Lecture to the 3rd World Congr of Cell and Mol Biol, 8.-13.Oct.2000, Jena:

ELECTROPHYSIOLOGY AND THERMODYNAMICS OF MITOCHONDRIA

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The research performed during the last 20 years lead inevitably to the formulation of the mitochondrial F_OF_1 -complex as coupled K^+/H_2O-pump (similar to the nAChR) with H^+/P_i^--inducible ATPsynthase as well as to the respiratory chain substrate driven K^+/H^+-antiport system. These systems are linked together in anticyclic energy driven K^+/H_2O, H^+/P_i^--movements and oscillations (swelling plus contraction of the mitochondrial matrix space by osmotically active K^+-ions), controlled by O_2 and the free Mg^{2+} - and Ca^{2+} -concentrations in the cytosol of the cells.The systems is responsible for the thermoregulation of our body. - The cyclic hydrolysis/synthesis of ATP and the concomitantly cyclic release/binding of Mg^{2+} in the "steady state flow system" releases heat (q) and the temperature (ΔT) is permanently raised. The released heat is constantly distributed throughout the entire body by the oscillating mitochondria, as well as the pumping heart, and is used up by the normal body functions. Disturbances of this system are normally compensated for by lower/higher respiration rates. The essentially by iron and its state of oxidation dependent H^+/e^- displacements, current (i), lead to high local voltages (ΔV) over the membrane with corresponding magnetic fields (H). The entire system is dependent on oxidized and reduced glutathione.

Starting point - to the results I am presenting today - was my appointment with Theodor Wieland at the MPI for medical research, Heidelberg, and his work on oxidative phosphorylation – model systems to the synthesis of ATP:

Angew. Chem. internat. Edit. / Vol. 7 (1968) / No. 11

Formation of Adenosine Triphosphate (ATP) from Adenosine Diphosphate (ADP) and Phosphate during Oxidation of Mercaptoacetate by Bromine [1]

By *Th. Wieland* and *E. Bäuerlein* [*]

We have converted oxidation energy into the energy of the "energy-rich" diphosphate bond, *e.g.*, that in ATP, by oxidation of *S*-alkylmonothiohydroquinones [2], *N*-acetylhomocysteine thiolactone [3], thiazolidones [4], monoacetyldurohydroquinone [5], α-tocopherol [6], and *S*-*p*-tolyl thioacetate [6] by bromine in pyridine in the presence of ADP and phosphate (P). We have now found that under these conditions mercaptoacetate also gives ATP from the tetrabutylammonium (TBA) salts of ADP and P, and that yields exceed 30%.

In several batches, 13.33 ml of 0.1 N (TBA)-OH solution in 2-propanol/methanol (Merck, Darmstadt) was treated with 0.67 ml of 1 N solution of *ca.* 90% phosphoric acid in dioxane; 0.107 g of $ADPH_3$-3 H_2O (C. F. Boehringer und Soehne, Tutzing) was dissolved therein, and then 0.016 ml of freshly distilled mercaptoacetic acid was added. The solution was evaporated under vacuum, and the residue was dried over P_4O_{10} for 20 min at 0.1 torr and then dissolved with stirring in 9 ml of anhydrous pyridine, whereupon a deep yellow color developed. (A solution of the TBA salt of mercaptoacetic acid in anhydrous pyridine is colorless.) The solution was then treated, by dropwise addition within a few minutes, with 5/9 mmole (0.028 ml), 2/9 mmole (0.011 ml), or 0.133 mmole (= 1.2×1/9 mmole) (0.007 ml) of bromine, each amount in 1 ml of anhydrous pyridine.

This caused a color change from deep yellow through deep red to pale yellow; a precipitate was formed after a few minutes. After 1 h each batch (several repeats) was worked up for determination of ATP (results in Table 1).

No more than conjectures can be made at present about the mechanism of this coupled reaction. It may be that a sulfenyl bromide is first formed by bromination of the thiol and then combines with the phosphate ion to give a mixed sulfenic phosphoric anhydride R–S–O–PO_3H^-, which is the actual phosphorylating agent.

Table 1. Yields of ATP (calc. on the 84% ADP used on simultaneous oxidation of equivalent amounts of $(TBA)_2HPO_4$, $(TBA)_2ADP$, and TBA mercaptoacetate by 1.2, 2.0, and 5.0 equivalents of bromine.

Br_2 (equiv)	1.2		2.0		5.0		0	
Mercaptoacetate	with	without	with	without	with	without	with	without
Yield (%) of ATP	5.8	0	12.2 10.0	0 0	36.4 37.6	3.2 1.6	0	0
			11.2	0	37.0	2.4		
Net yield (%) of ATP	5.8		11.2		34.6		0	

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German version: Angew. Chem. *80*, 915 (1968)

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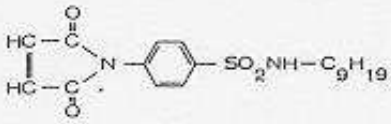
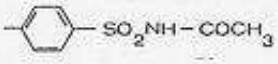
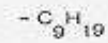
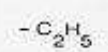
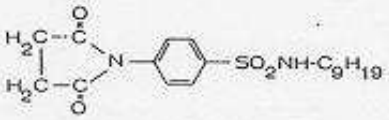
[1] Model Experiments on Oxidative Phosphorylation, Part. 10.– Part 9: [4].
[2] *Th. Wieland* and *E. Bäuerlein*, Mh. Chem. *98*, 1381 (1967).
[3] *Th. Wielnnd* and *E. Bäuerlein*, Chem. Ber. *100*, 3869 (1967).
[4] *Th. Wieland* and *H. Aquila*, Chem. Ber. *101*, 3031 (1968).
[5] *Th. Wieland* and *H. Aquila*, Angew. Chem. *80*, 190 (1968); Angew. Chem. internat. Edit. *7*, 213 (1968).
[6] *E. Bäuerlein* and *Th. Wieland*, Chem. Ber., in press.

The model, I will present today, arises out of experimental results – obtained by my groups - and known physical and biophysical basic (natural) laws: first published results during the year 1976 = effects of lipophilic maleimides, thiouracils and thioureas = lipophilic thiol – and sulfenylgroup reagents – on mitochondrial energy linked functions: incl.RCR (thesis, FEBS Letters 61, 68-71 and 72, 24-28):

Table 1

Effects of various N-substituted maleimides on the respiration of ox heart mitochondria with glutamate + malate as substrates

No.	Formula	Abbr.	Inhibition of state 4 → state 3 transition (nmoles/mg protein)	Respiration inhibition (nmoles/mg protein)
Ia		NSPM	12 – 16	90 – 110
Ib		ASPM	without effect up to 50	110 after addition of ADP
III		NNM	60 +30 % stimulation of state 4 respiration	–
IV		NEM	without effect up to 50	200 (50 %)
II		NSPS	without effect up to 50	120

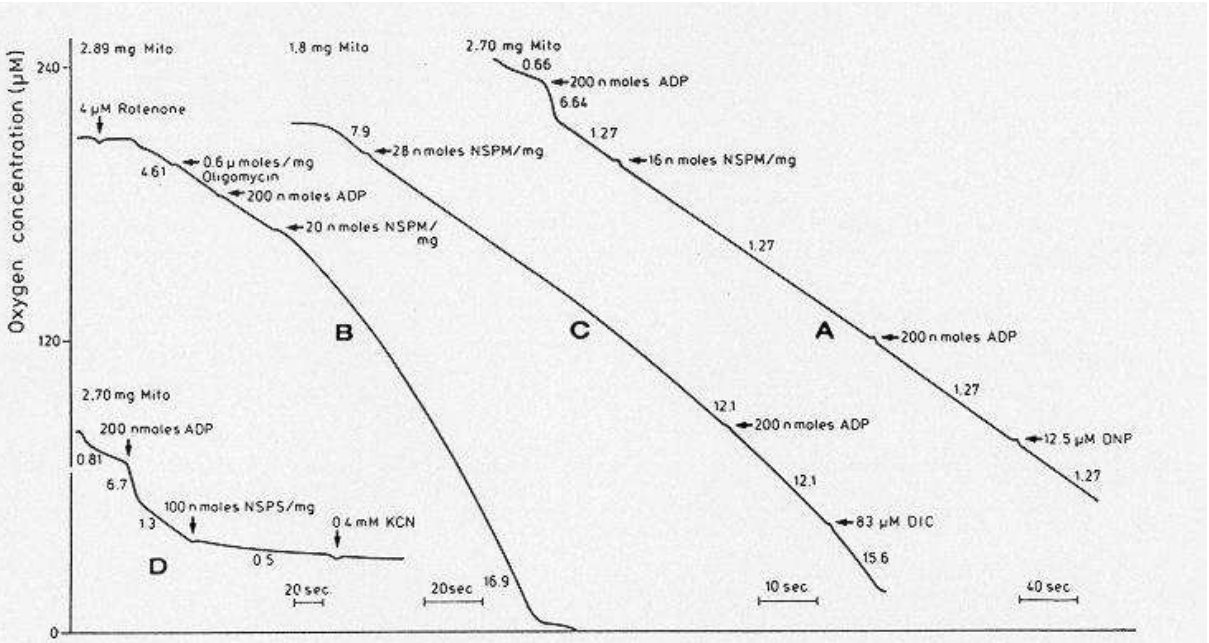
The conditions are the same as described in fig.1 for the experiments A and D. The concentrations indicate maximal inhibition, unless otherwise indicated. Maximal inhibition of respiration means the same degree of inhibition as with 0.6 mM KCN.

Table 2

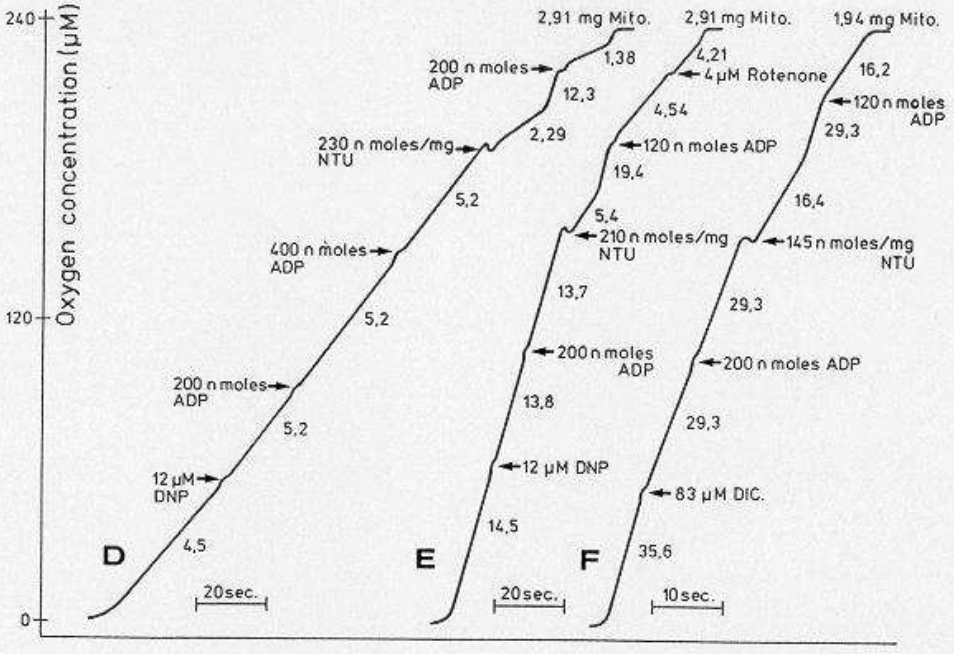
Effects of various N-substituted maleimides on the respiration of ox heart mitochondria with succinate

No.	Abbreviation	uncoupling (nmoles/mg protein)	Inhibition of state 4 → state 3 transition (nmoles/mg protein)
Ia	NSPM	20–26	–
Ib	ASPM	no effect	110
III	NNM	120	–
IV	NEM	without effect up to 300	
II	NSPS	no effect	170 +50% stimulation of state 4 respiration

The conditions are the same as described in fig.1 for experiment B. The concentrations indicate either maximal uncoupling, i.e. state 3 respiration, or maximal respiration inhibition, i.e. inhibition as with 0.6 mM KCN.



Effect of NSPM and the correlated succinimide NSPS, on the coupled respiration of ox heart mitochondria. The lines represent the output from an oxygen electrode. The numbers on the lines are respiration rates, µmoles of oxygen mg^{-1} protein h^{-1} at 25°C. Experiment A and D: ox heart mitochondria (2.70 mg) were added to a reaction mixture consisting of 2.4 ml 0.25 M sucrose containing 2.5 mM glutamate, 2.5 mM D,L-malate, 5 mM malonate, 20 mM KCl, 5 mM $MgCl_2$, 10 mM phosphate and 20 mM Tris–HCl, pH 7.4. Experiment B: ox heart mitochondria (2.89 mg) were added to a reaction mixture consisting of 2.4 ml 0.25 mM sucrose containing 10 mM succinate, 20 mM KCl, 5 mM $MgCl_2$, 10 mM phosphate and 20 mM Tris–HCl, pH 7.4. Experiment C: ox heart mitochondria (1.80 mg) were added to a reaction mixture consisting of 2.4 ml 0.25 M sucrose containing 2.5 mM ascorbate, 0.25 mM TMPD, 20 mM KCl, 5 mM $MgCl_2$, 10 mM phosphate and 20 mM Tris–HCl, pH 7.4.



Effect of NTU on the coupled respiration of mitochondria. The lines represent the output from an oxygen electrode. The numbers on the lines are respiration rates, µmoles of oxygen mg^{-1} protein h^{-1} at 25°C. Experiment A: ox heart mitochondria (2.91 mg) were added to a reaction mixture consisting of 2.4 ml 0.25 M sucrose containing 2.5 mM glutamate, 2.5 mM D, L-malate, 5 mM malonate, 20 mM KCl, 5 mM $MgCl_2$, 10 mM phosphate and 20 mM Tris–HCl, pH 7.4. Experiment B: ox heart mitochondria (2.91 mg) were added to a reaction mixture consisting of 2.4 ml 0.25 mM sucrose containing 10 mM succinate, 20 mM KCl, 5 mM $MgCl_2$, 10 mM phosphate and 20 mM Tris–HCl, pH 7.4. Experiment C: ox heart mitochondria (1.94 mg) were added to a reaction mixture consisting of 2.4 ml 0.25 M sucrose containing 2.5 mM ascorbate, 0.25 mM TMPD, 20 mM KCl, 5 mM $MgCl_2$, 10 mM phosphate and 20 mM Tris–HCl, pH 7.4.

One result obtained – beside other results - was the finding of the involvement of glutathione in the energy transduction of mitochondria (thesis76):

Beim isolierten, verunreinigten Reaktionsprodukt handelt es sich wahrscheinlich um das gemischte Disulfid des Glutathions mit ^{35}S-NTU (nach Totalhydrolyse gespalten,S.56). Ähnlich wie die DCCD-Reaktionsprodukte findet man dieses NTU-Reaktionsprodukt bei MG 10000 (El.,S.51). Dieses 10000 MG-Protein spielt wahrscheinlich eine zentrale Rolle bei der ATP-Synthese. Folgende Reaktionen sind denkbar (analog [59]):

$$\text{Glutathion-S-S-Protein}\ (\delta+\ \delta-\ \text{10000 MG}) + {}^{\delta-}\text{S}^{*}\text{-Thiouracil} \longrightarrow \text{Glutathion-S-}\overset{\text{H}}{\text{S}}\text{-Protein} \quad {}^{*}\text{S-Thiouracil}$$

$$\left(\xrightarrow{H_2O} \text{Glutathion-S-}\overset{*}{\text{S}}\text{-H} + \text{Uracil}\right).$$

Now, I will show some morphological pictures of mitochondria – electron mikroscopic details – show the methods used in tables, present some few measurements, point to the optical probes used, ... and finally explaine the model.

The next slide shows epithel cells of a kidney tubulus

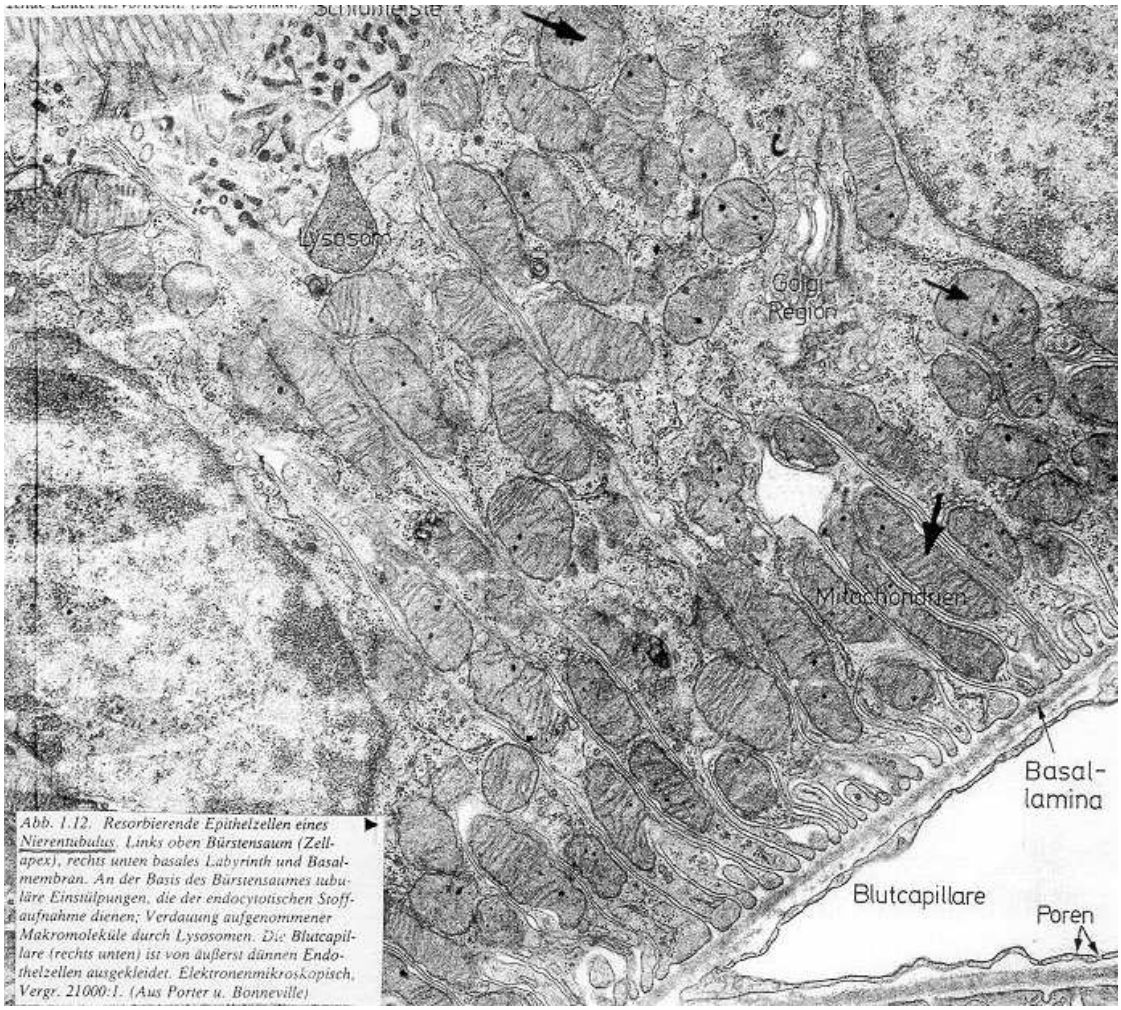
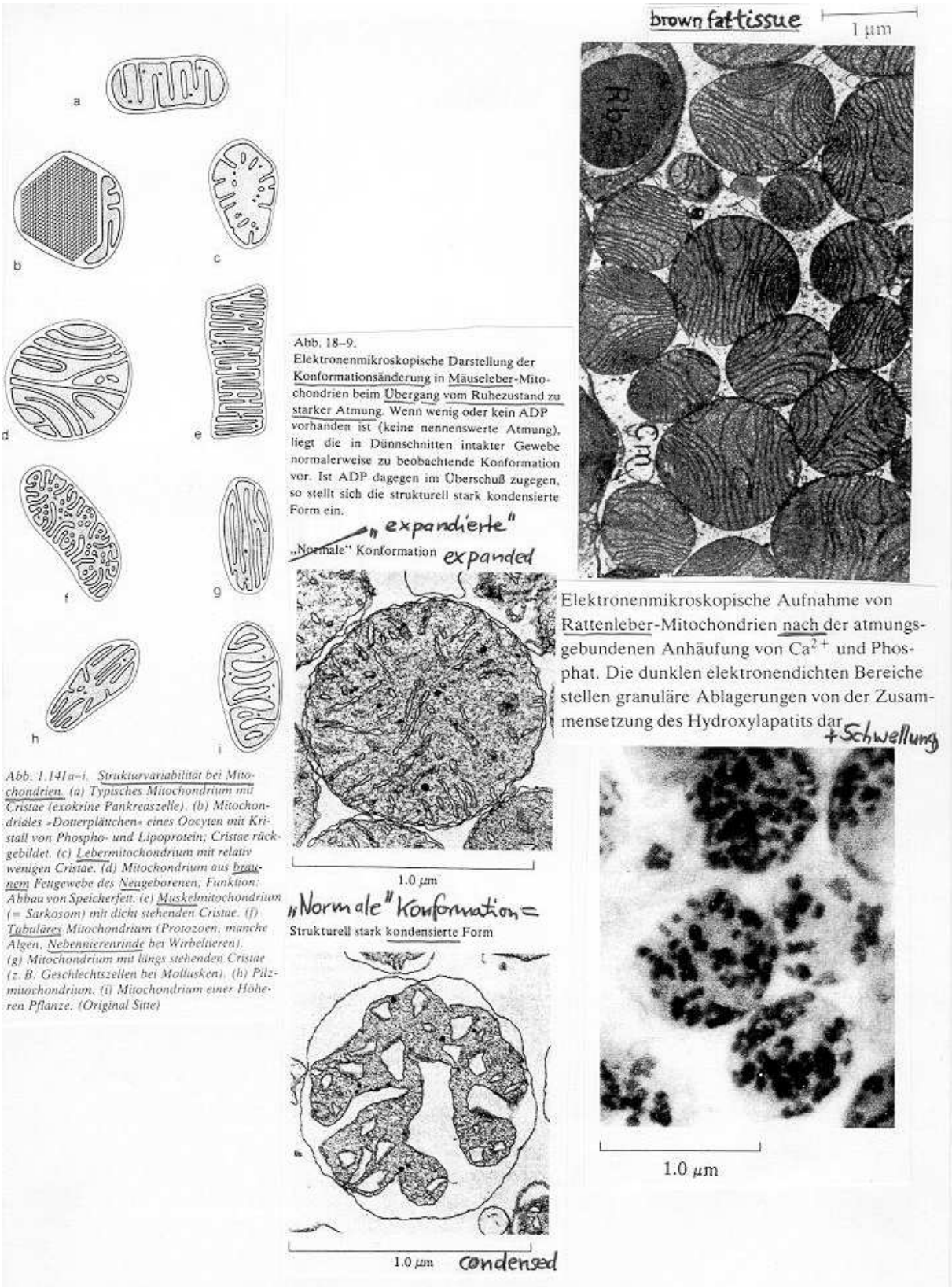


Abb. 1.12. Resorbierende Epithelzellen eines Nierentubulus. Links oben Bürstensaum (Zellapex), rechts unten basales Labyrinth und Basalmembran. An der Basis des Bürstensaumes tubuläre Einstülpungen, die der endocytotischen Stoffaufnahme dienen; Verdauung aufgenommener Makromoleküle durch Lysosomen. Die Blutcapillare (rechts unten) ist von äußerst dünnen Endothelzellen ausgekleidet. Elektronenmikroskopisch, Vergr. 21000:1. (Aus Porter u. Bonneville)

Mitochondria with cristae (arrow), - partly damaged mitochondria: cristae/condensed plus extended – extended mitochondria; extended mitochondria – cristae parallel to the basal lamina – in heart muscle cells: parallel to the muscles



- liver mitochondria – expanded and condensed
- •• brown fat tissue mitochondria of the new born: burn of fat for the production of heat

•• liver mitochondria, expanded - with heavy Ca (P_i) deposits/hydroxyapatite = swollen (extended)
••• structur variation of mitochondria

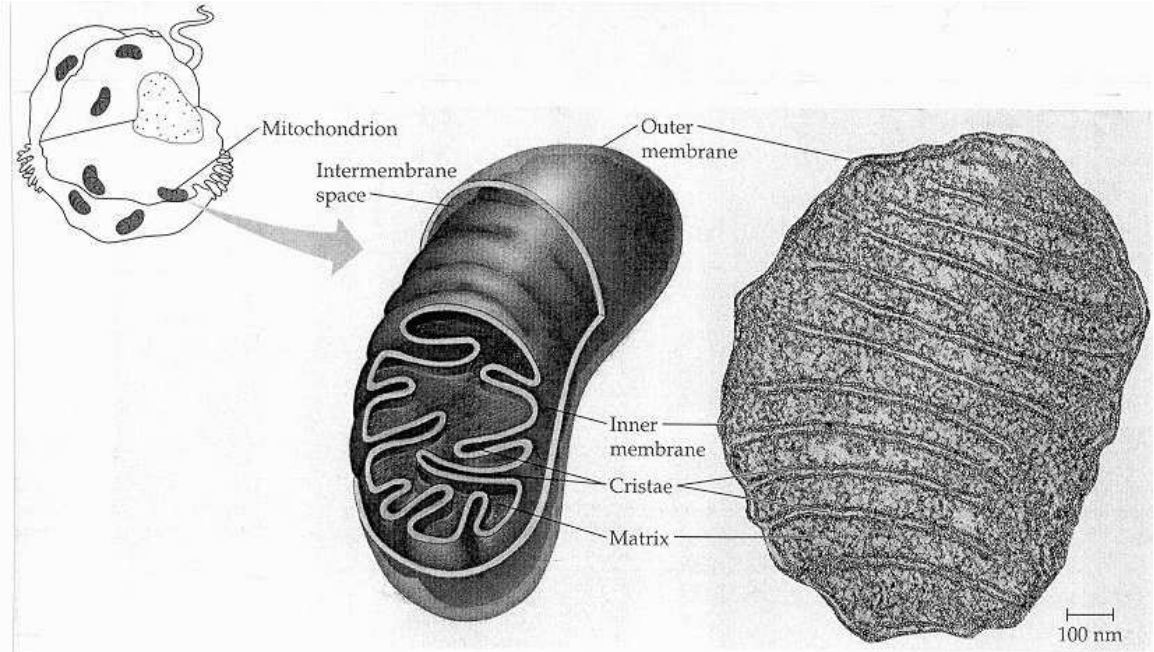
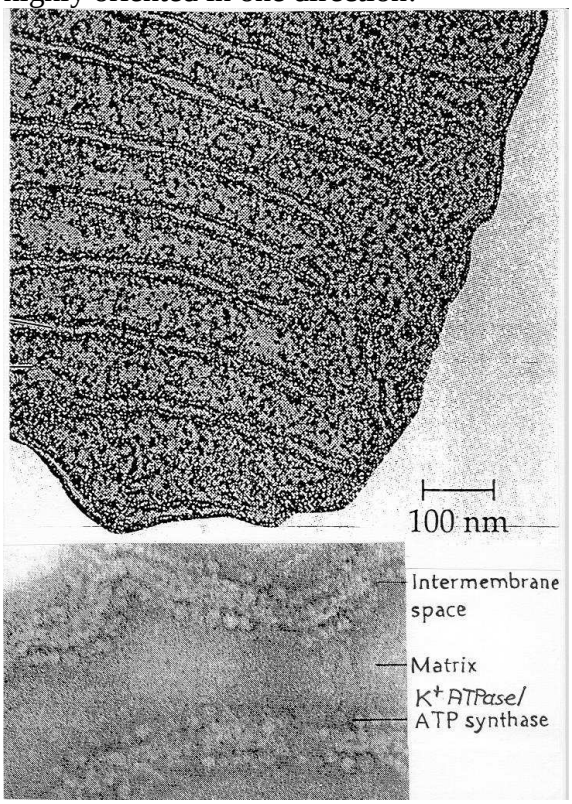


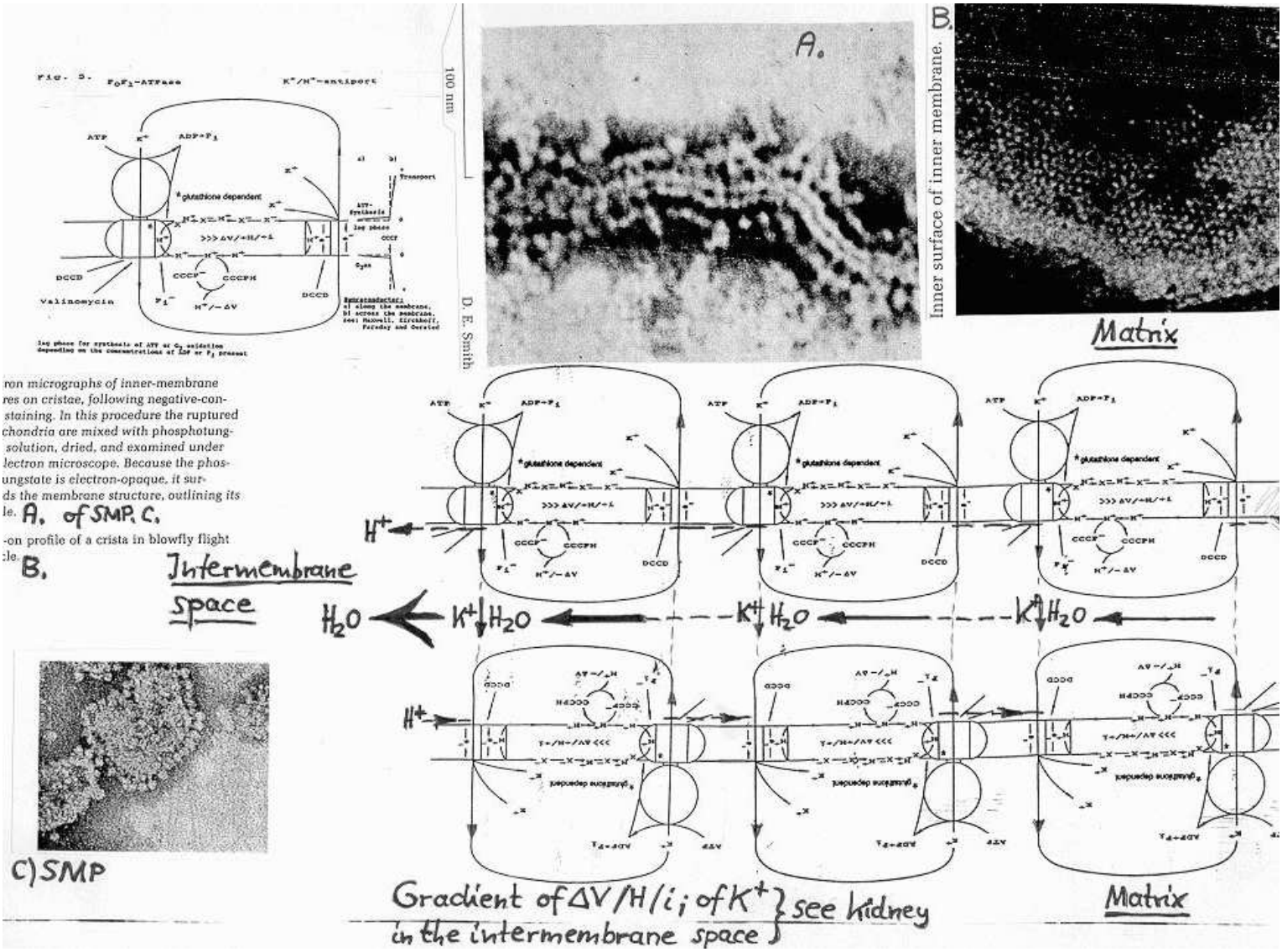
Figure 7.21
The mitochondrion. The double membrane of the mitochondrion is evident in the drawing and the micrograph (TEM). The cristae are infoldings of the inner membrane. The three-dimensional drawing emphasizes the relationships between the two membranes and the compartments they bound: the intermembrane space and the mitochondrial matrix.

Mitochondria with outer membrane and highly structured/folded inner membrane or cristae – highly oriented in one direction!



Magnified cristae: you can see the „knobs“ on the surface of the cristea –

Cristea further unlarged: double membrane with „knobs“ = the K^+-ATPase/ATPsynthase – not in line, very dense packed → directly neighbared – physically in contact!



A) double membrane/cristae with ATPase

B) the inner surface of the inner membrane – with densely packed ATPase

C) submitochondrial particles/sonicated mitochondria – isolated cristae

- **arrangement of the K^+-ATPase along the cristae and its function (– remember the kidney): heat/water pump with gradients of K^+/voltage, magnetic field and current**

→ this model will be shown again -

At first to the methods employed in order to get the model:

(I.)

Methods employed: all methods possible on mitochondria, particles, complex I-V, F_1, incl.

Material: rat liver mitochondria, beef heart mitochondria

Methods: • electron microscopy

- O_2 /RCR - Clark electrode
- K^+/(Na^+)(ion movements) - turbidity change, /swelling exp. - 750nm
- K^+ - transport - Beckmann K^+-electrode (Kiehl; Hanstein)
- Ca^{2+} - transport - atomic absorption/RCR - turbidity changes
- Phosphate - transport - $^{33}P_i/P_i$
- DNP - transport - [^{14}C]DNP, binding (Kiehl)
- NPA - transport/binding - [^{3}H]NPA, competition exp. (Hanstein)
- pH-jump/ΔpH - pH-electrode (P. Mitchell, incl.)
- Picrate uptake/binding (Hanstein, Kiehl, BBRC 1981)
- Glutathione - (Ellmann 6, 1959; Tietze F, 1969) (Kiehl)
- Nucleotides/phosphate/ATPase - } (Kiehl, Schuermann, Hanstein,
- $\Delta\psi$ - [^{14}C]thiocyanate (SMP) } Biochem (Life Sci. Adv.) 1988,
- ΔpH - [^{14}C]methylamine (SMP) } ultra fuge)

[• + • $\Delta\psi$ - Di S-C_3-(5) fluorescence - 667nm/exc. 622nm (mito),
• + • ΔpH - 4-methylumbelliferon (4-MU) fluorescence - 450nm/exc. 356nm (mito)

[• + • $\Delta\psi$ - oxonol VI - absorbance increase - 630nm - 602nm,
• + • ΔpH - 9-amino-6-chloro-2methoxyacridine (ACMA) - 480nm/exc. 410nm,
SMP and CV; conformational changes/ion channel: CV, F_1

(II.)

→ to methods:

---- $\Delta\Psi$, Ox VI; ΔpH, ACMA – SMP, CV, F_1 plus change of conformation on K^+, Na^+, Nucleotides: appl.
(Kiehl, Hanstein, Vect. reactions on ... 1981;
BBA 766, 1984;
3rd EBEC 1984;
Kiehl, Hanstein, Biochem (Life Sci Adv) 7, 1988).

- NTU, [^{14}C]NTU and [^{35}S]NTU – sulfenyl group reagents/ lipophilic membrane probes
- NSPM, [^{14}C]NSPM – thiol group reagent/lipophilic membrane probes
- DCCD, [^{14}C]DCCD – carboxyl group reagent/lipophilic membrane probes
- Picrylacetate (PA), [^{3}H]PA – membrane probes
- •• NTU, NSPM, DCCD, PA – probes for conformational changes, incl.

Kiehl, Hatefi, Biochemistry 19, 1980;
Kiehl, FEBS Letters 109, 1980;
Kiehl et al, 2. EBEC 1982;
Kiehl, Hanstein, BBA 766, 1984; 3. EBEC 1984
Kiehl, various congress presents.

- •• PAGE, pH 5.0, ± DTE, and others,
2D-PAGE / proteomics; coop.: Hatefi, D. Fayle (australia), Hanstein (1977 – now)
- spez. extraction of radioactive trapped products / tlc – amino acid analysis, incl.

Now some examples/a selection of results –

The distribution of some ions between plasma, cytosol and mitochondria:

Sodium- und potassium-gradients between plasma/ cytosol and mitochondria –

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.

^{14}C-DNP-accumulation in the presence of NSPM → inhibition by the lipophilic thiol reagent:

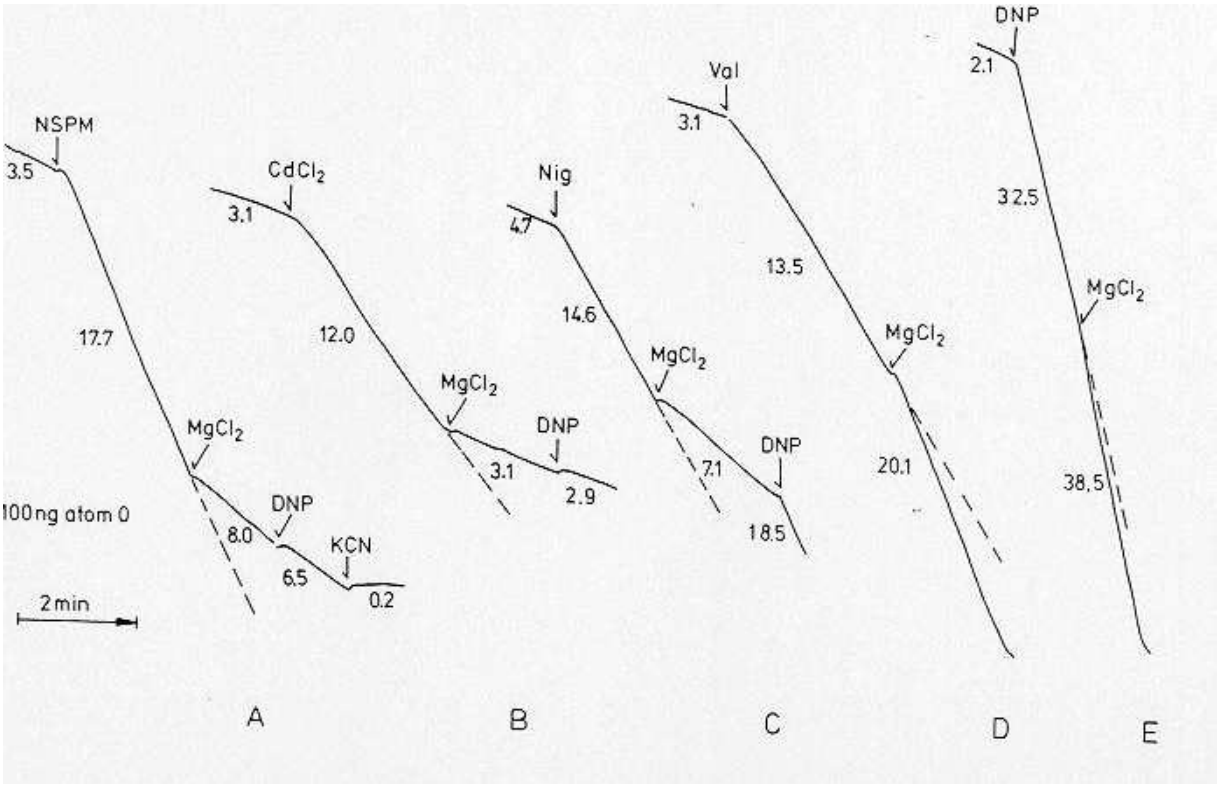
^{14}C-DNP-accumulation in the presence of NSPM

Conditions		^{14}C-DNP, Pellet nmol/mg	Accumulation nmol/mg	%
95 µM	14C-DNP	13.30 ± 0.33 (3)	6.63	100
95 µM	14C-DNP, + 223 nmoles Triton X 100/mg [a)]	6.67 ± 0.10 (3)	0	0
95 µM	14C-DNP, + 20 nmoles NSPM/mg	8.75 ± 0.15 (3)	2.08	31.4
20 nmoles NSPM/mg, + 95 µM	^{14}C-DNP	11.60 t 0.10 (3)	4.93	74.4

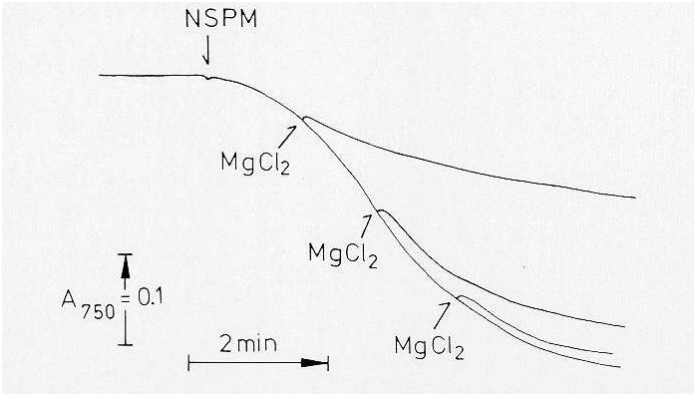
a) amount of Triton resulting in uncoupling; inhibition of RCR at 90 nmoles/mg

Effect of various uncouplers on the coupled respiration of rat liver mitochondria:

- Inhibition of the NSPM-, Cd^{2+}-, Nigericin-stimulation by Mg^{2+}
- Further stimulation of valinomycin- and DNP-stimulation by Mg^{2+}:

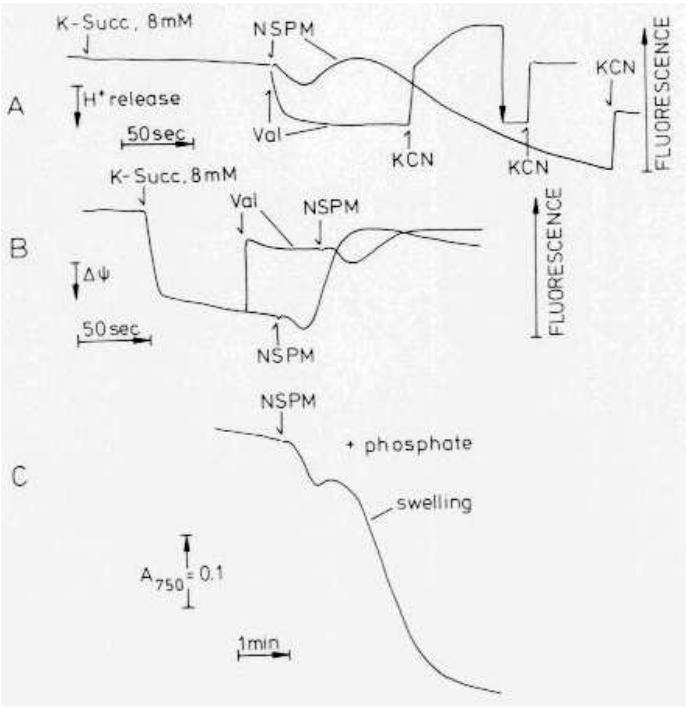


In correlation: inhibition of NSPM induced mitochondrial swelling (turbitity change = ion movement) by Mg^{2+}:



and

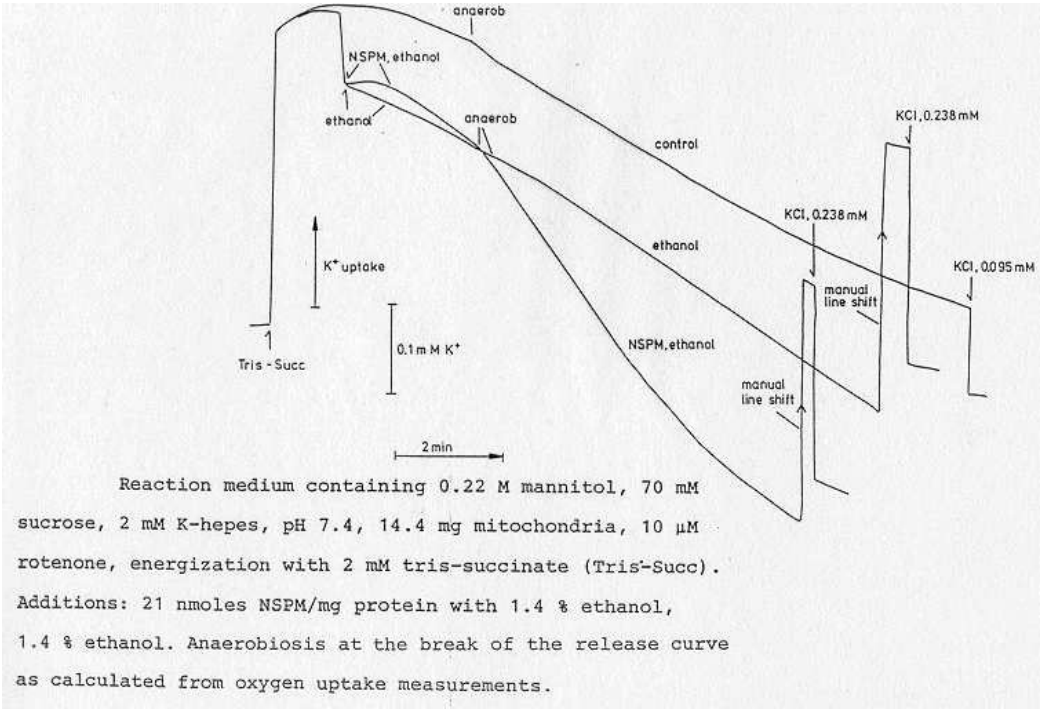
pH-gradient (4-MU), membrane potential (DiS-C_3-(S)) and swelling (turbitity change) of mitochondria in the presence of NSPM or valinomycin:



A) proton release

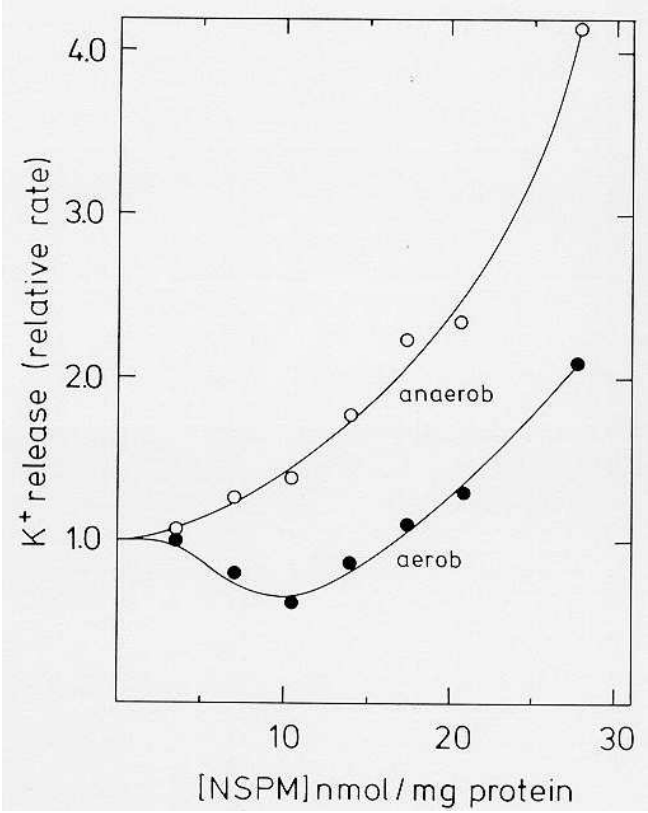
A) + B) potassium cycling across the K^+/H^+-exchange system induced by succinate oxidation and the activated K^+-ATPase

C) in correlation: swelling – water uptake → oscillations by the countermovement of ions



Reaction medium containing 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 (Tris-Succ). Additions: 21 nmoles NSPM/mg protein with 1.4 % ethanol, 1.4 % ethanol. Anaerobiosis at the break of the release curve as calculated from oxygen uptake measurements.

Typical curves out of K^+-transport-measurements with a K^+-Beckmann-electrode +/- NSPM

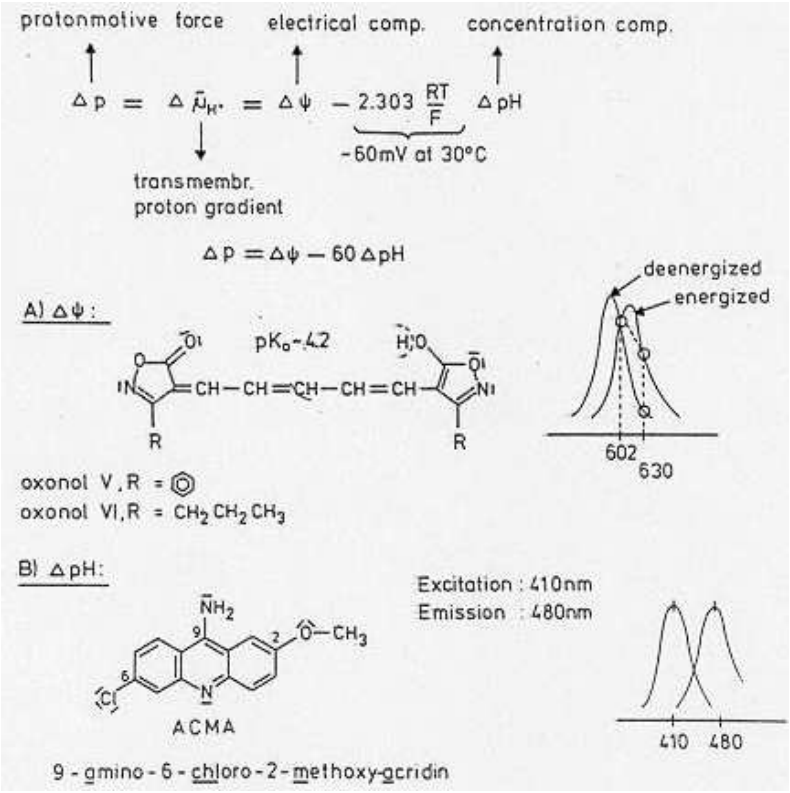


Summation of the results obtained with the K^+-Beckmann-electrode:

Potassium release in the presence of NSPM ($-P_i$) +/- O_2 = aerob/anaerob

= uptake/release → balance

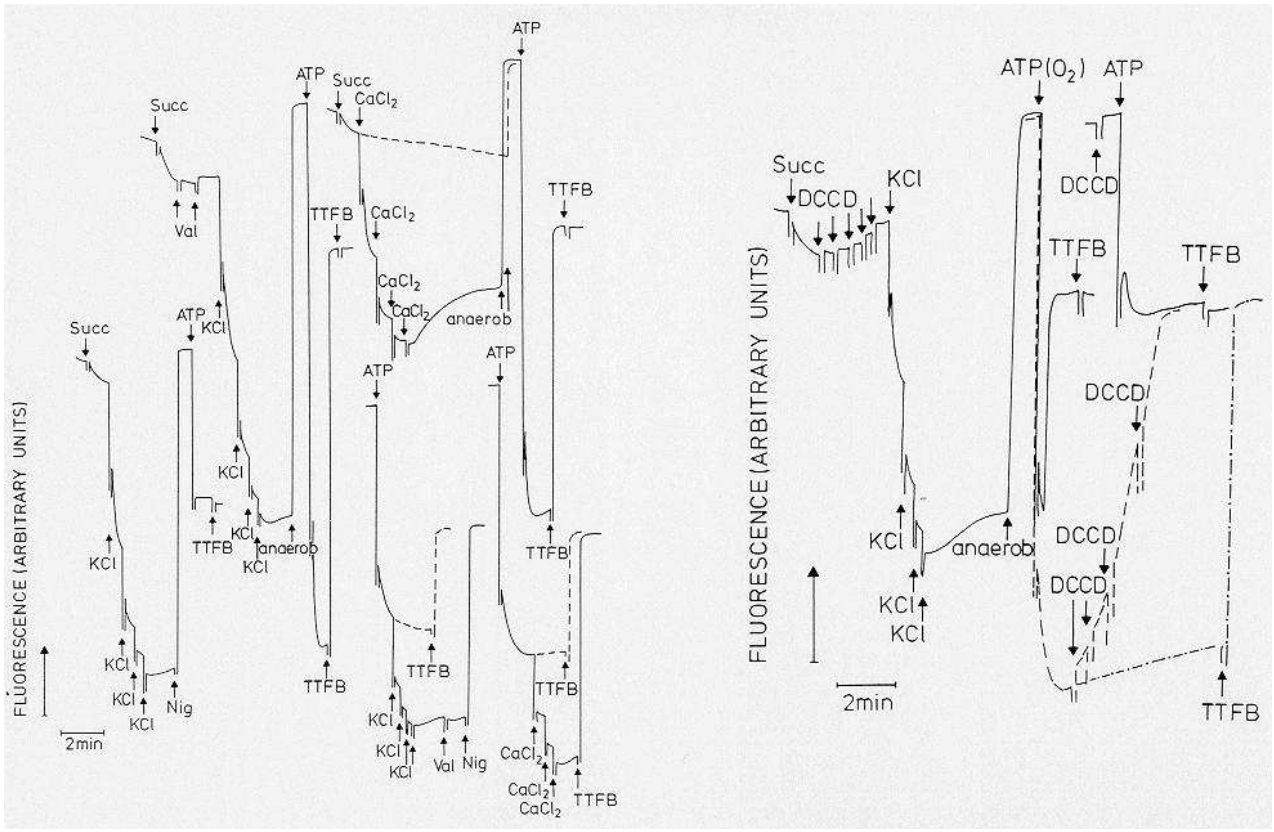
Two probes used for measurements on SMP, CV, F_1:



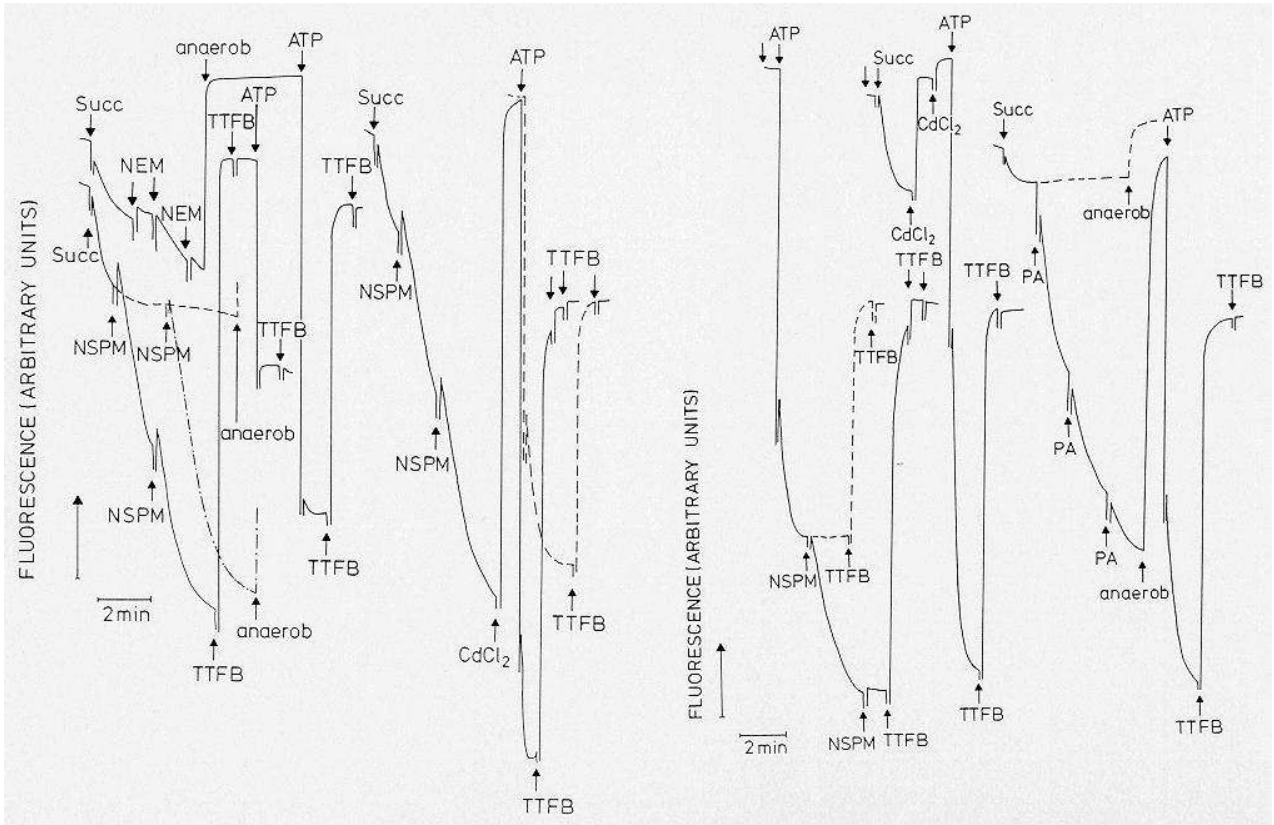
a)oxonol VI for effects on Δφ/conformation-
on energization shift of absorption from 602 to 630 nm

b)ACMA for ΔpH-changes-
on energization fluorescence change from 410 to 480 nm

Some measurements using the energy dep. fluorescence quenching of ACMA in SMP:

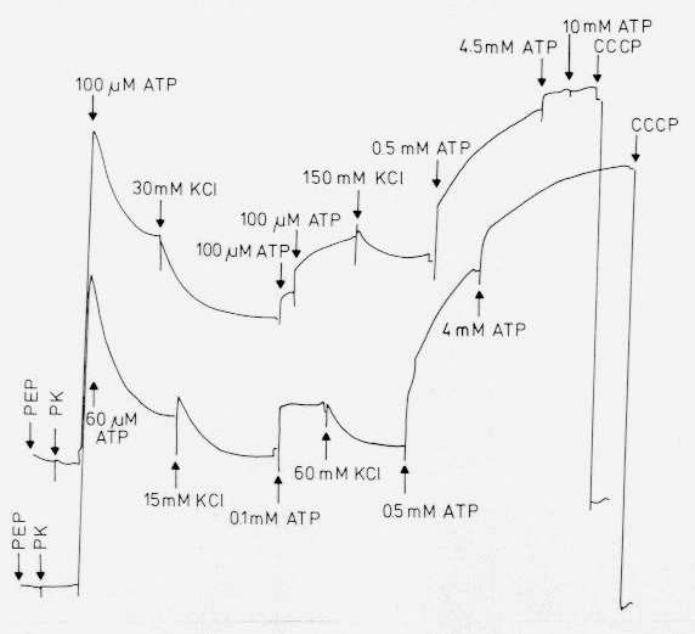


Effects of K^+,Ca^{2+}(chlorids) and Nig/Val/TTFB: K^+ and Ca^{2+} show effects related to their osmotic activity relations = law of physics (osmotic activities)



.....of NSPM (similar to K^+, Ca^{2+}), Cd^{2+}, NEM (without effect)...

ATP-dependent shift /response of oxonol VI in complex V with an ATP regenerating system:



Effect of K^+ and ATP –

detection of conformational changes in CV (complex V):

ATP-dependent shift/response of OxVI in complex V using PEP/PK as ATP regenerating system

Labeling of SMP by [^{14}C]-NSPM in the presence of Diamide or CAT (=proteomics):

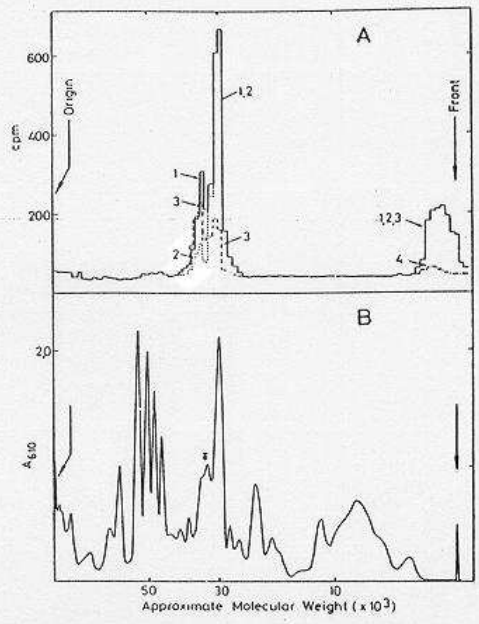


Fig.1. Distribution of radioactivity due to [^{14}C]NSPM in 117 µg submitochondrial particles (A), and distribution of the particle protein (B). Submitochondrial particles were incubated as follows: 0.88 mg particles were added to 1 ml solution containing 0.25 M sucrose, 50 mM Tris–acetate, 30 mM $MgCl_2$ 40 mM phosphate, 12.5 mM ATP (pH 7.5) at 0°C. (1) (solid line) [^{14}C]NSPM was added after 10 min incubation at 0°C and 1 min at 37°C to 20 nmol/mg protein and the reaction stopped after 2 min at 37°C with 1 mM cysteine. (2) (dotted line) as (1) but 10 min preincubation of the particles at 0°C and 1 min at 37°C with 230 nmol diamide/mg protein. (3) (dashed line) as (2) but preincubation with 26 nmol CAT/mg protein. The protein distribution pattern of submitochondrial particles is unchanged by the incubation procedures, the scan shown in fig.1B corresponds to fig.1A, trace 3.

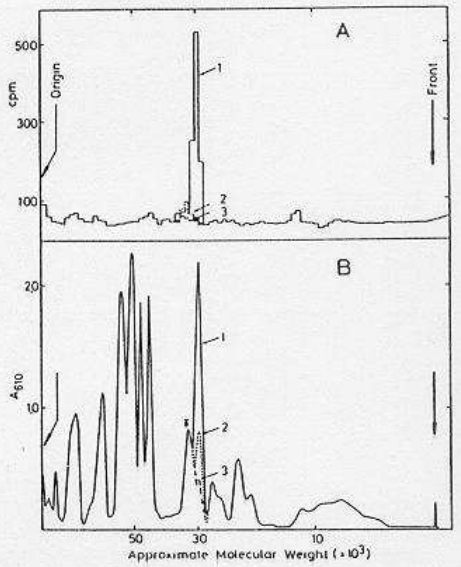
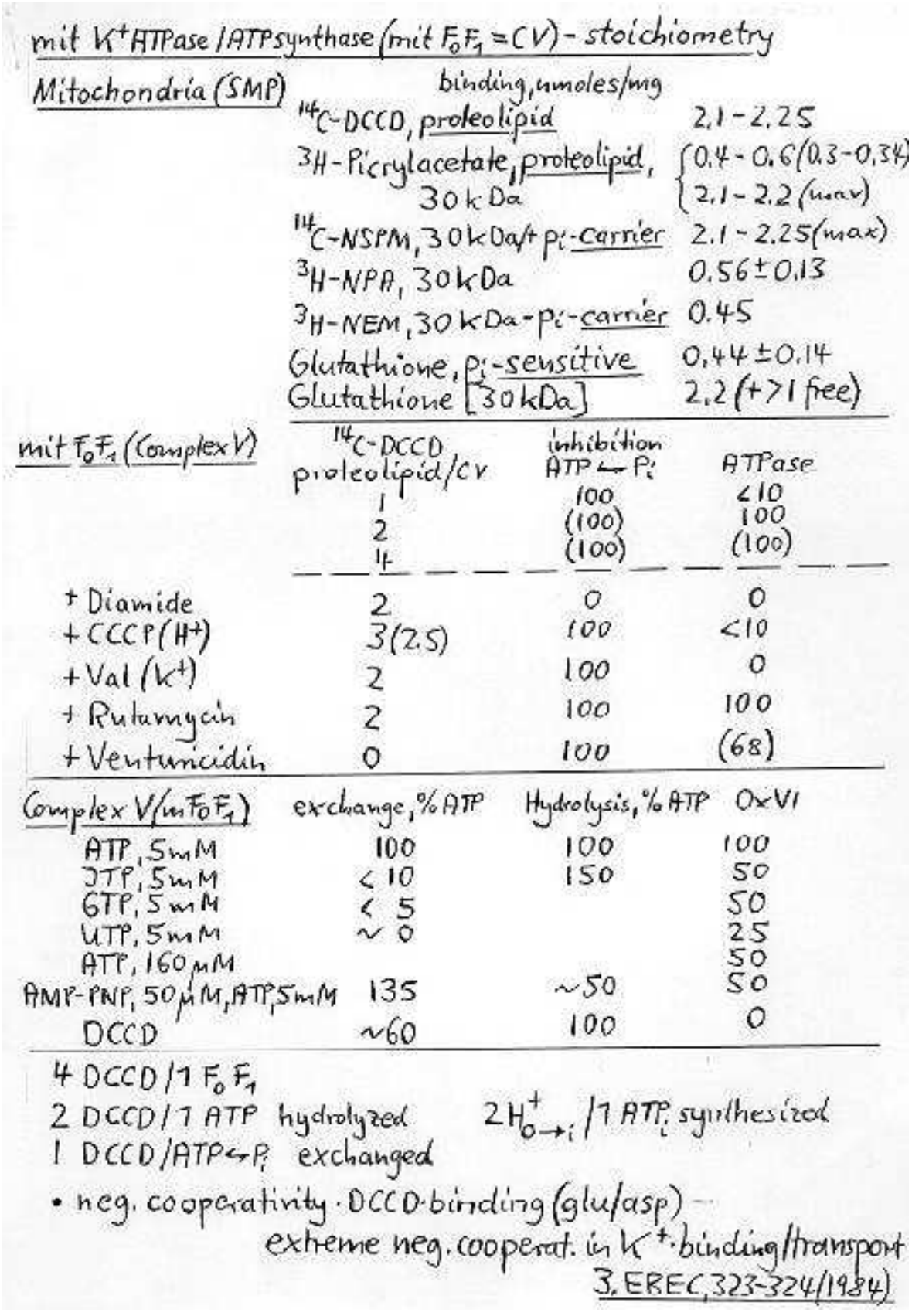


Fig.2. Distribution of radioactivity due to [^{14}C]NSPM in 93 µg ATPase protein (A), and distribution of ATPase protein (B). Conditions as in fig.1. (a) (solid line) 50 nmol [^{14}C]NSPM/mg Serrano-type ATPase protein. (2) (dotted line) as (1) but preincubation of the ATPase with 60 nmol CAT/mg protein. (3) (dashed line) as (1) amount of radioactivity and mol. wt 30 000 band in complex V preparations.

Summation of some measurements on the ATPase:

- neg.cooperativity in DCCD-binding
  - •• extrem neg.cooperativity in K^+-binding/transport (3.EBEC)...



mit K^+ATPase/ATPsynthase (mit F_0F_1 = CV) - stoichiometry

Mitochondria (SMP)

	binding, nmoles/mg
^{14}C-DCCD, proteolipid	2.1 - 2.25
^{3}H-Picrylacetate, proteolipid, 30 kDa	0.4 - 0.6 (0.3 - 0.34) / 2.1 - 2.2 (max)
^{14}C-NSPM, 30 kDa/P_i-carrier	2.1 - 2.25 (max)
^{3}H-NPA, 30 kDa	0.56 ± 0.13
^{3}H-NEM, 30 kDa - P_i-carrier	0.45
Glutathione, P_i-sensitive	0.44 ± 0.14
Glutathione [30 kDa]	2.2 (+?1 free)

mit F_0F_1 (Complex V)

	^{14}C-DCCD proteolipid/CV	inhibition ATP ↔ P_i	ATPase
	1	100	< 10
	2	(100)	100
	4	(100)	(100)
+ Diamide	2	0	0
+ CCCP (H^+)	3 (2.5)	100	< 10
+ Val (K^+)	2	100	0
+ Rutamycin	2	100	100
+ Venturicidin	0	100	(68)

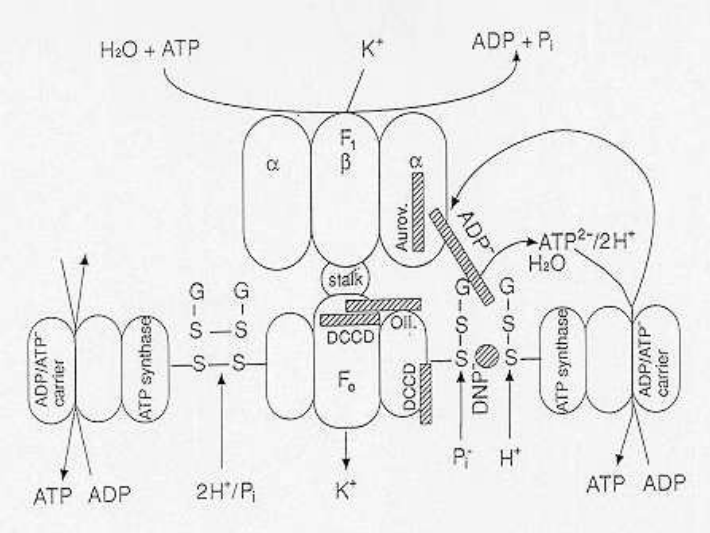
Complex V (mF_0F_1)	exchange, % ATP	Hydrolysis, % ATP	OxVI
ATP, 5 mM	100	100	100
ITP, 5 mM	< 10	150	50
GTP, 5 mM	< 5		50
UTP, 5 mM	~ 0		25
ATP, 160 µM			50
AMP-PNP, 50 µM, ATP 5 mM	135	~50	50
DCCD	~60	100	0

4 DCCD / 1 F_0F_1

2 DCCD / 1 ATP hydrolyzed $2H^+_{o\rightarrow i}$ / 1 ATP_i synthesized

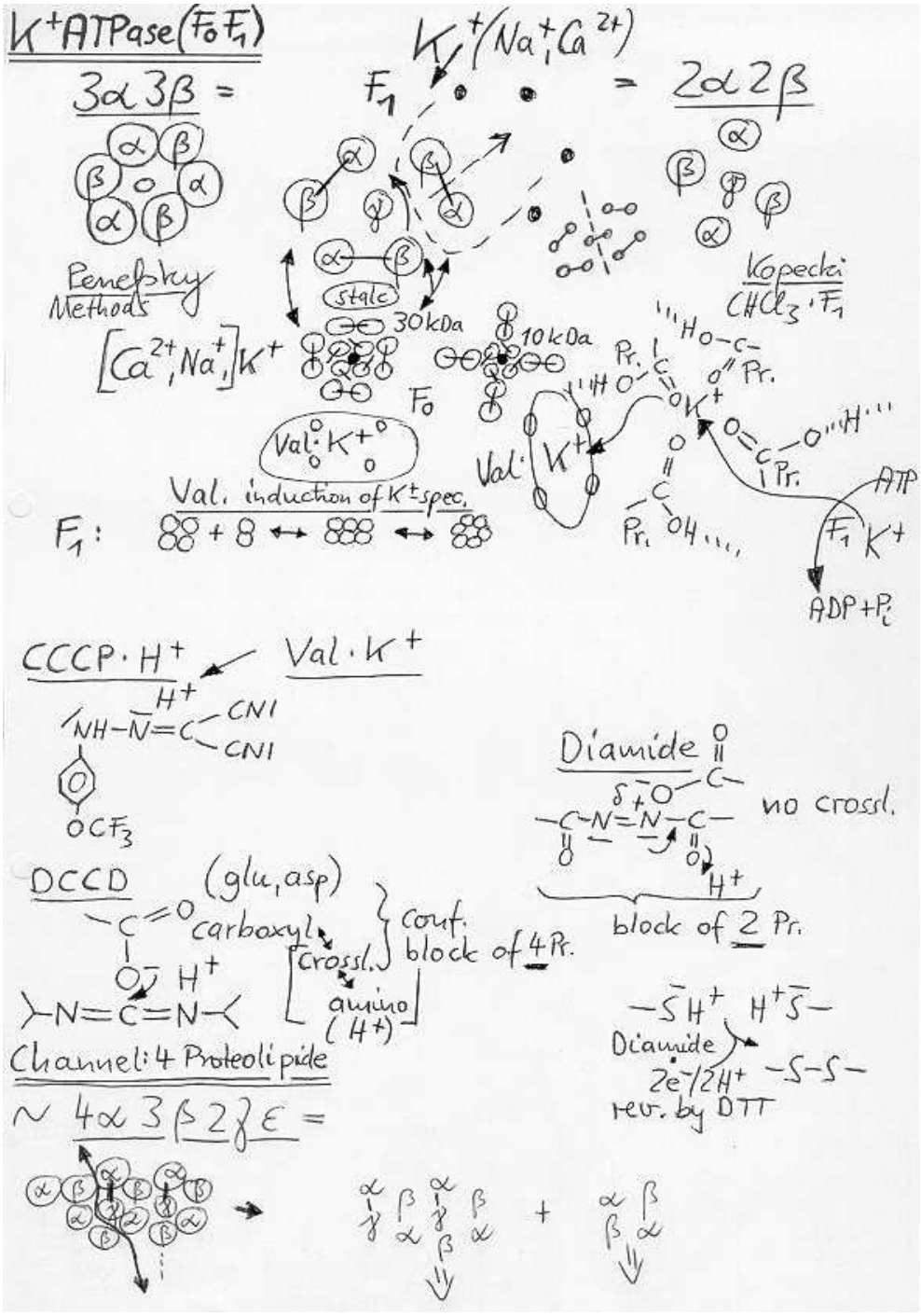
1 DCCD / ATP ↔ P_i exchanged

- neg. cooperativity · DCCD-binding (glu/asp) –
  extreme neg. cooperat. in K^+-binding/transport
  3. EBEC, 323-324/1984)

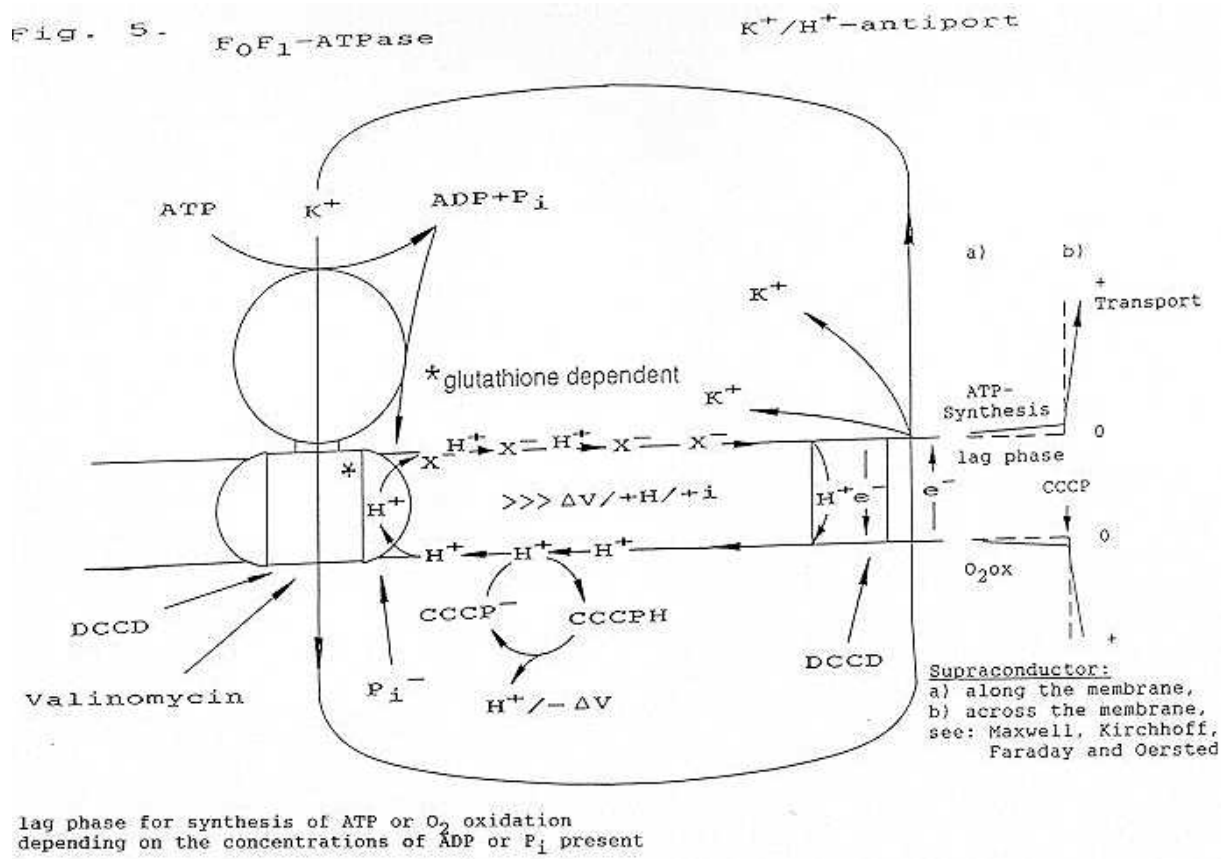


The model shows the coupling between the K^+-ATPase (F_1), membrane part of the ATPase (F_0), ATPsynthase and the nucleotide carrier

Movement of K^+ along the F_o-part of the ATPase: Induction of K^+-specifity by valinomycin

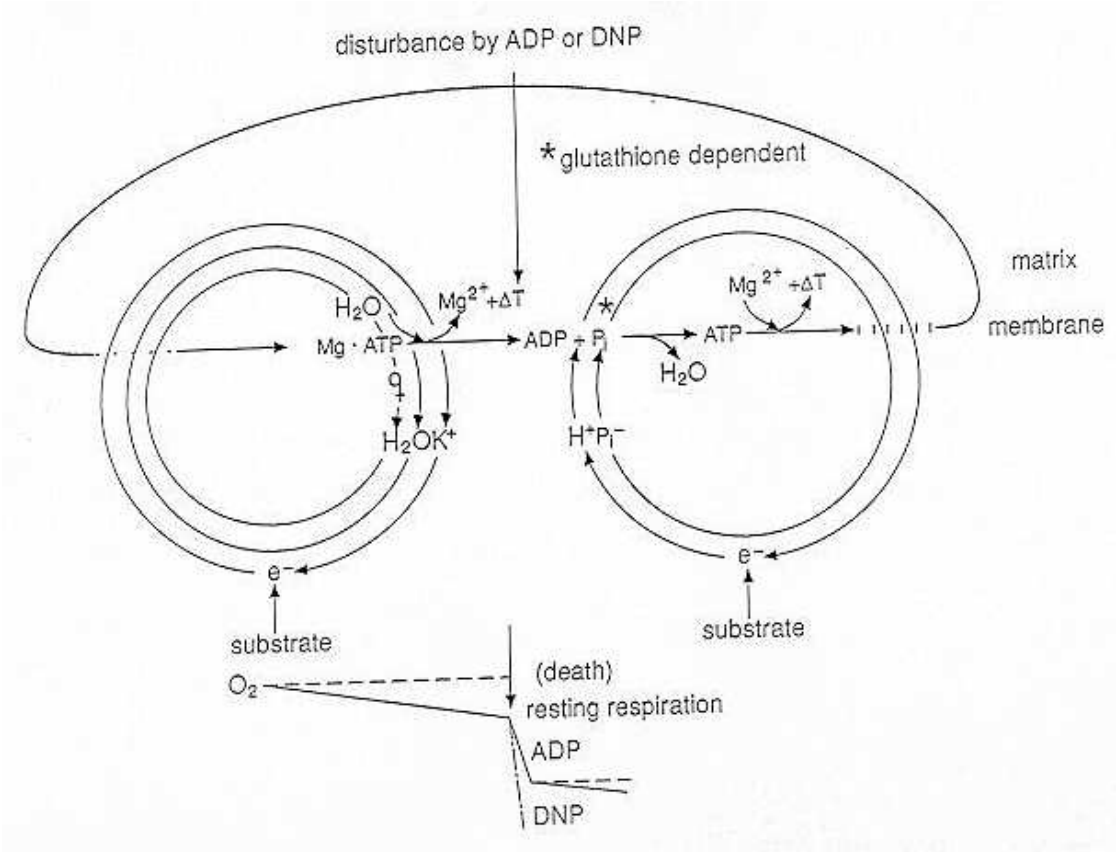


Coupling between the K^+-ATPase and the K^+/H^+-antiport system: sensitivity of voltage/field/ current to for instance $CCCP^-$: → on mitochondria from the outside...



lag phase for synthesis of ATP or O_2 oxidation depending on the concentrations of ADP or P_i present

Thermoregulation by mitochondria: heat exchange by the water pump/K-ATPase



The kidney again: cristae oriented along/paralel to the basal lamina – heart muscle cells paralel to the muscles:

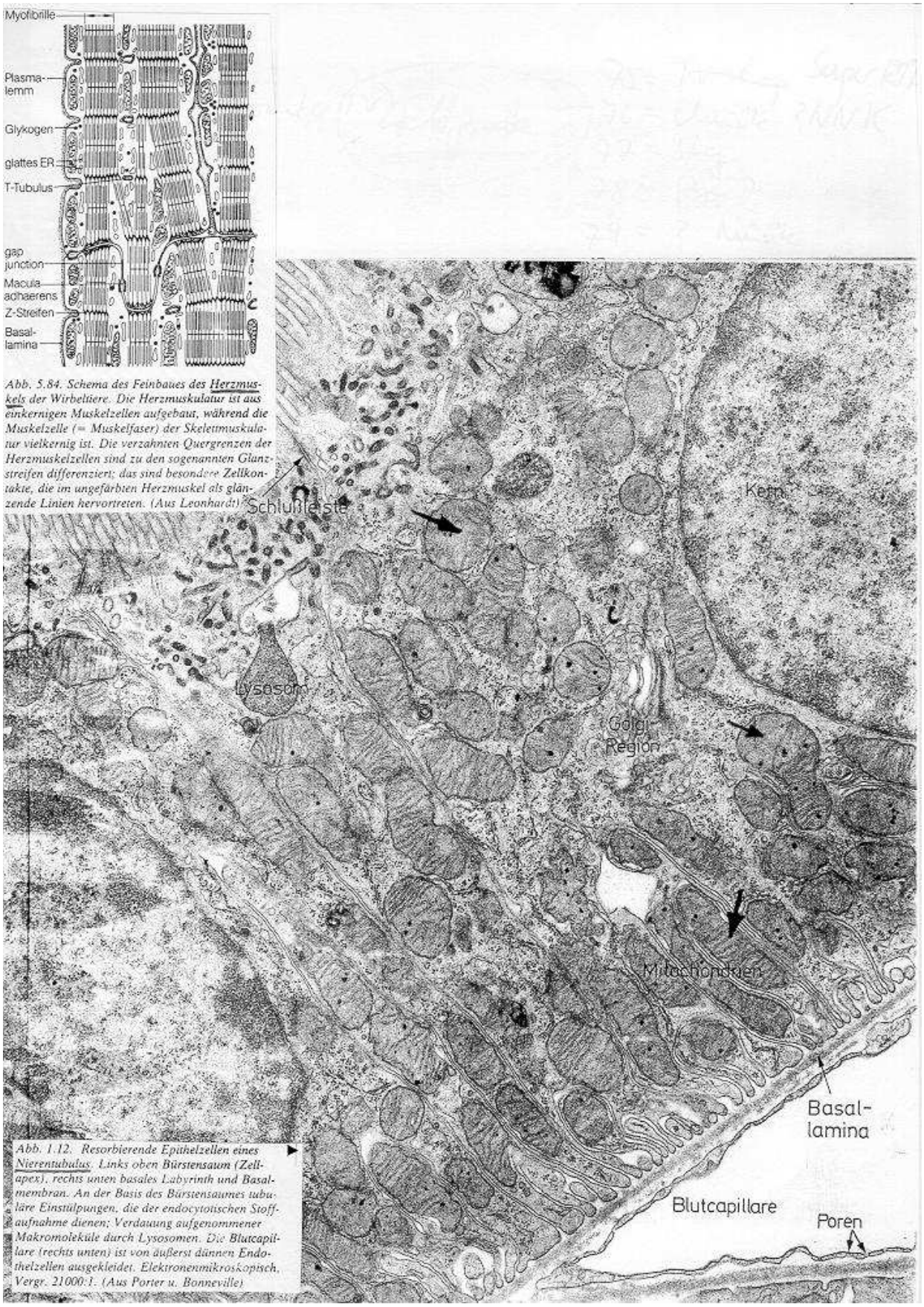


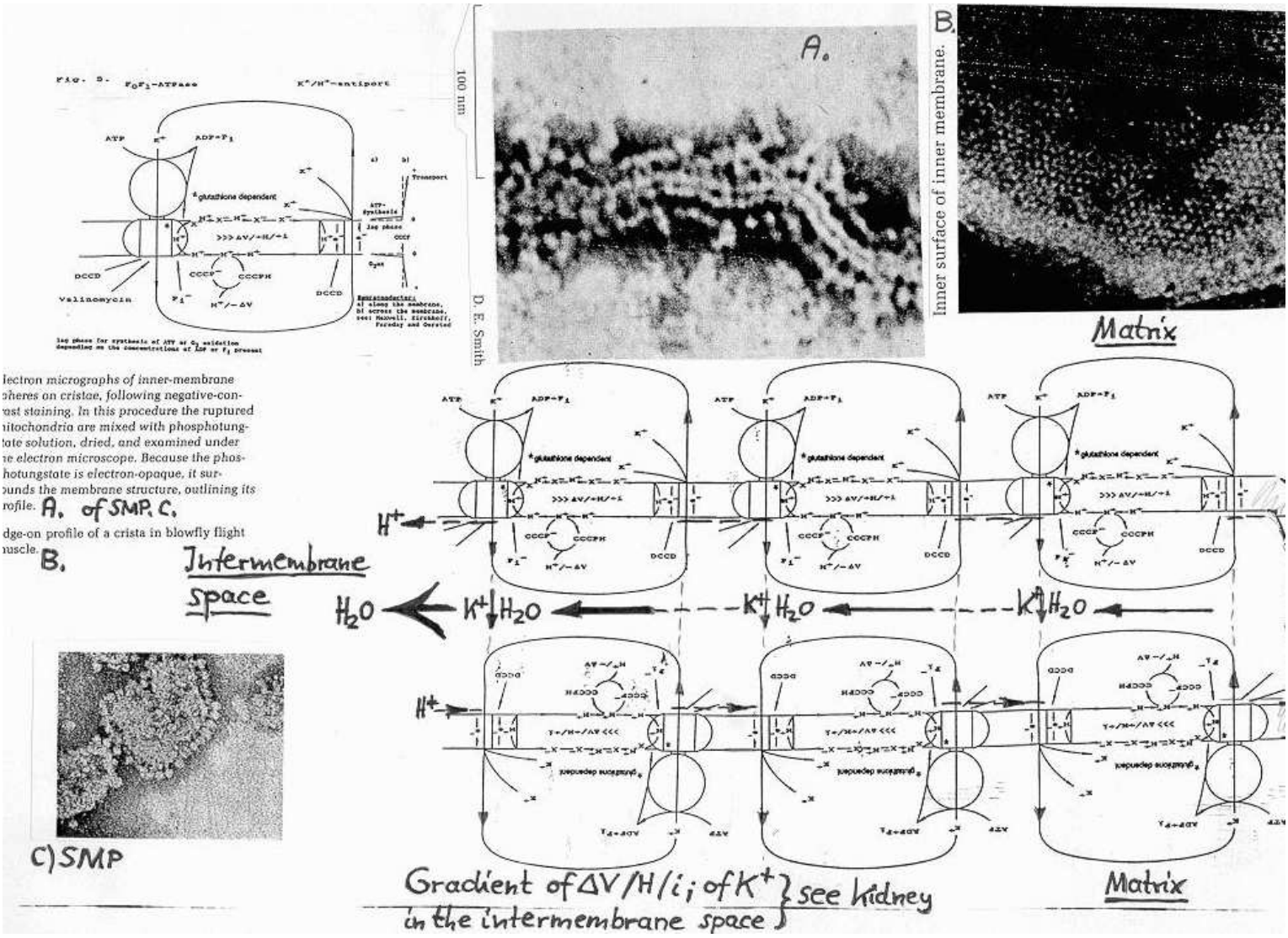
Abb. 5.84. Schema des Feinbaues des Herzmuskels der Wirbeltiere. Die Herzmuskulatur ist aus einkernigen Muskelzellen aufgebaut, während die Muskelzelle (= Muskelfaser) der Skelettmuskulatur vielkernig ist. Die verzahnten Quergrenzen der Herzmuskelzellen sind zu den sogenannten Glanzstreifen differenziert; das sind besondere Zellkontakte, die im ungefärbten Herzmuskel als glänzende Linien hervortreten. (Aus Leonhardt)

Abb. 1.12. Resorbierende Epithelzellen eines Nierentubulus. Links oben Bürstensaum (Zellapex), rechts unten basales Labyrinth und Basalmembran. An der Basis des Bürstensaumes tubuläre Einstülpungen, die der endocytotischen Stoffaufnahme dienen; Verdauung aufgenommener Makromoleküle durch Lysosomen. Die Blutcapillare (rechts unten) ist von äußerst dünnen Endothelzellen ausgekleidet. Elektronenmikroskopisch, Vergr. 21000:1. (Aus Porter u. Bonneville)

Summation of the results on these cristae:

Gradient of voltage, magnetic field, current and K^+ (and temperature) between and in the intermembraneous space:

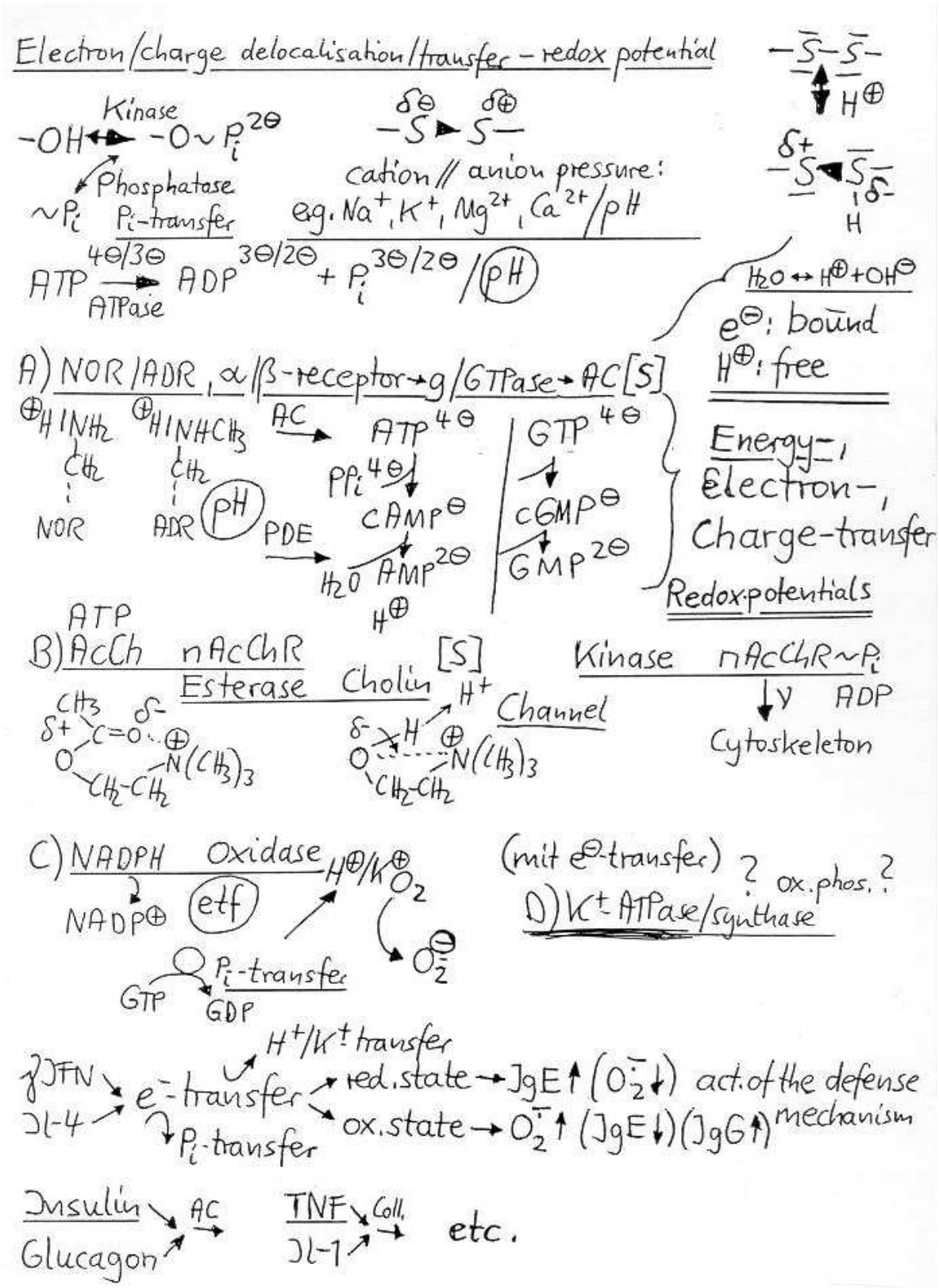
the result: ...pumping of water and heat out of this space...



I hope this overview gives you an impression of the complexity of the mitochondrial thermodynamics and electrophysiology and its involvement in the proper functions of the human body.

Appendix:

To this matter, see also the lecture given at the 23.Jahrest.DGZ 1999, 14.-18.3,Rostock, Eur.J.Cell.Biol.78 (Suppl.49), p.89, P 247 and the GBM-Tagung on BioMembranes, 8.-10.April 1999, Jena, Abstract at the end and at www.rki-i.com **.**



Eur.J.Cell Biol. 78 (Suppl.49), p.89, P 247:

247 **CNS / Psyche - Blood / Immune system / Cells - Acupuncture / Drugs - Electrophysiology and Thermodynamics: The redox potential / electron transfer is responsible for stress protein or O_2^- - synthesis / transport and proliferation**

Reinhold Kiehl, Laboratory and Research for Molecular Medicine/Biology, Saliterweg 1, D-93437 Furth i. Wald,Germany, Fax 0049-9973-801057.

The plasma membrane NADPH oxidase is a rather complicated electron transfer system, which resembles probably the mos important crossover-, end- and starting point of various signal transduction pathways: Ca^{2+}/Mg^{2+}-sensitive phosphorylation an dephosphorylation (incl. JAK-STAT-g-protein-pathway) of the complex regulates the electron transfer (incl. thiol / disulfid interchange-FeS-protein) between mitochondria / plasma and nucleus / ER (NADH / ATP-NADPH / K^+-O_2 / O_2^- - DNA / IgE) Another control is played for instance by arachidonic acid (delivered by PLA_2): The NADPH oxidase belongs then to enzym systems like insulinR, nAcChR, adenylate cyclase, mitochondrial K^+ ATPase / ATP-synthase presenting a universal principle o nature: The connecting logistics between CNS (adrenal cortex) and blood / immune system / cells is played by electron transfe or redox potential and may be visualized by the successful treatment of various diseases and psychological disorders wit acupuncture / homeopathy / relaxation therapy or drugs. We try to connect natural sciences in order to get involved into thi logistic for prediction of effective new treatments, which includes the modeling of interfering drugs for instance. Literature: Kieh R (1976) Dissertation, MPI for Med. Res. Heidelberg; Kiehl R and Hanstein WG (1984) 3. EBEC, 323; Kiehl R et al. (1987 BBRC 147, 1251; Kiehl R (1993) Dear Colleague, New in dermatology 2(2), 4; Kiehl R (1994) Int. Alk-Ciba Corning Join Symp, Benzheim; Kiehl R (1995/1996) Habilthesis LMU Munich Med. Fak.; Kiehl R (1997) Bioforum 12, 686; Amino Acid 13, 50; Kiehl R (1998) Proc., 17th Int. Symp., Electrolyte / Blood Gas, Intercontinental Working Group on the Confluence o Crit. Care Analysis and Near Patient Testing, Nice; Biotechnology Int., Universal Med Press Inc; 2nd Joint Meeting, Signa Transduction: Receptors, Mediators and Genes, Langen.

GBM-Tagung an BioMembranes, April 8-10, 1999; Jena:

The redox potential/electron transfer is responsible for stress protein or 02'-synthesis/Transport and proliferation p.103, P13

R. Kiehl, Lab and Research for Mol Med/Biology, Saliterweg 1, D-93437 Furth i.W./FRG.

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Kiehl, R. (1995/96). Habilthesis, LMU Munich, Med.Fac.; Kiehl, R.(1997). Amino Acids 13, 50/51.
Kiehl, R (1998) Proc,17.Int Symp, Electr/Blood Gas, Int.Work. Group on Confl.of CritCareAnal. and NearPatientTesting, Nice; Kiehl, R. (1998). Biotechnology Int. (Universal Med.Press).

Further References under www.rki-i.com "publications"/Publikationen - Literaturliste/ Dateien, Book under "materials"/Materialien.

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