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DIFFERENCE IN THE METABOLISM OF COLD- AND WARM-BLOODED ANIMALS AND ITS MITOCHONDRIAL MECHANISMS

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ABSTRACT
It was studied the intensity of the oxygen consumption by whole organisms in cold and warm-blooded animals. It was revealed that cold-blooded species of animals (steppe turtle, water snakes etc.) have a very low metabolism, the level of which is about 10 times lower than in warm-blooded rats. These data are consistent with literature data obtained on other animals. The authors asked themselves about the mechanisms of such a high difference between animals. In particular, it was found that the tissues mitochondria of warm-blooded animals, in contrast to cold-blooded ones, carry out coupled respiration with the ATP synthesis, as well as with uncoupled respiration. It was shown that the functioning of uncoupled respiration is a thermogenic mechanism that determines the warm-blooded state of organisms.

KEYWORDS: Metabolism, cold- and warm-blooded animals, heat production, uncoupled respiration, oxygen consumption, ATP.

INTRODUCTION
It is discussed in this article about the metabolism issues of cold- and warm-blooded animals living in Uzbekistan. According to the available literature data, these groups of animals differ significantly (5-10 times) in a metabolism level and heat production.[1,2,3,4,5,6] Perhaps the indicated metabolic difference between animals is a common feature for all animals living in different climatic conditions, including Central Asia.

Further, in this work, an important question is raised about the physiological significance of metabolic differences between these groups of animals. It should be considered that warm-blooded organisms consume about 10 times more energy than cold-blooded ones. What is this high cost used for? One of the possible reasons may be heat production that is used to create the warm-blooded status of the body. However, to date, neither physiologists nor biochemists have shown this possibility.

However, researchers pay little attention to the mechanism of this difference. Usually, heat production in the body of animals is explained by the low efficiency of biological processes.[7,8,9] Only a few works have noted the existence of a special form of respiration – the uncoupled respiration at the mitochondrial level, which converts oxidation energy directly into heat.[10,11,12] This is still a single case and further research is needed in this direction. As a result, the biological mechanism of thermogenesis can be established, which is responsible for the consumption of 80 - 90% of the metabolic energy in the body, and only about 10-20% of the energy of the body of warm-blooded animals can be used for vital functions. In the body of cold-blooded animals, little heat is generated, so little oxygen is consumed. Moreover, the efficiency of using the energy of metabolism in them is much higher than that of warm-blooded animals.[13,14,15] It is possible that at the subcellular level, these groups of animals have different energy metabolic pathways. This issue has not been widely considered in the literature and it is possible to cite the works of individual authors who applied theirs' original approaches to solve this issue. In the available works[10,11,12,16], it is believed that this issue is associated with mitochondria of warm-blooded animals. Their' mitochondria are able to carry out not only coupled ATP-synthesizing respiration, but also uncoupled respiration, which was the subject of additional research in this work. The possibility of participation in the thermogenesis of high proton leakage in all mitochondria in the tissues of warm-blooded animals was also considered.[17,18,19] An uncoupling protein was isolated from mitochondrial membranes, which causes high membrane permeability to the proton.[17,18]

MATERIALS AND METHODS

Installation for measuring of oxygen consumption by the whole organism. In this case, the intensity of the general (gas-oxygen) exchange was measured by the polarographic method using a platinum electrode. The measuring (respiratory) installation included a chamber (plastic can) with a volume of 200-1000 ml of air. The animal was placed in this chamber and hermetically sealed. This chamber is connected to a micropump through plastic tubes, and communicates through tubes with a platinum electrode that measures the oxygen content in the chamber with the animal. The micropump allows air to circulate in a measuring installation isolated from the outside air.[20,21]

As the animal breathes in the measuring chamber, oxygen will decrease, which will be measured by the polarograph. The intensity of oxygen consumption depends on the weight of the animal, as well as on the warm and cold-blooded status of the animals used in the experiment.

Local used animals. White laboratory rats were used in the experiments. The rest of the animals were caught in the Karshi steppes and Namangan: Ground squirrels (*Spermophilus xanthoprymnus*), Steppe turtles (*Testudo horsfieldii*), Glass lizard (Sheltopusik, *Pseudopus apodus*), Dice snakes (*Natrix tessellata*) and Marsh frogs *(Pelophylax ridibundus*).

After capturing animals were kept in a vivarium with an indoor temperature of 25°C. In the vivarium, conditions were created for all animals close to those of the steppe. Feeding was also carried out taking into account the nutritional characteristics of each animal. The animals were caught in spring. Studies on these animals were carried out within a month after their capture.

Isolation of mitochondria from various animal tissues and the study of their respiration. Mitochondria from different tissues were isolated by differential centrifugation.[22] After decapitation of the animals according to the Ethical Guidelines for the Use of Animals in Research of Namangan state university, the necessary tissues were removed from the cavity and placed in a cooled isolation medium containing 300 mM sucrose, 10 mM Tris-HCl (pH 7.5). This medium also contained 2 mM of EDTA and 1 mg/ml bovine serum albumin (BSA).

After preliminary grinding with a Micro press, the tissue was homogenized in a homogenizer with a Teflon pestle[22,23] in a 10-fold volume of isolation medium. The homogenate was centrifuged at 700×g for 7 min. Mitochondria were precipitated from the supernatant at 6000 x for 20 min. The mitochondrial sediment was suspended in the same isolation medium (about 30–40 mg protein per ml) and stored in the cold at 0–2°C. Mitochondrial protein was determined according to Lowry et al.[24] Oxidation of various substrates in mitochondria was measured polarographically using a rotating platinum electrode.[25]

The incubation medium contained 120 mM KCl, 5 mM KH_2PO_4, 2 mM EDTA, 10 mM Tris-HCl, pH 7.5. The following substrates were used: 5 mM succinate, 1 mM NADH, NADH + cytochrome c 1 mg, 20 mM ascorbate + 2.5 mg cytochrome c per ml, ADP was added to the chamber in portions of 100 μM. The phosphorylation process in mitochondria was assessed according to Chance and Williams (1955). The following symbols are used: V3 - respiration during phosphorylation, V4 - respiration after phosphorylation, Polarographic recordings of mitochondrial respiration were made at 25°C.

RESULTS

Characteristics of animals and the study of gaseous exchange intensity. First, the weight of the animals was determined. We used laboratory rats for comparison with field animals. From the table 1, it was shown that from the field animals we used 5 species caught in Uzbekistan. These animals differ in mass from two to four times, where marsh frog has the smallest mass.

Table 1: The intensity of metabolic processes in warm and cold-blooded animals.

Animal species	Average body mass, g	Body temperature °C	Metabolism intensity, ml O_2 kg/h
Rats	245.6	37.2	1393.3 ± 281.4
Ground squirrel (*Spermophilus xanthoprymnus*)	325.7	36.9	920.5±97.4
Steppe turtle (*Testudo horsfieldii*)	438.3	25.3	57.9 ± 4.6
Sheltopusik (*Pseudopus apodus*)	215.6	25.4	81.8 ±7.2
Dice snake (*Natrix tessellata*)	150,3	25.3	92.7±7.3
Marsh frog *(Pelophylax ridibundus*)	55.3	25.2	102.4±5.4

Note: Basal metabolism was measured in a respiratory chamber, where the oxygen content in the air was taken as 21%, which closely corresponds to the air oxygen content

The next rate measured in animals is their body temperature. It must be said that these animals were kept in a vivarium, the temperature of which during the study period was maintained at about 25°C. Body temperature measurements showed a large difference between warm and cold-blooded animals; in the former, it was at 37°C, while in cold-blooded individuals it was about 25°C. As indicated in the Table 1, the body temperature in cold-

blooded individuals closely corresponds to the ambient temperature. In warm-blooded animals, it is much higher than the ambient temperature and is approximately 37°C.

These data show that body temperature is one of the important criteria for establishing the warm-blooded status of animals. Their body temperature is higher than the ambient temperature.

Subsequently, the metabolism was studied in a comparative aspect in different animals, where the indicator was the intensity of oxygen consumption by the body. In this case, the study was carried out at the same and optimal temperature of the environment for all animals, which is 25°C. Under the used conditions, we found that different animals have different metabolic intensity (Table 1). However, we were interested in the issue of the difference between warm and cold-blooded organisms for this indicator. Such a research was not performed previously on Central Asian animals living in arid zones. In general, it is interesting to what extent these animals correspond to the established principles of metabolic physiology, made earlier[1,2,3,4,5,6], according to which cold-blooded organisms have a lower metabolic rate than warm-blooded ones. Our results showed that in cold-blooded animals, the metabolic rate is much lower than in warm-blooded species.

The graphical comparison of the difference in metabolism between the studied animals (Fig. 1) from which it appeared that in cold-blooded organisms the metabolic rate is 10 or more times lower than in warm-blooded ones. The metabolism in desert turtles is especially low (17.3 times lower) in comparison with the metabolism of ground squirrels.

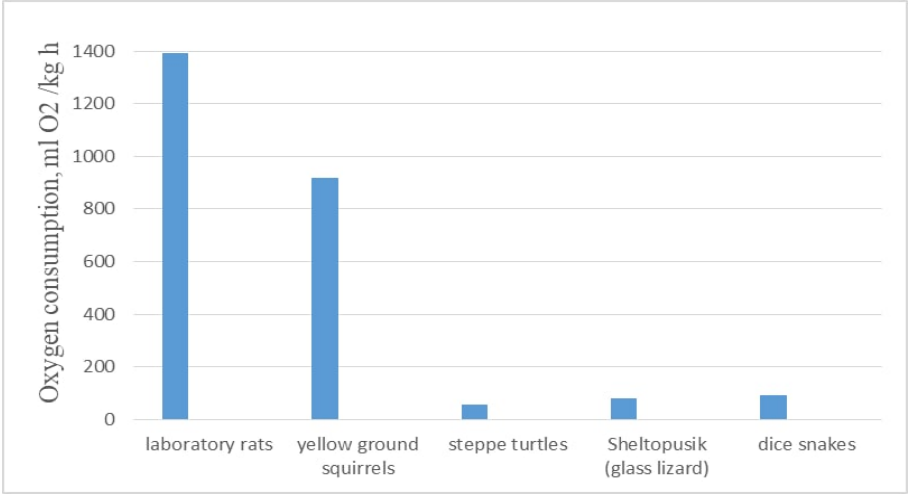

Figure 1: Differences in percentage in metabolic rate between warm- and cold -blooded animals.

When compared with a laboratory warm-blooded rat, it is noted a higher difference. In particular, the difference between a rat and a turtle reaches 23.01 times, and the difference between a rat and a glass lizard reaches 16.9 times.

As can be seen from the data (Figure 1), the metabolism of cold-blooded organisms is significantly lower compared to warm-blooded ones. The presented data indicate the possibility of differences in the intracellular energetic, and more likely, in the mitochondrial energetic.

Respiration of tissue mitochondria of cold and warm-blooded animals. Earlier there were a number of studies on the respiration of isolated mitochondria in different groups of animals. According to literature data.[26,27,28], mitochondria in these groups of animals do not have qualitative differences, but they have quantitative variations in the respiratory and phosphorylating activity. It must be stated that the previous literature studied the phosphorylating respiration of mitochondria, coupled with the ATP synthesis, in the tissues of different groups of animals. However, later, another form of mitochondrial respiration was established - not coupled with ATP synthesis.[16,21,23] This form of respiration was very active in mitochondria of warm-blooded animals and was low in the mitochondria of cold-blooded ones. It could be associated with tissue thermogenesis and could participate in the ensuring the warm-bloodedness of the body. However, uncoupled respiration can result from mitochondrial damage when they are isolated.

Lehninger warned about this in his work[29], which negatively influenced the course of research in this direction. Proving the nativeness of this form of respiration was not an easy question. To establish the nativeness of this respiration, studies of uncoupled respiration were carried out on a cellular preparation - on cardiocytes.[10,30] In such a preparation, mitochondria are not damaged by homogenization procedures. It turned out that mitochondria inside intact cardiocytes also have uncoupled respiration, like isolated mitochondria.[10,20,30], and the intensity of both phosphorylated and uncoupled respiration does not differ quantitatively.

The foregoing research provided the basis for further research in this direction using different animals.

As a continuation of these studies, this work compares the respiration of mitochondria of different animals living in our area.

Table 2 shows the results of these studies, from which it can be seen that the mitochondria of tissues (liver) of a warm-blooded rat have active phosphorylating respiration, as indicated by high respiration control and ADP/O on succinate.

Table 2: Phosphorylating respiration of mitochondria of various tissues in marsh frogs and rats (substrate - succinate 4 Mm).

Tissue mitochondria	V_3	V_4	RC	ADP/O
Rat tissue mitochondria				
Liver	88,2±4,6	21,3±2,4	4.2	1,8±0,2
Heart	194.2±8,7	86,4±3,5	2,25	1,6±0,1
Skeletal muscle	117,±12,1	57,0±5,8	2.,1	1,6±0,3
Tissue mitochondria of marsh frog				
Liver	68,2±1.8	16.1±2,3	4,2	1,8±0,3
Heart	108,4±5,8	28,2±3,2	3,8	1,7±0,1
Skeletal muscle	41,6±3,4	11,9±2,1	3,5	1,7±0,21
Tissue mitochondria of turtle				
Liver	46,4± 3,1	14,4± 1,8	3,27	1,8±0,3
Heart	52,4±4,4	14,5±1,2	3,7	1,7± 0,6
Skeletal muscle	30,2±3,2	8,4±1,6	3,6	1,7±0,1

Note: V2, V3, V4 - respiration rates of mitochondria in nanograms of oxygen atoms per minute per milligram of protein - (ng-at O / min mg of protein).

However, in mitochondria of the heart and skeletal muscles, RC has a lower level than liver mitochondria. Consequently, in mitochondria of muscle tissues, it is manifested on succinate as substrate; both phosphorylating and high uncoupled respirations are manifested.

A comparative study of tissues' mitochondria of cold-blooded marsh frogs and steppe turtles showed that in this case the oxidation of succinate proceeds more slowly. In particular, in liver mitochondria of marsh frogs less than twice, in the liver of turtles - more than two times lower than in mitochondria of rat liver. In the cardiac mitochondria, it is about three times lower; in mitochondria of skeletal muscles, it is about four times lower than in warm-blooded rats. A difference between the animals increases due to the increase in uncoupled respiration in warm-blooded organisms.

Further, uncoupled respiration was studied on the NADH as a substrate, which usually does not penetrate into phosphorylating mitochondria and is oxidized by highly permeable non-phosphorylating mitochondria.

Table 3: Uncoupled oxidation of NADH and ascorbate in liver and cardiac mitochondria of different animals.

Different tissues	NADH	NADH+ cytochrome c	Ascorbate+ cytochrome c
Mitochondria of rat tissues			
Liver mitochondria	32.8±3,4	62.6±5,7	84.2±6,5
Cardiac mitochondria	105.7±8,5	165.4±9,4	204.2±12,8
Mitochondria of skeletal muscles	52.6± 3,2	85.8±5,4	115.7±7,5
Mitochondria of marsh frog tissues			
Liver mitochondria	10.21±0,8	14.81±1,2	22.31±1.6
Cardiac mitochondria	24.21±1,9	38.21±2,5	51.51±4,6
Mitochondria of skeletal muscles	15.21±1.4	21.81± 1,8	32.61±2,6
Mitochondria of steppe turtle tissues			
Liver mitochondria	4.41±0,6	6.41±0,9	13.31±1.6
Cardiac mitochondria	9.2±1,2	19.6±1,6	42.6±3,5
Mitochondria of skeletal muscles	6.4±0,8	11.3±1,1	18.6±1.6

According to our data, turtles and ground squirrels differ by up to 17 times in reduced metabolic rate. When compared to laboratory turtles with rats, the differences increase more than 20 times. It is possible that such a difference in steppe turtles is due to the ecological characteristics of theirs life. In general, our results are consistent with the above literature and show that cold-blooded organisms are characterized by their existence with a low metabolic rate.

A number of physiologists[7,8,9] did not pay attention to cold-blooded animals for a long time and believed that, in general, the metabolism in the animal world proceeds with a low-efficiency.[7,8,16,10] However, the data obtained on the low level of metabolism in cold-blooded organisms[1,2,3,4,5,6] had important consequences - on the possibility of using metabolic energy with high-efficiency in the body of cold-blooded animals than in warm-blooded ones. Experiments have shown[13,14,15] that the efficiency of energy use in cold-blooded animals when performing work was 2-4 times higher than in warm-blooded animals. Consequently, their low metabolism can have a certain physiological significance. In particular, in cold-blooded animals, the metabolism may not be associated with heat production. In warm-blooded animals, on the contrary, a high level of metabolism can be associated with heat production. Our additional analyses made it possible to reveal that phosphorylating respiration and uncoupled respiration are carried out in different populations of mitochondria.[10,20]

Considering the importance of the role of mitochondria in metabolism, we studied mitochondrial respiration. Our findings showed that mitochondria of warm-blooded animals are characterized by the functioning of highly active uncoupled respiration (without ATP synthesis), which is about 8 times higher than in cold-blooded organisms. Such a large difference is an indication that such an uncoupled respiratory system can participate in such an energy-intensive process as providing animals with a warm-blooded state. As it is known[31,32,33,34], endothermic organisms consume approximately 8-10 times more oxygen and they emit more heat than cold-blooded organisms. Therefore, it can be assumed that the established system of uncoupled respiration is directly related to thermogenesis that provides the endothermic state.

CONCLUSION

In the conclusion, considering the importance of the role of mitochondria in metabolism, the mitochondrial respiration are shown. Authors' findings showed that mitochondria of warm-blooded animals are characterized by the functioning of highly active uncoupled respiration without ATP synthesis), which is about 8 times higher than in cold-blooded organisms. Such a large difference is an indication that such an uncoupled respiratory system can participate in such an energy-intensive process as providing animals with a warm-blooded state. As it is known endothermic organisms consume approximately 8-10 times more oxygen and they emit more heat than cold-blooded organisms. Therefore, it can be assumed, that the established system of uncoupled respiration is directly related to thermogenesis that provides the endothermic state.

CONFLICTS OF INTERESTS

The authors declare that they have no any conflict of interests.

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