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ISSN 2391-8306
Formerly ISSN 1429-9623 / 2300-665X

Journal of Education, Health and Sport

formerly Journal of Health Sciences

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Vol 8 No 2 2018

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation.
Part B item 1223 (26.01.2017).
Indexed in Index Copernicus Journals Master List. ICV 2016: 84,69; ICV 2015: 93.34 IC Value 2014: 89.51 Standardized Value: 8.27
http://jml2012.indexcopernicus.com/Journal+of+Education+Health+and+Sport,p24782242,3.html
Universal Impact Factor 1.78 for year 2012. (http://www.uifactor.org/AppliedJournals.aspx)
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ISSN 2391-8306
2391 8306

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Instytut Kultury Fizycznej Uniwersytet Kazimierza Wielkiego w Bydgoszczy
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ISSN 2391-8306
Formerly ISSN:1429-9623 / 2300-665X

Bieniak Monika, Łuczyk Robert, Wysokiński Mariusz, Fidecki Wiesław. Health behaviour of patients with unstable angina pectoris. Journal of Education, Health and Sport. 2018;8(02):196-213. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1174001 http://ojs.ukw.edu.pl/index.php/johs/article/view/5286

Piech Piotr, Polak Paweł, Kozioł Maciej, Obierzyński Paweł, Staśkiewicz Grzegorz, Neścior Małgorzata. The influence of thickness of ACL tendon graft on long-term results of treatment. Journal of Education, Health and Sport. 2018;8(02):214-230. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1174039 http://ojs.ukw.edu.pl/index.php/johs/article/view/5288

Vlasova O.V. Risk of developing congenital malformations in infants whose mothers live under unfavourable geochemical conditions. Journal of Education, Health and Sport. 2018;8(2):231-234. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1175175 http://ojs.ukw.edu.pl/index.php/johs/article/view/5289

Potapchuk Olga. Application of Web-technologies in the educational process of higher educational institutions of Ukraine. Journal of Education, Health and Sport. 2018;8(2):235-242. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1175249
http://ojs.ukw.edu.pl/index.php/johs/article/view/5290
https://pbn.nauka.gov.pl/sedno-webapp/works/855746

Guty Edyta, Puszkarz Irena, Stefaniak Iwona. Mental Disorders related to stroke. Journal of Education, Health and Sport. 2018;8(2):243-249. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1182707
http://ojs.ukw.edu.pl/index.php/johs/article/view/5299

Rynzhuk Larisa Vasylivna, Rynzhuk Vasyl Yefremovych, Gresko Marina Dmitryina, Bulik Tatyana Sergyivna, Sheremet Anastsia Mihaylivna. Peculiarities of the colon microflora condition in pregnant women with asymptomatic bacteriuria. Journal of Education, Health and Sport. 2018;8(2):250-255. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1182741
http://ojs.ukw.edu.pl/index.php/johs/article/view/5302
https://pbn.nauka.gov.pl/sedno-webapp/works/856616

Tiupka T. I., Zalyubovska O. I., Zlenko V. V., Avidzba Yu. N., Litvinenko M. I., Minaieva A. O. Disorders of water-electrolyte metabolism in pulmonary edema under inhibition of the renin-angiotensin-aldosterone system. Journal of Education, Health and Sport. 2018;8(2):256-262. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1185341
http://ojs.ukw.edu.pl/index.php/johs/article/view/5313
https://pbn.nauka.gov.pl/sedno-webapp/works/858082

Dikal M. V., Ferenchuk Ye. O., Bilous T. M., Grytsiuk M. I., Kopchuk T. G. Biochemical and histochemical chenges in the activity of succinate dehydrogenase and ATP-ase in mitochondria of kidneys rats by acute intoxication. Journal of Education, Health and Sport. 2018;8(2):263-271. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1185391
http://ojs.ukw.edu.pl/index.php/johs/article/view/5314
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http://ojs.ukw.edu.pl/index.php/johs/article/view/5314
https://pbn.nauka.gov.pl/sedno-webapp/works/858097

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26.01.2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

Received: 01.02.2018. Revised: 05.02.2018. Accepted: 26.02.2018.

"Pathological Physiology of Kidneys & Water Salt Homeostasis"

February, 15-16, Odessa
Materials of conference

UDC 577.152.1.008.5:616.61-008.64-099]-092.9

BIOCHEMICAL AND HISTOCHEMICAL CHANGES IN THE ACTIVITY OF SUCCINATE DEHYDROGENASE AND ATP-ASE IN MITOCHONDRIA OF KIDNEYS RATS BY ACUTE INTOXICATION

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Abstract

Object of the study was to evaluate activities of succinate dehydrogenase and ATP-ase in mitochondria of kidney under conditions of toxic influence of 2,4-dinitrophenol. The experiment was conducted on 60 male albino rats. Toxic damage was caused by 0.1% solution of 2,4-dinitrophenol intraperitoneally in the dose of 3 mg / kg. The histochemical, biochemical and statistical methods of research were used. Mitochondria isolation was performed using the method of differential centrifugation. Determination of mitochondrial succinate dehydrogenase activity consists in the restoration of potassium ferricyanide (K_3[Fe

$(CN)_6$]), whose solution has a yellow color, to the colorless ferricyanide of potassium (K_4[F $(CN)_6$]) succinate by the action of succinate dehydrogenase. The activity of ATP-ase was determined by the accumulation of inorganic phosphate.

The level of activity of succinate dehidrogenase was 39% lower than that of the control values. Whereas the level of activity of ATP-ase decreased by 48% in accordance with the control. Similar histochemical changes of the enzyme of the II complex of the mitochondrial respiratory chain were revealed.

The findings revealed that 2,4-dinitrophenol produced significant biochemical and histochemical changes in the kidney of the rats. This led to a decrease enzyme activities and as a result to a discrepancy between the energy needs of the cell and energy production in the system of mitochondrial oxidative phosphorylation.

Key words: **mitochondria, kidney, 2,4-dinitrophenol, succinate dehydrogenase, ATP-ase activity.**

Introduction. Mitochondria regulate key cellular processes, from energy production to apoptosis, and measuring their function is important for understanding different aspects of cellular metabolism. The inner membrane of the mitochondria comprises of the highest number of proteins per phospholipid moiety in a cell. These proteins are integral to the electron transport chain, ATP synthesis and transport. The inner membrane is also less permeable to ions and molecules and helps in compartmentalization through separation of the mitochondrial matrix from the cytosolic environment, there by acting as an electrical insulator and chemical barrier. This helps in leaving of the electron gradient across the membrane, which enables generation of universal value of energy – ATP [1].

In fact, the variety of reactive molecules is formed at all levels during the execution of physiological functions of the cell. Mitochondria are the source of most reactive oxygen species in cells, and the electron transport chain is responsible for most of the superoxide that is generated through partial reduction of oxygen. As the main sites of reactive oxygen species production are regarde mitochondrial complex I and III. But the mitochondrial complex II has been the subject of renewed interest over the past years. Newly some studies have demonstrated that succinate dehydrogenase (SDH) also contributes to mitochondrial reactive oxygen species production at the FAD-binding site through the monovalent electron reduction of O_2 [11, 13]. In addition, SDH has been recognized as an indirect modulator of superoxide production by complexes I and III. Recent research has provided irrefutable evidence that reactive oxygen is important mediator of cellular response to stress and they function through

several mechanisms including, modulation of autophagy, mitochondrial network, signaling and apoptosis [5].

Succinate dehydrogenase (SDH; succinate: ubiquinone oxidoreductase; mitochondrial complex II) plays a central role in mitochondrial metabolism, catalyzing the oxidation of succinate to fumarate and the reduction of ubiquinone to ubiquinol, thereby linking the tricarboxylic acid cycle andthe electron transport system.

Classically, SDH is composed of four subunits. SDH is the oxidative phosphorylation complex to lack subunits encoded by the mitochondrial genome and the only respiratory complex to not pump protons across the inner membrane during its catalytic cycle. The structure of the complex of SDH consists of a hydrophilic head that protrudes into the matrix compartment and a hydrophobic tail that is embedded within the inner membrane with a short segment projecting into the soluble intermembrane space [8].

The catalytic activity of SDH is modulated by post-translational phosphorylation and acetylation as well as active site inhibition.

SDH catalytic activity is also modulated by Krebs cycle intermediates including oxaloacetate, which is a potent inhibitor. Succinate promotes the dissociation of oxaloacetate from SDH there by activating the enzyme. The oxaloacetate inhibition may contribute to the known modulation of SDH activity by the metabolic status of mitochondria. Other specific inhibitors of the enzyme are malonate, mercurial compounds, maleic acid N-alkylamides, phenylglyoxal, 2,3-butanedione, and some carboxylic acid esters [5, 6].

We propose that the wide clinical spectrum of specific roles of the SDH in the respiratory chain in the mitochondria and in the Krebs cycle could confer to the SDH a specific function in kidneys under condition of intoxication processes.

Therefore, the study of the biochemical and histochemical enzyme activity of succinate dehydrogenase in the kidneys under conditions of toxic influence of 2,4-dinitro-phenol has an important diagnostic value as a marker of energy metabolism and oxidative phosphorylation under various pathological conditions.

To investigate the link between 2,4-denitrophenol and mitochondrial energy production, appropriate methods are needed to quantify changes both in mitochondrial form and function.

Objective. To determinate activities of mitochondrial enzymes by biochemical and histochemical methods.

Materials and methods. The experiment was conducted on 60 male albino rats with the body weight 0.16 – 0.2 kg.

The rats were divided into groups: 1) control rats (C); 2) experimental rats (E).Toxic damage was caused by 0.1% solution of 2,4-dinitrophenol intraperitoneally in the dose of 3 mg / kg [10]. All manipulations with animals were carried out according to European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and law of Ukraine "On protection of animals from cruelty". Rats were kept under the standard vivarium conditions at constant temperature and basic allowance. Animals were narcotized with chloroform and then sacrificed using cervical dislocation. All procedures were executed separately from other rats.

Mitochondria isolation was performed using the method of differential centrifugation in the buffer (250 mM sucrose, 1 mM EDTA, 10 mMTris-HCI, pH 7,4). The kidneys was washed with a cooled 0.9% solution of KCl (2-4 ° C), chopped and homogenized in 10 times the volume of buffer (mmol/l): sucrose - 250, EDTA - 1, tris-HCI - 25, pH 7.4. The homogenate was centrifuged at 700 g 10 min (4° C), and the supernatant - at 11000 g 20 min (4° C). The precipitate was resuspended in 5 ml of the same buffer (without EDTA) and centrifuged again under the same conditions. The resulting precipitate (mitochondrial fraction) was resuspended in buffer (mmol/l): sucrose – 250, EDTA – 1, tris-HCl – 25, pH 7.4, and used immediately in experiments [12]. Determination of mitochondrial succinate dehydrogenase activity consists in the restoration of potassium ferricyanide ($K_3[Fe(CN)_6]$), whose solution has a yellow color, to the colorless ferricyanide of potassium ($K_4[Fe(CN)_6]$) succinate by the action of succinate dehydrogenase. To incubation solution (10 mM phosphate buffer (pH = 7.8), 5 mM succinic acid, 1.25 mM EDTA, 7.5 mM sodium azide) was added to the suspension of mitochondria (0.2 ml). Samples were incubated at room temperature for 5 minutes. The reaction was initiated by the addition of 1.25 mM potassium ferricyanide solution to the samples. Samples were incubated for 10 minutes at + 30°C. After incubation, the reaction was stopped by lowering the sample temperature to 0°C. In control samples containing all components of the incubation mixture, CCl_3COOH was added before the introduction of the mitochondrial suspension. After stopping the reaction the sample was photometrised by ƛ=420 nm. The activity of the enzyme is proportional to the amount of ferricyanide [2]. The activity of ATP-ase was determined by the accumulation of inorganic phosphate. The activity was determined in an incubation solution containined 400 μmol of tris-HCI (pH 7.4), 5 μmol of ATP disodium salt, 7.5 μmol of MgSO4, $5 \cdot 10^{-2}$ μmol of 2,4-dinitrophenol, 7 , 5 μmol of $CaCl_2$, 120 μmol of NaCl, 20 μmol of KCl. The reaction was initiated by the addition of 50 μl of a mitochondria suspension containing 1 mg of protein,

incubated for 15 minutes at 37°C. The reaction was stopped by the addition of 1 ml of 10% CCl_3COOH. The contents of Pi were determined colorimetrically [4].

Some kidney biopsies were rapidly extracted, frozen at -15°C in cryostat and 10 um thick sectioned then incubated in equal volumes of 0.2 M phosphate buffer with 0.2 M sodium succinate to be mixed with equal volume of nitroblue tetrazolium in distilled water solution (1 mg/1 ml) for 10-20 minutes at 37°C for histochemical configuration of succinic dehydrogenase. Other kidney biopsies were fixed in 10% buffered formal saline, dehydrated in ascending grades of ethanol, cleared in xylene, infiltrated-impregnated in soft paraffin at 50°C and embedded in paraplast paraffin wax. Solid paraffin blocks were cut into 5 um thick sections by rotator microtome and then mounted on glass slides covered by albumin glycerin. Multiple histological and histochemical stains were utilized to ensure reliability of these techniques in explaining the parallel results whether confirmatory or contradictory.

Histochemical determination of enzyme activity of succinate dehydrogenase. In a cryostat, sections of 10 μm thick tissue are prepared and applied on a slide glass. The incisions are then incubated at a temperature of 20-25˚C. Mix the levels of 0,2 M phosphate buffer (pH 7,6) and 0,2 M solution of sodium succinate. To 10 ml of solution add 10 mg of nitrosine tetrazolium and incubate until intense violet color.

Statistical processing of the obtained data was performed with the establishment of mean values, standard errors, confidence intervals. To estimate the probability of differences in comparison of studied groups, Wilcoxon's coefficient was used. The difference between groups was considered to be significantat the level of $P<0,001$.

Results and discussion. The use of 2,4-dinitrophenol in the experimental group of animals results in damage to the mitochondria of the nephrocytes and electron transport by the respiratory chain, decrease in the activity of succinate dehydrogenase and, consequently, reduction of the compensatory potential of the energy supply system (Fig. 1). The violation of energy metabolism is indicated by a decrease in the activity of succinate dehydrogenase by 30% compared to the control group of animals.

This was also clear with histoenzymological survey through histochemical SDH test which showed marked decrease of enzyme activity among test animals
group especially in the proximal tubules areas (Fig. 2) and activation of the enzyme in the distal nephron in comparison with the control (Fig. 3). This could be explained by the use of the present study an modelof intoxication that made a rapid affection of enzymatic activity and metabolism.

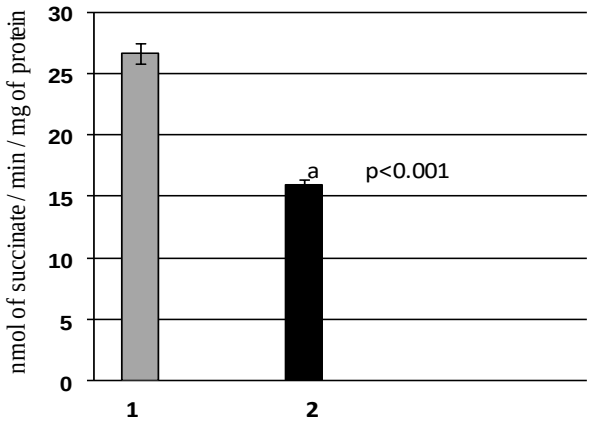

Fig. 1 The activity of succinate dehydrogenase in mitochondria of the kidneys under conditions of introduction of 2,4-dinitrophenol. 1 – control, 2 – experiment; a – significant changes compared to the control; p – the reliability of the difference compared with the control.

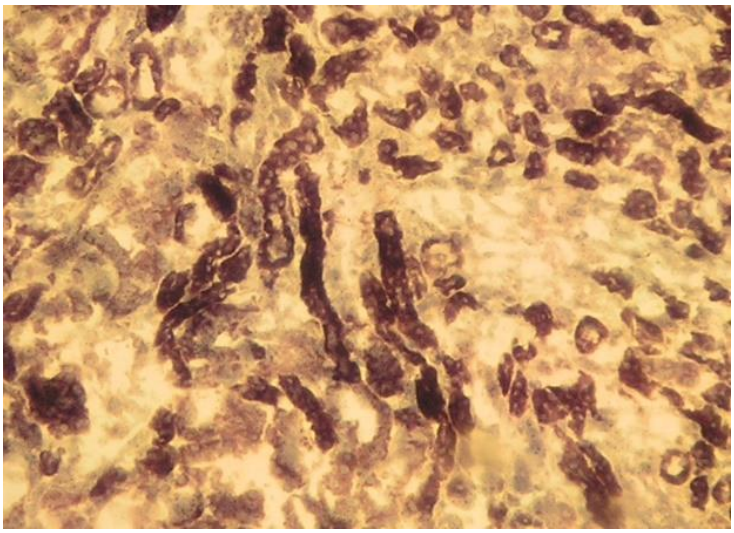

Fig. 2. Photomicrograph of SDH reacted kidney sections showed decrease of activity of succinate dehydrogenase in proximal tubules and its activation at the level of distal parts of nephoron in kidney cortical substance under conditions of introduction of 2,4-dinitrophenol (x56, SDH).

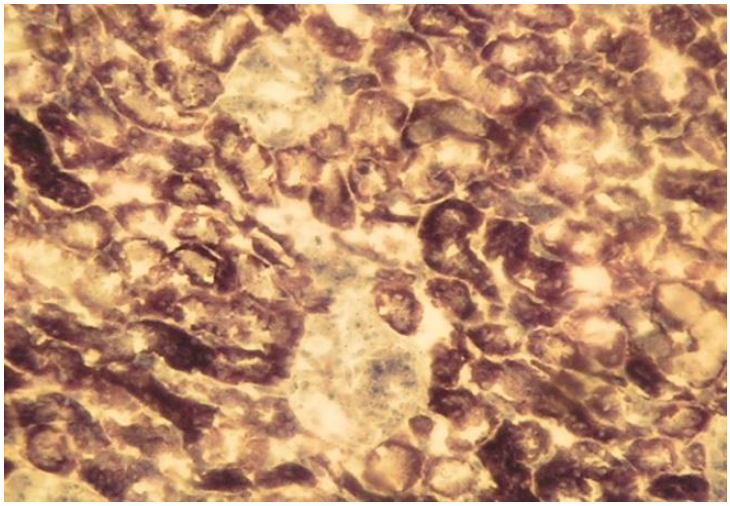

Fig. 3. Photomicrograph of SDH reacted kidney sections showed activity of succinate dehydrogenase in proximal and distal tubules in kidney cortical substance of control animals group (x56, SDH).

The reducing of the activity of succinate dehydrogenase activity causes inhibition of activity of ATP-ase in the kidney (Fig. 4). And under conditions of decrease of ATP the effective implementation of the kidneys is impossible as the functioning of the energy supply system of the mitochondria of the nephrocytes is impaired [3, 7, 9].

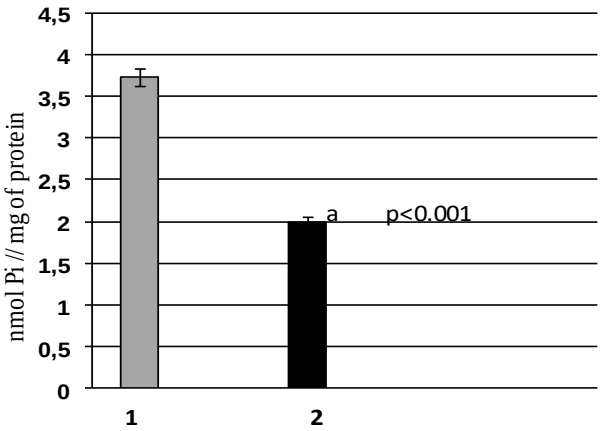

Fig. 4 The activity of ATP-ase in mitochondria of the kidneys under conditions of introduction of 2,4-dinitrophenol. 1 – control, 2 – experiment; a – significant changes compared to the control; p – the reliability of the difference compared with the control.

Consequently, the introduction of 2,4-dinitrophenol leads to a decrease in the activity of enzymes in the respiratory chain of the mitochondria of the nephrocytes and proximal tubules of the nephron, due to the splitting of oxidation and phosphorylation, and

hyperproduction of the reactive oxygen species caused the activation of peroxide oxidation of lipids and proteins.

Conclusions. Diseases of the kidneys are characterized by pathological changes in transport processes in the tubular sections of the nephron. By dissociation of the processes of oxidation and phosphorylation 2,4-dinitrophenol causes the development of acute tissue hypoxia. This led to a violation of the intra-mitochondrial transport of protons and electrons by the respiratory chain by reducing the succinate-dehydrogenase activity and a violation of the synthesis of ATP. There was a discrepancy between the energy needs of the cell and energy production in the system of mitochondrial oxidative phosphorylation.

References

1. Benard G, Bellance N, James D, Parrone P, Fernandez H, Letellier T & Rossignol R. Mitochondrial bioenergetics and structural network organization. J Cell Sci. 2007; 120: 838-48.

2. Elshennawy ATM, Sayed SR, et al. Histopathological and Histochemical Assessment of the Protective Effects of Zinc on Ethanol-Induced Acute Hepatotoxicity in Adult Albino Rats. J.Cytol. Histol. 2015; 6: 321.

3. Faccenda D. Molecular Regulation of the Mitochondrial F_1F_0-ATPsynthase: Physiological and Pathological Significance of the Inhibitory Factor 1 (IF1). Int. J. Cell Biol. 2012; 40 (12): 2698–701.

4. Gabibov MM. Influence of hyperbaric oxygenation on the activity of proton ATPase mitochondria of various tissues of rats. Ukr, biochem. journ. 1986; 58 (5): 68-71.

5. Gutman M, Silman N. The steady state activity of succinate dehydrogenase in the presence of opposing effectors.II. Reductive activation of succinate dehydrogenase in presence of oxaloacetate. Mol Cell Biochem 1975;7(3):177–185.

6. Hagerhall C. Succinate: quinone oxidoreductases. Variations on a conserved theme. Biochim Biophys Acta 1997;1320(2):107–141.

7. Hajjawi O.S.Succinate Dehydrogenase: Assembly, Regulation and Role in Human Disease. Eu. J. Sc. Res; 51 (1): 133 – 42.

8. King A, Selak MA, et al. Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. Oncogene 2006;25(34):4675–4682.

9. Mahran AA, Husam Eldien HO, Abd El-Mawla AMA, Attia AM. Protective effect of zinc (Zn) on the histology and histochemistry of liver and kidney of albino rat treated with cadmium. J Cytol Histol. 2011; (2):120 – 3.

10. Rogovy Yu.E., Belyavsky V.V., Dikal M.V. Modified proteins in the kidneys and the liver with intoxication with 2,4-dinitrophenol and the effects of melatonin in the experiment. Clin.Anat. and surgery. 2011; 10 (3): 18-21.

11. Sun F, Huo X, et al. Crystal structure of mitocho ndrial respiratory membrane protein complex II. Cell 2005;121(7):1043–1057.

12. Weinbach TC.A procedure for isolating stadle mitochondria from rat liver and kidney. Analyt.Biochem.1961; 2: 335-343.

13. Yankovskaya V, Horsefield R, et al. Architecture of succinate dehydrogenase and reactive oxygen species generation. Science 2003; 29 9(5607):700–704.