Living unicellular eukaryote Tetrahymena pyriformis as a model for study of mitochondrial energetics in mammalian cells under conditions of reduced oxidative metabolism.

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Abstract

Some animals are able to survive for long time under conditions of drastically reduced oxidative metabolism, called metabolic depression. The most investigated type of the metabolic depression is hibernation. Research into the basic properties of liver mitochondria energetics during hibernation is essential for fundamental biology and medicine. However, the absence of the suitable hepatocyte culture makes it impossible to study the characteristic features of mitochondrial metabolic states in living cells during hibernation. We proposed that under selected conditions, the unicellular eukaryote *Tetrahymena pyriformis* resembles hepatocytes under hibernation, as (i) both cell types survive under condition of restricted food supply, hypoxia, and accumulation of toxic products of metabolism; (ii) the mechanisms for survival during drastically reduced oxidative metabolism probably developed in the ancestors of the eukaryote mitochondria and may be conserved, in somewhat modified forms, in mitochondria of the ciliates and mammalian cells; (iii) mitochondria isolated from rat liver and *Tetrahymena pyriformis* are similar in their energetics properties The already published results of experiments with *Tetrahymena pyriformis* and with mitochondria isolated from the liver of hibernating animals are considered. In this paper to support and develop this suggestion. Sharp decrease in the maximal uncoupler-stimulated respiration rates in liver mitochondria isolated from the hibernating animals was described in many publications. The respiration recordings and Mito Tracker Red fluorescence observations in the ciliates were tentatively explained by low ΔΨ and high ΔpH. Prior to this study the strong decline in ΔΨ and the subsequent remodeling of mitochondria into the condensed configuration was found during earlier apoptosis induced by suppression of the respiratory activity. On the grounds of these and other data, a hypothesis is put forward that the prerequisite for reducing the oxidative metabolism and mitochondrial transition to hibernation resembles the early apoptosis, with initial ΔΨ decline with following mitochondrial matrix transition to a condense configuration. Correct comparison of ΔΨ of mitochondria isolated from liver of the active and hibernating animals is difficult as probably they have different matrix volumes and energy-independent binding with the ΔΨ probes.

Key words: Tetrahymena pyriformis; hibernation; metabolic depression, condensed configuration; mitochondrial electrical potential; ATP/ADP-antiporter.

Running title: mitochondria during metabolic depression.

Abbreviation: ΔP - proton motive force - difference in the electrochemical potential of hydrogen ions across the inner mitochondrial membrane; Δψ - electrical potential difference across the inner mitochondrial membrane; ΔpH - difference in hydrogen ion concentrations between the sides of the inner mitochondrial membrane; FCCP – *p*-trifluoromethoxycarbonylcyanide phenylhydrazone; DNP - 2,4-dinitrophenol; TP -*Tetrahymena pyriformis* cells sampled during stationary growth phase and washed with a substrate-free medium; ATP/ADP - antiporter - adenine nucleotide translocase; fatty acids - long chain free fatty acids.

Introduction

Some animals can survive for a long time under the conditions of metabolic depression which is characterized by drastic decrease in oxidative metabolism. Hibernation is the best studied type of such metabolic depression. The study in the regulation of oxidative metabolism during hibernation and transition from hibernation to the active state is important for both fundamental biology and applied medicine. The hibernating state in some animals can be induced by lowering the ambient temperature, hypoxia, fasting and some other factors. In all these cases, strong decline in oxidative metabolism was observed.

There is strong evidence that the metabolic processes during hibernation decreased not only due to reduction enzyme activity at low temperature and due to decreasing the energy-expensive processes [1]. During hibernation mitochondria used an evolutionarily conserved mechanism for metabolic control [1]. Numerous publications addressed the characteristic features of liver mitochondrial energetics during these processes [1-3 and references therein). Such research is considerably limited by lack of hepatocyte cultures suitable for the study of mitochondrial metabolic states in liver cells during hibernation. The already reported experiments were performed mainly with mitochondria isolated from the liver of hibernating animals; however, the properties of such mitochondria may considerably differ from the mitochondria functioning in vivo.

Previously we suggested that the “animal-like” unicellular eukaryotes *Tetrahymena pyriformis* when sampled in the stationary phase of growth and washed with a substrate-free medium (TP) may in many aspects model liver cells under the conditions of reduced oxidative metabolism [4]. In the present paper the features of the mitochondrial energetics in TP and of mitochondria isolated from liver of the hibernating animals were considered; the data published earlier are included. On the grounds a hypothesis is put forward concerning mitochondrial metabolic state during transition to hibernation and functioning in the hibernating state. In both objects the mitochondrial energetics should support cell survival under the conditions of food restriction, hypoxia and other unfavorable factors. I would like to underline, that hypoxia is useful during metabolic depression as hypoxia protects the cells from oxidative stress. Ancestors of the ancient eukaryote mitochondria evolved the program for surviving during metabolic depression. Such mechanisms may be conserved, in modified forms, in the mitochondria of TP and mammalian hepatocytes.

The previous studies of metabolic depression were mostly performed in the framework of cell biology, especially oncology; cell cultures and genetically modified animals were employed as a rule in these experiments. The basic features of the mitochondrial energetics in living cells with heavily reduced oxidative metabolism were not addressed.

Some unicellular eukaryotes, including *Tetrahymena pyriformis,* are widely used instead of mammalian cells for detecting various environmental toxic factors and, less frequently, in fundamental research. However, many publications demonstrate that the experiments with these organisms provide valuable information for solving fundamental problems in biology and medicine. *Tetrahymena pyriformis* cells are similar to the mammalian cells in many aspects, including functioning of the essential metabolic systems. The comprehensive characterization of these unicellular eukaryotes can be found in monograph of A.M. Elliott (editor) Biology of Tetrahymena [5]. These ciliates resemble mammalian cells even in their high sensitivity to very low hormone concentrations and in their response to stress at the hormonal level [6].

Mitochondria isolated from *T. pyriformis* and rat livers are very alike in their energetic characteristics: they are similar in the content of the cytochrome c-type components of the respiratory chain, and comparable in the values of the P/O ratio attesting to the effectiveness of the oxidative phosphorylation, etc [7]. Both cell types are similar even in the number of mitochondria per cell [8].

Hepatocytes during hibernation and *T. pyriformis* cells use lipid droplets as a fuel source [2, 9, 10]. Close contacts between lipid droplets and mitochondria were clearly detected in hepatocytes of the hibernating animals (see Fig 1B in [2]) and in *T. pyriformis* cells during stationary growth phase (see Fig. 17 in [9]).

Both liver mitochondria from hibernating animals and *T. pyriformis* cells do not utilize the long-chain fatty acids as substrates. During hibernation, long-chain fatty acids and in particular essential fatty acids in the liver mitochondria do not undergo the beta-oxidation cycle. They can be used for the membrane phospholipids rearrangement when adapting to low temperature, and/or for the synthesis of endogenous regulators of the cellular metabolism and other functions ([11-13] and references therein).

Mitochondria from *T. pyriformis* can oxidize short-chain fatty acids. The beta-oxidation of long-chain free fatty acids occurs mainly in peroxisomes [14].

The analysis of the published data led us to conclusion, that *T. pyriformis* cells can be used to model some important features of mitochondrial energetics in hepatocytes during hibernation and probably during some other forms of the metabolic depression.

It should be underlined that energetics of *Tetrahymena .pyriformis* have some features which are absent in hepatocytes during hibernation. For example, the autophagy of mitochondria is probably activated in *Tetrahymena pyriformis* during later growth phase; some mitochondria are incorporated into vacuoles containing acid phosphatase [9]. The moderate autophagy facilitates surviving of the cells under the metabolic depression; it defends mitochondria from apoptosis ([15] and references within). Such process was not observed in liver cells during winter slipping.

Unexpected difference in DNP- and FCCP-induced uncoupling in experiments with T.pyriformis

Proton-motive force (ΔP), the integral characteristic of mitochondrial energetics, is composed of the electrical component, Δψ, and the concentration component, ΔpH. In mitochondria of the eukaryotic cells, most of ΔP is stored in the form of Δψ ([16-18] and references therein). ATP synthesized in mitochondria must be rapidly transported to cytosol to support the energy-dependent processes providing for normal cell functions. The electrophoretic exchange of intramitochondrial ATP^{4-} for extramitochondrial ADP^{3-} is rapidly performed by the ATP/ADP antiporter only at high Δψ [19].

In addition to this main function, the ATP/ADP-antiporter is involved in the uncoupling effect of low concentrations of fatty acids and DNP ([17, 20-23]). This uncoupling, which is often called the protonophoric effect, can also occur only at high Δψ. However, some powerful uncouplers, such as FCCP, increase the proton permeability of the inner mitochondrial membrane via different mechanisms [24-25]

During experiments with TP we found that the maximal stimulation of endogenous cellular respiration by DNP (or oleic acid) was close to twofold whereas that achieved by FCCP was close to sixfold (Fig.1). Such result was quite unexpected; it was tentatively explained by low value of Δψ. In the case of low Δψ, the ATP/ADP-antiporter activity should be low.

The difference in uncoupling activity between FCCP and DNP (or oleic acid) remained nearly the same in the presence of 20-30 mM pyruvate (for details, see [4]). Strong stimulation of the respiration rate by FCCP attested to high ΔP in TP mitochondria. Thus the most part of the mitochondrial ΔP should be stored in the form of ΔpH.

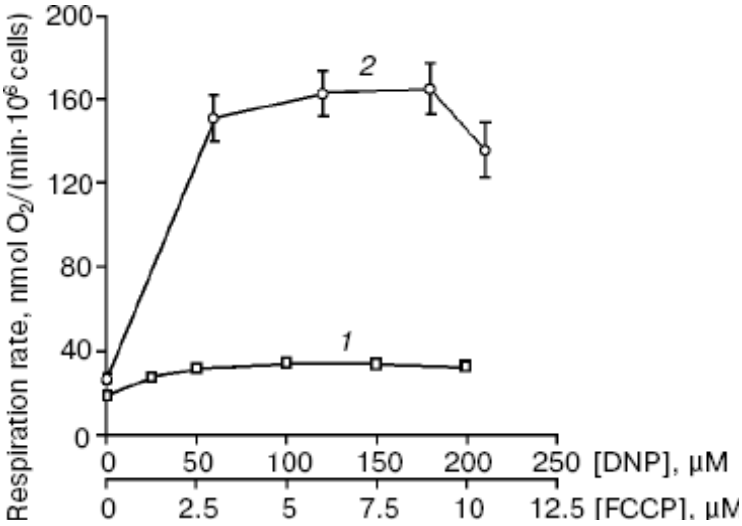

Fig. 1. Effects of DNP (1) and FCCP (2) on respiration of TP cells. Before recording respiration, cells were incubated for 1 day in the medium containing 30 mM KCl, 10 mM NaCl, 6 mM KH_2PO_4, and 5 mM Hepes, pH 7.4. The respiration rates are means (from 10 to 15 experiments) ± S. E. [4]. With permission of Pleiades Publishing, Ltd. in the RF.

Additional evidence consistent with our assumption of low Δψ in TP mitochondria was obtained in the experiments with a Δψ-sensitive probe Mito Tracker Red [4, 26]. In contrast to the experiments with other eukaryotic cells, in TP, we did not observe mitochondria with bright fluorescence. The weak intensity of Mito Tracker Red fluorescence in TP mitochondria was also reported by other authors [27].

It is unlikely that the unexpectedly low fluorescence of Mito Tracker Red in the intact TP mitochondria is due to their pumping out of the cells by the multidrug efflux pump. Mito Tracker Red, in contrast with another Δψ-sensitive probe rhodamine 123, did not usually clear out mitochondria during fixation. The bright fluorescence of Mito Tracker Red is clearly discerned in mitochondria-like organelles inside the fixed cells [4, 26], where this Δψ-sensitive probe is concentrated within the phospholipid area.

For proper interpretation of the data, it is important to perform similar experiments with *Tetrahymena pyriformis* cells taken at the different stages of the stationary and exponential growth phases. The results may clarify the question concerning Δψ value in these ciliates.

It should be added that in short series of experiments with mitochondria, isolated from cells of T.pyriformis, Δψ was recorded by safranin method. The mitochondria had Δψ, close to that of liver mitochondria during the several first consecutive recordings, and when mitochondrial suspension was incubated in the presence of EGTA, oxidative substrates, oligomycin and nigericin [23]. The last two reagents produced no any effect on the respiration rates when being added to the polarographic well after or before T.pyriformis. Possibly it was due to low permeability of the cell membranes to these substances.

Oleic acid (2-5 μM) produced a clear decrease in Δψ in the presence of oligomycin. However, in these experiments a powerful specific inhibitor of the ATP/ADP-antiporter, carboxyatractylate, did not produce a stable increase in Δψ when added after oleic acid. This effect was present in all experiments with rat liver mitochondria: carboxyatractylate always increased Δψ under similar conditions [.20]. It could not be excluded that the ATP/ADP-antiporter in living TP cells did non participate in the FFA-induced uncoupling, as the cells should not waste energy for heat generation

The ability to store most of ΔP in the form of ΔpH is essential for survival of free-living *Tetrahymena pyriformis* under unfavorable conditions such as hypoxia and food deprivation. Conversion of Δψ to ΔpH results in enhanced transport of some substrates into mitochondria. On the other hand, at low Δψ the Δψ-dependent functions would be drastically reduced. Probably, from time to time, the energy stored in ΔpH form is converted to Δψ form to support the functions essential for cell survival.

Insight into nature of liver mitochondrial energetics during switching into and living during hibernation

From the very beginning, living organisms were faced with a nutrient deficiency. Certainly, at first, trying to adapt to new conditions they chose diverse pathways. It is quite possible that

mitochondrial basic energetics during transition from active to hibernating state would resemble, in some features, an initial stage of apoptosis/necrosis/ autophagy induced by nutrient deprivation.

The recent research by E. Gotlieb and colleagues [28] is very important in this aspect. These authors found that early in apoptosis induced by suppression of the respiratory activity, an initial Δψ decrease led to remodeling the mitochondrial structure from an orthodox to a condensed configuration.

The following study of the relationships between the mitochondrial ultrastructure and Δψ during modification of Δψ in experiments with cell cultures and isolated mitochondria confirmed their proposal that the changes in Δψ controlled the matrix remodeling to the condensed configuration ([28] and references therein). The conclusion is understandable as Δψ decrease induces an efflux of cations, such as K^+ or Ca^{2+}, from matrix resulting in the matrix condensation. The initial Δψ decrease may result from reduction of the respiratory activity under substrate deficiency, hypoxia, from decline in temperature, etc.

It is desirable to reproduce these very important experiments with another Δψ-sensitive fluorescent probe of different properties; e.g., with a suitable cyanine dye, such as JC-1 [29].

I propose that the initial Δψ decrease with the following remodeling mitochondria to the condensed configuration during the above considered apoptosis [28] occurs in the liver mitochondria too during transition from the active to hibernating state. Earlier we suggested that the decrease of Δψ during hibernation is due to a partial conversion of ΔP from Δψ to ΔpH form, that such conversion prevents the dangerous exhaustion of the intramitochondrial ATP pool [4]. These and other features of mitochondrial energetics give a possibility to avoid a death from metabolic depression during hibernation.

In the condensed configuration, the respiration activity would be stably reduced due to the changes in the mitochondrial morphology, including an essential expansion of the intermembrane and intercristal space with the parallel decrease in the matrix volume. Features of decrease of respiration during incubation of rat liver mitochondria in hypertonic medium had been studied earlier [30].

In the condensed configuration, the number of contact sites between the inner and outer mitochondrial membranes is much lower than in the orthodox state. The activity of the ATP/ADP-antiporter and the processes associated with this anion carrier, as well as Ca^{2+} fluxes in mitochondria would significantly diminish (to illustrate, see the scheme in Fig. 5 in [31] and references therein).

After remodeling of the mitochondrial structure to a condensed configuration great changes occur in the structure and function of the supramolecular protein complexes located in the contact sites between inner and outer mitochondrial membrane. They include the ATP/ADP-antiporter and other proteins involved in the regulation of cellular energetics ([32] and references therein). These authors argue that such complexes control both energetics and cell fate [32].

Features of the oxidative metabolism in liver during entering into hibernating state had been studied in experiments with isolated mitochondria. When the hibernating state was imitated in the experiments with isolated mitochondria, EGTA was present in the incubation medium to avoid diverse ways of amplifying the oxidative metabolism by Ca^{2+}, especially in the presence of fatty acids.

In our experiments with isolated liver mitochondria, low, (5 -10) μM Ca^{2+} considerably affected the mitochondrial energetics in the presence of (5 -10) μM myristic acid. Depending on the experimental conditions, these reagents either lowered Δψ without stimulation of the respiration, or stimulated the respiration due to opening of the non-specific pore in the inner mitochondrial membrane [31, 33].

Calcium was probably bound during the stationary growth phase of *T. pyriformis* cells; the inclusions found in these cells were tentatively identified as Ca^{2+} complex with other substances [9].

When liver mitochondria were incubated in the EGTA-containing medium, carboxyatractylate, the specific inhibitor of the ATP/ADP-antiporter, lowered the succinate oxidation rate. The rate of the maximal DNP-uncoupled respiration was 5-6 times lower in hibernating animals as compared to that of arousing animals at body temperature of 25-30^{o} C. The rate of succinate oxidation was about twofold lower in hibernating animals as compared to that of arousing animals [34].

Unexpectedly, the degree of carboxyatractylate suppression of succinate oxidation was about similar in these groups. I therefore suggest that during hibernation, only some part of the mitochondria which have the orthodox configuration, make an essential contribution to the respiration.

Important results were obtained by parallel recording of the oxygen consumption and analysis of the mitochondrial ultrastructure [35]. It was found that mitochondria from both active and hibernating animals were in the condensed configuration after their isolation and storing in the cold. However, when their fixation was performed after 4-min incubation at 27^{o}C, mitochondria from the active animal had the orthodox configuration, and mitochondria from the hibernating animal remained in the condensed configuration with shrunken matrix, their respiration was reduced. Incubation of the mitochondria from the hibernated animal in a hypotonic medium also resulted in remodeling of their structure into the orthodox configuration; however, some structural differences between the samples of mitochondria from active and hibernating animals were still preserved (see Fig.3 in [35]).

Some methodological aspects of experiments with mitochondria in living T.pyriformis and isolated from liver of hibernating animals.

The energetics of isolated mitochondria and mitochondria in intact cells usually differs in many important features; especially regarding energy coupling between oxidation and phosphorylation. Recently a new method has been developed. The experimental conditions

were elaborated in order to preserve the mitochondrial reticulum during tissue homogenization and subsequent incubation [36]. Mitochondrial respiration rates were calculated from the homogenate oxygen consumption. Under such conditions, the metabolic state of mitochondria *in vivo* was close to the state *in vitro*. The method was validated using several physiological models. It is reasonable to employ this method at least in tentative experiments with mitochondria isolated from liver from active and hibernating animals.

Before beginning the experiments with liver mitochondria from hibernating animals, the suitable characteristics of mitochondrial metabolic state should be chosen. It is noteworthy that different metabolic state characteristics should be chosen for condensed and orthodox configurations. For condensed configuration, the relation between rates of succinate oxidation in the presence and absence of an uncoupler may depend both on the proton conductance of the inner mitochondrial membrane and the mitochondrial structure.

The following indices may be preliminary proposed: Vo (hib) / Vo (act) and Vunc (hib) / Vunc (act), where Vo (hib) and Vo (act) are the respiration rates in the presence of substrate whereas Vunc (hib) and Vunc (act) are the respiration rates in the presence of substrate and an uncoupler in mitochondria isolated from the liver of active (act) or hibernating (hib) animals.

In addition, it is useful to estimate tonicity of the incubation medium, when Vo (act) in the hypertonic medium is close to Vo (hib) in the isotonic medium, and Vunc (act) in the hypertonic medium is close to Vunc (hib) in the isotonic medium.

According to personal communication of Z.G. Amerkhanov, this tonicity was close to 500 – 600 osmM. A sucrose EGTA containing medium was used in the experiments.

The future experiments will correct this preliminary set of the indices and help to add additional ones. The following step is a study of the the mitochondrial ultrastructure under similar conditions.

As to Δψ estimation, an application of a TPP^+-sensitive electrode for Δψ comparison of liver mitochondria isolated from hibernating and active animals is questionable. One of the problems is the matrix volume estimation. Hepatocyte mitochondria have very complicated matrix structure [37-38 and references therein]. The ultrastructural features of liver mitochondria isolated from hibernating animals were not investigated by similar methods. In addition, the energy-independent binding of TPP^+ by the mitochondria isolated from hibernating animals was not assessed.

The other problem is heterogeneity of mitochondria. TPP^+ and similar Δψ –sensitive probes are accumulated mainly in mitochondria with highest Δψ value (according to the Nernst equation); so that the results obtained with TPP^+ - electrode refer to the mitochondrial fraction with the highest Δψ. Probably, the Δψ value obtained with TPP^+-sensitive electrode refers firstly to the mitochondria with the orthodox configuration.

Because of heterogeneity of mitochondria, the calculation of proton conductance by the common method is questionable. In many studies, the proton conductance in isolated mitochondria was estimated from the ratio of the respiration rate and Δψ during succinate

oxidation without and with of the successive malonate additions [1]. While TPP^+ or other Δψ – sensitive probes are accumulated mainly in mitochondria with the highest Δψ, the main contribution to the respiration rates comes from the mitochondria with the lowest energy coupling, with the highest respiration rates. Thus, different fractions of mitochondria may contribute to the ratio [39].

In experiments with rat liver mitochondria recording mitochondrial Δψ kinetics is often performed with various Δψ-dependent probes. In experiments with rat liver mitochondria we often used the safranin method [40]. In the experiments with rat liver mitochondria when ΔΨ kinetics was recorded either with a double-beam spectrophotometer by safranin extinction or with a TPP^+-sensitive electrode the same results were obtained in many parallels assays [31].

We prefer to use diS-C3-(5) rather than rhodamine123 for recording the mitochondrial ΔΨ kinetics in experiments with thymus lymphocytes. The solubility of diS-C3-(5) in lipids is high and it is localized mainly within phospholipids membranes. To avoid the rotenone-like effect of the carbocyanine, we generated Δψ by succinate oxidation in the experiments with mitochondria and by oxidation of ascorbate, in the presence of TMPD, in the experiments with cells. Our homemade fluorimeter (a tungsten lamp was used for fluorescence exitation) enabled us to work at low, 0.3-0.6 μM concentration of carbocyanine to avoid its uncoupling effect and to diminish its photodynamic damage [41].

For visual of observation of ΔΨ increase in thymocyte mitochondria with the help of a microscope we used rhodamine 123. To dissipate Δψ in the control experiments we added an inhibitor of the cytochtrome c oxidase, e.g., cyanide [42], but not FCCP. In the control, we always observed bright fluorescent within mitochondria of all the objects under study, except TP.

The experiments with TP have some specific features. The great advantage of working with TP is that they respond to some unfavorable environmental factors by changing their shape and movement ([4, 26] and references therein). In such way TP demonstrated that digitonin even at very low concentrations, used for membrane permeabilization, was toxic to these ciliates. Therefore we did not use digitonin in the main experiments with TP.

In the case of a careful treatment of TP during experiment (for details see [4]) shortly after recording the oxygen consumption, the cells restored their normal motion, and their shapes were not changed. It follows that the polarographic method of the oxygen consumption recording is not only informative but also noninvasive for TP. The respiration rate is closely related to cell concentration. Usually the concentration of the cells was chosen so to diminish the dispersion of .repeated measurements. It should be added that the experiments with *Tetrahymena thermophila* showed that the cells could not survive for long period if the suspension was too diluted [43].

Due to large size, *Tetrahymena pytriformis* cells can be easily observed under a regular microscope after staining or with side illumination .The only complication for microscopic observation is high cell sensitivity to bright light. Such sensitivity is common for a number of

unicellular eukaryotes [44]. Prolonged illumination even with a tungsten lamp changed cell behavior.

In the experiments with TP, Mito Tracker Red appeared to be the optimal Δψ-sensitive fluorescent probe [4]. It should be added that Mito Tracker Red and diS-C3-(5) produced strong photodynamic damage and in the experiments with TP [26].

It could not be excluded that and quite other factors exert influence on results of the metabolic depression study. During strong contraction of the matrix its viscosity may limit the diffusion of metabolites and add substances. This aspect was considered earlier in the publication of D.G. Nicholls and O. Lindberg O. [30] and some others [45]. A suitable experiments and consideration of published materials are needed to clarify this important aspects of the mitochondrial energetics related to metabolic depression induced by deficit of water.

Two following methods were not used in the discussed above data. However, these methods are very important for study of mitochondrial features during metabolic depression. One of them is recording the cytochrome spectra with the help of a special high sensitive differential Aminco-Chance type spectrophotometer, suitable for recording spectra of turbid suspensions – mitochondria, cells etc. The method enable not only to estimate the cytochrome concentrations but and to localize the limiting steps of the respiratory chain and to give other useful information [46, 47].

Addition of *Tetrahymena .pyriformis* cells at high concentration to the spectrophotometer cell did not result in any signal: possibly, the cells escaped the illuminated by light beam areas. In our previous experiments with low quantities of mitochondria we used a special device to maintain the frozen samples at close to liquid nitrogen temperature and to concentrate the scattered light on a light detector [48]. Such installation was constructed by V. N. Larionov following the prototype developed by Estabrook [49]. A thin plastic cell with mitochondrial suspension was immersed several times in liquid nitrogen. Its light scattering was greatly amplified. As a result, the optical path was increased as well, and very low concentrations of cytochromes within living cells could be estimated.

The oxidative metabolism of *Tetrahymena pyriformis* under the natural physiological conditions is best to be studied by using an infrared imaging camera with the corresponding accessories. The measurements are absolutely noninvasive; only the far infrared irradiation from the object is collected. The mitochondrial oxidative metabolism in TP may be estimated, as TP respiration is more than by 90 per cent suppressed by such inhibitor as cyanide [26]. It should be added that this method has been successfully applied to study of isolated liver mitochondria energetics [50].

infrared thermohgraphy may be also useful during the study of some aspects of metabolic depression in people. I speculate that the evolutionary ancient mechanisms of rearrangement in metabolic state of mitochondrial during the metabolic depression also exist in a modified form in our (human) organism. The decrease of metabolism may support surviving the cells within an area of blood interruption. Otherwise the cells may be damaged due to deficit of substrates and

hypoxia and not fully oxidized product of mitochondrial oxidative metabolism. It is unclear if the favorable effects of metabolic depression give a contribution to health improvement by relaxation during entering trance state in the course of meditation /autogenic training. Here such invasive informative method of far infra red thermography should be useful. The data also give information concerning functional state of inner organs [51].

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Conclusion

Consideration of the previous publications supports the earlier conjecture [4] that *Tetrahymena pyriformis* may be useful in a number of important aspects as a model of mitochondrial energetics of the liver cells under conditions of the reduced oxidative metabolism, e.g., during hibernation. On this ground a tentative hypothesis was proposed that a strong decline in Δψ and subsequent remodeling of mitochondrial matrix to a condense configuration are prerequisite for reducing the oxidative metabolism.

The considered methods may be useful to study the mitochondrial energetics in thymocytes from the capable to hibernation animals; thymocytes may be easily isolated in the intact state. Such experiments are especially important during the annual thymus reparation after its annual winter involution.

On the other hand, T.pyriformis and some other “animal-like” free living in water unicellular eukaryotes give a unique possibility to investigate some controversial points of water properties. Described above methods for research the mitochondria in living T.pyroiformis enable to elucidate diverse effects of water structure and low concentrations of dissolved in water substances on energetics of the living cells.

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References

1. Barger, J. L., Brand, M. D., Barnes, B. M., and Boyer, B. B. (2003) Tissue-specific depression of mitochondrial proton leak and substrate oxidation in hibernating arctic ground squirrels *Am. J. Physiol. Regul. Integr. Cop. Physiol.*, **284**, R1306-13.

2. Malatesta, M., Battistelli, S., Rocchi, M. B., Zancanaro, C., Fakan, S., and Gazzanelli, G. (2001) Fine structural modifications of liver, pancreas and brown adipose tissue mitochondria from hibernating, arousing and euthermic dormice. *Cell Biol. Int.,* **25**, 131-8.

3. Brustovetsky, N. N., Mayevsky, E. I., Grishina, E. V., , and Amerkhanov, Z. G. (1989) Regulation of the rate of respiration and oxidative phosphorylation in liver mitochondria from hibernating ground squirrels, Citellus undulates *Comp Biochem Physiol. B*, **4**, 537-41.

4. Prikhodko, E. A., Brailovskaya, I.V., Korotkov, S .M., and Mokhova, E .N. (2009) Features of mitochondrial energetics in living unicellular eukaryote Tetrahymena pyriformis. A model for study of mammalian intracellular adaptation. *Biochemistry (Mosc*), **74**, 371-76.

5. Biology of Tetrahymena (1973); Elliott, A. M., ed., Dowden, Hutchinson and Ross. Inc, Stroudsburg, Pennsylvania

6. Csaba, G., and Pállinger, E. (2008) A general response to stressors by the unicellular Tetrahymena: effect of stress on the hormone levels. Cell Biochem Funct., 26,797-800.

7. Kilpatrick, L., and Erecińska, M. (1977*)* Mitochondrial respiratory chain of Tetrahymena pyriformis. The thermodynamic and spectral properties *Biochim. Biophys. Acta,* **460**, 346-63.

8. Poole, R.K. (1993) Mitochondria of Tetrahymena pyriformis: enumeration and sizing of isolated organelles using a Coulter Counter and pulse-height analyzer. *J. Cell Sci*., **61,** 437-51.

9. Elliott, A. M., and Bak, I. J. (1964) Fate of mitochondria during aging in Tetrahymena pyriformis. *Cell Biol.,* **20**, 113-29.

10. Malatesta, M., Zancanaro, C., Baldelli, B., and Gazzanell, G. (2002) Quantitative ultrastructural changes of hepatocyte constituents in euthermic, hibernating and arousing dormice (Muscardinus avellanarius). *Tissue Cell*, **34**, 397-405.

11. Florant, J. L., (1998). Lipid metabolism in hibernation:the importance of essential fatty acids. *Amer.Zool*., **38**, 331-340.

12. Eddy, S. F., and Storey, K. B. (2004) Up-regulation of fatty acid-binding proteins during hibernation in the little brown bat, Myotis lucifugus. *Biochim Biophys Acta,* **1676,** 63-70**.**

13. Gerson A. R., Brown, J., C., Thomas, R., Bernards, M. A., and Staples J. F. (2008) Effects of dietary polyunsaturated fatty acids on mitochondrial metabolism in mammalian hibernation. *J. Exp. Biol.,* **211,** 2689-99.

14. Takei, M., Gotoh, K., Watanabe, T., and Suga, T. (1985). The regulation of peroxisomal enzyme systems of *Tetrahymena pyriformis* by fatty acid composition, glucose and oxygen in the medium. *J. Biochem.,* **79**, 1631-6.

15. Jin, S., and White, E. (2008) Tumor suppression by autophagy through the management of metabolic stress. *Authophagy*, **4,** 563-6.

16. Mitchell, P. (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism. *Nature*, **191**, 144-148.

17. Skulachev, V. P. (1988) *Membrane Bioenergetics*, Berlin: Springer-Verlag.

18. Skulachev, V. P. In: Semenza, G., Turner, A. J., editors. *Selected Topics in the History of Biochemistry: Personal Recollections VII (Comprehensive Biochemistry*) Vol. 42. London, NewYork, etc.: Elsevier Science B.V; 2003, 319-410.

19. Kramer, R., and Klingenberg, M. (1980) Modulation of the reconstituted adenine nucleotide exchange by membrane potential. *Biochemistry*, **19**, 556-560.

20. Andreyev, A. Yu., Bondareva, T. O., Dedukhova, V. I., Mokhova, E. N., Skulachev, V. P., Tsofina, L. M., Volkov, N. I., and Vygodina, T. V. (1989) The ATP/ADP-antiporter is involved in the uncoupling effect of fatty acids on mitochondria. *Eur. J. Biochem*., **182**, 585-592.

21. Skulachev, V. P. (1991) Fatty acid circuit as a physiological mechanism of uncoupling of oxidative phosphorylation. *FEBS Lett***., 294**, 158-16

22. Skulachev, V. P. (1998). Uncoupling: new approaches to an old problem of bioenergetics. *Biochim. Biophys. Acta*, **1363**, 100-124.

23. Mokhova, E. N., and Khailova, L. S. (2005) Involvement of mitochondrial inner membrane anion carriers in the uncoupling effect of fatty acids. *Biochemistry (Moscow*), **70**, 159-163.

24. Starkov, A. A., Dedukhova, V. I, and Skulachev, V. P. (1994) 6-ketocholestanol abolishes the effect of the most potent uncouplers of oxidative phosphorylation in mitochondria. *FEBS Lett*., ***355,*** 305-308.

25. Starkov, A. A. (2006) Protein-mediated energy-dissipating pathways in mitochondria. *Chem. Biol. Interact*., **161**, 57-68.

26. Brailovskaya, I. V., Kudryavtseva, T. A., Larionov, V. N., Prikhod'ko, E. A., and Mokhova, E. N. (2007) Changes in the shape of photodynamically damaged Tetrahymena pyriformis cells. *Doklady Biokhim. Biofiz*., **413**, 72-75.

27. Wloga, D., Strzyzewska-Jowko, I., Gaertig, J., and Jerka-Dziadosz, M. (2008) Septins stabilize mitochondria in Tetrahymena thermophila. *Eukaryot Cell*., **7**, 1373-86.

28. Gottlieb, E., Armour, S.M., Harris, M. H, and Thompson, C. B. (2003) Mitochondrial membrane potential regulates matrix configuration and cytochrome c release during apoptosis. *Cell Death Differ*., **10**, 709-17.

29.. Reers, M, Smith, T. W,, and Chen, L. B.(1991) J-aggregate formation of a carbocyanine as a quantitative fluorescent indicator of membrane potential. *Biochemistry*,**18,**:4480-6.

30. Nicholls, D. G., and Lindberg, O. (1972) Inhibited respiration and ATPase activity of rat liver mitochondria under conditions of matrix condensation. *FEBS Lett.,* **25**, 61-64.

31. Bodrova, M. E., Dedukhova, V. I., Samartsev, V. N., and Mokhova, E. N. (2000) Role of the ADP/ATP-antiporter in fatty acid-induced uncoupling of Ca^{2+}-loaded rat liver mitochondria. *IUBMB Life,* **50,** 189-94.

32. Brdiczka, D., Zorov, D. B., and Sheu, S. S. (2006)) Mitochondrial contact sites: their role in energy metabolism and apoptosis. *Review. Biochim Biophys Acta*, **1762**, 148-63.

33. Bodrova, M. E., Brailovskaya, I. V., Efron, G. I., Starkov. A. A., and Mokhova, E. N. (2003) Role of the ADP/ATP-antiporter in fatty acid-induced uncoupling of Ca2+-loaded rat liver mitochondria. *Biochemistry (Mosc),* **68**, 391-8.

34. Brustovetsky, N. N., Amerkhanov, Z. G., Egorova, M. V., Mokhova, E. N., and Skulachev, V. P. (1990) Carboxyatractylate-sensitive uncoupling in liver mitochondria from ground squirrels during hibernation and arousal. *FEBS Lett*., **272**, 190-2.

35. Brustovetsky, N. N., Egorova, M. V., Iljasova, E. N., and Bakeeva, L. E. (1993) Relationship between structure and function of liver mitochondria from hibernating and active ground squirrels, *Citellus undulates. Comp Biochem Physiol B*, **106**, 25-30.

36. Kondrashova, M. N., Zakharchenko, M. V., and Khunderyakova, N. V. (2009) Preservation of the in vivo state of mitochondrial network for ex vivo physioliogical study of mitochondria. Invited Review. The International Journal of Biochemistry and Cell Biology, **41,** 2036-2050.

37. Mannella, C. A. (2008) Structural diversity of mitochondria: functional implications. ***Ann. N. Y. Acad Sci.***, **1147**, 171-9.

38. Logan, D. C. (2006) The mitochondrial compartment. J. Exp. Botany, **57**, 1225-1243.

39. Duszyński, J., and Wojtczak, L. (1985) The apparent non-linearity of the relationship between the rate of respiration and protonmotive force of mitochondria can be explained by heterogeneity of mitochondrial preparation. *FEBS Lett.,* **182**, 243-8.

40. Akerman, K. E., and Wikstrom, M. K. Safranine as a probe of the mitochondrial membrane potential (1976) *FEBS Lett.,* **68**, 191-197.

41. Gulyaeva, N. V., Konoshenko, G.I., and Mokhova, E. N. (1985) Mitochondrial membrane potential in lymphocytes as monitored by fluorescent cation diS-C3-(5). Membr. Biochem. **6,**19-32.

42. Dedukhova, V. I., and Mokhova, E. N. (1987) *Biokhimiia*, **52,** 1324-34.

43. Christensen, S. T., Sørensen, H., Beyer, N. H., Kristiansen, K., Rasmussen, L., Rasmussen, M. I. (2001) Cell death in Tetrahymena thermophila: new observations on culture conditions. *Cell Biol. Int..,* **25,** 509-19.

44. Sobierajska, K., Fabczak, H., and Fabczak, S. (2006) Photosensory transduction in unicellular eukaryotes: a comparison between related ciliates *Blepharisma japonicum* and *Stentor coeruleus* and photoreceptor cells of higher organisms. .*J Photochem. Photobiol. B*., **83,** 163-71.

45. Copp, J., Wiley, S., Ward, M. W., and van der Geer, P. (**2005)** Hypertonic shock inhibits growth factor receptor signaling, induces caspase-3 activation, and causes reversible fragmentation of the mitochondrial network. ***Am. J. Physiol. Cell Physiol*., *288*, 403-15.**

- 2002, 1(2):70-76

46. Chance, B. (1952) The kinetics and inhibition of cytochrome components of the succinic oxidase system. II. Steady state properties and difference spectra. *J Biol Chem.* **197,** 567-76.

47. Chance, B. (1954) Spectrophotometry of intracellular respiratory pigments. *Science,* **120**, 767-75.

48. Mokhova, E. N., Taptykova, S. D., and Sharoyan, S. G. (1968) (In Russian). *Voprosi Medizinskoy Khimmii*, **14,** 437-439.

49. Estabrook, R.W., "The low temperature spectra of hemoproteins. I. Apparatus and its application to a study of cytochrome c." (1956) *J. Biol. Chem*., ***223,*** 781-794.

50. Kondrashova, M. N., Grigorenko, E. V., Khizhnyak, E. P., and Tyazhelov, V. 51. (88) Heat production in the mitochondria on oxidation of different substrates. *Biophysics*, **33**, 566-568.

51. A. Ovechkin**,** S. M. Lee, and K. S. Kim (2001) Termovisual evaluation of acupuncture points. *Acupunct. Electrother. Res*., **26**, 11–23.