Nagoya J. med. Sci. 36: 79-89, 1974

THERMOREGULATORY RESPONSES IN NORMAL AND COLD ACCLIMATED RABBITS

TETSUO NAGASAKA

The Research Institute of Environmental Medicine, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464

ABSTRACT

Metabolic rate (M), tissue conductance (k), rectal (T_{re}) and mean skin temperature ($\overline{T_s}$) were measured in normal (CONT) and cold acclimated (CA) rabbits after steady state for a given ambient temperature (T_a) from 5 to 35°C. Below T_a=15°C, T_{re} of CONT decreased considerably, while T_{re} of CA was maintained within a normal range. The rate of change in $\overline{T_s}$ for each degree change in T_a (below T_a=15°C) was smaller in CA. M, always higher in CA than in CONT at any T_a, increased more than twice the basal value (2.54 W/kg±0.06 SE for CONT and 2.85 W/kg±0.07 SE for CA at T_a=25°C) during cold exposure (T_a=5°C). Though k was relatively constant at T_a=25−5°C, it increased considerably at T_a=35°C. The value for CA was higher than that for CONT at any T_a. Perfusion of norepinephrine (NE, 3 μg/kg•min for 30-min) in curarized rabbits caused bradycardia, which was more conspicuous in CONT. NE caused an increase in M (*ca.* 18%) in CA without any change in CONT ($p<0.01$). The increase in M was not limited to the duration of NE perfusion. NE did not change body temperatures except for slight rise of $\overline{T_s}$ in CA. During cold exposure, body temperatures decreased continuously in the curarized rabbits. M increased slightly during the initial 30-40 min of cold exposure in CA. The results confirmed that the rabbit acclimates to cold by enhancing nonshivering thermogenesis mediated by NE, as well as by improving insulation.

INTRODUCTION

Rabbits, which have been used in studies of temperature regulation, can survive in severe cold for prolonged periods. In rabbits, nonshivering thermogenesis, mediated through norepinephrine, also appears to be augmented by cold exposures, but the magnitude of the calorigenic response to norepinephrine is not profound. According to Jansky *et al.*,[9] rabbits adapt to cold mainly by reducing heat loss from the skin. However, metabolic acclimation should be involved when the animal is exposed to more severe conditions in which homeothermy can not be maintained by insulation. In this study, rabbits were acclimated to 25°C and 0°C, and their thermal and metabolic responses

永 坂 鉄 夫
Received for publication July 14, 1973.

were measured in hot and cold environments to see how they acclimate to moderate cold. The role of norepinephrine in cold acclimation was also studied.

METHODS

Young male New Zealand white rabbits (Oryctolagus cuniculus), with an average weight of 2.2 kg at the beginning of this study, were used. The animals, provided *ad libitum* with water and commercial rabbit food (CR-1, NIHON CLEA Co.), were segregated into control (CONT) and cold acclimated (CA) groups. The CONTs were kept in a temperature controlled room in which ambient temperature was maintained at 25±2°C. Relative humidity (R.H.) in the room was 70-80%. The CA, composed of six rabbits, was placed in a cold environmental chamber (0±1°C, R.H. 90-100%). Before experiment, each group was exposed to its respective environment at least four months. Food consumption and body weight were checked once every week. The average growth rate was 0.38 kg/month in CA and 0.43 kg/month in CONT. Each rabbit was trained repeatedly to rest quietly in a canvas sling prior to the tests. In the canvas sling, the animal was allowed to freely move the fore- and hindlimbs without contact with the floor. All experiments were performed in winter (from January to March) from 9 : 00-16 : 00 hr. No food was allowed the morning of an experimental day.

In the first series of experiments, heat balance of the unanesthetized non-curarized rabbits was determined at an ambient temperature (T_a) of 5±1, 10±1, 15±1, 25±2 and 35±2°C. At 9 : 00 hr, each animal was transferred from each environment into a climate chamber in which temperature was first kept at 25±2°C. The velocity of the air, blowing from the ceiling, was approximately 1 m/sec near the animal. The animal was placed in the canvas sling and was allowed to equilibrate for at least one hour in the climate chamber before measurement. Metabolic rate was measured by a similar method described by Gonzalez *et al.*[5] A polythylene hood placed over the animal's head was continuously ventilated at the rate of 10-12 L/min by an air pump. The ears protruded from the hood without interfering with blood flow and heat loss from the ears. Oxygen consumption was measured with a paramagnetic O_2 analyzer (Beckman E_2). Heat production was calculated assuming an RQ of 0.74.[12] Respiratory heat loss was calculated from the rate of respiratory water loss of which measurement method was the same as reported previously.[13] Rectal (T_{re}) and skin temperatures were continuously recorded by means of copper-constantan thermocouples on an Ohkura potentiometer. The rectal thermocouple, enclosed in vinyl tubing, was inserted to a depth of 10 cm. The skin temperatures were recorded from the following four sites: the dorsal surface of the ear (T_e), the back (T_{ba}), forelimb (T_{fl}) and hindlimb (T_{hl}). After the fur of these sites was locally removed with a

depilatory, the skin thermocouples were placed with an adhesive tape (BAND-AID, Clear Tape). Mean skin temperature ($\overline{T}_s$) was calculated as $\overline{T}_s = 0.73\ T_{ba} + 0.07\ T_{fl} + 0.08\ T_{hl} + 0.12\ T_e$, the formula proposed by Gonzalez *et al.*[5] Heart rate was recorded on a Nikkor polygraph. In a single experiment, the animal was exposed to different ambient temperatures by either increasing or decreasing the chamber temperature in steps. After body temperature or oxygen consumption became constant, all data were collected in a given ambient temperature.

In the second series of experiments, thermoregulatory responses to norepinephrine (NE) were measured in the curarized rabbits. Either CONT or CA rabbit was brought into the laboratory (of which temperature was kept at 25±2°C) and anesthetized with sodium pentobarbital (25 mg/kg, i.v.). Following anesthesia, a tracheotomy was performed for artificial respiration. A small polyethylene catheter was inserted into the marginal vein of the ear for infusions. After the surgical procedures, the animal was placed in the canvas sling and transferred into the climate chamber, in which temperature was also kept at 25±2°C. One hour after anesthesia, the animal was paralyzed with gallamine triethiodide (1 mg/kg, every 20-min) and resuscitated. After 20 minutes resuscitation period under gallamine triethiodide, 3 μg/kg·min of NE, diluted with normal saline, was perfused intravenously for 30 minutes with an infusion pump (ATOM-LG 2). The volume of the NE solution perfused was 0.17 ml per minute. Oxygen consumption, heart rate and $\overline{T}_s$ and T_{re} were also measured every 10 minutes during the experiments. After 150 minutes at T_a of 25°C, the animals were exposed to an air temperature of 5±1°C for 70 minutes. Changes in oxygen consumption and T_{re} were also measured during this cold exposure.

RESULTS

Body temperatures. T_{re} and $\overline{T}_s$ under steady state conditions were plotted as a function of T_a in Fig. 1. In CONT, T_{re}, which was 38.94°C±0.11 SE at T_a=25°C, fell considerably in the cold environments. At 5°C, T_{re} was 37.39°C ±0.18 SE. In CA, T_{re} was regulated within the range of 38.6−38.3°C at T_a of 5-25°C. T_{re} increased significantly in hot environments in both CONT and CA ($p<0.01$). In the 15-35°C range of T_a, T_{re} of CONT was significantly higher ($p<0.05$) than that of CA. In CONT, $\overline{T}_s$ decreased linearly as T_a was decreased. The rate of change in $\overline{T}_s$ for each degree change in T_a was 0.35°C. In CA, however, this rate decreased to 0.26°C in the cold environments.

Heat production. The metabolic rate (M) of rabbits in conditions under present investigation are shown in Fig. 2. M, which was minimum (2.54 W/kg±0.06 SE for CONT and 2.85 W/kg±0.07 SE for CA) at T_a=25°C, increased steadily with reduction of T_a from 25°C to 5°C. The average metabolic rate

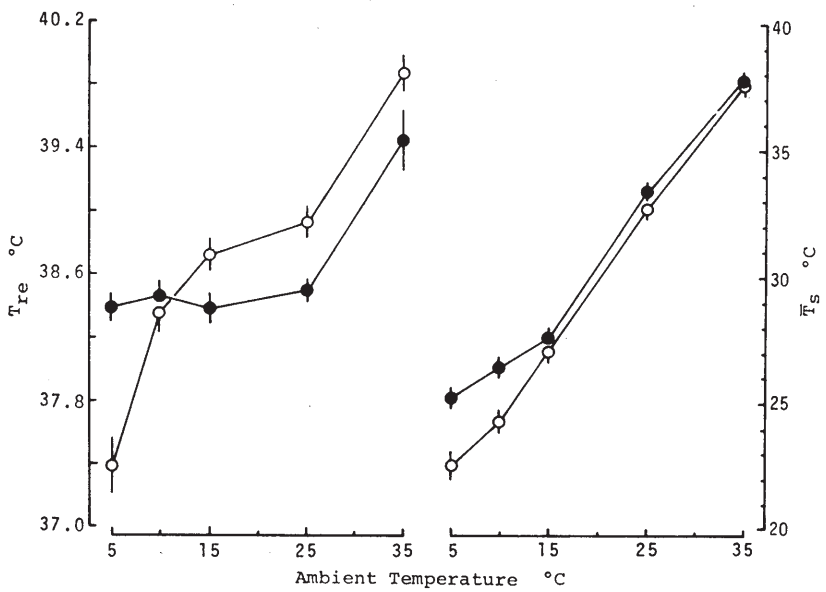

FIG. 1. Rectal (T_{re}) and mean skin temperature ($\overline{T}_s$) in cold acclimated (●, $n=6$) and control rabbits (○, $n=8$) plotted against ambient temperature. Vertical lines are ±SE.

was higher in CA than in CONT. At T_a of 15-5°C, M increased proportionately more with decreasing T_a in CA. The level of metabolism was slightly higher at 35°C than at 25°C. The heart rate was lowest at 25°C and increased in both the hot and cold environments. In CONT, however, the heart rate decreased considerably at T_a of 5°C. Shivering was observed in both CONT and CA animals in the environments maintained at temperatures lower than 15°C, but CA appeared to be shivering less than CONT.

Respiratory evaporative heat loss (E_{res}) *and tissue conductance* (k) (Fig. 3). At T_a=25°C, E_{res} was 0.43-0.47 W/kg in both CA and CONT. Panting was observed in hot environment of 35°C, and E_{res} increased by approximately 100% at this temperature. Heat loss from the respiratory tract fell slightly with decreasing T_a in both groups. However, there was no significant difference in the E_{res} in these two groups at T_a of 25-5°C. Body tissue conductance was calculated by the following equation: $k=(M-E_{res})/(T_{re}-\overline{T}_s)$ × surface area in W/m²•°C.[5)16)] Surface area was converted from body weight (1 kg= 0.07 m²).[5)] At any given T_a, k was higher in CA than CONT. At lower T_a of 5-15°C, the difference in k was not significant in both groups. At T_a=35°C, k increased greatly in CA (27.9 W/m²•°C). On the other hand, k in CONT was rather low even at T_a of 35°C.

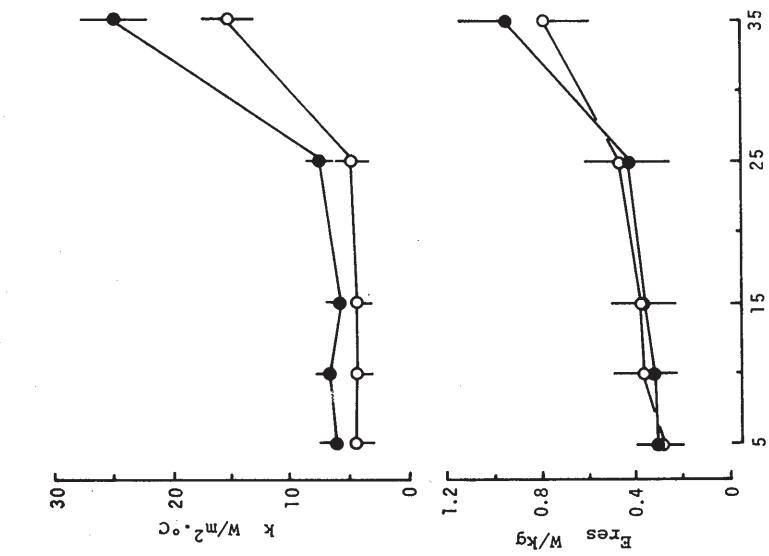

FIG. 3. Respiratory heat loss (E_{res}) and tissue conductance (k) in cold acclimated (●, $n=6$) and control rabbits (○, $n=8$) plotted against ambient temperature. Vertical lines are ±SE.

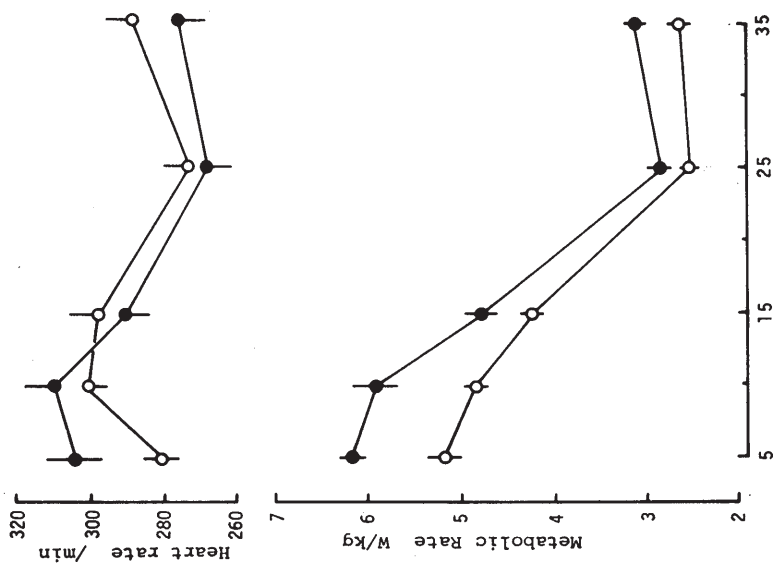

FIG. 2. Metabolic rate and heart rate in cold acclimated (●, $n=6$) and control rabbits (○, $n=8$) plotted against ambient temperature. Vertical lines are ±SE.

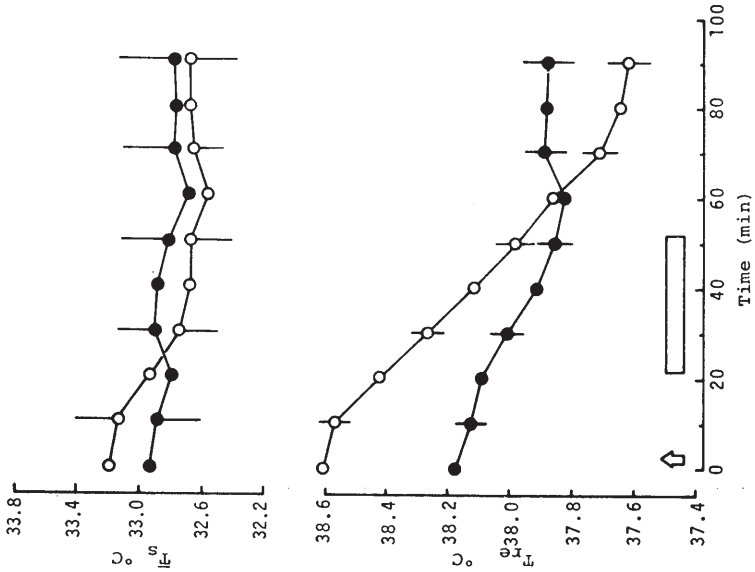

FIG. 5. Response of rectal (T_{re}) and mean skin temperature ($\overline{T}_s$) to perfused norepinephrine (3 μg/kg·min) in cold acclimated (●, $n=5$) and control rabbits (○, $n=6$). Arrow shows starting point of gallamine triethiodide infusion. Open bar indicates perfusion of norepinephrine. Vertical lines are ±SE.

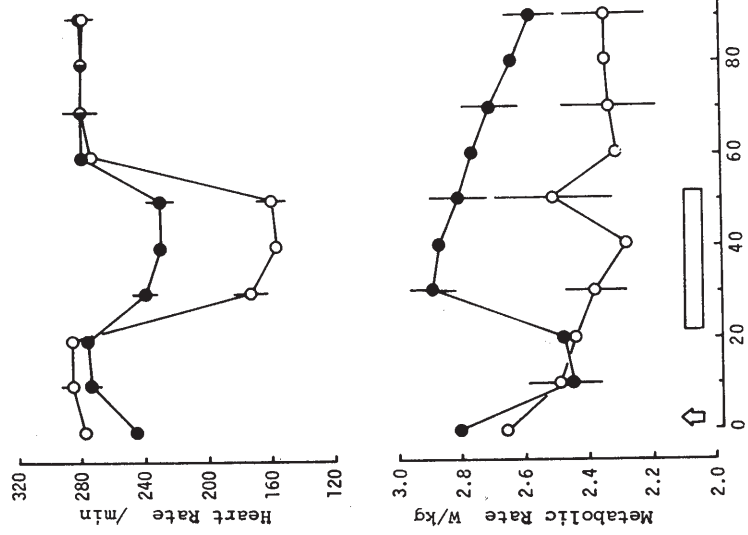

FIG. 4. Response of metabolic rate and heart rate to perfused norepinephrine (3 μg/kg·min) in cold acclimated (●, $n=5$) and control rabbits (○, $n=6$). Arrow shows starting point of gallamine triethiodide infusion. Open bar indicates perfusion of norepinephrine. Vertical lines are ±SE.

Effects of norepinephrine after curarization at 25°C (Fig. 4 and 5). Before curarization, average heart rate was 278/min±4 SE in CONT and 256/min±4 SE in CA. The infusion of gallamine triethiodide caused an increase in heart rate (HR) in both groups. The increase in HR with infusion of gallamine triethiodide was statistically significant in CA ($p<0.05$). The perfusion of NE caused a marked bradycardia in both groups. However, the decrease of the heart rate was more conspicuous in CONT ($p<0.01$) than CA. The bradycardia was limited to the duration of the NE perfusion in both groups. M, which was 2.66 W/kg±0.06 SE in CONT and 2.82 W/kg±0.07 SE in CA, decreased to 2.49 W/kg±0.11 SE and 2.45 W/kg±0.09 SE respectively by curarization. The decline of M after curarization was significant in CA ($p<0.05$). The NE perfusion caused an increase in M (ca. 18%) in CA with no effect in CONT. The increase in metabolism in CA was not limited to the duration of the drug perfusion. A higher metabolic rate was still maintained for 30-40 minutes

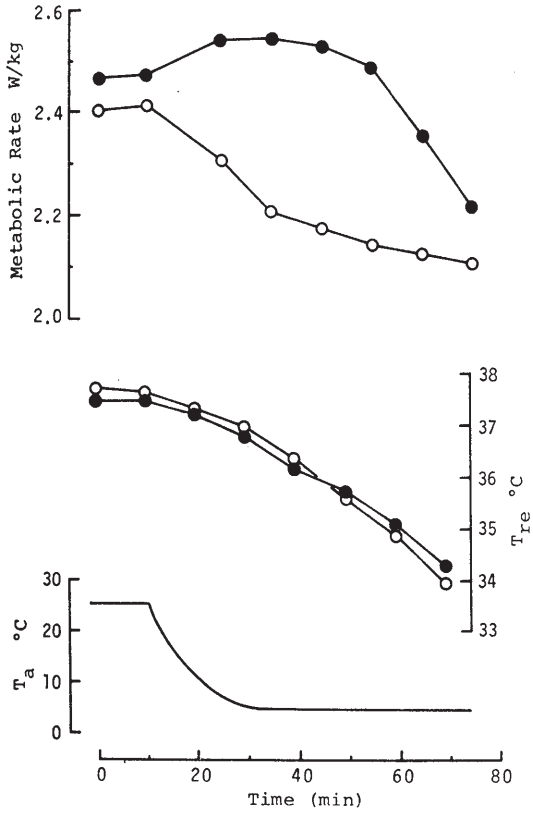

FIG. 6. Changes in metabolic rate and rectal temperature (T_{re}) in curarized cold acclimated (●, $n=4$) and control rabbits (○, $n=4$) to lowered ambient temperature ($T_a=5°C$).

following the end of the perfusion. During this period, M stayed at a new level lower than that before the NE perfusion in CONT.

T_{re} in both CONT and CA fell after the infusion of gallamine triethiodide. The rate of decrease in T_{re} was considerably larger in CONT. The perfusion of NE did not lessen the rate of fall in T_{re} in CONT. After the end of the NE perfusion, T_{re} increased very slightly in the CA animals. In CA, $\overline{T_s}$ increased slightly during the NE perfusion.

Effects of acute body cooling after curarization (Fig. 6). M of CA increased slightly during the initial 30-40 minutes of acute body cooling, and then decreased. On the other hand, M of CONT started to fall immediately following the body cooling. The difference between M of the two groups became greater during this initial period of body cooling than in $T_a=25°C$. T_{re} continued to fall during the period of body cooling. The thermal steady state was not obtained during this 70-min cold exposure in both groups.

DISCUSSION

At neutral environmental temperatures the internal body temperature was considerably lower in the cold acclimated rabbits than in the control rabbits reared at 25°C. The difference in T_{re} could not be explained by a difference in heat production because the metabolic rate was consistently higher in the cold acclimated rabbits. The thermal tissue conductance was, however, larger in the CA animals at these neutral environmental temperatures, so that the lower body temperature of the CA animals may be partly explained by this increased heat loss from the body surface. Below $T_a=15°C$, normal heat balance can not be maintained in the control rabbits. In this group, the internal body temperature decreased considerably at lower ambient temperatures. An increase in rectal temperature at lower ambient temperatures, as reported by Gonzalez *et al.*[5] in normal rabbits, was not observed here. In this investigation, the air velocity was approximately 1 m/sec near the animal, so that the discrepancy between the results obtained in "still air" by Gonzalez *et al.* and the results obtained here could be attributed to the difference in heat dissipation from the body surface due to the different air movement.

In the cold acclimated rabbits, body temperature was maintained at a level close to that at $T_a=25°C$. The rate of heat production increase was greater in the animals displaying somewhat reduced muscular activities. Since the evaporative heat loss from the respiratory tract did not differ from that in the control animals, and the difference between the T_{re} and $\overline{T_s}$ grew less and less, the tissue conductance must have increased greatly in these animals. Hart[6] summarizes the conductance in relation to heat conductivity of the fur in rodents. For most species, there is a direct correlation between conductance and fur conductivity. The results in this investigation may also support the

conclusion offered by Jansky *et al.*[9] that rabbits can adapt to cold by increasing insulation.

When the animal was exposed to T_a=35°C, T_{re} increased by approximately 1°C in both control and cold acclimated rabbits. Metabolic rate and tissue conductance were elevated more in the CA animals in this hot environment. As suggested by Gonzalez *et al.*,[5] the increase in heat production was greater than could be expected from a Q_{10} effect. Why the metabolic rate increased more in this group, and whether the increase in the central heat storage plays a role in facilitating heat dissipation as reported for squirrel monkeys[16] should be studied further. The lower critical temperature for metabolic increase appears to lie between 15-25°C of T_a, which corresponded to $\overline{T_s}$=27-33°C. However, in contrast to the data reported by Kockova and Jansky,[10] the metabolic heat production in the CA animals was always higher at any given T_a below this critical ambient temperature. Change in the heart rate parallels the change in the metabolic rate except at T_a=5°C. At this temperature, heart rate decreased considerably in CONT rabbits. This fall in heart rate may be due to the direct influence of hypothermia on the sinus node.

The dose of norepinephrine (3 μg/kg•min for 30-min) was chosen in this study because it was about maximum physiological dose beyond which the animal showed a marked cardiac arrhythmia. Perfusion of NE caused an increase in the metabolic rate in the cold acclimated rabbits, but not in the controls. The magnitude of the calorigenic action to norepinephrine was found to be 18% in this experiment. This value for nonshivering thermogenesis, approximately the same as that reported by Cottle,[3] was much lower compared with the increase in heat production in cold acclimated rats.[8,9] The failure to respond to NE in normal rabbits and the small values for nonshivering thermogenesis after cold acclimation were again confirmed.[4,14] Brown adipose tissue, which is a principal source of nonshivering thermogensis in newborns disappears gradually as the animal grows. After cold acclimation, however, Chaffee *et al.*[2] have observed brown fat in the adult squirrel monkey which has probably no such tissue when reared in neutral temperature environments. It is speculated that brown fat may be also the source of the nonshivering thermogenesis in cold acclimated rabbits. LeBlanc *et al.*[11] reported an enhanced metabolic response to catecholamines with a decrease in epididymal white fat in rats after cold acclimation. The role of white fat should be studied in cold acclimation in this species. Anatomical studies on the adipose tissue have not been performed in this experiment, so that it is not possible to conclude which tissue is more responsible for the increased calorigenic response during cold acclimation in rabbits.

The increased calorigenic response to NE was not limited to the perfusion period. These post-perfusion increases in heat production, also observed in dogs,[15] may be due to residual calorigenic effects of NE uptake in the various

tissues. Perfusion of NE did not raise body temperature after curarization. Enhanced heat production by 18% was not sufficient to maintain core temperature as observed in cold acclimated animals. The reasons why body temperature of curarized rabbits fell further during NE perfusion at a neutral temperature are unknown. Although sodium pentobarbital was injected at least one hour before measurements, this anesthetic may have interfered with temperature regulatory mechanisms in rabbits. Further fall in the metabolism in CONT animals during NE perfusion may be partly due to a Q_{10} effect. The mean skin temperature increased in CA animals but not in CONT, during NE perfusion. As reported previously,[4)7)14)] the decrease in heart rate was minimum in the cold acclimated rabbits, while in the control animals it was very conspiouous. Bartunkova and Jansky[1)] suggested that activation of β-adrenergic receptors in the cardiovascular system plays a role in cold acclimation. Repeated stimulation of β-adrenergic receptors by isoproterenol facilitated tachycardia and peripheral vasodilatation during NE infusion in rats.[11)] The changes in cardiovascular system, such as vasodilation and chronotropic effects of NE on the heart, may have been also enhanced by increased sensitivity of β-receptors to catecholamines through cold exposure in rabbits. The cold-induced nonshivering thermogenesis was observed when the curarized CA rabbits were exposed to 5°C, although the increase in T_{re} was not consistent.

REFERENCES

1) Bartunkova, R. and Jansky, L., Effect of cold and noradrenaline on cardiovascular changes in cold acclimated rats, *Intern. Congr. Physiol. Soc., 25th, 1971*, p. 9.
2) Chaffee, R. R., Allen, J. R., Brewer, M., Horvath, S. M., Mason, C. and Smith, R. E., Cellular physiology of cold- and heat-exposed squirrel monkeys (Saimiri sciurea), *J. Appl. Physiol.*, **21**, 151-157, 1966.
3) Cottle, W. H., Calorigenic response of cold-adapted rabbits to adrenaline and to noradrenaline, *Can. J. Biochem. Physiol.*, **41**, 1334-1337, 1963.
4) Gonzalez, R. R., Thermoregulatory responses to norepinephrine after inhibition of its synthesis in rabbits, *J. Appl. Physiol.*, **33**, 341-345, 1972.
5) Gonzalez, R. R., Kluger, M. J. and Hardy, J. D., Partitional calorimetry of the New Zealand white rabbit at temperatures 5-35°C, *J. Appl. Physiol.*, **31**, 728-734, 1971.
6) Hart, J. S., Rodents, In: Comparative physiology of thermoregulation, Vol. II, ed. C. C. Whittow, Academic Press, New York, pp. 74-81, 1971.
7) Honda, N., Judy, W. V. and Carlson, L. D.. Effects of adrenaline and noradrenaline on ear vessels in cold- and warm-adapted rabbits, *J. Appl. Physiol.*, **17**, 754-758, 1962.
8) Jansky, L., Bartunkova, R. and Zeisberger, E., Acclimation of the white rat to cold: noradrenaline thermogenesis, *Physiol. Bohemoslov.*, **16**, 366-372, 1967.
9) Jansky, L., Bartunkova, R., Mockova, J., Mejsner, J. and Zeisberger, E., Interspecies differences in cold adaptation and nonshivering thermogenesis, *Federation Proc.*, **28**, 1053-1058, 1969.
10) Kockova, J. and Jansky, L., Cold acclimation in the rabbits, *Physiol. Bohemoslov.*, **17**, 309-316, 1968.

11) LeBlanc, J., Vallieres, J. and Vachon, C., Beta-receptor sensitization by repeated injections of isoproterenol and by cold adaptation, *Am. J. Physiol.*, **222**, 1043-1046, 1972.
12) Lee, R. C., Size and basal metabolism of the adult rabbit., *J. Nutr.*, **18**, 489-500, 1939.
13) Nakayama, T., Hori, T., Nagasaka, T., Tokura, H. and Tadaki, E., Thermal and metabolic responses in the Japanese monkey at temperatures of 5-38°C, *J. Appl. Physiol.*, **31**, 332-337, 1971.
14) Nagasaka, T., Effects of daily infusions of noradrenaline on metabolism and skin temperature in rabbits, *J. Appl. Physiol.*, **32**, 199-202, 1972.
15) Nagasaka, T. and Carlson, L. D., Responses of cold- and warm-adapted dogs to infused norepinephrine and acute body cooling, *Am. J. Physiol.*, **209**, 227-230, 1965.
16) Stitt, J. T. and Hardy, J. D., Thermoregulation in the squirrel monkey (Saimiri sciurea), *J. Appl. Physiol.*, **31**, 48-54, 1971.