<table>
<thead>
<tr>
<th>項目</th>
<th>内容</th>
</tr>
</thead>
<tbody>
<tr>
<td>項目</td>
<td>内容</td>
</tr>
<tr>
<td>項目</td>
<td>内容</td>
</tr>
<tr>
<td>項目</td>
<td>内容</td>
</tr>
<tr>
<td>項目</td>
<td>内容</td>
</tr>
</tbody>
</table>

タイトル：保温適応と寒冷期におけるアフガニスタンオショモキサギの体温リズムの消失

著者： Luo, Zong-Wei; Matsumoto, Takaaki; Ohwatari, Nobu; Shimazu, Munenori; Kosaka, Mitsuo


URL：http://hdl.handle.net/10069/4732

NAOSITE：長崎大学学術研究発表成果リポジトリ
Insulative Adaptation to Cold and Absence of Circadian Body Temperature Rhythm in Afghan Pikas
*(Ochotona rufescens rufescens)*

Zong-Wei LUO, Takaaki MATSUMOTO, Nobu OHWATARI, Munenori SHIMAZU and Mitsuo KOSAKA

*Department of Environmental Physiology, Institute of Tropical Medicine, Nagasaki University, Nagasaki 852, Japan*

**Abstract:** The pika inhabiting cold climate and high mountains is thought to be adapted to its environment. The autonomic characteristics of the high body temperature, high metabolism and poor heat loss ability such as poor panting, small ear pinnae and absence of thermal salivation as well as the behavioral characteristics, i.e. living in burrows under the ground or in the shelter among rocks and hoarding grasses for food to survive winter have been reported. However, insulative cold defense in pikas have not been studied and the pika's circadian activity rhythm remains controversial. In this study, the skin thickness and hairs length were histologically studied and the body temperature and locomotive activity were monitored in the freely moving pikas with a bio-telemetry system to clarify the pika's circadian rhythm. The hairs in the pikas were longer than the rats and more dense and 2 times longer on the dorsal region than the ventral region, though the skin was thinner. 20 mm of the hair length of the pikas was estimated to provide 3 Clo of insulative property. In contrast with the robust nocturnal rhythms in the rabbits and the rats, neither diurnal nor nocturnal body temperature rhythm was observed in the pikas, except for a slight increase at dawn and dusk consistent with the previous field observations. The pika is considered to be adaptive to cold not only ecologically and autonomically but also due to the insulative cold defense mechanisms. Though further investigation should be done, the absence of circadian body temperature rhythm in the pika is quite unique and attractive for the research field of circadian rhythm.

**Key words:** Pika, Circadian rhythm, Body temperature, Telemetry, Skin and hairs

**INTRODUCTION**

The pika, genus *Ochotona*, (for review see Smith *et al.*, 1990) lives in cold climate and in high mountains and is an ideal animal model for cold (Kawamichi, 1985; Yang *et al.*, 1988;
Yang, 1990) and high-altitude adaptation studies (Sakai, 1988; Sakai et al., 1994). It is considered that the pikas appeared in the cold area in the Pliocene and expanded southward during cold glacial periods, and retreated to the high mountains during warm interglacial periods in the Pleistocene. The current distribution of the pika ranges widely from low to extremely high altitude, about 10 to 6,100 m above sea level (Sakai et al., 1988), indicating that temperature not altitude is the limiting factor in the distribution of pikas. The pikas prefer low temperature. The temperature of their burrow is very low even in summer, about 12°C to 14°C (Haga, 1960). The pika is intolerant to heat (Smith, 1974; Yang, 1990). The ratio of ear surface area to body surface area is very small (Yang, 1990) and thermal panting is poor (Yang et al., 1988) compared to rabbits, though heat radiation through the ear pinnae and thermal panting are major heat loss responses in rabbits. The pikas do not spread saliva on their body surface when exposed to heat (Matsumoto et al., 1992). Body temperature and metabolic rate in pikas are higher than rabbits (Kosaka et al., 1985). Pikas do not hibernate, but survive severe cold winters. They live in burrows under the ground or in the shelter among rocks and boulders, and hoard grasses for food during winter. Thus due to autonomic (poor heat loss responses and high metabolism) and behavioral characteristics the pika is heat-intolerant and adaptive to cold.

Until now, however, the mechanisms of cold tolerance in pikas in not fully understood. In this study, the skin and hairs of the pikas were analyzed from the view point of insulative adaptation to cold. Circadian activity rhythm in pikas is controversial, although rabbits and hares, which belong to the same order Lagomorpha as the pika, are known to be nocturnal. Previously, circadian activity of the pika was studied mainly by an observation in the field. Telemetry recording of the body temperature is an excellent tool to investigate circadian rhythm. Matsumoto et al. (1995) recorded the body temperature of wild Mongolian pikas (Ochotona daurica) in their natural burrows with a telemetry device in Mongolia. The recording period was however short as several hours and they failed to reveal the body temperature rhythm, because the transmitter was relatively heavy for the wild pikas. Therefore, in this study, the body temperature and locomotive activity were monitored in the freely moving pikas in the laboratory with a bio-telemetry system to clarify the pika’s circadian rhythm.

MATERIALS AND METHODS

Since 1985, Afghan pikas (Ochotona rufescens rufescens) supplied from the Central Institute for Experimental Animals, Kawasaki, Japan were reared and bred in the Animal Research Center for Infectious Tropical Diseases, Institute of Tropical Medicine, Nagasaki University. Originally, they were collected in Afghanistan by Puget in 1969 (Puget, 1973a, b). The Afghan pika lives mainly in Afghanistan and Iran, and the distribution area is mainly located at high altitudes: 1,800–3,000 m above sea level. The pikas were reared in individual stainless steel cages at 22°C with a 12L: 12D light-dark photoperiod, with lights on at 0800 a.m. Tap water and special feed (Matsuzaki et al., 1980) were provided ad libitum.

Exp. 1: Anatomical study of the skin and hairs
Eleven Afghan pikas (5 males and 6 females, 185-210g, 18-19 months old) and 6 male Wistar rats (200-230g, 4 months old) were used in this experiment. The animal was sacrificed by an overdose of ether inhalation. After haircutting with electrical clippers, in 1.0cm × 1.0cm of skin was cut off from the chest, abdomen, back and waist. The skin specimens were fixed in 10% paraformaldehyde in 0.1M phosphate buffer, embedded in paraffin and stained with hematoxylin and eosin. The thickness of the skin, from the epidermis to the dermis, was measured with micrometer under a light-microscope. The density of the hairs in an area of $1.03\text{mm} \times 0.74\text{mm}$ were counted in the horizontal skin sections under a light-microscope. Several strands of hairs were pulled out from each region of the chest, abdomen, back and waist. The length of the hairs, from the hair papilla to the tip, were measured.

Exp. 2: Telemetry-recording of the circadian rhythm in the body temperature and locomotive activity

Six Afghan pikas (3 males and 3 females, 180-210g, 14-23 months old), 2 rabbits (one male and one female, 1.2kg, 3 months old) and 7 male Wistar rats (200-230g, 4 months old) were used in this experiment. Each animal was reared in an individual plastic cage (30 × 35 × 18 cm) with a wire-mesh top in a chamber set at 25°C and 60% rh with a 12L: 12D light-dark photoperiod, with lights on at 0600 a.m. A battery-operated transmitter (TA10TA-F20, Data Sciences Inc., St. Paul, MN, USA), 12 × 9 × 17 mm in size and 3.2g in weight, was intraperitoneally implanted under ketamine hydrochloride (35mg/kg body weight) anesthesia, 7-14 days before the experiment began. The body temperature and locomotive activity were recorded every one minute using a bio-telemetry system (Dataquest II, Data Sciences Inc.). Output of the transmitters was monitored by a mounted antenna (RLA2000, Data Sciences Inc.) placed under each animal’s cage and fed into a peripheral processor (BCM100, Data Sciences Inc.) connected to a personal computer (Prolinea 4/66, Compaq). The raw data saved in a disk was transformed to the reduced average of 30-min period. For each animal, circadian rhythm in the body temperature and locomotive activity were obtained by averaging the data for 7-14 days period. Mean pattern of circadian rhythm for each species was finally obtained by averaging the group.

The “Guidelines for Animal Experimentation” (Nagasaki University, 1989) and the “Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences” (The Physiological Society of Japan) were followed in the experiments.

Statistical analysis was performed with Students’ t-test and analysis of variance (ANOVA).

RESULTS

Exp. 1: Anatomical study of the skin and hairs

The mean skin thickness (±SD) in pikas were $0.293 \pm 0.070\text{mm}$, $0.214 \pm 0.027\text{mm}$, $0.488 \pm 0.083\text{mm}$ and $0.451 \pm 0.068\text{mm}$ on the chest, abdomen, back and waist, respectively. Those in rats were $0.673 \pm 0.117\text{mm}$, $0.657 \pm 0.141\text{mm}$, $1.000 \pm 0.104\text{mm}$ and $1.025 \pm 0.108\text{mm}$ on the chest, abdomen, back and waist, respectively (Fig. 1). The skin thickness of pikas was significantly thin compared to that of rats on the all of the 4 determined regions ($P<0.01$, unpaired t-test).
The mean hair length (±SD) in pikas were 10.38±1.72mm, 8.75±1.28mm, 20.15±1.61mm and 20.13±1.99mm on the chest, abdomen, back and waist, respectively. On the other hand, those in rats were 7.88±1.21mm, 7.70±1.04mm, 9.60±1.05mm and 9.49±2.61mm on the chest, abdomen, back and waist, respectively (Fig. 2). The hairs on the back and waist were longer in pikas than in rats (P<0.01, unpaired t-test).
The mean density (±SD) of the hair on the chest, abdomen, back and waist in pikas were 34.4±9.3mm⁻², 26.7±10.5mm⁻², 53.6±14.0mm⁻² and 61.55±16.0mm⁻², respectively (Fig. 3).

![Hair density](image)

**Fig. 3.** Comparison of hair density among the regions in the pikas. Vertical error bar is SD (n=11).

Exp. 2: Telemetry-recording of the circadian rhythm in the body temperature and locomotive activity

Circadian changes in the body temperature and locomotive activity in the pikas, rabbits and rats are shown in Fig. 4. The rabbits and rats showed the nocturnal rhythm of the body temperature. The mean body temperature (±SD) was 39.62±0.02°C during light-period and 39.94±0.01°C during dark-period (P<0.05, unpaired t-test) in rabbits and 37.05±0.13°C during light-period and 37.67±0.15°C during dark-period (P<0.01, unpaired t-test) in rats. The amplitude of the daily oscillation was 0.70±0.01°C in rabbits, and 1.19±0.22°C in rats. The acrophase was 12.25±0.01 hours after the lights were turned on in rabbits and 15.75±1.65 hours in rats. In rats, the locomotive activity also showed a significant difference between photo-periods, 0.4±0.1 (relative unit) during light-period and 1.7±0.2 during dark-period (P<0.01, unpaired t-test). On the contrary, the pikas did not show diurnal nor nocturnal rhythm in the body temperature. Mean body temperature was 39.05±0.13°C and 39.04±0.18°C during light-period and during dark-period, respectively (P>0.05, unpaired t-test). In pikas, however, the body temperature seemed to slightly rise at dawn and dusk accompanying with a distinct increase in the locomotive activity at least at dawn. The difference in the locomotive activity between photo-periods was not statistically significant. The mean difference (±SD) between the highest and lowest body temperature was 0.43±0.14°C in the pikas, and significantly smaller than the amplitude of the daily oscillation in the rats' body temperature (P<0.01, ANOVA). Daily variations of the body temperature and locomotive activity in the individual pikas were shown in Fig. 5. In the pikas-01, 03, 04 and 05, two-peak pattern, a slight rise at dawn and dusk, or mono-peak pattern at dawn in the
body temperature seems to be observed, however unclear in the pikas-02 and 06. There was a significant differences between the body temperatures during light-period and during dark-period, higher during dark-period in pika-03 and higher during light-period in pika-04, but not in the others.

Fig. 4. Circadian changes in the body temperature and locomotive activity (relative units) in the pikas, rabbits and rats. Each dot represents a mean value (n=6 for pikas, n=2 for rabbits and n=7 for rats) and vertical error bar is SD. Shaded columns represent dark-period. Robust nocturnal circadian rhythm in the body temperature was observed in the rabbits and the rats. The pikas did not show any circadian rhythm in body temperature, except for a slight rise at dawn and dusk. The low activity in rabbits may have been influenced by the narrowness of the cages.

Fig. 5. Daily variations in the body temperature and locomotive activity (relative units) in individual pikas. Each dot represents a mean value during the period (7-14 days) and vertical error bar is SD.
DISCUSSION

In human beings and animals without hairs, subcutaneous fat plays a role as the insulative barrier against cold. In animals with fur and hairs, however, insulation depends on the fur not on the subcutaneous fat (Hori et al., 1977). In the present study, the skin thickness was thinner but the hair length was longer in the pikas than those in the rats (Figs. 2 and 3). Furthermore, the hair length on the 4 determined regions were nearly identical in the rats, however, the hair length on the dorsal region (back and waist) was about 2 times longer than those on the ventral region (chest and abdomen) in the pikas (Fig. 3). Additionally, hair density on the dorsal surface of the pikas was also higher than those of the ventral surface (Fig. 4). These results indicate that the pika is more adaptive to the cold than the rat from insulation point of view.

Animals living in cold area develop thick fur to prevent heat loss from the body to the environment. Fur insulation is a function of fur thickness, from a fraction of an inch in thickness in shrew to nearly 3 inches of the thickness in sheep (Scholander et al., 1950). According to their report, 20mm of the hair length of the pikas provides 3 Clo of insulative property, nearly the same as those of the squirrel and the lemming but less than those of the rabbit and dog. The present study was performed with summer furs in the pika, but they studied the winter furs. Recently, Ohwatari et al. (1996) reported that the pikas’ fur is composed of two layers, inner layer with the short dark hairs and outer layer with the longer brawn hairs, and that such structure of the fur is convenient for protection against UV rays.

The relation between surface area and mass plays an important part in the effectiveness of insulation. Small animals with a large surface-to-mass ratio loose more heat in the cold compared to large animals. The large animals shows the large increase in insulation with their thick winter fur, however, the small animals do not show such a large increase in insulation (Hart, 1956). Small mammals less than 2kg with a small seasonal variation of the fur thickness need to develop some behavioral thermoregulation such as gathering together or living in the burrow during cold winters (Tokura, 1981).

Some animals living in the cold area hibernate to survive winters. The hibernators such as marmots, chipmunks, golden-mantled squirrels and ground squirrels are the neighbors of the pikas in North America (Broadbooks, 1965). The pikas do not hibernate (Haga, 1960, Dawson, 1981), therefore, the pikas should develop characteristic strategies to survive in the cold, other than hibernation. They live in the burrows or in the shelter among rocks and boulders, and hoard grasses for food during winter. Thus the pikas are thought to be ecologically adapted to cold environments.

Another focus in this study is the circadian rhythm in the pikas. The pikas have been reported to be diurnal, most active at dawn and dusk (Kawamichi, 1985, Smith et al, 1990), mainly diurnal (Broadbooks, 1965), active in day and night, especially in dawn and dusk (Haga, 1960, Kawamichi, 1969) after the field observation, and nocturnal (Kosaka et al., 1988) after the recording of locomotor activity in the laboratory. In this study, the body temperature and locomotive activity in freely moving pikas under a 12L: 12D light-dark photoperiod were moni-
tored with a bio-telemetry device to investigate circadian rhythm. The pikas did not show any circadian temperature rhythm, except for a slight rise at dawn and dusk, in contrast with the robust nocturnal rhythms observed in the rabbits and the rats (Fig. 4). A tendency of higher temperature at dawn and dusk confirms the previous observations in the field, however, absence of circadian rhythm in the body temperature in the pikas is quite unique. Birds and mammals maintained in a constant-temperature environment exhibit a daily rhythm of body temperature with the approximate shape of cosine wave and an amplitude of 1–3°C (Refinetti & Menaker, 1992). Some of the pikas in this study showed a crepuscular pattern, two-peak at dawn and dusk or mono-peak at dawn in the body temperature (Fig. 5), however, the amplitude of the daily variation in the body temperature was quite low as 0.4°C.

A reduction in the amplitude of the circadian rhythm of body temperature in old age was reported in rodents (Refinetti et al., 1990) as well as humans (Touitou et al., 1986). Life span of the pika was reported to be around 100 weeks in the Afghan pikas, Ochotona rufescens rufescens (Yang, 1990), and 3–7 years in the American pikas, Ochotona princeps (Smith, 1974). Relatively old age in the pikas used in this study may have influenced the absence or low amplitude of circadian body temperature rhythm in the pikas, however, no distinct circadian rhythm in the body temperature was observed even in the relatively young (14–16 months old) pikas, (pikas-02, 03 and 05 in Fig. 5). The body temperature rhythm is masked by the social cues and physical activities. The plasma melatonin rhythm is a more reliable reference of the circadian pacemaker than the body temperature (Honma et al., 1995). Plasma melatonin rhythm and also circadian rhythm under the freerun condition should be examined to further confirm absence of circadian rhythm in the body temperature of pikas.

In conclusion, insulative adaptation to cold was demonstrated in the pika. The pika is considered to be adaptive to the cold not only ecologically and autonomically but also due to the insulative cold defense mechanisms. The second important finding is absence of circadian rhythm in the body temperature and locomotive activity, except for a slight rise at dawn and dusk in the pika. Although further investigation should be done to confirm, the present findings in pikas are quite unique and might be a novel focus in the research field of circadian rhythm.

ACKNOWLEDGMENT

The authors thank Prof. H. Itakura and Dr. M. Senba, Department of Pathology, Institute of Tropical Medicine, Nagasaki University, for their guidance in the histological analysis. This work was partly supported by Grants-in-Aid for Scientific Research (No. 04304029, 06271253, 06557007) from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES

clopedia Britannica, Inc., Chicago.

Altitude Medical Science. Shinshu University, Matsumoto, Japan.


