ISSN 1810-5033

НОВОСТИ МЕДИКО-БИОЛОГИЧЕСКИХ НАУК

NEWS OF BIOMEDICAL SCIENCES

Минск 1 2005

УДК 616-001.8:577.158:576.311.347

E. P. TITOVETS [1], V. V. KOSHKIN [2], and L. P. PARHACH [1]

OXYGEN-FLUX CONTROL OF MITOCHONDRIAL RESPIRATION AND ENERGY FUNCTION

[1] *Research Institute of Neurology, Neurosurgery and Physiotherapy, Ministry of Health, Minsk, Belarus*
[2] *Departments of Medicine and Physiology, University of Toronto, Toronto, Canada*

Oxygen has an unchallenged priority over many other factors regulating mitochondrial respiration and energy function. Mitochondrial respiration becomes oxygen-flux controlled whenever the kinetically limiting step is determined by the oxygen delivery rate. Control of mitochondrial respiration by oxygen flux is realized through multiple physiological and biochemical mechanisms regulating oxygen delivery from the air to cytochrome *C* oxidase (COX), the terminal oxygen-consuming enzyme of the mitochondrial respiratory chain.

A dysfunction or breakdown at any step in the sequence of oxygen cascade, from the respiratory system and the systemic circulatory disorders, the microvasculature and the extracapillary oxygen mass transfer, might have an impact on the oxygen delivery rate to COX. Ensuing tissue hypoxia has, in its metabolic basis, a reduced electron transfer rate in the mitochondrial respiratory chain, accompanied by energy deficit, which thus triggers the development of the pathogenic mechanisms of hypoxia. The oxygen delivery rate to mitochondria has physiological significance, attuning tissue energy demand and supply through feedback mechanisms.

A modern advanced non-destructive method of near-infrared spectroscopy (NIRS) of tissue COX, used for monitoring tissue oxygenation in clinical and experimental research, is challenged with a difficult problem of interpreting the steady-state oxidation-reduction levels of this terminal component of the mitochondrial respiratory chain in terms of the physiologically significant respiration rates of mitochondria *in situ* [8, 14]. This problem is closely linked to finding a solution for quantitative relationship between the oxygen-flux-controlled respiration of the mitochondria and the COX data available through NIRS. It is important to investigate what impact the oxygen flux control produces on mitochondria in terms of the response of the mitochondrial respiratory chain and coupling of oxidative phosphorylation. This is the area that has been covered by much research [12] but there are still many problems waiting solution.

The aim of the present work was to develop, using a strictly quantitative approach, a technology for modeling *in vitro* oxygen flux controlled states of the mitochondrial respiration and characterize them in terms of the respiration rates, oxidation-reduction levels of cytochrome C and COX, and coupling stoichiometry of oxidative phosphorylation.

Materials and Methods. White Wistar rats were used in these experiments. The liver mitochondria were obtained by differential centrifugation essentially as described elsewhere [11]. The mitochondria, isolated in isotonic saccharose medium, were kept in cold before use. Mitochondrial proteins were determined by biuret test [10], and orthophosphate by that of Saheky et al. [21]. The concentration of mitochondria in terms of the protein used was 4.5±1 mg/ml, respiratory control ratio (RCR) was 3-4 with succinate and 6–7 with glutamate +malate.

A respirometer employed in the present research, and belonging to open-system devices [26], was developed in our laboratory and described elsewhere [25]. Below, we give some of its features relevant to the present study. Essentially, the respirometer incorporates a respiratory and a gas compartment separated by an oxygen-permeable membrane (usually 5-10μm polypropylene). The solution in the respiratory compartment (1.19 ml volume), where the mitochondria are placed, is thoroughly stirred with an electric-motor-driven plastic mixer having specially fashioned blades to ensure homogeneity of the reaction medium over the whole volume of the compartment. This approach virtually excludes formation of the oxygen partial pressure gradients in the respiratory compartment and mitochondrial microenvironment. Oxygen tension in each compartment was monitored using Clark oxygen sensors. Routinely, calibration of the Clark electrodes was carried out using a zero solution (with oxygen having been washed away with pure nitrogen) and a saturating oxygen concentration determined from the oxygen solubility tables with reference to the temperature, atmospheric pressure, and composition of the solutions. The experiments were carried out at room temperature (21± 1C°).

During an experiment the gas compartment of the respirometer was continuously flushed with oxygen-nitrogen mixtures of known composition.

A flow of oxygen through the intervening membrane is described by Fick's first law for isothermal unidirectional diffusion:

$$J_{O_2} = K\alpha(P^g_{O_2} - P^r_{O_2}), \qquad (1)$$

where J_{O_2} – oxygen mass transfer rate, K – membrane permeability coefficient [constant] having the dimension of reversed time., α – Henry's oxygen solubility coefficient, $P^r_{O_2}$ and $P^g_{O_2}$ stand for oxygen partial pressure in the respiratory and the gas compartments, respectively.

A large oxygen partial pressure difference across the membrane is routinely maintained to satisfy the inequality:

$$P^g_{O_2} \rangle\rangle P^r_{O_2} \qquad (2)$$

With cytochrome oxidase apparent for oxygen being as low as 0.5-0.1 mmHg O_2, this condition is readily fulfilled [5, 23, 24] within a practically acceptable range of oxygen partial pressure in the gas compartment, with total gas pressure there being equal to that of atmospheric pressure.

In view of (2) Eqn.1 becomes:

$$J_{O_2} = K\alpha P^g_{O_2} \qquad (3)$$

The membrane permeability coefficient, *K, is* found from the inclination of the oxygen trace graph on semi logarithmic transform of the oxygen inflow curves. The oxygen-concentration VS time curves are obtained on creating the oxygen partial pressure gradient across the membrane and monitoring oxygen kinetics in the respiration compartment.

The initial condition for establishing the oxygen flux control of mitochondrial respiration is $J^{mit}_{O_2} \rangle J_{O_2}$

where $J^{mit}_{O_2}$ is the rate of mitochondrial respiration. In an established oxygen-flux-controlled steady state

$$J_{O_2} = j^{mit} \quad , \qquad (4)$$

where j^{mit} is oxygen-flux-controlled steady-state of the mitochondrial respiration.

To quantify the oxygen-flux-controlled respiration of mitochondria a dimensionless parameter η, efficiency, is introduced. It represents a ratio of an oxygen-flux-controlled respiration rate of mitochondria to that of the mitochondrial respiration with no oxygen-diffusion control:

$$\eta = j^{mit} / J^{mit}_{O_2} = J_{O_2} / J^{mit}_{O_2} \qquad (5)$$

By definition, the efficiency assumes the values of $0 \le \eta \le 1$. At $\eta = 0$ the mitochondria are anoxic, while at $\eta = 1$ the oxygen flux is equal to the rate of mitochondrial respiration and there is no oxygen-flux control of respiration.

The respirometer was made to be used together with other instruments to monitor simultaneously several selected parameters. The instruments employed in the present research were: a differential dual-wave spectrophotometer Model UV-30 (Shimadzu); a Specol ZV fluorimeter (Karl Zeiss-Jena), two PHA934 modules (Radiometer, Copenhagen) for measuring pH and oxygen partial pressure. The analogue outputs from the Radiometer modules were fed via an ADC into a personal computer and displayed on the monitor and observed in real time. The results were memorized on the HDD in the text ASCII file format to be later processed using other software (Microsoft Excel and TableCurve).

The respiratory compartment proper was designed to carry out either spectrophotometric or fluorescence measurements and to accommodate other electrodes. Thus, pH in the incubation medium was monitored with a glass microelectrode, oxygen partial pressure – with Clark oxygen electrodes. The electric potential difference across the mitochondrial membrane was measured a TPP-sensitive electrode in the presence of tetra phenyl phosphonium cation (TPP^+) [29].

The reagents Tris-chloride, bovine serum albumin (BSA), EDTA, ADP were from Reanal (Hungary), Rotenon, succinate, glutamate, and malate from Sigma Chemical Co.; hexokinase (E.C.2.7.1.1) from Ferrak

(Germany). TPP was from Merck (Germany). All other reagents were analytical grade. Glass bidistilled water was used throughout.

Results. Fig.1 demonstrates an experimental approach used to study a relationship between the oxygen-flux-controlled rates of mitochondrial respiration and the steady-state oxidation-reduction levels of COX. The oxygen tension both in the respiratory and gas compartments was simultaneously monitored using Clark oxygen sensors. As seen in this figure, the mitochondria, with the gas compartment flushed at the start with pure nitrogen, freely respire till all oxygen is consumed in the respiratory compartment. The transition to anoxia is accompanied by a sharp reduction of COX. Shortly after this, oxygen-nitrogen mixtures are introduced into the gas compartment to ensure graded oxygen fluxes into the respiratory compartment. These fluxes are now governed by the relationship presented by Eqns. 3 and 4 above.

As seen in Fig.1, the oxidation-reduction levels of cytochrome oxidase closely mirror the oxygen tension values in the gas compartment that are linearly related to the value of a corresponding oxygen flux.

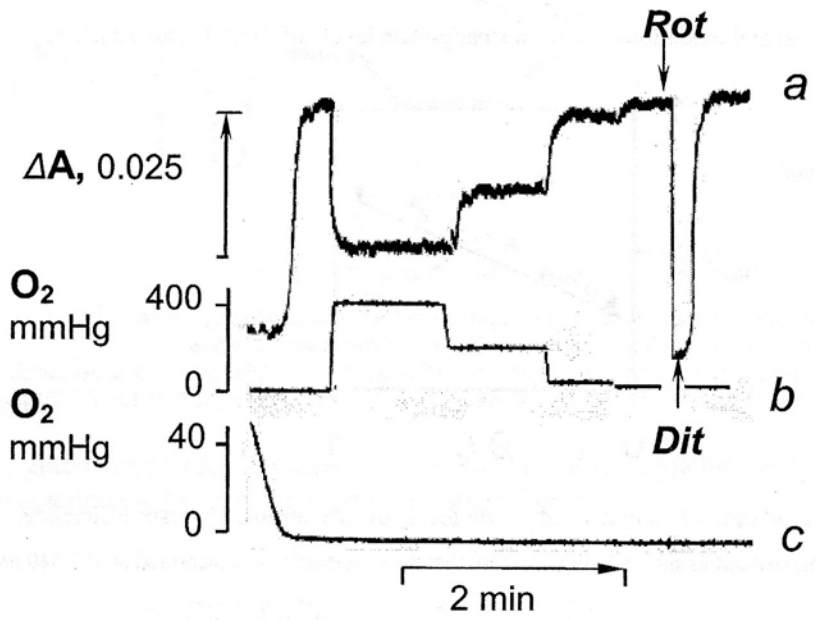

Fig.1 Oxidation-reduction steady states of COX under oxygen-flux control of mitochondrial respiration

a – absorbency at 605-630 nm; b, c – oxygen partial pressure in the gas and respiratory compartment, respectively, *Ro t*– 6 nM Rotenon, *Dit* – 0.5 mM sodium dithionate

Composition of the reaction medium: 0.25 M sucrose, 5mM Tris-chloride buffer pH 7.4, 5 mM KH_2PO_4, 1mM EDTA, 2.5 mM $MgCl_2$, 10 mM glucose, 0.2 mg/ml hexokinase, 0.02 mM ADP. 6 mM glutamate + malate.

Inhibition of electron transfer in the respiratory chain with Rotenone resulted in fast oxidation of cytochrome oxidase. This is mostly due to the traces of oxygen being introduced into the respiratory compartment with the Rotenone solution. Addition of dithionate brought about a complete reduction of cytochrome oxidase and made it possible to determine the total concentration of COX.

It should be noted that in these experiments, after the mitochondria have entered the anoxic state and further on, oxygen partial pressure in the respiratory compartment apparently stays at zero levels with no detectable changes over the whole experiment. This is hardly surprising in view of the fact that cytochrome oxidase Km for oxygen is below one mmHg [5], bordering on the sensitivity limit for the Clark-type oxygen sensors.

In the same experiments we also monitored the oxidation-reduction levels of Cyt C.

The results of these experiments are presented in Fig.2 and 3. A linear dependence of the cytochrome oxidase steady-state oxidation-reduction levels and those of Cyt C on the efficiency factor is shown. These levels are very sensitive to variations in the oxygen flux. The corresponding equations are as follows:

$$COX_{ox}/COX_{total} = 0.04 + 1.19\,\eta \qquad (6)$$

$$CytC_{ox}/CotC_{total} = 0.1 + 0.46\,\eta \qquad (7)$$

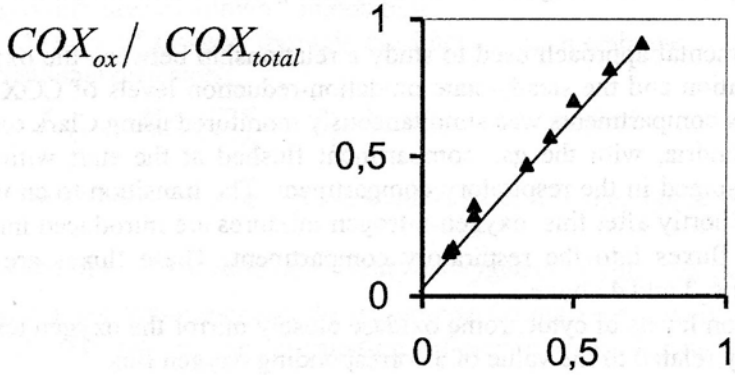

Fig.2. Normalized oxidation-reduction steady-state levels of COX versus efficiency.

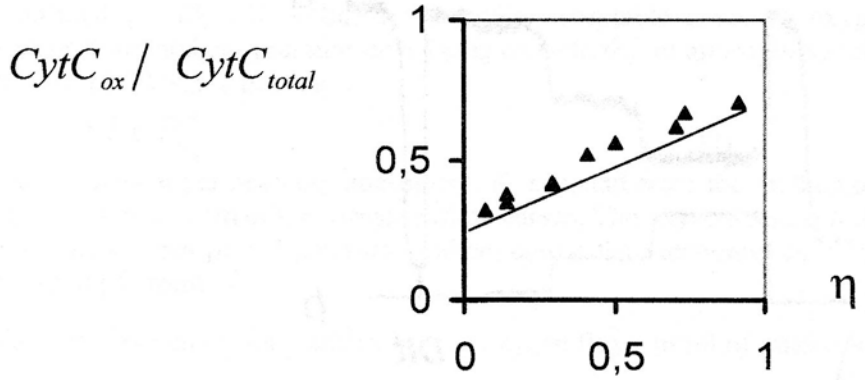

Fig.3. Normalized oxidation-reduction steady-state levels of cytochrome C versus efficiency.

The experiments were carried out as shown in Fig.1 except that the absorbency was measured at 550-540 nm

Eqn.6 is inclusive of eighty-eight determinations carried out with eight preparations of rat liver mitochondria, as shown in Fig.1.

The constants in the above equations may be partly accounted for by the fact that a small moiety of either cytochrome still stays oxidized due to small oxygen leaks into the respiratory compartment with η approaching zero.

An effect of oxygen-flux – induced inhibition of electron transfer on oxidative phosphorylation was studied in experiments where the phosphorylation rate was either monitored with a pH electrode or through direct measurements of inorganic phosphate in the incubation medium.

The kinetics of ADP phosphorylation in the oxygen-flux-controlled states based on pH measurements in the incubation medium is shown in Fig. 4. In these experiments, the mitochondrial pyridine nucleotides (PN) fluorescence was also measured to visualize, through this carrier, the response of respiratory chain to the changes in the oxygen influx rates. Addition of 400 μM ADP to the mitochondrial suspension in the fully aerobic phase activates mitochondrial respiration to State 3 with a corresponding oxidation of PN. On entering the oxygen-flux-controlled state, PN respond with various degrees of reduction that generally is higher at lower oxygen influx rates. The rate of oxidative phosphorylation, as revealed through the changes in pH, is lowered on lowering oxygen influx rates.

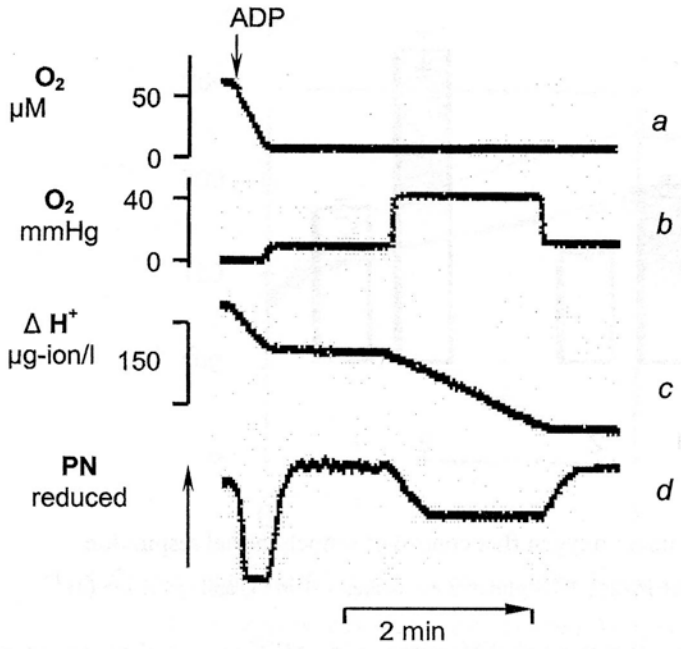

Fig.4. Oxygen-diffusion control of respiration.

a– oxygen electrode trace for the respiratory compartment, b– oxygen tension in the gas compartment, c– pH-electrode trace, d– PN fluorescence. Composition of the reaction medium: 0.12 M KCl, 4 mM Tris-chloride buffer, 2 mM KH_2PO_4, 2 mM histidine, 0.5 mM EDTA, 0.1 % BSA, glutamate + malate 5 mM each, pH 7.2. ADP–600 mM. Rat liver mitochondria –3.2mg

These experiments make it possible to assess the ΔP/ΔO stoichiometry for the initially fully coupled mitochondria entering the oxygen-flux-controlled states Fig. 5.

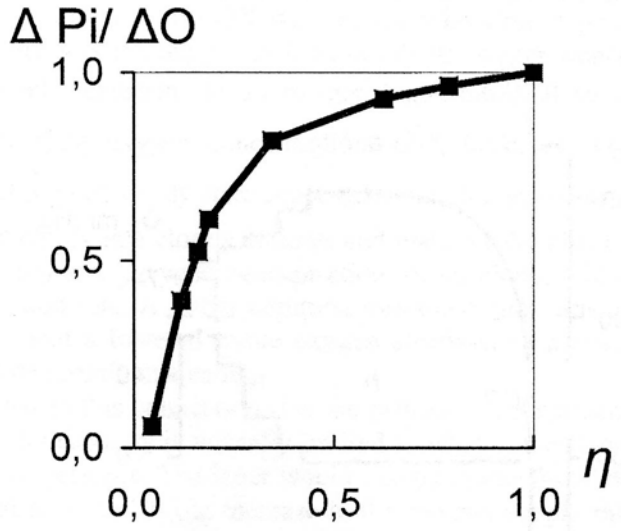

Fig.5. Normalized curve of ΔP /ΔO versus efficiencyExplanations in text.

This figure represents a normalized plot of ΔP/ΔO ratios VS the efficiency factor. The curve clearly demonstrates that overall stoichiometry of oxidative phosphorylation depends non-linearly on the oxygen flux rate. With lowering oxygen flux rates, the extent of uncoupling of oxidative phosphorylation increases. The oxygen-flux-dependent uncoupling of oxidative phosphorylation is also demonstrated in Fig.6, presenting the results of the ΔP /ΔO assessment based on direct measurement of orthophosphate in the incubation medium.

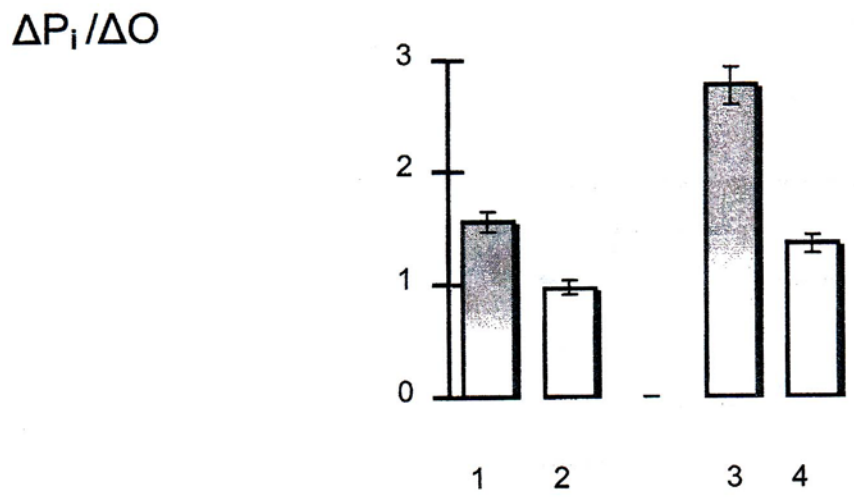

Fig.6. Coupling stoichiometry under oxygen flux control of mitochondrial respiration

1, 2 – succinate, η=1 in (1) and η= 0.1 in (2) ; 3,4 – glutamate+malate, η=1 in (3) and η=0.13 in (4).

Measurements of the membrane electrical potential difference with TPP^+ revealed an eager response of the potential to changes in the oxygen flux rates (Fig.7). Fig. 8 presents a plot of the electric potential difference for both the steady head (State 4) and State 3 as a function of the efficiency. At η =1 the electric potential difference is 230 mV for the static head and 195 mV for the State 3 respiration. In the presence of either ADP or the glucose-hexokinase acceptor system a linear decline in the electrical potential with diminishing η is observed till about η=0.08 after which a non-linear response follows. Both acceptor systems give identical results. The electric potential drop within the linear piece of the curve is about 40 mV. These data are generally in line with those obtained with the use of respiratory inhibitors to control mitochondrial respiration where a comparative stability of the electric potential difference is observed even at low η [29]. On the other hand, this stability is in opposition to the observed changes in the stoichiometry of oxidative phosphorylation implying some nonlinear effects in the coupling system, as discussed in the next section.

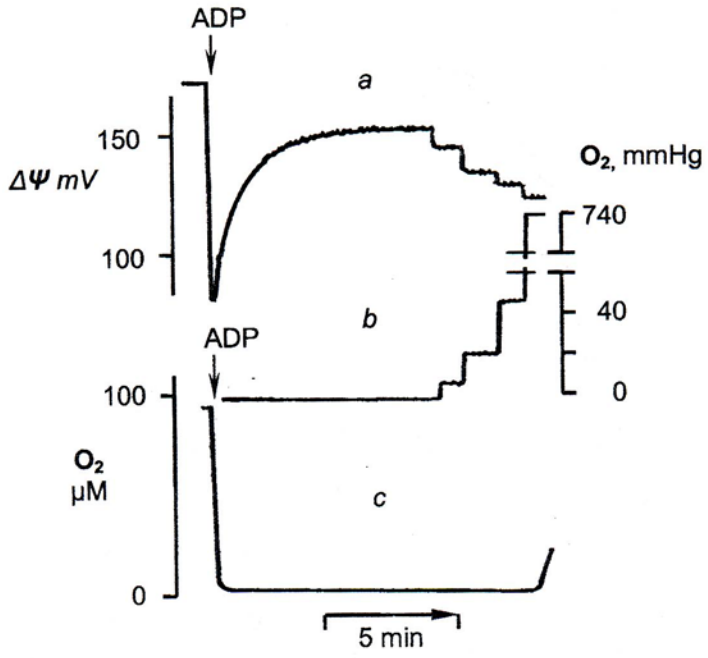

Fig.7 . Oxygen-flux-control of mitochondria respiration and distribution of TPP

Composition of the reaction medium : 0.12 M KCL, 5 mM Tris· chloride, 10 mM KH_2PO_4, 0.5 mM EDTA, 0.1% BSA, 20 mM TPP , 10 mM succinate, (a) TPP -sensitive-electrode trace, (b) oxygen electrode trace for the diffusion compartment, (c) oxygen electrode trace for the test compartment. Rat liver mitochondria 4.5 mg/ml. ADP-850mM.

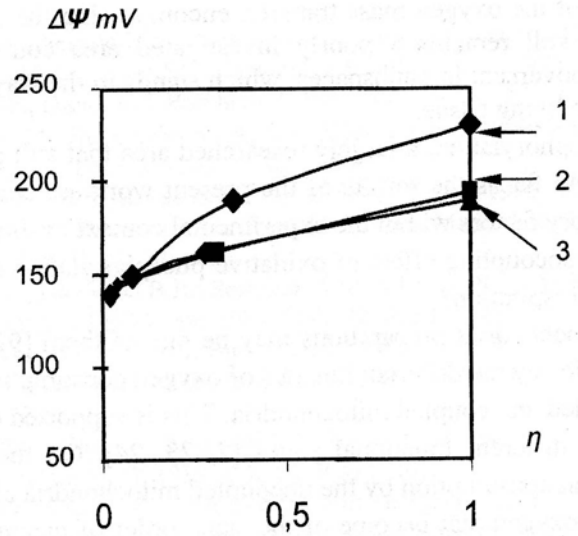

Fig.8. Electric potential difference across the mitochondrial membrane against the efficiency.

1 – static head (State 4); 2 – State 3 (ADP); 3 – State 3 (glucose-hexokinase).

Discussion. The experimental approach described in this paper makes it possible to simulate oxygen-flux-controlled respiration of mitochondria as it occurs in tissue environment. Thus, the respirometer is an open-system device where an oxygen-permeable membrane offersa diffusion resistance barrier equivalent to that of the combined resistance of the capillary wall, the interstitial space and the housing cell – all forming an oxygen supply route from the capillary lumen to COX. Thermodynamically, the gas compartment, presenting an oxygen source, corresponds to the capillary lumen while the respiratory compartment with mitochondria serves as an oxygen sink.

The technology used here corresponds to the orthodox mechanism of tissue oxygenation [13] and, at the same time, gives an opportunity to demonstrate the limitations of the orthodox theory. As a simplistic approach, we may assume hyperbolic kinetics for COX with the mitochondria respiring, for example, on succinate. Then $v=V_{max}O_2/(K_m+O_2)$, where v is the oxygen reduction rate at oxygen concentration – O_2, V_{max} is the maximal rate, and K_m is a Michaelis constant. In an oxygen-flux-controlled steady state $v = J_{mit} = J_{O_2}$. Then the expression for a steady state oxygen concentration, O_2*, becomes: $O_2^* = K_m / (V_{max}/J_{O_2} - 1)$. From this expression it follows that oxygen steady state concentration will stay the same as long as $V_{max}/J_{O_2} = const$.

When the oxygen supply rate closely follows and matches the changes in the oxygen consumption rates, then the value of the steady state oxygen concentration, taken alone, will carry no sensible information about the mitochondrial respiration rate. A rather common misconception, arising from the diffusion mechanism of oxygen mass transfer, is that a lowered tissue oxygen steady-state partial pressure means a reduced oxygen consumption rate. It can be grossly misleading.

It should be stressed in this connection that the orthodox mechanism leaves little room for regulation of the oxygen supply rates. Its capacity is virtually limited to what comes from an increased capillary blood flow and luminal oxygen partial pressure. The latter would rise by moderate 50 % or so [15], which falls short of the mitochondrial demand of an over 10-fold increase in the oxygen supply rates upon transition from resting and active states [5].

This pinpoints one of the main flaws of the orthodox diffusion theory. Basically it admits only a thermodynamic regulation of oxygen supply administered through the changes in the overall oxygen partial pressure gradient spanning from the capillary lumen over to the terminal oxygen consumer, i.e. COX. Within the framework of the orthodox theory, it is difficult to visualize some kinetic regulation mechanism. Indeed, the diffusion cross-section area, the pathway length, temperature, and oxygen diffusion coefficient–all that could introduce a kinetic control – do not change significantly in the tissue to produce an impact on the oxygen supply rates. Besides, there are no physiological means regulating the above factors of oxygen mass transfer.

A conceptual solution of the problem, which would accommodate for the parity in the oxygen supply rates and the mitochondrial oxygen demand, should rather be a convective mechanism of oxygen mass-transfer incorporating a physiologically controlled kinetic step. Such a mechanism, where the rate-limiting step lies with

aquaporins, has been presented elsewhere [27]. Within the framework of this mechanism, a fast cell-to-cell communication for the up-regulation of the oxygen mass transfer, encompassing the microvasculature and the interstitial space, is possible. There still remains a poorly investigated area concerning the kinetics and thermodynamics of interstitial water movement in nanospaces, which stands in the way of better understanding the mechanism of oxygen supply in the living tissue.

The regulation of oxidative phosphorylation, a largely researched area that still poses many questions, is brought about by many factors [12]. As far as the results of the present work are concerned, it is possible to disregard many of the multiple regulatory factors within the experimental context of this research.

What could be the cause of the uncoupling effect of oxidative phosphorylation observed in the oxygen-flux-controlled states of mitochondrial respiration?

A possible heterogeneity of mitochondrial preparations may be one of them [9] and the changes in the coupling efficiency may be accounted for by the different kinetics of oxygen consumption at low oxygen partial pressures, demonstrated by the uncoupled and coupled mitochondria. This is supported by diversity of Km s for oxygen, reported for mitochondria in different functional states [2, 23, 24]. On the other hand, a general tendency in the kinetic pattern of oxygen consumption by the uncoupled mitochondria and those in state 3 is the convergence of their apparent Km for oxygen that become of the same order of magnitude [28]. This puts all species practically on the same level of competition so that no priority in the oxygen consumption rates during the oxygen diffusion controlled states would occur to account for the lowering of the overall coupling stoichiometry at a low oxygen influx. In view of this, the changes in the coupling stoichiometry observed in the present research are considered to be related to some other events taking place in the complex coupling system

Some insight into the mechanism underlying the changes in the overall coupling stoichiometry on introducing oxygen diffusion control of the electron transfer rate can be provided when considering the proton motive stoichiometry at the proton pumps.

It is dioxygen, a reducible substrate of COX, that represents here the only variable factor of control. Its control is realized through a disbalance between oxygen supply and mitochondrial oxygen demand. As the oxygen flux is set below the free mitochondrial respiration rate the steady-state oxygen partial pressure during oxygen-flux-control of mitochondrial respiration stays below effective Km for COX and varies in accordance with the oxygen influx. Thus the regulatory effects of oxygen can be visualized through (a) inhibition of electron transfer in the respiratory chain with resulting changes in proton motive force and (b) oxygen concentration (partial pressure) in the reaction medium.

According to P. Mitchell, uncoupling of oxidative phosphorylation generally occurs through a proton leak through the inner mitochondrial membrane, which thus reduces the proton flux through complex V [16]. That is interpreted as a natural leak of protons across the inner mitochondrial membrane [3, 4, 20].

A slip for COX in rat liver mitochondria by high proton motive force (where $\Delta\Psi$ is the major component) with a decrease in H+/e stoichiometry on increasing $\Delta\Psi$ was demonstrated [17]. Contrary to these findings, the uncoupling of oxidative phosphorylation demonstrated in the present paper increases with a diminished oxygen flux, becoming more pronounced at lower $\Delta\Psi$. This requires some other explanation for the observed changes in coupling stoichiometry at low oxygen flux.

It is well established that COX is particularly involved in regulation of the rate and efficiency of oxidative phosphorylation through intrinsic uncoupling [12]. A diminished P/ O ratio might be the result of intrinsic uncoupling or ''slip'' of COX [1, 18, 19]. One may hypothesize that dioxygen might act as an allosteric effector of COX, modulating its activity via conformational modification and regulating the efficiency of oxidative phosphorylation through intrinsic uncoupling at complex IV. With this approach, allosteric COX modulation is expected to appear at subsaturating oxygen concentrations as is the case in our experiments. This view enjoys an experimental support [6].

The results presented in this paper have some important implications as far as the NIRS data on monitoring *in vivo* the oxidation-reduction states of COX are concerned. Cytochrome oxidase acts as an important intracellular marker of tissue oxygenation while a standing problem with NIRS is finding a way of explaining the obtained spectrophotometric data on the COX oxidation-reduction levels in meaningful terms of the mitochondrial oxygen consumption rates. This paper describes a functional relationship between the oxidation-reduction levels of COX and the rates of mitochondrial respiration, which offers a new approach to solving the problem of interpretation of the NIRS data on tissue respiration.

References

1. Azzone G.F., Zoratti M., Petronilli V., Pietrobon D. // J. Inorg. Biochem. 1985. Vol.23. P.349– 356.
2. Bienfait H.F., Jacobs J.M. C., Slater E.C. // Biochim. Biophys. Acta .1975. Vol. 376. P. 446–457.
3. Brand M.D. Chien L.-F., Diolet P. // Biochem. J. 1994. Vol. 297. P. 27–29.
4. Brand M.D., Brindle K.M., Buckingham J.A. et al. // Int. J. Obes. 1999. Vol. 23 (Suppl. 6) P.S4–S11.
5. Chance B. // J.Gen.Physiol. 1965. Vol. 49. P. 163–185.
6. Chandel N.S., Budinger G.R.S., Schumacker P.T. // J.Biol. Chem. 1996. Vol. 271(31). P. 18672–7.
7. Degn H., Lundergaard J.S., Petersen L.C., Ormicki A. // In: Methods of Biochemical Analysis (Click D., ed.) New York, Wiley. 1980. Vol.26, P. 47–77.
8. Desoky El., Delpy D.T., Davidson B.R., Seifalian A.M. // Liver. 2001. Vol. 21. P. 37–44.
9. Dussynsky J., Wojtczak L. // FEBS Lett. 1985. Vol.182. P. 243–248.
10. Gornall A., Bardawill C., David M. // J. Biol. Chem. 1949. Vol. 177. P. 751–766.
11. Hogeboom C., Schneider H. // In: Methods in Enzymology. 1955. (Colowick S.P., Kaplan N.C., eds.), Vol.1. P. 16, New York: Academic Press.
12. Kadenbach B. // Biochim. Biophys. Acta. 2003. Vol. 604. P. 77–94.
13. Krohg A.J., // J. Physiol.(London). 1919. Vol. 52. P 391–405.
14. Kang K.A., Bruley D.F., Chance B. // Biomed Instrum Techno (U S). 1997. Vol.31 (4). P. 373–86.
15. Lubbers D.W. // In: Oxygen transport in blood and tissue. Stuttgart.1968.P.124–139.
16. Mitchell P. // J. Biochem. 1985. Vol. 97 (1) P. –18.
17. Murphy M.P., Brand M.D. // Eur. J. Biochem. 1988.Vol. 173. P. 645–651.
18. Pietrobon D., Azzone G.F., Walz D. // Eur. J. Biochem. 1981. Vol. 117. P. 389–394.
19. Pietrobon D., Zoratti M., Azzone G.F. // Biochim. Biophys. Acta. 1983. Vol.723. P. 317– 326.
20. Rolfe D.F.S., Brand M.D. // Biosci. Rep. 1997. Vol. 17. P. 9 –16.
21. Saheki Sh., Takeda A., Shimadzu T. // Analyt. Biochem. 1985. Vol.148.P. 277–281.
22. Stuart J.A., Cadenas S., Jekabsons M.B. et al..// Biochim. Biophys. Acta. 2001. Vol. 1504P. 144–158.
23. Sugano T., Oshino N., Chance B. //Biochim. Biophys. Acta. 1974. Vol. 368. P. 298–310.
24. Sugano T., Oshino N., Chance B. // Biochim. Biophys. Acta. 1974 . Vol. 347. P. 340–358.
25. Titovets E. //Analyt. Biochem. 1987. Vol. 166. P. 79–82.
26. Titovets E., Lunets E.F.// In: Manual to the Study of Biological Oxidation by Polarographic Method (Frank G. M., and Kondrashova M.N., eds.) Moscow: Nauka. 1973. P. 162–172.
27. Titovets E., Stepanova T. // News of Biomedical Sciences. No. 2. 2004.P. 127–134.
28. Wilson D.F., Rumsey W.L., Green T.G., Vanderkooj G.M. // J. Biol. Chem. 1988. Vol. 263 (6). P. 2712–2718.
29. Zoratti M., Pietrobon D., Azonne G.F. // Eur. J. Biochem. 1982.Vol.126. P. 443– 451.

E. P. TITOVETS [1], V. V. KOSHKIN [2], and L. P. PARHACH [1]

OXYGEN-FLUX CONTROL OF MITOCHONDRIAL RESPIRATION AND ENERGY FUNCTION

[1] *Research Institute of Neurology, Neurosurgery and Physiotherapy, Ministry of Health, Minsk, Belarus*
[2] *Departments of Medicine and Physiology, University of Toronto, Toronto, Canada*

Summary

A technology has been developed for modeling *in vitro,* on quantitative basis, oxygen-flux-controlled steady-states of mitochondria. A linear dependence of the steady-state oxidation levels of cytochrome *c* and cytochrome *c* oxidase on the respiratory efficiency was demonstrated. Reduction by oxygen flux of the rate of electron transfer in the respiratory chain brought about reversible uncoupling of the stoichiometric efficiency of oxidative phosphorylation in the initially tightly coupled rat liver mitochondria. This phenomenon is explained in terms of allosteric modulation of cytochrome *c* oxidase by oxygen, resulting in intrinsic uncoupling at complex IV.