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The Cytologic Investigation of Brown Adipose Tissue of *D.laniger* (Felten & Storch, 1968) (Mammalia: Rodentia) in Hibernation

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ABSTRACT

This study was conducted on the *D.laniger* samples maintained in the laboratory condition after collecting from the natural environment. Investigations were performed in the experimental groups in the active period at the time of hibernation and at the period of intermediate awakening during hibernation. The brown adipose tissue (BAT) of these animals investigated was removed by dissection. The tissue samples dissected were prepared for the analysis at Transmission Electron Microscopy (TEM) and photographed. It was observed for the *D. laniger* BAT cells in the active period that there are high amount mitochondria and whereas the scarce amount of the lipid droplets. However, it was drawn attention to BAT cell of animals in hibernation that there plenty of capillary vessels and lipid droplets between them. The contact between lipid droplets with each other in the cytoplasm of animal at intermediate awakening in the winter during hibernation and cytoplasmic material loss and melting of mitochondria cristae partly were observed. These results of this study suggest that it may be useful to understand the importance of the brown adipose tissue of some hibernating mammals.

Key Words:
Dryomys Laniger; Brown Adipose Tissue; Mitochondria; Hibernation.

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INTRODUCTION

Hibernation and torpor

All living things show various adaptations to cope with the challenges in the environmental conditions. With the changes in the temperature at winter, the challenges arise such as to find food to meet the required amount of energy. Some of the behavioral characteristics of the animals are intended to prevent or to reduce the heat loss from the body (1). The selection of suitable feeding grounds with the desired thermal property is one of the behaviors that reduces heat loss. Nest construction and also behaviors to come together are the behaviors that are aimed at maintaining the body temperature. Most living things are somnolent and minimize their energy requirements by lowering metabolism in order to overcome these kinds of difficulties (1). Mammals has been evolved as having the proficiency to keep the body temperature higher than that of the environment (ambient) and at that level constant using the heat produced by internal origin (endogenous) (2). The hibernation is a kind of lethargy and sleepiness state to conserve energy and to get rid of unsuitable conditions in winter. It is a seasonal activity routinely and estimated that begins with the arrival of winter. The warm-blooded animals have an inherent response mode for cold winter conditions. These animals show long sleep periods, reducing metabolic rate and lowering the body temperature (3-7). Their metabolic rate at the time of deep sleep is much slower than that of the normal daily life(8,9).

The period of inactivity and sleepiness at which the metabolism is extremely slow, and thus the body temperature decreases, hereafter the heart rate decreases is called torpor. The hibernation also known as seasonal sleep consists of the total of torpor phases in a specific subsequent order (7,10,11).

In winter, at regular intervals, the short awakening

times are observed to eat the nutrients stored in the nest and to excrete. The body temperature is raised to survive during these awakening times. These short awakening times become increasingly rare towards the middle of winter, the duration of torpor increases, whereas the torpor durations decreases towards the spring and the animals remain awake for longer periods of time. This model is available for all of the living things having real hibernation (9, 11, 12).

The cytology of adipose tissue in mammals

There are 2 types of adipose tissue known with different location, structure and color. Their distribution in the body, the color, the relationship with the veins and the metabolic activity are different (13). White (unilocular) adipose tissue (adipose tissue made up of cells with a single space), when fully developed, are made up of cells containing a yellow big lipid drop in the middle of cytoplasm. Brown adipose tissue (multilocular adipose tissue) is made of cells including a plenty of lipid droplets and copious amounts of brown mitochondria. The amount of BAT owned by animals hibernating or surviving in a cold environment is quite high as compared to others (13). Both types of the adipose tissues mentioned have also a rich blood flow.

There are many reports published by many researchers reporting the distinguishing features between white and brown adipose tissue. The most important difference between these two tissues is that brown adipose tissue has more than one (multiloculer) lipid droplets, whereas the white adipose tissue has only one (uniloculer).

The BAT cells are rich in terms of mitochondria. White adipose tissue has mitochondria in the cell size of 0.3 μ and a small number of, on the contrary brown lipid cell has the one in the cell size of 0.5 μ and many. The cristae extends along the entire width of the mitochondrion. The mitochondrion has tightly arranged cristae with the amount of approximately 8-15 extending along the mitochondria which are 1 μ in lengths. The cristae is slightly curved. This case is the phenomenon observed in the mitochondrion of many active tissue cells. The mitochondrion is oval or rounded in shape (14).

In addition to a big lipid droplet observed via light microscope, the presence of small lipid droplets in each adipose cell was proven by the electron microscopy studies. There is no membrane around the small lipid droplets. There is basal lamina around each adipose cell (15). It was reported that there are a plenty of free ribosomes in the brown adipose cells of young and adult animals, and polysomes can be monitored partly (16.17).

Physiology of brown adipose tissue

All mammalian brown adipose tissue has the same structural appearance. The physiology of this tissue was understood comprehensively for hibernating animals (18). Brown adipose tissue is used especially by hibernating animals as a source of energy for the continuity of life during sleep. This tissue located especially in the dorsa and neck area of hibernating animals is vital for living things. It is usually located around the shoulders of rats and a few other mammals. This tissue is seen in a few places in the human embryo and newborn babies, moreover, it is known that this tissue remains in some places after birth. It becomes crucial because of the fact that it protects newborn against the cold by creating heat in the first few months usually after birth. Brown adipose tissue cells generate heat by the oxidation of fatty acids stored in lipids (19). The main function of the brown adipose tissue is to produce heat. It is estimated that the maximum respiratory capacity of that tissue is 10 times greater than that of skeletal muscle (20).

In the case of a real hibernator, the body temperature at the time of deep sleep can fall into the values of 3-4°C. Increasing the body temperature from the lower level to the previous higher level (36-37°C) during awakening takes a considerable time (9). The location of this heat production is the brown adipose tissue. The body is heated to a certain extend by the heat released from the brown adipose tissue stimulated (21,22). The protein named by thermogenin enables the release of energy from ATP as in the form heat energy. Later, the animal begins to tremble, heart beat and respiration rate rise and the animal wake up. (23,24).

When the studies are examined, there are more or less a few studies on hibernation and brown adipose tissue found, but most of these studies are about the physiology of this tissue (18,25,26). Nonetheless, ultrastructural studies on brown adipose tissue are fewer. In some of these studies, some important organs were examined using light and electron microscopy, morphometric, cytochemical and immunocytochemical approaches (27). Our cytological study on the BAT will contribute to literature with lack of such studies about the hibernation of mammals.

MATERIAL AND METHODS

This study was carried out on the *D.laniger* samples collected from the natural environment in Antalya, Elmalı district and stored in the laboratory. The first step in the investigation was to form different experimental group cared separately in different cages to determine the hibernation biology of specie. The animals were fed

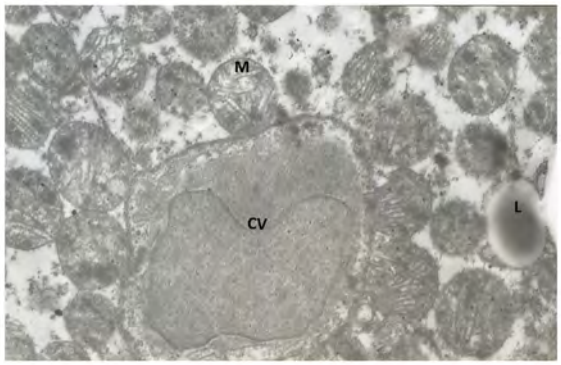

Figure 1. The brown adipose tissue of *Dryomys laniger* in active period. M**:** Mitochondria, **L:** Lipid Droplet, **CV:** Capillary Vessel (TEM, x 10.000)

on regular during controls carried out daily. *D.laniger* specimens were hosted in steel cages (37x17x12 cm) individually during the time they were in the laboratory. The odorless litter and cotton were placed in the cages as a bedding and nest material, respectively. In addition, enough water and feed were given to each of *D.laniger* specimens (28). The samples were taken via dissection from BAT of these animals. Dissection procedures were conducted through 3 different times as when some specimens were in active period, when some were in torpor and when some in intermediate awakening in winter.

The hibernation of *D.laniger* samples

During the experimental period, the temperature of the laboratory where the *D. laniger* samples were placed was ranged between 24°C-7°C. By the start of hibernation, the illumination period was set so as to have constant dark conditions and this condition was continued until the end of the heterothermal period. During this period, the experimental animals were not given food and water. This application, i.e. no to give food and water throughout the heterothermal period to the animals brought to the laboratory from the nature, is a standard for research on hibernation (28,29).

The preparation of slides for transmission electron microscope

First fixation was performed in phosphate buffered 3% glutaraldehyde (pH = 7.2, +4°C) for 4 hours for the *D.laniger* samples, removed by the dissection in the laboratory. Later on, it was washed every half hour for 2 hours only with the buffer solution used for fixation solution. Second detection operations were performed for 1 hour in 1% osmium tetroxide solution freshly prepared in phosphate buffer. After fixation, the block was prepared in resin. Block resin were mixed in an order and performed as follows:

Araldite CY – 212……..……..……..10cc
HY-964 (DDSA)…… ……..……..10cc
DY-064 (DMP-30)……..……..0,5cc
Dibutyl phthalate……..……..0,5cc

The polymerization process was performed at 45°C for 24 hours and at 60°C for 24 hours by placing samples into the resin. As a following process, thin and semi thin section were obtained using ultra microtome from these hardened blocks. After thin sections were taken on the grid, they were stained using uranyl acetate-lead citrate dye. Section prepared in this way were examined and photographed via JEOL 100 CX-II Transmission Electron Microscope.

RESULTS AND DISCUSSION

BAT has been investigated by several researchers with great interest for various animal species in terms of ontogenetic, histological, histochemical, electron microscopic, physiological and biochemical view (13,18). Brown adipose tissue is significantly important due to its role in regulating body temperature during hibernation. The color of this tissue is coming from blood vessels and cytochromes in mitochondria those it contains. Although white adipose tissue spreads throughout the body, brown adipose tissue is concentrated in the body's specific locations (20). In our study, the BAT has attracted markedly great attention as the brown color on the back of the animal in hibernation.

In our study, it was determined as a result of the examination of brown adipose tissue of *D.laniger* in active period that there are mitochondria in various sizes, capillary vessel and rarely present lipid droplets. Despite the abundance of mitochondria, it was observed a few lipid droplets in the cell. The most conspicuous formations in the *D. laniger* BAT cells are lipid droplets and mitochondria. The lack of cytoplasmic material in cell cytoplasm was drawn attention (Figure 1). During the active period, despite the excess mitochondria in the cells, the existence of a lack of the lipid droplets may be thought to be related with the energy usage in the active period (30). Similarly, some other authors have interpreted that this shortage is because of the usage of glycogen and lipid content of these cells and so reduces of the volume as a first response of brown adipose cells against cold effects (31).

There are mitochondria observed located between the spherical lipid droplets in BAT cell of *D.laniger* samples in torpor during the winter (Figure 2a and 2b). The number of lipid droplets in the cells of animals in torpor was higher as compared to those in the active period. It was observed that there are plenty of capillary vessels between the cells in the

general structure of the tissue (Figure 2a). The abundance of capillary vessels carrying blood to the brown adipose tissue has been expressed in other studies performed in rats (13).

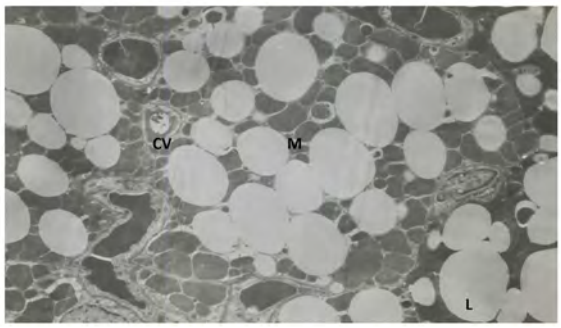

Figure 2a. The brown adipose tissue of *D.laniger* in torpor period. **M:** Mitochondria, **L:** Lipid Droplet, **CV:** Capillary Vessel (TEM, x 1900)

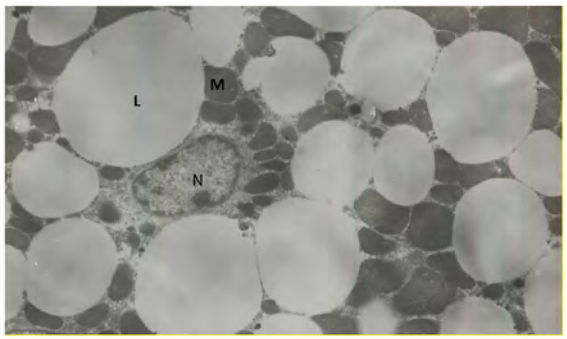

Figure 2b. The brown adipose tissue of *D.laniger* in torpor period. **M:** Mitochondria, **L:** Lipid Droplet, **N:** Nucleus (TEM, x 4800).

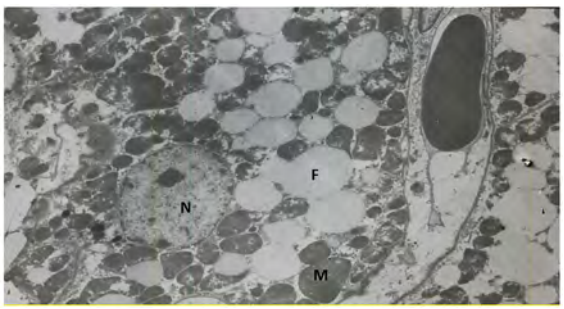

Figure 3a. The brown adipose tissue of *D.laniger* in intermediate awakening period in winter **N:** Nucleus, **F:** Fused lipid droplets, **M:** Mitochondria (TEM, x 2900).

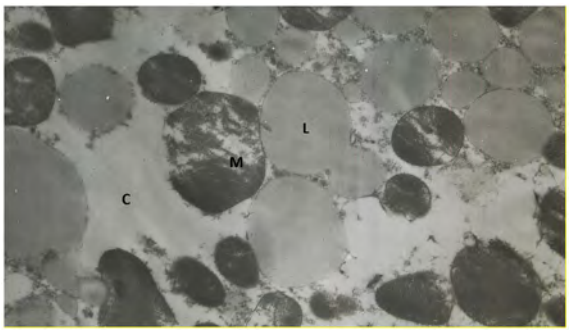

Figure 3b. The brown adipose tissue of *D.laniger* in intermediate awakening period in winter. **L:** Lipid droplets, **M:** Mitochondria, **C**: Cytoplasmic material loss (TEM, x 4800).

It was observed that the lipid droplets within the cytoplasm of adipose cells of *D. laniger* in intermediate awakening period during winter were in contact with each other and the nucleus has a spherical shape (Figure 3a). There are partly cytoplasmic material loss and lysis in the cristae of mitochondria observed (Figure 3b). It was found that brown adipose tissue of *D. laniger* sample in an intermediate awakening period of the hibernation has mitochondria in various sizes. With a higher magnification ratio in the SEM studies, it can be seen if an adipose cell considered in detail that there are damages in the cells attracted more attention (Figure 3b). Similarly, Çiftçi (32) has reported in a study conducted in rats that lipid droplets in the BAT cells are not wrapped with a membrane and these lipid droplets are combined with each other. The lipid droplets in BAT cells of the rat in his study were so great sometimes so that two or three of these droplets can cover the entire optic field of electron microscopy. In BAT of *D. laniger*, the big, rough and notched nucleus located near the center of the cell had no significant difference in terms of chromatin distribution as compared to those of the animals in active period. Çiftçi (1989b) has reported in another study he worked that there are no differences between lipid droplets and mitochondria of control group rats to which he has injected physiological saline solution and those of the experimental group.

There are some differences between the findings of the researchers working on the KYD of rats in the cold environment. Many of these differences may be dependent on the various factors such as the age of the animal in the cold, living conditions in the cage etc.

CONCLUSIONS

In this study, *D.laniger* samples were examined in uncontrolled laboratory temperature. Some of the animals have been observed during the summer in active period, some of them during winter in hibernation period and some others during the winter in the intermediate awakened periods. Brown adipose tissue fragments taken from the interscapular part of these samples were photographed in the Transmission Electron Microscope. When the *D. laniger* samples in active period were analyzed via images taken from electron microscopy it was determined that there are plenty of mitochondria in the BAT cells, but rarely the lipid droplets. The lack of cytoplasmic material of the cell cytoplasm was drawing attention. There were mitochondria observed located between spherical lipid droplets in various sizes due to the features of the adipose tissue in BAT cells of *D.laniger* samples in hibernation (torpor) period. There are plenty of capillary vessels observed between the cells in the general structure of the tissue. There were lysis of

cristae in mitochondria, contact between lipid droplets and partly cytoplasmic material loss observed in the *D.laniger* cells during the intermediate awakening period of hibernation.

The significant part of the biological richness of a country constitutes mammals. This study is important due to the fact that *D. laniger* is chosen because of being an endemic specie and there is no such study conducted before in the literature. The findings obtained from this study has enabled us to have information about brown adipose tissue of *D. laniger* in our country. Moreover, this study is also important because it gave us new information about hibernation encountered in some mammals. The work we have conducted here is an original study. The information obtained from this study is intended to form the basis for future hibernation related studies on this species.

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