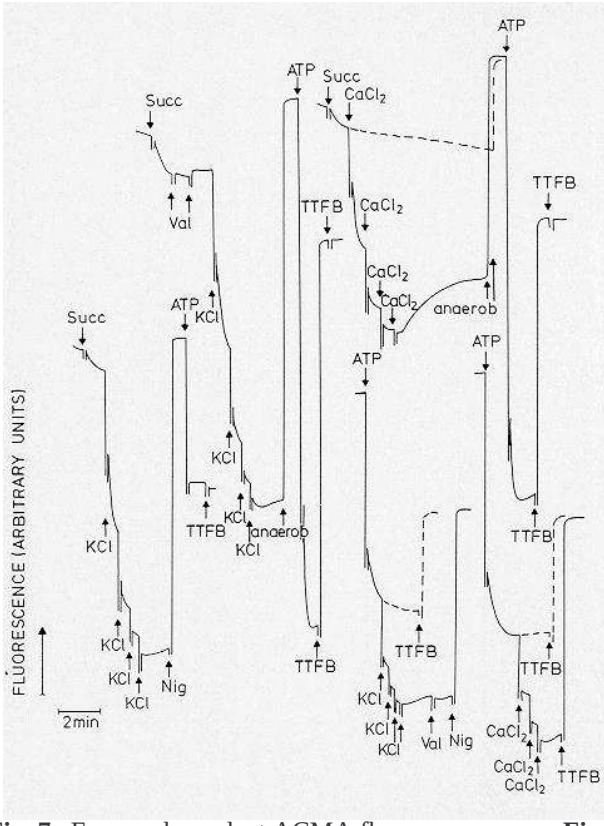

Fig. 7. Energy-dependent ACMA fluorescence quenching in the presence of KCl and $CaCl_2$. Conditions as described in Fig. 4. Additions (each): 12 mM KCl, 6 mM $CaCl_2$, 1 µg nigericin (Nig), 1 µ valinomycin (Val), 2 µM TTFB.

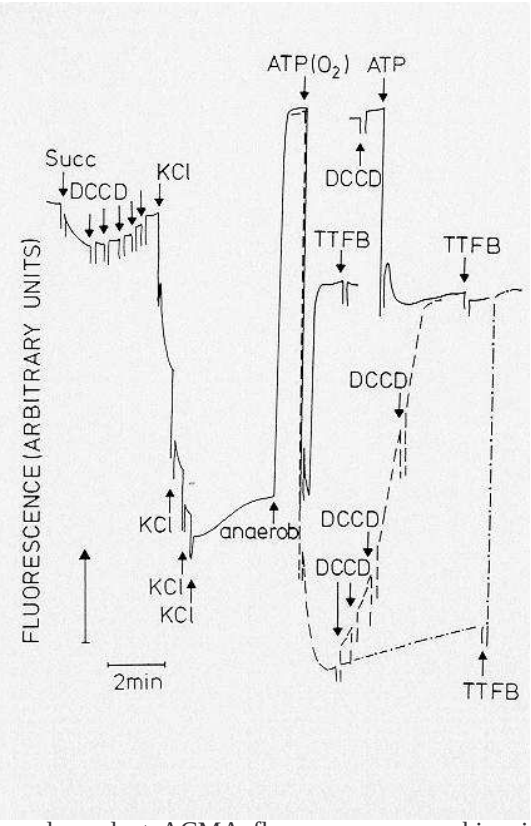

Fig. 8. Energy-dependent ACMA fluorescence quenching in the presence of KCl and DCCD. Conditions as described in Fig. 4. Additions (each): 2 nmol DCCD/mg (10 nmol/mg before ATP), 12 mM KCl, 2 µM TTFB. M Tris-sulfate, 10 mM $MgSO_4$, pH 7.5, 0.4 mg SMP, 3.8 µM oxonol VI, energization with 5 mM Na-succinate or 5 mM Na-ATP, respectively.

The effects of the described compounds on the energy-dependent absorbance increase of oxonol VI have been summarized in Fig. 9. With essentially the same conditions as described for the experiments on the ACMA fluorescence quench (Fig. 5 to Fig. 8) 60 to 80 µM picrylacetate, 20 nmoles NSPM/mg SMP, 8 to 12 mM or 0.4 to 1 mM $CdCl_2$, respectively, abolish the ATP- or succinate-supported absorbance increase of oxonol VI. The absorbance increases are therefore 2 to 4 times less sensitive to picrylacetate or $CdCl_2$, but equally sensitive to NSPM if compared

with ACMA fluorescence quenches. N-ethylmaleimide (500 nmoles/mg SMP) has almost no effect on the energized oxonol VI response, which is similar to the results obtained for ACMA fluorescence quenches. All oxonol VI responses are, or remain, fully sensitive to 2 μM CCCP and/or O_2 depletion.

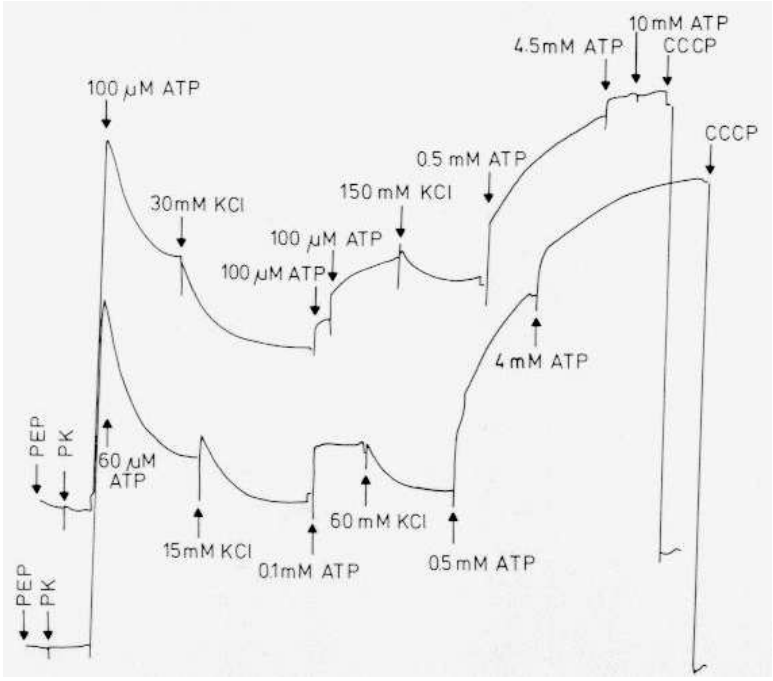

Fig. 10. ATP-dependent response of oxonol VI in complex V. Conditions as described [8]. Additions: 2 mM phosphoenol-pyruvate (PEP), 20 μg pyruvate kinase (PK), 10 μM CCCP.

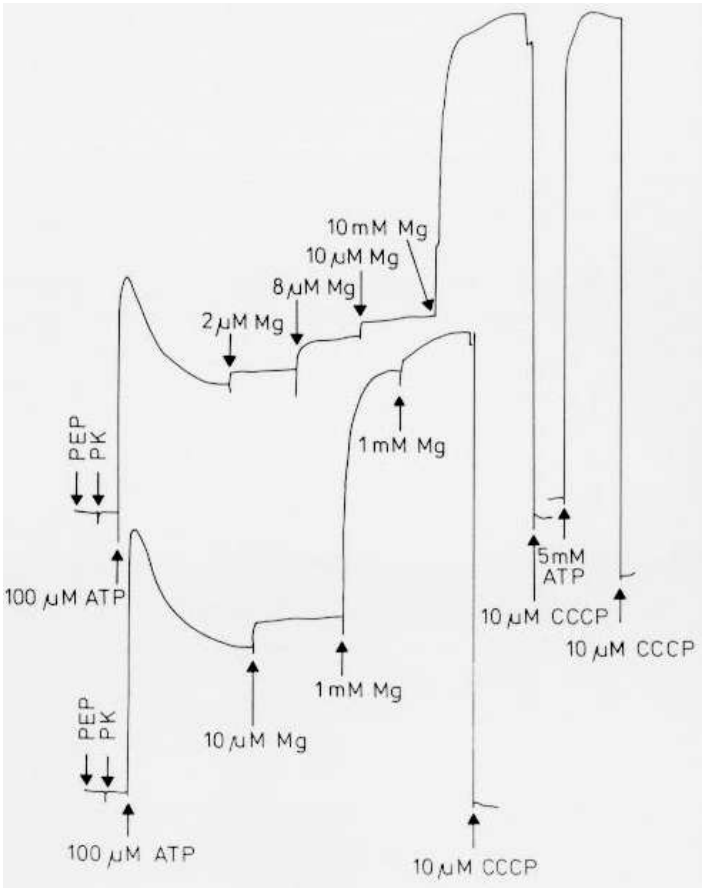

Fig. 11. Mg-ATP-dependent response of oxonol VI in complex V. Conditions as described [8], but without Mg^{2+}. Additions: 2 mM phosphoenolpyruvate (PEP), 20 μg pyruvate kinase (PK), Mg as $MgSO_4$, 10 μM CCCP. Controls show: PK is active, although not maximal at the applied starting conditions.

Figs. 10 and 11 show K^+- and Mg^{2+}-modulations of the ATP-dependent absorbance increases of oxonol VI on complex V. In the ATP regenerating system at low steady-state ATP, the response can be modulated by K^+ (Fig. 10), Mg^{2+} (Fig. 11) or Ca^{2+}-ions (not shown). K^+ (Ca^{2+}) lowers, Mg^{2+}, in contrast, enhances dye response [9].

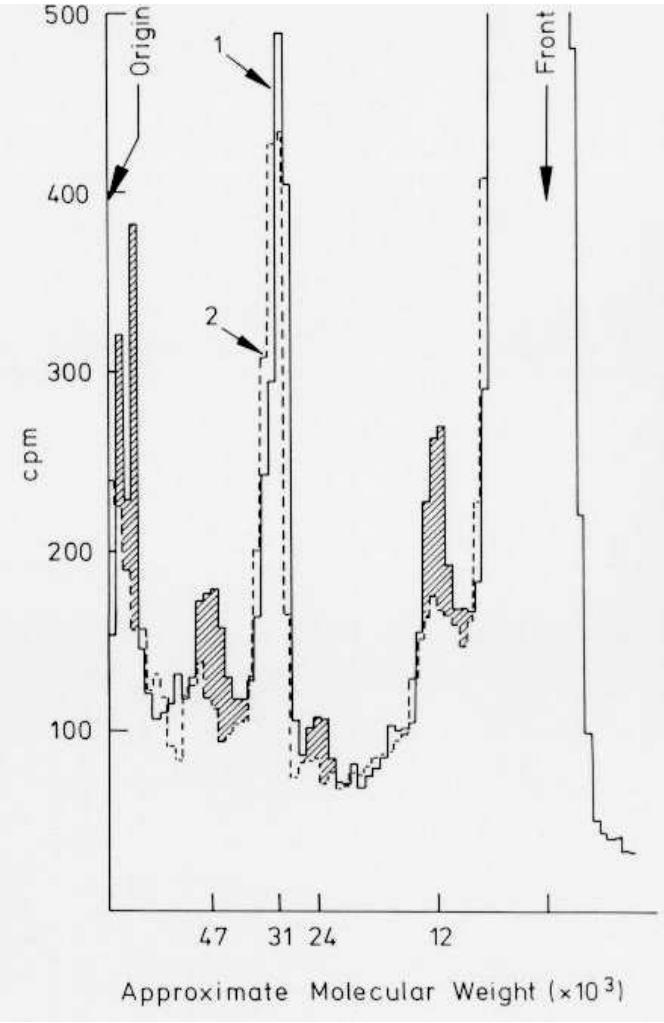

Fig. 12. Distribution of radioactivity due to 40 μM ^{3}H-picryl acetate-induced acetylation of SMP electrophoresed on pH 5.0 acetate NaDodSO$_4$-acrylamide gels (-DTE, continuous curve), and on 12.5 % pH 7.0 Weber-Osborn NaDodSO$_4$-acrylamide gels (+ 5 mM DTE, interrupted line).

Labeling patterns of ^{14}C-NSPM-treated submitochondrial particles, complex V and soluble F_1-ATPase are described and shown [1-3, 35], plus ^{3}H-picrylacetate-treated submitochondrial particles and F_1 [2, 3, 5]. 40 μM ^{3}H-picrylacetate acetylate about 0.4 to 0.6 nmoles 30 kDa polypeptides, as well as 0.2 to 0.3 nmoles DCCD-binding peptide. At 120 μM ^{3}H-picrylacetate there is label incorporation of about 1 to 1.5 nmoles/mg into the 30 kDa polypeptides, and 0.6 to 0.8 nmoles/mg into the proteolipids. This label incorporation raises to about 2 nmoles/mg or 1.2 nmoles/mg into the 30 kDa – respectively 12 kDa peptides on treatment of SMP in the normal assay medium with 200 μM ^{3}H-picrylacetate. Label incorporation into the proteolipids in SMP is dependent on pH and reducing agents (Fig. 12). As may also be seen in Fig. 12, the proteolipids obviously appear in aggregated multiple forms under the applied conditions. The labeling behavior of SMP towards ^{3}H-picrylacetate at the 30 kDa polypeptides resembles the ^{14}C-NSPM label incorporation into the 33/30 kDa polypeptides. ^{3}H-picrylacetate acetylates in F_1 the α-, β- and γ-subunits [5].

Discussion

We do not intend to review all literature data presented over the last 30 years. Instead, we will briefly summarize the results described above and the information related to the aim of our study (i.e. the K^+/Ca^{2+}-transport activities of mitochondria). Figures will be presented showing K^+ (Ca^{2+})-fluxes obtained, which demonstrate the effects of our "aids" in evaluating transport activities. Then we will discuss some of the most important literature data, in our opinion, in relation to our flux schemes, and will finally describe some newly-discovered mitochondrial functions.

The results of our experiments may be rationalized as shown in Fig 13. It is obvious that we are dealing with the K^+/H^+-antiport system of mitochondria [32]. This K^+/H^+-antiporter is driven with the energy derived from substrate oxydation by the respiratory chain. It is sensitive to Cd^{2+}, NSPM, Ca^{2+} or DCCD (all stimulate) from the outside and is regulated by Mg^{2+} and quinine. Cd^{2+} and Ca^{2+} sensitivity are prevented by ruthenium red. As expected, K^+/H^+ exchange is stimulated, Mg^{2+} sensitively, by nigericin-supported K^+/H^+-cycling. It is promoted by valinomycin/$\pm$ Mg, presumably through direct interaction with the F_0F_1-ATPase/ATPsynthase [4], and by 2.4-dinitrophenol at the μM_0 or mM_i concentrations applied, respectively, and its direct interaction with the F_0F_1-ATPase/ATPsynthase [2-4].

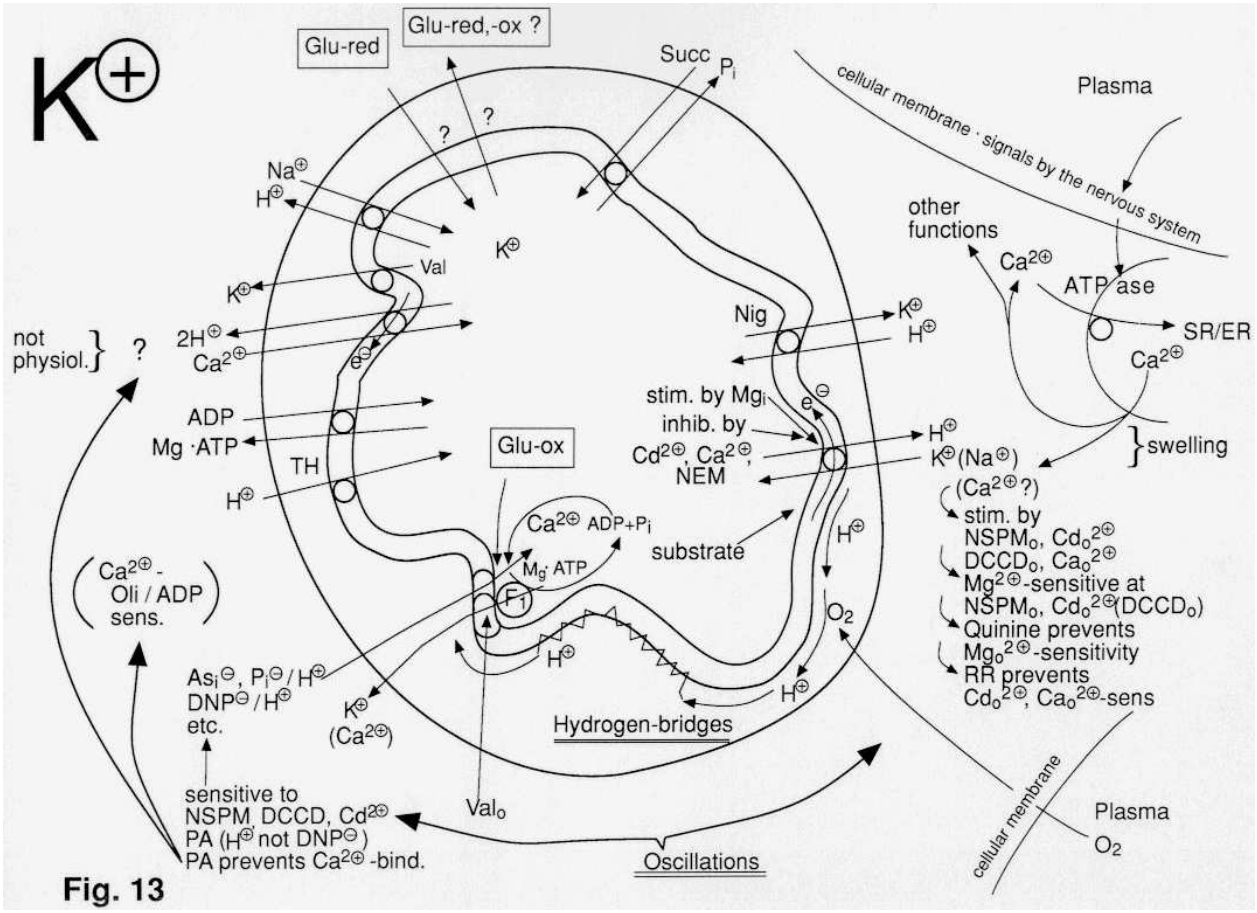

Fig. 13. Summation of various mitochondrial energy-linked functions. Location and effects of energy transfer inhibitors and uncouplers on K^+/H^+-antiporter, K^+-pump and P_i/H^+-symporter. Mitochondrial coupling to the nervous system. Glu = glutathione, TH = transhydrogenase, Oli = oligomycin, PA = picrylacetate, Val = valinomycin, RR = ruthenium red, Nig = nigericin, SR/ER = sarcoplasmic/endoplasmic reticulum.

The coupling modes between e^--transfer and the K^+/H^+-antiport system are unclear to us. How is it possible to link a K^+/H^+-antiporter to a respiratory chain just pumping protons as described by Li et al [32], assuming we are dealing with this antiporter (which seems to be the case based on the presented results, although Li et al used sonicated uncoupled mitochondria for their study resulting in different DCCD effects compared to our study)? Green [36] demonstrated active and opposite H^+- and K^+-transport activities by cytochrome oxidase (complex IV) reconstituted in phospholipid vesicles. His results and the model derived from his studies agree much more with our data than the current descriptions of electron transfer coupling, and should thus be considered when explaining our results.

The modulations of the K^+/H^+-antiporter activity described above lead to corresponding swelling or contraction of the mitochondrial matrix space by the osmotically active K^+-ions and in modified oligomycin-sensitive (valinomycin, NSPM, Cd^{2+}, Ca^{2+}) or ruthenium red-sensitive (Cd^{2+}, Ca^{2+}) ATPase activities. NSPM and Cd^{2+} - at the low concentrations applied - act from the outside, since β-hydroxybutyrate or NADH-dehydrogenase respectively, and complex III activities were not modified by the compounds. Swelling/contraction and ATPase activity were therefore indirectly influenced via osmotically-active K^+-ions. The oligomycin sensitivity of the changed ATPase activities suggests mitochondrial F_0F_1-ATPase involvement, most probably by the release of K^+-ions. The osmotic back-pressure [37] at high K^+-load (see description of the oscillatory phenomena in results) may also open up the ATPase for K^+-release, in which the ATPase inhibitor IF_1 could be involved [38]. Ruthenium red prevents Cd^{2+}-, Ca^{2+}-induced K^+-uptake and accompanies ATPase modulations together with K^+-release.

Cd^{2+} and Ca^{2+} under 10 μM bind (Mg^{2+}-sensitive) at high affinity binding sites (~ 1 nA/mg) [2, 3, 6, 39], thereby inducing Mg^{2+}-sensitive K^+-uptake, but without being accumulated itself. The free active cytosolic Mg^{2+} concentration should be low in contrast to our applied concentrations (~ 6 to 10 mM). In fact, free concentrations of cellular cytosolic Mg^{2+} have been estimated to be < 1 mM [40]. Respiratory activity, K^+-cycling, swelling/contraction phenomena and ATPase activity are thus controlled by available O_2, and most importantly by the free Ca^{2+} and Mg^{2+} concentrations!

A high noradrenaline/adrenaline-ratio in plasma leads in low glycolysis rates and as consequence lowered ATP concentrations in the cell. ADP-, P_i- and free Mg concentrations in the cytosol, as well as ATP synthesis [41, 42] and oxidized glutathione concentrations in mitochondria, are concomitantly raised, but the reduced glutathione concentrations in the cells lowered. The changed ATP/ADP or Ca/Mg ratio, respectively, determines, in this case, the respiratory rates (Table III). Our results and conclusions are based on extrapolations from summarized results on mitochondrial oscillations [44] and are, furthermore, one way to explain the molecular mechanism leading to these phenomena. Longer noradrenaline exposure of cells leads to constantly lowered cAMP-concentrations and the consequences described [43].

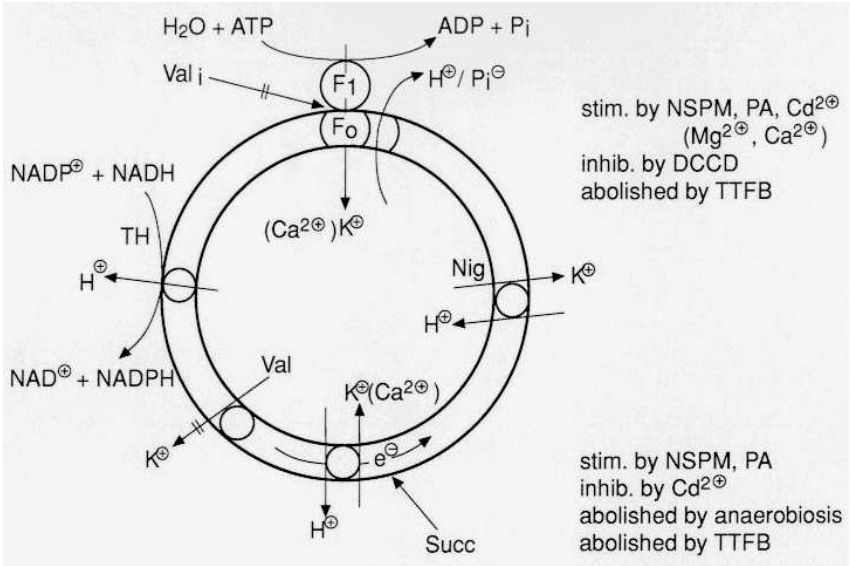

Fig. 14. Summation of various submitochondrial energy-linked functions. Location and effects of energy transfer inhibitors and uncouplers on K^+/H^+-antiporter, K^+-pump and P_i/H^+-symporter.
Val = valinomycin, Nig = nigericin, PA = picrylacetate, TH = transhydrogenase.

A rise in cytosolic Ca by extracellular signals leads to muscle contractions, myofibril mobility, stimulation of respiration, etc. with liberation of ADP, P_i and Mg, resynthesis of ATP by creatine phosphate [42] and rebinding of Mg. As a consequence, there is a constantly changing Ca/Mg-ratio with oscillating mitochondria. The changing Ca_{cyt} determines the Ca/Mg or ATP/ADP ratio, respectively (Table III).

Table III. Distribution of various ions between plasma, cytosol, and mitochondria

Ion	Plasma/mM[a)]	Cytosol/mM[b)]	Mitochondria/mM [b)]
Na	150	10	< 0,1 - 10[d)]
K	5	175	< 2 - 175
Mg	2	10[c)]	> 10[e)]
Ca	1 - 2	1 - 10 µM	< 2 - > 30
P_i	1 - 2	5 - 10	< 2 - > 30
Cl	110	3	nd
SO_4	0.5	10	nd
ADP	<< 0.1	0,7	6-8
ATP	<< 0.1	4,0	5-7
Glutathione red.	< 0.1	1- 10[f)]	< 0.1 - 10
Glutathione ox.	nd	0,5	< 0.4 - > 2.1

a) clinic values. b) clinic values; own values; Elbers et al (1974) Hoppe-Seyler's Z. Physiol. Chemie 355: 378-393; Klingenberg and Heldt (1982) in Metabolic Compartmentation, Sies, Ed., Acad. Press, London, 101-122; Wahlländer et al (1979) FEBS Letters 97: 138-140. c) free plus bound. d) in the presence of K^+ as calculated from the K^+ values; the natural Na-reservoir in the cell is the nucleus. e) above ATP conc. f) Meister and Anderson (1983) Am Rev Biochem 52: 711-760.

At Cd^{2+} or Ca^{2+} concentrations above 10 µM, both ions were taken up. But Cd^{2+}, at < 10 µM, prevents a Ca^{2+} (P_i)-accumulation by binding to the P_i/H^+- and Ca^{2+}-binding sites. Taken together, Ca^{2+}-ions behave similarly in many ways to K^+-ions (uptake, swelling/contraction, ATPase, etc.). This is not surprising since the Ca^{2+} concentrations in the cytosol of our cell is only about 1 to 10 µM under normal physiological conditions (Table III) and its K^+-cycling system may, therefore, also be able to handle high "abnormal" Ca_o^{2+} concentrations (as well as Ca_i^{2+}/P_i) (Fig. 13).

Submitochondrial particles can be compared to mitochondria: in both systems we are dealing with energy-driven K^+/H^+-exchange activities, driven either by substrate-supported electron transfer or by ATP-hydrolysis (Fig. 14). As may be seen in the summation of our results, the succinate-driven K^+/H^+-antiporter works opposite to mitochondria in SMP under the applied conditions. The arising nigericin-sensitive (valinomycin-insensitive) K^+ and H^+ gradients become maximal and stimulated by succinate oxidation at low NSPM and picrylacetate, and at high K^+ or Ca^+ concentrations, but are abolished at moderate Cd^{2+} concentrations, at anaerobiosis, or on TTFB addition.

The abolishment by Cd^{2+} can be explained in detail by the action of this heavy metal ion on complex III activity, and thereby the inhibition of the energy transfer from the high potential to the low potential cyt b (resulting in inhibition of the forward, succ $\rightarrow O_2$, but not the back reaction, reverse e^--transfer, Table II). However, the question of why Cd^{2+} inhibits succ $\rightarrow O_2$, but not NADH $\rightarrow O_2$, remains to be answered. We think that a possible answer may be found in the ubiquinone pool.

The direction of the succinate-driven K^+/Ca^{2+}(and H^+)-gradients are obviously dependent on the forces the K^+/H^+-antiport system is exposed to in the SMP-membranes (opposite bending to the mitochondrial membrane). Otherwise, we do not have a plausible explanation for the different directions of the gradients in SMP or mitochondria. There is definitely a relationship to the forces effective on the membrane proteins during the membrane transitions (oscillations) in mitochondria.

ATP-hydrolysis (similarly to succinate oxidation) produces large nigericin-sensitive/ valinomycin-insensitive (site-directed binding to F_0 [4]) K^+/H^+-gradients, which become maximal and almost identical to the succinate-supported gradients at low levels of NSPM, picrylacetate and Cd^{2+}, and at high levels of K^+, Ca^{2+}, but which disappear at low concentrations of dicyclohexylcarbodiimide or TTFB, in contrast to mitochondria and isolated complex V [8]. In fact, the ATP-supported K^+- or H^+-gradients in our SMP particles are highly DCCD sensitive.

This kind of DCCD sensitivity has not been observed so far for the other activities performed by SMP [8], mitochondria [32] or even complex V-preparations [8, 23, 45]. DCCD abolishes ATP synthetic activity (RCR)/P_i-transport > K^+-transport >>> ATPase in mitochondria, presumably by first modifying the phospholipids [32] before trapping any proteolipid or P_i-carrier. In SMP, the sequence of the DCCD-sensitive reactions is K^+-transport > ATP-P_i-exchange or P_i-transport >>> ATPase and proves 1) side-specific reaction of DCCD and 2) separation of the various reactions by DCCD. Modification of the ATPase leads to abolishment of the K^+-gradients, although not of their establishing force, the Mg-ATPase. Our complex V-preparations show no ATP-dependent ACMA-responses because they are not vesicularized [8].

Before discussing data on oxonol response, some points about valinomycin have to be added. Valinomycin is able to abolish K^+-gradients in mitochondria, which leads to K^+-cycling and a high proton gradient (by stimulation of the K^+/H^+-exchange and the ATPase), together with stimulated respiration (uncoupling, as shown). This is not the case with submitochondria, which could be demonstrated with ACMA. If one considers all the results for the explanation of this fact, including the binding of valinomycin to the F_0-part of the ATPase, one reaches the only possible conclusion: Valinomycin stimulates the ATPase activity (K^+-pump) of mitochondria

(oligomycin-sensitive) from the outside. For these measurements, all the ions present (including $Tris^+$) are important. It should be added that the movement of valinomycin through pure phospholipid membranes is already very difficult, and should be almost impossible through mitochondrial membranes. Pressman's final conclusions [46] are mainly derived from phospholipid bilayer measurements and are different from his original ones [47, 48].

Why should all the different microorganisms produce these highly complex "ionophores" just for phospholipid bilayer membranes, when their antibiotic activity is directed against systems containing only minimal or no phospholipid bilayers? A synthesis directed directly against the ion-transporting units is much easier to imagine.

The cyclic decapeptide antamanide for instance has been described by Pressman as neutral “ionophore” because he found stimulation of K^+-uptake by mitochondria and complexation of $Na^+ > K^+$ by this compound [46]. However, studies by Ovchinnikov [49] demonstrate that the biological action of antamanide is not connected to ionophoric action, and in accordance with this finding, photoaffinity labelling studies [50] demonstrate specific binding of this compound to membrane polypeptides. Our results on "protonophoric" uncoupling agents [2] match perfectly the experimental findings on "ionophoric" compounds.

Normally a model system has to be proven in every aspect and dismissed if not valid in all points. In the case described above, it is obviously easier to keep a wrong model rather than to perform difficult new experiments.

The oxonal VI response of SMP is due to the membrane potential and has been extensively discussed [8]. The absorbance increase of oxonol VI is less sensitive to picrylacetate or $CdCl_2$, but equally sensitive to NSPM if compared with the sensitivity of the ACMA fluorescence quench to these compounds. The difference between the oxonol VI responses described [8], and this paper is due to the use of a $Tris^+$-buffer, which replace K^+ or H^+, respectively, in the experiments. The oxonol response in the isolated ATPase complex reflects a more localized, ATP-dependent and energy-related process in contrast to SMP [4, 8, 9], showing, most likely, K^+ (Ca^{2+})-induced conformational changes of the ATPase [9]. The oxonal VI response in SMP thus might reflect K^+-pumping activities.

The K^+-pumping activity cannot be located on the P_i/H^+-symporter, which should be inhibited for P_i-translocation at the high ADP/ATP and/or low NSPM concentrations applied during the experiments. The protons cannot be pumped by F_0F_1 because of the direction in which they are forced. They are most likely delivered by ATP hydrolysis, or $Tris^+$ (used as a buffer), possibly by involvement of the P_i/H^+-symporter. Only the F_0F_1-ATPase is then able to operate as a K^+-pump.

Furthermore, we add four additional points to those listed in [4], which imply that the F_0F_1-ATPase acts as a K-pump:

1. Mg^{2+} is not required for ATP synthesis [51], but for ATPase activity. Our proposed mechanism for ATP synthesis does not require Mg^{2+} [4].

2. Concentrations of our "aids" (NSPM, etc.) leading to maximal K^+- and H^+-gradients and moderately-stimulated, oligomycin-sensitive ATPase acitivities, are not very effective on the ATP/O-ratio or ATP-P_i-exchange.

3. Sandri et al [52] required monovalent cations, including K^+, besides F_6 for membrane binding of F_1; a point possibly important for a K-pumping activity of F_0F_1.

4. Schneider et al [53] found K^+-translocation on EF_0 by performing conductance studies on black lipid membranes.

The summation of all described points concerning K^+-translocation – other than by the electron transfer linked K^+/H^+-exchange (Fig.13, 14) proves that mitochondria contain a valinomycin- and oligomycin-sensitive, ATP-driven K^+-pump as originally suggested [47, 48], which is identical to the well-known oligomycin-sensitive F_0F_1-ATPase, but not with the ATP synthase (Fig. 13, 14). Also, the results obtained on reconstituted CF_0F_1liposomes [54-56] are in agreement with the ones presented here. ATP-P_i^{33}-exchange/ATP synthesis may be due to avidin-sensitive propionyl-CoA-carboxylase [8, 57], to (coupled) AP_5A-sensitive adenylate kinase [8, 54], or should be compared to the sarcoplasmic reticulum Ca^{2+}-pump ATP synthesis [58]. Our hypothesis for ATP synthesis [4] is therefore not only probable, but indirectly proven and requires only a few more experiments to be directly proven.

The results of Lardy et al [4, 31, 33] become clearer in light of our results: the anti-swelling activity of ATP and the contraction of swollen mitochondria by ATP are due to the described ATP-driven K-pump or F_0F_1-ATPase, respectively. Under normal physiological conditions, this pump is responsible for the establishment and maintenance of a high K^+-gradient, which means the K^+ concentration is very low inside (matrix), but high outside (cytosol). The physiological K^+ concentrations in the mitochondrial matrix space are normally in the range of about 1 mM_i , in the cytosol about 175 mM_o (the maximal K^+ concentrations for the matrix space under deenergized/uncoupled conditions) (Table III). Under normal physiological conditions, no protons are either moving from the K^+/H^+-exchanger to the P_i/H^+-symporter (ATP synthase) along the membrane via hydrogen bridges [59], or the K^+/H^+-exchanger and the P_i/H^+-symporter (ATP synthase) are directly linked [61] (Fig. 13). The conclusion for a delocalized, protonmotive force as presented in [43] is thus, in light of our results, incorrect.

The results presented above imply a direct connection (and regulatory function) between the nervous system (brain) and mitochondria (body and brain). Our body temperature may then be regulated by mitochondria via nervous signals from the brain. The normal substrate oxidation and O_2-uptake/-reduction in our bodies (anorexia/dystrophia) is directly linked to our nerve impulses, and thus essentially to the brain. Substrate overload leads to a rise in acetyl-CoA concentrations, and consequently also to a rise in cholesterol, hormone and Vitamin (D3) concentrations, which as a result, can in turn lead to such diseases as arteriosclerosis [60], heart attack and cancer.

Oxygen deficiency, or even depletion in our cells, results in an imbalance of the Na^+/K^+ concentrations across the cellular and mitochondrial membranes, and to osmotic imbalances resulting in H_2O decreases with damage to mitochondria and cells. As a consequence, the build-up of edema during anoxia/hypoxia, or shock and dilusion with coma (death) can occur.

The above examples may be just some of the possible interactions between nervous system and mitochondria. Mitochondria are in the true sense of the word the “hearts” of our cells.

A technical realization of bioenergy production (via molecular biotechnology) is made possible by our results. Mitochondria are ideal oscillators and predestined, through mutual coupling with the plasma membranes, to function in the brain for memory and thinking. Such new materials could thus lead to the development of thinking computers.

Acknowledgements

I thank Prof. E. Neumann, in whose laboratory I learned more about plasma membrane receptors and also about some of the possible problems which may arise during interdisciplinary research efforts, and Prof. K. Altendorf for allowing us to use his fluorescence spectrophotometer. We also thank Prof. Th. Wieland, Mrs. E. Dirscherl and Mrs. C. Cavanna for their help in preparing this manuscript.

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Transport and ATP synthesis in mitochondria
IV. K+-transport: Evidence for mitochondrial F0F1-ATPase being a K+-pump

by

Kiehl Reinhold

Editor: Pettersson

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