On Different Views On The Mechanis Of Tissue Thermogenesis And Endothermity Of The Body

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ABSTRACT

The review discusses the mechanisms of thermogenesis in animal tissues, including mitochondria, which provide the endothermic state of the body. Previous views on thermogenesis were largely based on the idea of a low efficiency of biological processes. Subsequently, data were gradually accumulating on the possibility of a sufficiently high efficiency of these processes. Otherwise, there would be no cold-blooded organisms with a low level of metabolism and high efficiency.

The role of Na, K-ATPase and mitochondrial proton leakage in thermogenesis is currently being elucidated. Uncoupled proteins (UCP) have been isolated from the inner membrane of the mitochondria of warm-blooded tissues, which increase proton leakage of membranes, reducing the efficiency of the respiratory chain. Membrane aniontraslocators can also be involved in proton leakage, which in general can increase basal metabolism by 20 to 50%. In another group of MX studies, a large number of oxidation substrates were used. The results showed the possibility of the existence of a subpopulation of mitochondria in the tissues of conjugated and non-conjugated ATP synthesis, as well as the intensity of non-conjugated respiration, since it is detected in the isolated cells of rat cardiocytes. Unconjugated respiration proceeds more intensively in the mitochondria of endothermic tissues than in ectothermic organisms.

In general, this review critically analyzes the issue of low efficiency of biological processes, and considers alternative pathways of tissue thermogenesis.

Keywords: thermogenesis, endothermy, coefficient of efficiency, mitochondria, proton leakage,
List of abbreviations: mitochondria – MX, efficiency – efficiency, uncoupled proteins – UCPs.

Introduction. The subject of our review is the mechanisms of thermogenesis in warm-blooded organisms. To maintain a warm-blooded state, mammals and birds must generate heat throughout their lives, regardless of the temperature conditions of the environment. This process is associated with the use of large energy reserves of the body, the study of which is one of the fundamental problems of modern biology. This circumstance, in all likelihood, served as a prerequisite for the development of mechanisms of tissue thermogenesis that ensure the warm-blooded state of the body.

Bioenergetics of the last century had important successes in elucidating the mechanisms of energy formation and its utilization at the cellular and intracellular levels.

The discovery of oxidative phosphorylation in MX made it possible to further investigate the role of this system in physiological processes, at the tissue level and in the whole organism. It should be noted that along with the achievements of that period, in our opinion, some issues remained not fully worked out. to say that in that era, the main object of research was warm-blooded organisms, in which metabolism is high (Leninger, 1966; Reker, 1967; Ivanov, 1972). This factor may have influenced some of the researchers' conclusions, particularly when considering the efficiency of energy processes (or COPs), which some researchers have estimated to be low. Thermodynamic calculations about the possibility of low efficiency of energy processes were also consistent with this opinion. However, the determination of metabolism in cold-blooded organisms demonstrated the presence of a low level of metabolism in them (Hemmengsen, 1960; Schmidt-Nielsen, 1982; Kern, 1985), and the efficiency of locomotion turned out to be higher (Moberly, 1968; Taylor, Schmidt-Nielsen,1969, 1970; Taylor, et al, 1970; Tucker, 1970; Bakker, 1972; Schmidt-Nielsen, 1972; Bennett, Ruben, 1979) than in endotherms. These contradicted the results obtained on warm-blooded animals and indicated the possibility of low energy cost of vital processes. Changes in the efficiency of energy transformations remain out of sight of individual bioenergetics to the present day. Meanwhile, the bioenergetic assessment of vital processes continues in the literature, which, at the level of tissues and MX, concretizes the energy bases for ensuring the warm-bloodedness of organisms. Moreover, such an assessment is made mainly on the basis of old positions (Hulbert, 1980; Rolfe et al.,1999; Son'kin et al., 2010; Busello et al., 2015).

This review focuses on heat production in the body under thermoneutral conditions and taking into account new developments in bioenergy. In particular, taking into account proton leakage studies (Brand et al., 1991; Ricquier, 2017) and nonconjugate breathing (Akhmerov, 1986; Akhmerov et al. 2016). These developments significantly change the understanding of the mechanism of biological thermogenesis, they are based on the principles of creating a disconnecting system in mitochondrial membranes. The system includes specialized uncoupling proteins (UCPs) in the inner membrane and regulators of their activity, mainly guanosine diphosphate (HDP).

This review does not touch upon the issues related to thermogenesis under the conditions of cold, which were considered earlier in the works of V.P. Skulachev (Skulachev, 1962; 1969, 1985). His work was the first serious step in the field of mitochondrial thermogenesis, and the discovery of free (non-conjugate) respiration was the first concrete mechanism of thermogenesis of the dissociating type.

Studies of thermogeny-specialized brown fat mitochondria have led to the identification of a specialized uncoupling protein in the inner membrane of MX as the basis of proton leakage and thermogenesis (Brand et al., 1991; Ricquier.2017). Analogues of this protein have been found in the mitochondria of other tissues (Porter, 2001). Uncoupling proteins have become the main object of research in the analysis of the problem of biological thermogenesis. A more complete disclosure of the proton leakage mechanism was achieved in the study of nonconjugate mitochondrial respiration in warm- and cold-blooded organisms (Akhmerov, 1986; Akhmerov et al. 2016).

Warm-bloodedness of the body and metabolism. In terms of the intensity of heat production at optimal ambient temperature, vertebrate organisms differ significantly (Prosser, 1972; Schmidt-Nielsen, 1987; Jansky, 1972; Bennett, Ruben, 1979; Hulbert , 1980). The results showed that warm-blooded animals have a high level of metabolism and maintain a constant and high (about 36 - 40°C) body temperature. The other group includes ectothermic animals, which have about ten or more times lower metabolism and variable body temperature than those of endotherms. These advances can radically change the existing ideas about the cost of vital processes and the efficiency of biological processes. to determine a new attitude to research in this direction. In modern literature, the concepts of "warm-blooded and cold-blooded", "homoiothermic and poikilothermic", "endothermic and ectothermic" are widely used to classify animals by temperature status (Prosser, 1972; Schmidt Nielsen, 1987).

However, it took considerable time and effort to understand to some extent the tissue mechanisms of the differences between cold- and warm-blooded organisms. The data obtained in this regard showed that the studied groups of organisms have certain differences in tissue energy. In particular, a comparative analysis of proton leakage in the mitochondria of warm- and cold-blooded organisms was carried out (Hulbert, Else 1981; Brand et al.,1991; Duong et al.,2006; Mendez-Romero et al.,2019). More fruitful were studies of nonconjugate respiration, which showed great differences between the mitochondria of warm- and cold-blooded groups of animals (Akhmerov, 1981; Akhmerov 1986; Akhmerov et al, 2018). Further research in our direction made it possible to form certain views on the nature of tissue thermogenesis and this will be discussed below.

The role of body weight and locomotion in metabolism. It turned out that the exchange rate among endothermic and ectothermic organisms depends on their body weight and decreases with increasing mass, and the slope of the regression curve is approximately the same for these groups of animals (Hemmengsen, 1960; Schmidt-Nielson, 1982; Kern, 1985). This regression is expressed by the formula $VO_2 = k\cdot M^{\text{is } 0.72}$, where VO2 is the intensity of metabolism, M is the mass of the organism, and k is the constant of proportionality. The dependence of metabolism on body weight is generally recognized as true for warm-blooded vertebrates (Schmidt-Nielsen, 1982), although some aspects have been discussed for a considerable time (Heusner, 1982).

When conducting comparative studies of cold-blooded organisms with warm-blooded organisms, the equality of their mass should be taken into account. In the intensity of heat production, endo- and ectothermic vertebrates differ by 5-10 times at the same body temperature (Dolnik, 1981; Schmidt-Nielsen, 1982; Bennett, Ruben, 1979; Else, Hulbert, 1981; Hulbert, Else, 1989). At the optimal temperature under ambient conditions (about 20°C), the difference between them reaches 40-50 times (Hemmengsen, I960; Schmidt-Nielsen, 1982); at 10°C ambient temperatures differ by a factor of about 100 (Bennett and Ruben, 1979). In these cases, different environmental temperatures have little effect on the body temperature of warm-blooded organisms, but strongly affect the body temperature of cold-blooded organisms, and their body temperature is similar to the temperature of the environment. When conducting studies under the above conditions, differences in the body temperature of animals were taken into account.

Separate studies are devoted to the analysis of the energy value of locomotion (speed of movement/kg of body weight) of endo- and ectothermes. According to the data obtained (Schmidt-Nielsen, 1982; Moberly, 1968; Taylor, Schmidt-Nielsen,1969, 1970; Taylor, et al, 1970; Tucker, 1970; Bakker, 1972; Schmidt-Nielsen, 1972; Bennett, Ruben, 1979), the price of locomotion is 2-10 times higher in endothermic than in ectothermic organisms.

From the above, the most significant difference between ectothermic and endothermic organisms is the ability of the former to spend significantly less energy resources on life at rest and when activating physiological processes. Therefore, if we proceed from the studies of cold-blooded organisms, life activity seems to be an energetically less wasteful process and proceeding with a higher level of efficiency. However, when considering warm-blooded animals isolated from cold-blooded animals, life support seems to be associated with the greatest energy costs. In the evolution of organisms, apparently, certain changes in the mechanisms of energy supply of the main functions of vital activity could occur, in particular, the appearance of a new energy function in the organism – ensuring the warmth of the organism. This circumstance could be one of the main reasons for the qualitative difference between endo- and ectothermic organisms in the intensity of metabolism.

The results of physiological studies of ecto- and endothermic animals served as a prerequisite for the development of the biochemical foundations of biological thermogenesis. As a result, a number of points of view were proposed on the origin of endothermity in organisms. In particular, we will consider such problems as the role of low efficiency of energy processes, the amount and composition of MX, as well as the role of disconnecting MX proteins, proton leakage and non-conjugate breathing.

Efficiency (COP) of the functioning of the respiratory chain and thermogenesis. For a long time, the point of view about the important role of low efficiency of energy reactions in thermogenesis was very popular among researchers.

Based on the data on the value of the standard free energy of ATP hydrolysis (ΔGo), which corresponds to about 7 kcal/mol, and the real free energy (ΔG) of ATP under cell conditions is approximately 13-16 kcal/mol. Therefore, many researchers believed that about half of the energy of oxidation substrates at the stage of ATP synthesis in MX is dissipated in the form of heat, and the energy-dependent processes that utilize ATP dissipate about half of the remaining energy. This portion of the energy was thought to be conserved in beneficial biological processes (Leninger 1966; Ivanov, 1972; Leninger, 1974; Marshell, 1981; Reker, 1967; Prusiner, Roe, 1968; Hill, 1974).

The free energy (ΔG), determined by the content of ATP, ADP and phosphorus in the tissue, its value turned out to be significantly higher than the standard free energy of ATP hydrolysis (ΔGo) and approximately corresponded to the values of 13-16 kcal/mol ATP (Leninger, 1974; Marschell, 1981; Weech, 1970; Whitmer,1978; Hassinen, Hiltunen, 1975; Wilson, Dilton, 1980; Lamesters et al., 1984)These results indicate that the organization of the cell energy system is adapted to maintain a high level of free energy of ATP synthesis and hydrolysis in tissues. With cellular values of ATP hydrolysis energy (13-16 kcal/mol) and the ratio of reactants close to equilibrium, the efficiency of the respiratory chain, i.e. efficiency, can be more than 80%. At present, not all researchers consider the synthesis and hydrolysis of ATP as an important source of thermogenesis. For the correct use of the thermodynamic approach in bioenergetics, it is also necessary to attract data on free energy in the tissues of ectothermic organisms, which is little used in this aspect. In the literature, there is currently a certain reorientation in this area, taking into account the existing achievements on this problem.

Wikström and Krab (1979), studying energy transfer in a model system with liposomes, obtained data that the respiratory chain can function with efficiency close to 100%. The possibility of high efficiency in energy transfer during ATP synthesis is still being considered. In particular, new molecular models of energy transfer in the mitochondrial respiratory chain with efficiency up to 100% are being created (Nath, 2016, 2017). In addition to the above, theoretical prerequisites do not exclude the possibility of the presence in tissues of such reactions that occur with the absorption of heat (endothermic reactions). In particular, Hard (1984) suggests that during the synthesis of ATP, the ΔpH count in MX must absorb heat from the medium, since under these conditions the electrochemical potential produces chemical work. The question of the efficiency of energy reactions is currently facing unexpected aspects. The data mentioned above give reason to believe that the efficiency of these reactions may be much higher than 50%, therefore, the system of oxidative phosphorogenation and energy-dependent processes may not be the main source of heat in the body.

It should also be remembered that these energy processes also take place in the body of cold-blooded organisms, in which these processes take place at a low level of metabolism, which contradicts the concept of low efficiency of biological processes considered above. Provided that biological reactions with low efficiency take place, it is unlikely that cold-blooded species of organisms existed in the animal world, since they would have a high level of metabolism. Currently, researchers are analyzing energy processes in biological tissues, taking into account current trends in this field (De Meis et al., 2012; Son'kin et al.,2010; Busiello, Savarese, 2015; Porter et al, 2017; Pohl, 2018; Treberg et al.,2018; Sepa-Kishi et al.,2019).

The phenomenon of aerobic metabolism activity. Dolnik and Bennett, Reuben (Dolnik, 1981; Bennett and Ruben, 1979), when interpreting the nature of goimothermia, much attention is paid to the phenomenon of the "activity" of exchange. In particular, the authors believe that warm-bloodedness is due to high aerobic metabolism, the level of which is up to 5-10 times higher than in cold-blooded animals, when tested under the same conditions of the body temperature of the compared animals. According to them (Dolnik, 1981; Bennett, Ruben, 1979), aerobic activity is the basis for increasing the energy capacity of the body, which is necessary for the rapid movement of mammals (Bennett, Ruben, 1979) and for the flight of birds (Dolnik, 1981). Discussing this issue, the evolution of blood circulation, the respiratory system in vertebrates, as well as the increase in the concentration of mitochondria in tissues are considered as a factor in the activation of aerobic metabolism (Tenney, Remmers, 1963; Bennett, Ruben, 1979). It should be noted that "aerobic activity" in warm-blooded organisms is most likely associated with an increase in energy expenditure for vital activity and an increase in heat production in endotherms. The main metabolic processes, including thermogenic processes, occur at the MX level. In our opinion, the evolutionary transformations of the MX caused the rise of the entire energy of the organism and the emergence of endothermic organisms. Research in this regard continues in various aspects (Affourtit et al., 2018; Cadenas, 2018).

The role of transport Na, K-ATP-ase in tissue thermogenesis. Given that ATPase is the membrane link of all biological cells, its role in the energy of tissues of endothermic and ectothermic animals has been investigated (Natochin et al., 1975; Ismail-Beigi, Edelman, 1970, 1973). According to the data obtained (Edelman, 1976), tissue respiration dependent on sodium transport is much higher in endothermic organisms, and the effect of uabain, an inhibitor of Na^+, K+-ATPase, is also more pronounced in endothermic organisms. The authors suggest that the sodium pump may be the main factor of heat production in the body of endothermic organisms and is controlled by thyroid hormones. A similar point of view is shared by Hulbert and Elze (1981). that the intensity of respiration in the tissues of mice is 2-5 times higher than in reptiles, which can cause Na+, K+-ATPases.

According to the calculations of Von Russum (1970), the share of transport-conjugated respiration in the functioning of Na+, K+-ATPase in liver sections accounts for only about 8% of the total energy expenditure of the tissue. Approximately the same values of energy consumption of Na+ and K+-ATPase are given in other works (Zorov, Mokhova, 1973; Crease, 1968; Clausen, Hansen, 1982). According to the results of Ismail-Beigi and Edelman (1970, 1973), the process can take up about 40% of the total energy consumption. The sodium pump and uncoupling proteins are thought to be the main thermogenic systems in tissue (Jarmuszkiewicz, Szewczyk, 2019).

Along with the analysis of energy processes, much attention is paid to the protein phospholipid composition of tissues in different vertebrates. The results of the studies showed a predominance of the content of proteins and polyunsaturated phospholipids in the membranes of endotherms than in ectotherms. According to the authors, polyunsaturated phospholipids activate the mobility of molecules of the enzymes Na+, K+-ATPase and increase the flow of sodium through the cell membrane (Hulbert, Else, 1989; Wu et al., 2004; Else et al., 2004). These findings suggest that the sodium pump is associated with increased tissue energy expenditure and is a pacemaker of cellular metabolism (Else et al, 2004; Wu et al.,2004). A number of determinations of the energy costs of the sodium pump have been carried out (Rolfe, Brown, 1997; Clarke et al.,2013; Else, 2016), which showed the possibility of its unequal contribution to the energy of different tissues. According to calculations, the work of the sodium pump consumes about 20% of the basal metabolic rate of endotherms (Brown, Brand, 1991; Porter, Brand, 1995; Rolfe, Brown, 1997; Else, 2016).

The sodium pump in cold-blooded organisms has low activity (Natochin et al., 1975), and the energy expenditure is much less than in warm-blooded organisms and is about 8% (Nilsson et al., 2000). At the moment, endothermic organisms have been little studied in terms of the energy efficiency of tissue processes. Further research is needed, as the existing data have a certain variation, the reasons for which remain unclear.

The amount of MXs, their structural features and the activity of enzymes of the main respiratory chain of endo- and ectotherms. The shown low activity of metabolism in ectothermic cells may depend on the number and structural organization of MX in different animals. Electron microscopic study of skeletal muscle cells in endotherms and ectothermes showed that the volume density of MX in the latter is 2-3 times less than in ectotherms (Mobley, Eidenberg, 1975). Hulbert, Else, 1981). These endothermic features could be the cause of low metabolic activity in ectothermic organisms to a certain extent. As it turned out, the enzymes of the MX respiratory chain quantitatively differ little in the homogenates of lamprey and reptile tissues (Savina, 1992; Coulson, Hernandez 1983). Moreover, the amount of MX protein, determined by their marker cytochromes, turned out to be similar in different groups of animals per 1 g of wet liver weight (Savina, 1992; Sutton, Pollak, 1978; Wilson et al.,1980). Only in the muscle tissue of lampreys there is almost three times more MX protein than in endotherms (Savina, 1992). In all likelihood, the excessive amount of MX in the skeletal muscles of lampreys is not realized in vivo in energy reactions, but performs other metabolic functions, since in lampreys the metabolic rate is only about 4% of the level of endotherms. In vitro study of MX respiration of this lamprey tissue did not show a noticeable difference from MX respiration of warm-blooded muscles. The totality of the accumulated data showed that in evolutionary terms, the MX apparatus in vertebrate animals did not undergo a significant change in the enzymatic-metabolic relationship (Savina, 1992; Coulson, Hernandez 1983). Nevertheless, we would like to draw attention to one circumstance that will be important in the further analysis. In particular, Savina M.V. (1992, p.27) notes that the ratio of cytochromes of the MH respiratory chain has changed in evolution, and it was accompanied by a 2-3-fold increase in the level of cytochrome *b* and the ratio of cytochromes *b/a* in terrestrial endothermic tissues. According to the available data (Mersmann, Cordes, 1964; Smith, 1973; Skoog et al, 1978), there is a low oxidation rate of exogenous NADH in the MX tissues of ectotherms, since oxidation of exogenous NADH occurs at a high rate in the MX tissues of endotherms (Blanchaer et al, 1966; Rusmussen, 1969; Rusmussen, Rusmussen, 1985; . Nohl, Schonheit, 1996; Akhmerov, 1981). Low NADH oxidase, in our opinion, was a prerequisite for the evolutionary development of non-conjugate respiration of MX (Akhmerov, 1994), as the main thermogenic mechanism of endothermic vertebrates.

Protein and phospholipid composition of MX endo- and ectotherms. In the evolution of vertebrates, there has been a significant reorganization of the protein and phospholipid composition in the tissues of endothermic organisms, as evidenced by the results of research in this direction. For example, a 60% increased protein content and phospholipid content in endothermic tissues was revealed in contrast to ectothermic vertebrates (Hulbert and Else, 1989). At the same time, semi-unsaturated forms prevail among the phospholipids of endotherms, which are associated, in particular, with the functioning of Na+-K+, ATPases and increase the permeability of the cell membrane to monovalent cations (Hulbert, Else, 1989; Porter, Brand,1995). Compositional rearrangement also occurs at the MX level (Hulbert, Else.1989), which could be a prerequisite for proton leakage activation.

The effect of protein and phospholipid composition is shown on the cell membrane, as a result of which the permeability of membranes to sodium increases. As a result, the sodium cycle is activated, and accordingly, cell metabolism is increased (Else et al., 2004; Hulbert, Else, 1989), which ultimately increases tissue thermogenesis. The sodium cycle, together with other cycles, is considered as pacemakers of metabolism (Wu, 2004; Busiello et al.,2015).

Adenine nucleotide pool as a factor in the regulation of energy in organisms. According to the results of Savina M.V. (Savina, 1992), the pool of adenine nucleotides (total content of ATP, ADP, AMP) can be of great importance in tissue respiration. The author develops this idea on the basis of his results on lamprey tissues, which are in a low-energy state for a long time during migration. It is shown that under these conditions, there is a significant decrease in the pool of adenine nucleotides in tissues, although the amount of MX in the muscle remains still higher. than in warm-blooded animals. The results of these experiments emphasize the important role of adenine nucleotides in the regulation of the low-energy state of the body, which is also important in the hibernation or estivation of endothermic and ectothermic animals (Akhmerov et al., 1995).

The obtained data on the large amount of MX in tissues in combination with a low level of metabolism in these organisms indicate that it is MX that contains the key to understanding such important problems as the mechanisms of regulation of metabolism and the efficiency of energy processes and the key to the mechanisms of turning on and off.

Proton leakage, nonconjugate MX respiration, and thermogenesis. One of the promising approaches in this direction is the study of the proton leakage process of MX, due to the increased permeability of their inner membrane. Proton leakage is caused by specific membrane proteins - uncoupled proteins (UCPs) localized in the inner membranes of MX. At present, several species of such proteins have been identified in different tissues, which are designated as UCP1, UCP2, UCP3, UCP4 (Bouillaud et al., 2001). They contribute to the dispersion of the proton gradient on the MX membrane, which was created due to the oxidation energy of the substrates. The scattering of the proton gradient is a source of biological heat that ensures the endothermity of the body (Hulbert , Else 1989; Brand, 2000; Clarke, Porter et al., 2010; Ricquier, 2017).

Uncoupling proteins are localized on the surface of the inner membranes of MX and work as proton carriers, making MX membranes permeable to protons. As a result of the action of uncoupling proteins, there is a constant scattering of the proton gradient on the MX membrane, created due to the oxidation energy of the substrates and serves as a constant source of heat.

Unfortunately, the idea of proton leakage as the main mechanism of thermogenesis does not always receive comprehensive confirmation in experimental terms. One of the reasons for this may be the experimental flaw in the problem of proton leakage. In particular, its study is carried out using only one substrate - succinate, which significantly limited the possibilities of this area of research. Thus, a comparison in the phylogeny of different groups of animals showed that in reptiles proton leakage in hepatocytes and their MXs has a lower level than in mammals, although the difference is negligible (Hulbert et al., 2002). These data, together with others (Duong et al., 2006), were poorly consistent with the idea of the thermogenic importance of UCPs and the maintenance of endothermity of the body. It is possible that later the leading role in this matter was assigned to anion translocators, in particular, adenine nucleotide translocase (ANT). When comparing endo- and ectothermic organisms, it was shown that the value of proton conduction correlates well with the number of MX transporter anions, in particular, with the amount of ANT (Brand et al, 2005; Jastroch et al, 2010), which may account for about 75% of the basal proton leakage. Authorsbelieve that ANT cannot detect all basal proton leakage and that other MX anion translocators may be involved. These ideas are based on an experimental comparison of the proton conduction of mammalian and reptilian hepatocytes. The rate of oxygen consumption is 4-5 times weaker, which correlated with a 4-fold lower level of the latter's ANT. Therefore, the amount of ANT in the MX of reptilian hepatocytes is considered to be an important modulator of thermogenesis at the cellular level (Brand et al. , 1991; Brand et al., 2005), there is no comprehensive evidence for this view. It should also be noted that in this group of studies it is postulated that the entire population of MX participates in disconnected breathing, and the proton leakage process passes through several stages. Moreover, UCPs have been found in ectothermic and other organisms, with a few exceptions (Porter, 2001; Mendez-Romero et al.,2019).

The involvement of UCPs in thermal regulation has also been shown (Hulbert et al., 2002; Mark et al., 2006; Bal et al., 2012; Busiello et al., 2015), obesity, disease, aging, as well as in the regulation of superoxide levels (Brand et al., 2004; Jastroch et al.,2010; Mendez-Romero et al., 2019). New methodological approaches are also being introduced to study the energy bases of thermogenesis using tissue models close to in vivo conditions (De Meis et al., 2012; Calderon-Dominguez et al., 2017; Bertholet, Kirichok , 2017).

A number of studies indicate that the contribution of proton leakage to the basal tissue metabolism is about 20%, and in skeletal muscle its level reaches 50% (Jastroch et al, 2010). The indicated contribution of UCPs proteins does not always explain the difference in exchange between endo- and ectothermic organisms, which differ in the level of metabolism by a factor of ten or more. is more likely due to methodological shortcomings, since in studies succinate is mainly used as an oxidation substrate on MX. In order to obtain a more complete characterization of the proton leakage of MX and to expand the possibilities of research, it is desirable to use different substrates.

It should be said that the idea of proton leakage has played a major role in the study of the bioenergetic mechanisms of MX and the understanding of the mechanisms of thermogenesis. The study made it possible to approach the problem of thermogenesis in a new way and to attract new reactions, which significantly expanded the possibilities of thermobiology.

The study of MX values in proton leakage and tissue thermogenesis occupies an important place in another group of studies. In this series of studies devoted to the study of MX thermogenic respiration, different oxidation substrates were used and additional, higher opportunities for heat production were identified (Akhmerov, 1981; 1982; 1983, 2018), which may occupy a significant share of MX respiratory activity. studies of proton leakage and non-conjugate respiration were conducted using different substrates on MX endo- and ectothermic organisms. Interesting results were obtained, which made it possible to put forward an alternative concept of the existence of special thermogenic MXs in tissues, the function of which is to carry out proton leakage, respectively, and thermogenesis to ensure the warm-bloodedness of the body. These experiments proved the existence of two types of MX in the study drug; one type of MX synthesized ATP, and the other type of MX carried out non-conjugate respiration (proton leakage), the consequence of which is heat production (or thermogenesis).

In order to judge the sufficient objectivity of the results obtained, the experiments were carried out on a cell preparation (cardiocytes) (Akhmerov et al, 2016) using different oxidation substrates and on MX endo- and ectothermic animals (Akhmerov, 1986). It should be noted that in our experiments, in addition to NAD-dependent substrates, succinate and exogenous NADH (+ cytochrome c) and ascorbate (+cytochrome c) also served as oxidation substrates. The last two substrates are not popular among bioenergetics, and they are rarely used in experiments. The study of their oxidation together with other substrates in our experimental conditions turned out to be a very useful approach for the analysis of non-conjugate reactions of MX. The results of the experiments indicated the existence of disconnected (thermogenic) MXs inside intact cells, functioning independently of ATP synthesizing MXs.

Analysis of differences in oxidation of different substrates. Conjugated and non-conjugate ways of oxidation of MX. The analysis of the oxidation features of the used oxidation substrates is of interest. On the succinate, the heart MX respiration rate occurred at an elevated rate in state 4 and with low respiratory control. This well-known fact is interpreted differently in literary sources (Kondrashova, 1972; Chance, Hollunger, 1961). In contrast to the literature, we assumed that this is a manifestation of non-conjugate oxidation, in the isolated MXs some of these organelles are non-conjugated (disconnected), which can increase the rate of respiration in state 4. Unconjugated MXs have a particularly strong effect on the oxidation of succinate, this process does not require the addition of coenzymes, and electrons from succinate transfer their electrons directly to the MX respiratory chain – at the level of the second complex (FAD-dependent enzyme). Two subpopulations of MXs can be involved in the oxidation of succinate at once: conjugated ATP-synthesizing and non-conjugated thermogenic MXs. As a result, there is a decrease in the parameters of the MX interface. Such an unusual way of oxidation of succinate in MX is also noted by other authors (De Meis et al., 2012), which is consistent with our concept. An interesting fact was obtained during the study of Mg+2-ATPase in MX muscle tissues (Cho and Davis, 1972; Akhmerov, 1983). The relationship of this enzyme to thermogenesis has not been studied.

In the course of experiments on cardiac MX, it was shown that NAD-dependent substrates – glutamate, pyruvate + malate (without NAD and cytochrome *c* in the incubation medium) were oxidized in the used MXs with greater respiratory control and ADP/O compared to succinate. These facts are consistent with numerous literature data. Oxidation of NAD-dependent substrates and succinate occurs in different ways, as noted by other authors (De Meis et al., 2012).

Non-conjugated MXs do not noticeably affect the oxidation of NAD-dependent substrates, the substrates first reduce the NAD coenzyme, which then enters the respiratory chain. However, the water-soluble coenzyme NAD comes out of the mitochondrial space when the membrane permeability of MX is increased by a dissociating protein, and its concentration is greatly reduced inside the MX, which inhibits the oxidation of NAD-dependent substrates of non-conjugated MXs. These substrates are oxidized only in ATP-synthesizing MXs and have high conjugation parameters with ATP synthesis. To activate their oxidation along the unconjugated pathway, it is necessary to introduce a coenzyme NAD up to 1 mM into the medium, which really increases the rate of nonconjugated respiration of MX in state 4 (Akhmerov, 1981, 1983). Usually, experimenters do not use such a combination of substrates, but limit themselves to the separate use of NAD-dependent substrates to obtain a better coupling of the oxidation process with ATP synthesis.

Among the substrates used, the oxidation of the NADH substrate under our experimental conditions is of particular interest. In the literature (Luzikov et al., 1967; Almatov, Akhmerov, 1976), NADH is widely used in the study of the respiratory enzyme (complex 1) of NAD-oxidase MX under conditions of artificial damage to MX membranes by their one-time freezing and thawing. isolated with extreme care: EDTA, BSA and other emollient ingredients were used in the working environment to release MX qualitatively, so that there is no damage to MX by isolation conditions. It turned out that in MX, exogenous NADH (+ cytochrome *c*) or ascorbate (+cytochrome *c*) is oxidized very intensively by preparations of isolated MXs along the rotenone-sensitive pathway (Akhmerov, 1993, Akhmerov, 1986).

The results obtained showed that the suspensions of the isolated MX contain two types of MX: one of which synthesizes ATP, the other performs non-conjugated thermogenic respiration. Interestingly, these two types of MX are detected quite easily by manipulating different substrates of oxidation. Previously, these reactions were not in the field of view of physiologists, thermobiologists, who used phylogenetic and ontogenetic approaches, and can specify their connection with thermogenesis. have been studied by researchers (Porter, 2001) and have received a positive response.

In the literature, the phenomenon of intense and non-conjugate oxidation of NADH in the MX of the heart and muscles was described long ago (Blanchaer et al., 1966; Rasmussen, 1969; Rasmussen, Rasmussen, 1985; **Nohl** and Schönheit 1996), which is considered to be an active property of MX and a regulatory link in the cytoplasmic level of NAD/NADH.

Under our conditions, it has been shown that the process of oxidation of NADH also proceeds without phosphorylation added by ADP, and oligomycin or carboxyatractyloside does not have a noticeable effect on the dynamics of their oxidation. Moreover, respiration in the mitochondria of the rat heart is inhibited by rotenone (1 µg/ml) on NADH, by about 90%, as well as sodium cyanide (1 mM) on both substrates. Consequently, their oxidation occurs along the main respiratory chain of mitochondria, but is not associated with the synthesis of ATP. In rat liver mx, NADH oxidation is only partially sensitive to rotenone (Akhmerov, Allamuratov, 1994), and this part flows along the main respiratory chain, and there is also a rotenone-insensitive fraction that flows along the redox chain with cytochrome b5 (Skulachev, 1969; Agureev and Mokhova, 1979).

It should be noted that we used two substrates (NADH and ascorbate), which are usually little used in experiments with MX. Their intensive and non-conjugate oxidation is considered by many researchers as a sign of damage to isolated MXs, i.e. as an artifact of research (Leninger, 1966; Reker, 1967). Indeed, many factors are known to damage MX during isolation or experimentation (Leninger 1966; Reker 1967). In order to show the improbability of an artifact in our studies, we studied non-conjugate respiration on an intact cell preparation - cardiocytes, MX that do not come into contact with damaging environmental factors.

Isolated cardiocytes, as well as isolated mitochondria, showed the functioning of non-conjugated mitochondria with conjugated mitochondria. Isolated cardiocytes extensively oxidized exogenous NADH and ascorbate in the presence of cytochrome c (Kokoz et al., 1987; Akmerov et al., 2016). Cardiocytes had a higher respiration rate in state 4 with oxidation of succinate, good conjugation with oxidation of NAD-dependent substrates. Isolated cells actually duplicate isolated mitochondria in the nature of respiration on different substrates, and this is considered by us as one of the main criteria for the motivation of nonconjugate MX respiration. In the isolated mitochondria of the heart under a light microscope, after introducing succinate and rhodamine 123 into the medium, two groups of MXs can be seen, one of which has a membrane potential and the other without a potential.

It should be noted that the study of the MX of the thermogenic organ of brown fat has shown that in them NADH and ascorbate (+cytochrome *c*) are oxidized very intensively along the non-conjugated pathway, along with low conjugation during oxidation of succinate (Akhmerov, Makhmudov, 1988). Nonconjugate respiration is also intensively present in the kidneys and skeletal muscles, and in moderate activity it is present in other tissues (Akhmerov, 1981; 1982; 1983; 1994).

To further substantiate this possibility, we conducted a comparative study of MX tissues of warm-blooded rats with cold-blooded frogs, turtles, and agamas (Akhmerov et al., 1995), which showed low activity of nonconjugate respiration in the tissues of cold-blooded organisms.

Experiments in this regard have shown that in the mitochondria of lake frog tissues, non-conjugate oxidation of both NADH and ascorbate (+cytochrome *c*) occurred at a low rate, and succinate, and especially NAD-dependent substrates, were oxidized with a high conjugation with ATP synthesis. Consequently, cold-blooded mitochondria are characterized by a low level of nonconjugate respiration, which can cause low metabolic activity in the body of frogs and their cold-blooded status. The corresponding results were obtained on the tissues of desert turtles and agamas (Akhmerov et al., 1995).

The results discussed above are highly likely to indicate the motivation for the oxidation processes described here, including non-conjugate respiration during the oxidation of exogenous NADH and ascorbate. Based on these results, it can be assumed that nature itself has learned to increase the permeability of a group of mitochondria in order to create warm-blooded organisms. As a result of such an evolution of the energy system, organisms had the opportunity to reduce the efficiency of the functioning of the energy system and sharply increase the intensity of metabolic processes, which, in turn, led to the emergence of warm-blooded mammals and birds (Akhmerov, Allamuratov, 1994).

Discussion. Speaking of the bond with uncoupling proteins (proton leakage ((Brand et al., 1991; Brand et al., 2005) by non-conjugate breathing (Akhmerov, 1981, Akhmerov, 1986), we believe that the process of proton leakage of MX is not limited to oxidation of succinate and oxidizes other substrates (Akhmerov, 1981, Akhmerov, 1986). According to the results of studies by other authors, this form of oxidation includes NADH and ascorbate (+cytochrome *c*). The inclusion of these substrates requires a reconsideration of the proton leakage mechanism and consideration of the involvement of the two types of MX mentioned above, which significantly increases the thermogenic efficiency of the cell's energy system.

To date, a lot of data have been obtained on the different levels of metabolic processes in different classes of vertebrates. It should be noted that the differences in the level of metabolism between organisms are quite high (Dolnik, 1981; Prosser, 1982; Savina, 1992; Schmidt-Nielson, 1992) from three to a hundredfold or more. Under the condition of low efficiency of energy processes, organisms with a low level of metabolism could not exist. A low level of metabolism is not consistent with the position of low efficiency. The course of life processes is most likely associated with a high efficiency of energy generation and utilization. In addition, under extreme conditions, a number of organisms have an additional decrease in the intensity of metabolism, in particular, in hibernating animals (Akhmerov et al. 1995) or in fish during long-term migration (Savina, 1992). These data have little agreement with the idea of a low efficiency of biological processes in the body. On the contrary, the living world can be dominated by efficient ways of using energy with their own unique mechanisms. The application of the idea of low-efficiency energy conversion in biology came from thermodynamics, about entropy as an integral part of any energy conversion process (Pevsner, 1977; Marschell, 1981). The biological world has the ability to improve itself and over millions of years could develop an ideal version of energy transfer from one reaction to another, with high efficiency. Many biologists do not always take this circumstance into account and, when considering warm-blooded organisms, proceeded from the thermodynamic principle of the low efficiency of energy transformations as a universal phenomenon, the biological sphere. However, to date, a number of data have accumulated that do not agree with this situation. Biologists view thermodynamics from the wrong point of view, physical or chemical, and mechanically transfer their principles to biology.

It is obvious that conditions are ripe for a revision of previous views in this field of research and for the analysis of the accumulated data, which are partially carried out by modern research. In the future, new methodological approaches are being introduced that will make it possible to analyze controversial issues that arise in this direction (De Meis et al., 2012; Porter, Herndon, 2016; Calderon-Dominguez et al., 2017).

This review critically analyzes the issue of low efficiency of biological processes and proposes a new consideration of the issues of mitochondrial thermogenesis and ensuring the warm-bloodedness of the body.

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