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Effect of Environmental Heat Exposure on Physiological Responses, Blood Constituents and Parameters of Blood Glucose Metabolism in Sheep

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Summary

Three clipped sheep were exposed to an ambient temperature of 30°C (relative humidity 70 per cent) for 10 days in the climatic chamber and their physiological responses, blood constituents and parameters of blood glucose metabolism were measured during the exposure period. The isotope dilution method using U-^{14}C-glucose was applied for the determination of glucose metabolism. The rate of food intake by sheep tended to decrease after the heat exposure. Two of three sheep ate up all the ration, but the last refused to eat on and after the 8th day of the heat exposure. The respiration rate increased remarkably (138 resp./min). Blood pH increased to 7.49 and Pco_2 decreased to 25 mmHg, indicating a typical respiratory alkalosis. Rectal temperature increased to 40.5°C, and heart rate, water intake and urine volume tended to increase. Plasma glucose and FFA concentrations did not exhibit clear changes. Plasma thyroxine decreased rapidly after the heat exposure, but after the third day the values slightly increased and maintained about 70 per cent of the control level. The haematocrit value tended to decrease. In the environmental temperature of 20°C, the pool size and turnover rate of blood glucose were 504.4 mg/kg$^{3/4}$ and 7.13 mg/kg$^{3/4}$/min, respectively. These values decreased to 474.8 mg/kg$^{3/4}$ and 5.50 mg/kg$^{3/4}$/min on the 4th day, and 445.8 mg/kg$^{3/4}$ and 5.92 mg/kg$^{3/4}$/min on the 10th day after the heat exposure. These results indicate that glucose utilization tends to decrease under the environmental condition of 30°C (R.H. 70 per cent).

During summer season, the milk yield decreases by 20 per cent compared with that of other seasons particularly in the southwestern district of Japan. This is one of the major problems which hinder to run the dairy farming successfully.

In order to clarify the cause of a decrease in the milk yield during summer, a considerable number of studies have been conducted on the effect of heat stress in relation to physiological responses, blood constituents, food intake, hormone secretions and other factors affecting the milk production in dairy cows (1, 2).

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Despite such studies, many questions still remain to be solved. In particular, little has been reproted concerning the effects of heat stress on the intermediary metabolism of glucose in the ruminant. It is well recognized that one of the nutritional differences of the ruminant from the nonruminant is that the ruminant must rely for its carbohydrate requirements almost entirely upon gluconeogenesis since the ingested carbohydrates are fermented largely to volatile fatty acids by the rumen microorganisms.

In considering the cause of the depression of milk production due to the heat stress, our attention focused on the fact that blood glucose plays a rate limiting role for the milk formation in the mammary gland of the ruminant. The present experiment was conducted to investigate the pattern of glucose metabolism and to make observations of changes in physiological responses and blood constituents in sheep exposed to high environmental temperature.

Materials and Methods

Experimental animals

Three Corriedale ewes, weighing 35.0 to 38.5 kg, were clipped closely (under 1 cm) before the experiment. The animals were kept in metabolic cages placed in an environment controled chamber with a temperature of 20°C for more than 20 days. Subsequently, the temperature in the chamber was elevated to 30°C with a relative humidity of 70 per cent, and the animals were exposed to this condition for 10 days. Each 200 g of alfalfa hay cube and commerical concentrate were given twice daily at 9:00 and 18:00. Therefore, each sheep was fed a ration of 800 g per day. Water was given *ad libitum.*

Experimental procedure

The observations of physiological responses and the collection of blood samples were performed just before the morning feeding. Blood samples were collected through polyethylene catheters inserted into the jugular vein. Immediately after the collection of blood samples, blood pH, Pco_2 and haematocrit value were measured. The residual blood samples were centrifuged and separated plasmas were frozen at –20°C until analysis.

The measurement of blood glucose metabolism using the isotope dilution method was made on the 4th and 10th day after 30°C exposure, and the results were compared with the value obtained in 20°C. The pool size and turnover rate of blood glucose were measured by a single injection of U-^{14}C-glucose. On the experimental day, 0.9 per cent NaCl solution containing 25 μCi of U-^{14}C-glucose was administrated at 13:00 within 10 seconds into the jugular vein through the catheter. After injection, blood samples were taken with intervals of 20 min for the first one hour and 30 min for the next one hour from the jugular vein through the catheter of the other side.

Analytical methods

Blood pH and Pco_2 were measured by the blood gas analyzer (Radiometer, BMS3–MK2). Plasma samples were analyzed for glucose (glucose oxidase method (3)), free fatty acids: FFA (Itaya & Ui method (4)) and thyroxine (columnar chromatography method (5)).

Isolation of plasma glucose: The plasma was added an equal volume of 12 per cent perchloric acid, and the protein precipitates were removed by centrifugation in the cold followed by the neutralization (pH 5.8) of the supernatant with 30 per cent KOH. After removal of the potassium perchlorate by centrifugation in the cold, the supernatant was treated twice with 1.0 g of a mixture (2:1 by weight) of Amberlite IR–4B and Dowex 50X8 for at least 30 min with occasional stirring. The resin mixture was removed by filtration.

Measurement of radioactivity of glucose: The resin filtrate (1.0 ml) was transfered to the counting vials, dried up in a desiccator, and dissolved in 0.5 ml of 1M Scintilamine-OH in methanol. After standing for more than 30 min at room temperature, 10 ml of scintillation solution (PPO: 2,5-diphenyloxazole 5.0 g., POPOP: 1,4-bis[2-(5-phenyloxazolyl)]-benzen 100 mg and toluene 1 liter) were added to the vials, and radioactivity of U-^{14}C-glucose was measured by liquid scintillation spectrometer (Aloka Model LSC 601). The amount of glucose in the resin filtrate was determined by the glucose oxidase method (3).

Calculation of parameters of glucose metabolism: The specific radioactivities of glucose were plotted on a semilogarithm graph, and plasma glucose pool size and turnover rate were calculated by the values of the specific radioactivity at zero-intercept which were obtained by extrapolation of specific activity-time lines and the values of the fractional turnover rates.

Results

1. *Physiological responses*

The rate of food intake by sheep tended to decrease after the heat exposure. Two (sheep A and B) of them ate up all the ration, but the last one (sheep C) refused to eat on and after the 8th day of the heat exposure.

Changes in physiological responses of the three sheep are shown in Fig. 1 and 2. Respiration rate increased remarkably from 19±1.2 in 20°C to 138±31.6 resp./min on the 7th day of the heat exposure. Heart rate slightly increased from 66±1.9 to 70±3.5 at the first half, and clearly increased to 89±28.5 beats/min at the second half of the heat exposure period. Rectal temperature increased about 0.5°C within 24 hrs. after the heat exposure, and maintained the same level of 39.4±0.1°C during the first half and increased further to 40.2±0.2°C during the second half of the exposure period.

Body weight changed little between before and after the heat exposure,

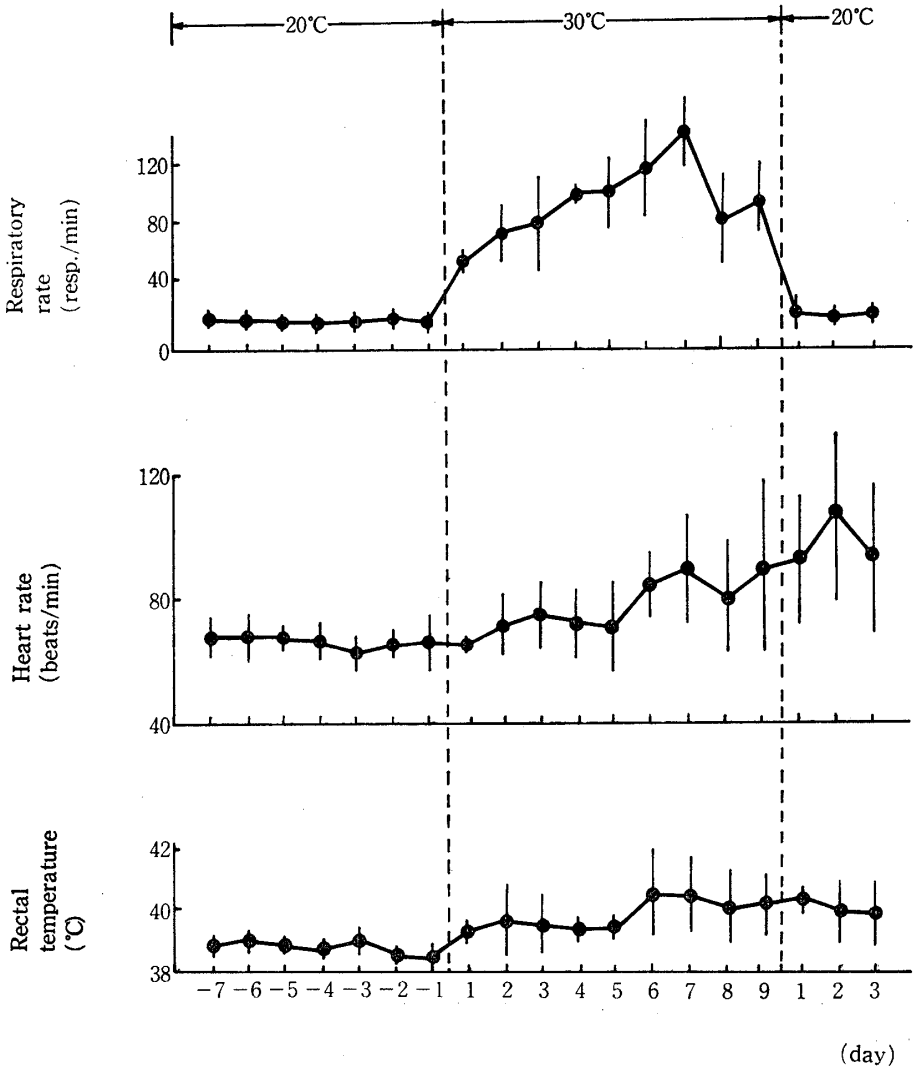

FIG. 1. Changes in physiological responses following the heat exposure in sheep. Data are expressed as means±SD.

except that of sheep C in which 2 kg of decrease was observed. Water intake and urine volume tended to increase from 2.2±0.6 and 1.3±0.2 to 4.2±1.0 l/day and 2.4±0.7 l/day, respectively, but no significance was found between the values.

2. Blood pH, Pco_2 and haematocrit value

Changes in blood pH, Pco_2 and haematocrit value are shown in Fig. 3.

Blood pH increased from 7.42±0.03 in 20°C to 7.54±0.03 within 24 hrs. after the heat exposure, and maintained a level of 7.49±0.02 after that time. On the other hand, blood Pco_2 gradually decreased from 34±1.3 in 20°C to the minimum value of 25±1.4 mmHg on the 7th day of the exposure. The haematocrit value gradually decreased from 27±1.7 in 20°C to the minimum value of 22±1.0 per cent on the 5th day of the exposure. The value of haematocrit in sheep C increased

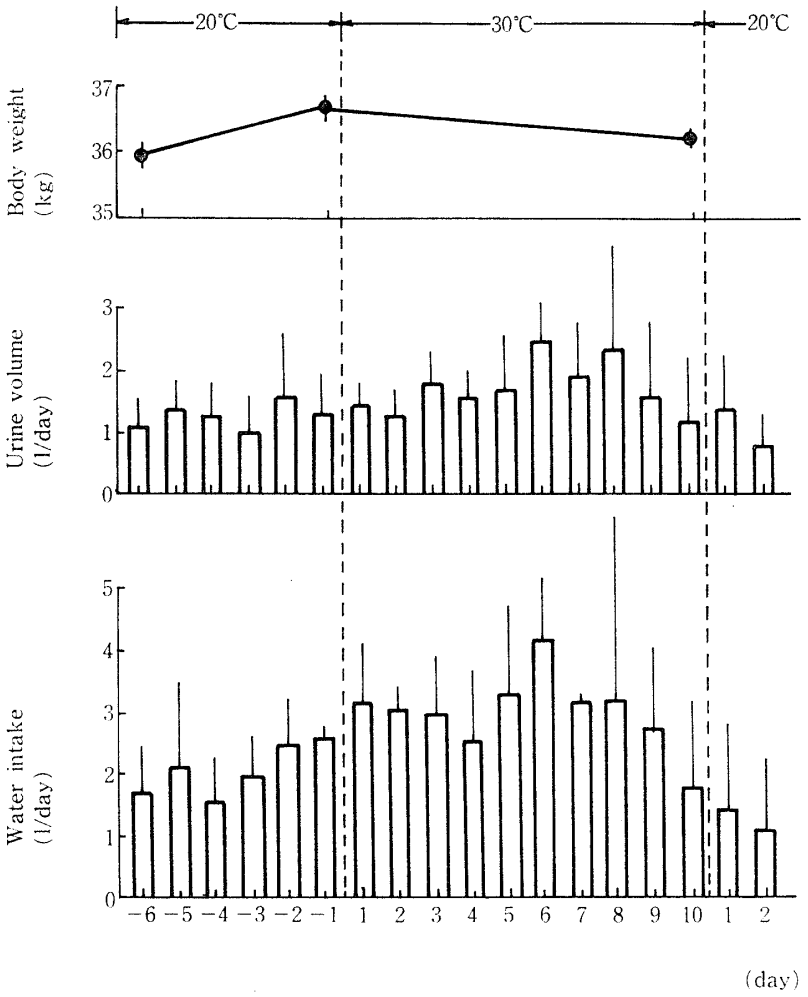

FIG. 2. Changes in body weight, water intake and urine volume following the heat exposure in sheep. Data are expressed as means±SD.

after the refusal of the ration.

3. *Blood constituents*

Changes in blood concentrations of sheep during the heat exposure are shown in Fig. 4.

The plasma glucose concentration exhibited no change from before (56.4±1.8 mg/dl) to after (56.8±1.9 mg/dl) the heat exposure. The plasma FFA concentration did not change clearly, although each value itself was variable. But in sheep C, the FFA increased remarkably after the refusal of the ration. After the heat exposure plasma thyroxine concentration decreased rapidly from 3.7±0.8 in 20°C to 2.1±0.7 μg/dl, but after the third day the values slightly increased and maintained about 2.5 μg/dl during the exposure period. Thyroxine concentration recovered to the same levels as in 20°C within 3 days after returning to 20°C.

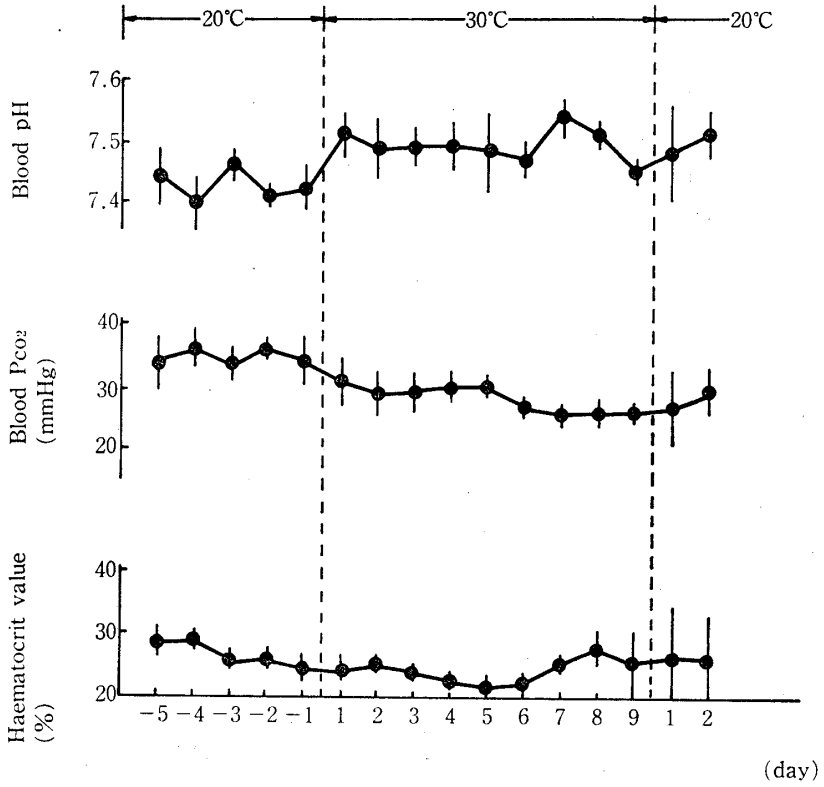

FIG. 3. Changes in blood pH, P_{CO_2} and haematocrit value following the heat exposure in sheep. Data are expressed as means±SD.

4. Parameters of blood glucose metabolism

The representative result of specific activity and concentration of plasma glucose following single injection of U-^{14}C-glucose are shown in Fig. 5. The nearly constant glucose concentration indicates that the glucose flux in the blood was kept at a steady-state condition, and a linear relationship between specific activity and time plotted on a semilogarithmic scale indicates that the decay of ^{14}C-labelled glucose in the blood followed first order kinetics during the experimental period.

The measurements on glucose pool size and turnover rate are listed in Table 1.

Plasma glucose concentrations were 59 at 20°C, and 61 and 55 mg/dl on the 4th and 10th days after the exposure to 30°C, respectively. In the environmental temperature of 20°C, the mean values of pool size and turnover rate were 504.4± 92.9 mg/kg$^{3/4}$ and 7.13±1.23 mg/kg$^{3/4}$/min, respectively. During the heat exposure, the pool size and turnover rate of glucose both tended to decrease in comparison with those of 20°C. However, no statistically significant difference was observed between two environmental conditions. In sheep C which refused the food intake after the 8th day both pool size and turnover rate of glucose on the 10th day decreased clearly.

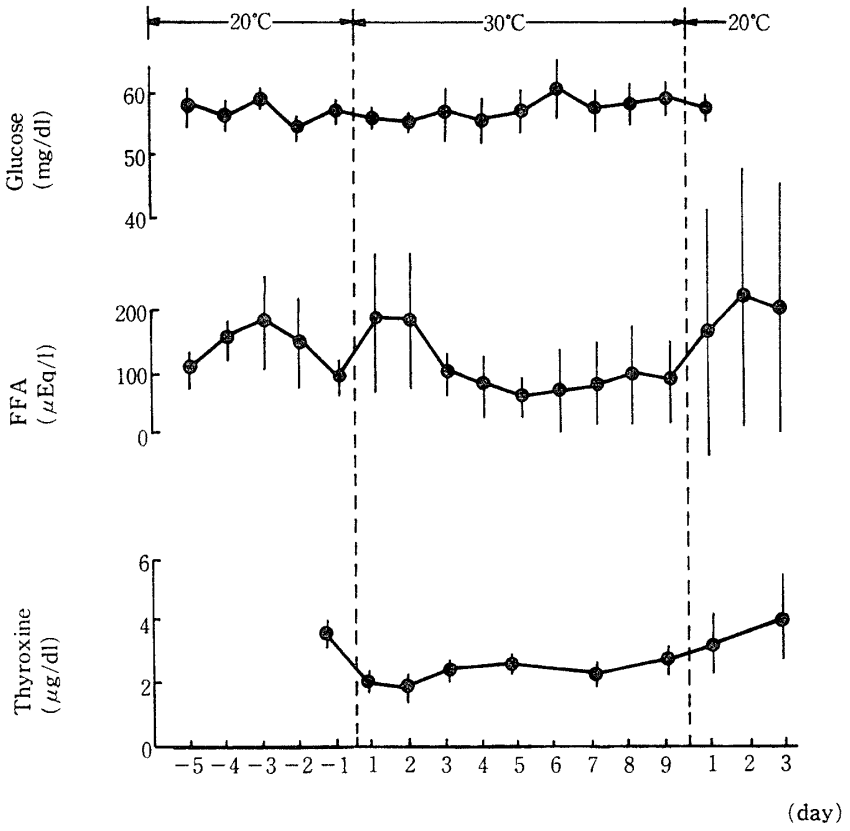

FIG. 4. Changes in plasma glucose, FFA, thyroxine concentrations following the heat exposure in sheep. Data are expressed as means±SD.

TABLE 1. *Body Weight, Plasma Glucose Concentration, Pool Size and Turnover Rate of Blood Glucose in Control (20°C) and Heat (30°C) Exposed Sheep*

Sheep	Body Wt. *kg*	Plasma glucose concentration* *mg/dl*	Plasma glucose pool size *mg/kg$^{3/4}$*	Plasma glucose turnover rate *mg/kg$^{3/4}$/min*
Control (20°C)				
A	35.0	59.5	611.1	7.62
B	35.0	58.4	460.5	8.04
C	38.0	58.3	441.7	5.73
Mean±SD	36.0±1.7	58.7±0.7	504.4±92.9	7.13±1.23
Exposed to heat (30°C) for 4 days				
A	35.5	60.1	547.4	5.61
B	36.5	66.8	477.4	5.47
C	38.5	56.1	399.5	5.43
Mean±SD	36.8±1.5	61.0±5.4	474.8±74.0	5.50±0.09
Exposed to heat (30°C) for 10 days				
A	36.0	61.6	549.9	6.74
B	36.5	49.8	459.9	7.64
C	36.5	53.9	327.6	3.39
Mean±SD	36.3±0.3	55.1±6.0	445.8±111.8	5.92±2.24

* Glucose concentration is mean of 6 estimates in each experimental period.

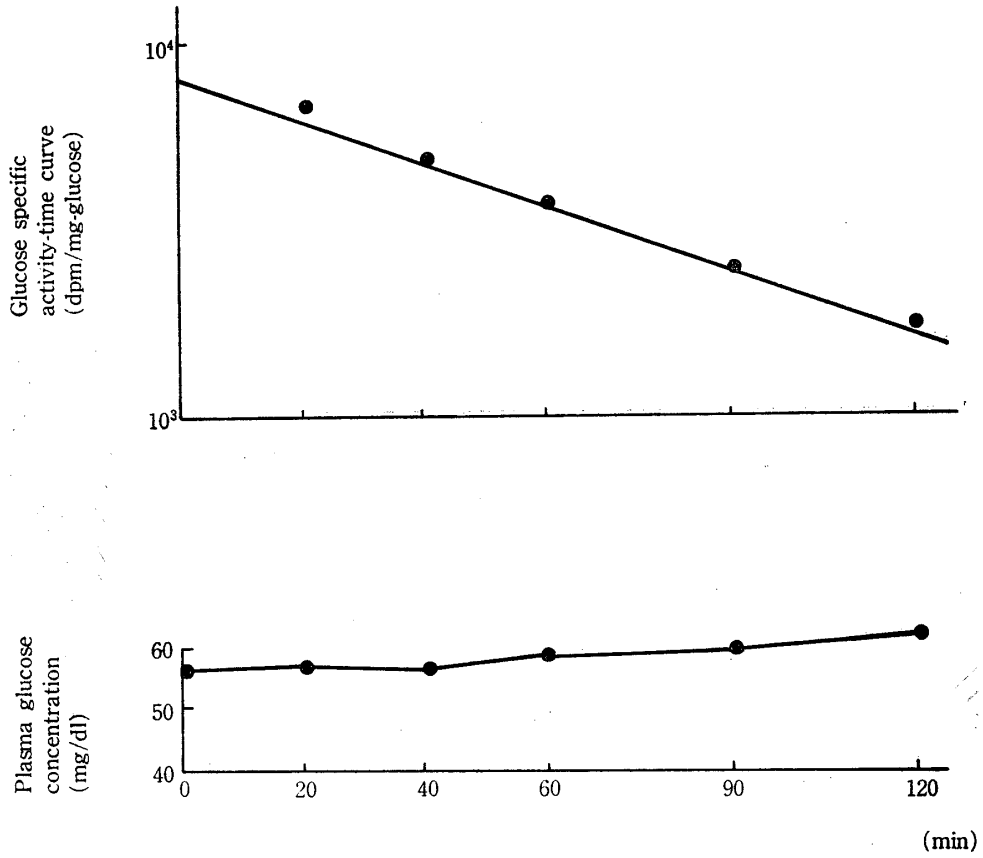

FIG. 5. Representative specific activity-time curve and concentration of blood glucose following the single injection of 25 μCi of U-^{14}C-glucose.

Discussion

For ruminants, panting is an effective means for increasing the heat loss from the body. In the present experiment, a remarkable increase in respiration rate was exhibited soon after the exposure to 30°C. Blood pH increased and blood Pco_2 decreased following the increase of respiration rate. These indicated a typical respiratory alkalosis. The heart rate also tended to increase. In this regard, Yamagishi (6) observed that the heart rate and cardiac output of clipped sheep increase under the 35°C (R.H. 70 per cent) environment and concluded that these responses permit the increase of circulating blood volume, which enhance the heat loss from the respiratory tract and skin surface, though the heat production also increases.

Despite physiological functions actively responded so as to increase the heat loss under the heat exposure, the body temperatures of all sheep gradually increased from 38.8°C in 20°C to 40.5°C on the 6th day of 30°C. Concerning the effect of the heat exposure on body temperature of clipped sheep, Yamagishi (6) showed that body temperature does not always increase under the environment of 25°C (R.H. 70 per cent) and clearly increases under the environment of 35°C (R.H. 70 per cent).

It is likely that the environment of 30°C (R.H. 70 per cent) which was set for the present experiment might be out side of the critical point of the thermoneutral zone for the experimented sheep.

Water intake and urine volume tended to increase after the heat exposure. The increase of water intake might have occured to conpensate for the deficit of body water which was caused by the increase of evaporation through the respiratory tract and skin surface. The urine volume increase might be due to the increased water intake which somewhat exceeded the loss of body water. The haematocrit value slightly decreased during the first 6 days of the heat exposure. This result was not consistent with the report by Poman-Ponce *et al.* (7), who have shown that the short term exposure to heat (32°C) in ewes leads to a temporary dehydration symptom, in which an increase of haematocrit value occurs. The environmental temperature and the experimental period might be related to the difference between the two results. In the present experiment, the especially long term heat exposure of 10 days may have occured the haematocrit value to be decreased because of increased water intake, even though the evaporation of water from the body increased.

Thyroxine has been considered as one of the important hormones controlling glucose and lipid metabolisms. Many reports have shown that the plasma thyroxine concentration is apparently higher in a cold environment than in a hot environment (8, 9). In the present experiment, the plasma thyroxine concentration decreased to about 50 per cent of the pre-exposure mean value by the second day of exposure, and stabilized at about 70 per cent of the pre-exposure value after the third day.

The plasma glucose and FFA concentrations did not change between before and after the heat exposure, except for the FFA value of sheep C. Sasaki *et al.* (10) reported that the urinary excretion of catecholamines which have the function of mobilizing FFA in sheep do not exhibit a clear change under either environment of 20°C or 30°C. These indicate that sympathicotonia would not develop in the sheep during the heat exposure in the present experiment.

However, several reports showed that despite no clear blood glucose concentration changed, significant alterations of glucose turnover rate were revealed under such physiological situations as pregnancy (11), lactation (12, 13), cold (14) and starvation (15). To know the kinetic pattern of glucose metabolism, therefore, the labelled glucose dilution method was applied in addition to the blood concentration measurement.

Since it is recognized that glucose metabolism is largely influenced by the levels of energy intake, the decrease in pool size and turnover rate of blood glucose in sheep C on the 10th day of the heat exposure might be related partially with the refusal to feed. To exclude this possibility, about 4 weeks after the present experiment, sheep C was restricted in feed amount to the same level as be eaten

on the 8th day after 30°C exposure for one day and was given no feed thereafter for 2 days under 20°C environment. On the last day, blood glucose metabolism was determined by the same way as before. In this additional experiment, the pool size and turnover rate of blood glucose were 368.8 mg/kg$^{3/4}$ and 4.72 mg/kg$^{3/4}$/min, respectively. These results suggest that a marked decrease of glucose metabolic rate in sheep C during the latter half of the heat exposure was principally due to the effect of high temperature, though the possible effect of feed refusal was not completely excluded.

It is not clear in such a small scale experiment as the present one whether the tendency of decrease in metabolic activity of glucose is related to any changes of physiological functions, i.e. respiratory alkalosis, hyperthermia or hypothyrodism. From the curves in the figures, however, the glucose turnover rate has a negative correlation to body temperature, and a positive correlation to blood thyroxine concentration.

Chayoth and Cassuto (16–21) who conducted a series of experiments on the heat stress to hamstar, observed that the depression of thyroid function during the heat stress inhibits both glycogenolysis and gluconeogenesis, and proposed the hypothesis that these changes are a part of the process of the metabolic adaptation, by which the heat production may consequently maintained at low level. While the heat production of sheep was not determined in the present experiment, the lack of sympathicotonia and the observations of the decreases in blood thyroxine concentration and in glucose metabolism may suggest the decrease of heat production.

References

1) Okamoto, S., Ishii, S., Mukai, A., and Inudo, Y., *Bull. Kyushu Agr. Expt. Sta.*, **10**, 183 (1964) (in Japanese)
2) Hafez, E.S.E., "Adaptation of Domestic Animals", ed. by E.S.E. Hafez, Lea & Febiger, Philadelphia, p. 74, (1968)
3) Hugget, A.G., and Nixon, D.A., *Biochem. J.*, **66**, 12, (1957)
4) Itaya, K., and Ui, M., *J. Lipid Res.*, **6**, 16, (1965)
5) Pileggi, V.J., and Kessler, G., *Clin. Chem.*, **14**, 339, (1968)
6) Yamagishi, N., *Proc. Jap. Soci. Anim. Nutr. Metab.*, **15**, 25, (1971) (in Japanese)
7) Roman-Ponce, H., Thatcher, W.W., Caton, D., Barron, D.H., and Wilcox, C.J., *J. Anim. Sci.*, **46**, 167, (1978)
8) Yousef, M.K., Kiber, H.H., and Johnson, H.D., *J. Anim. Sci.*, **26**, 142, (1967)
9) Premachandra, B.N., Pipes, G.W., and Turner, C.W., *Mo. Agr. Exp. Sta. Res. Bull.*, No. 727, (1960)
10) Sasaki, Y., Oshiro, S., Miura, M., and Tsuda, T., *Jap. J. Zootech. Sci.*, **44**, 248, (1973)
11) Bergman, E.N., *Am. J. Physiol.*, **204**, 147, (1963)
12) Bergman, E.N., and Hogue, D.E., *Am. J. Physiol.*, **213**, 1378, (1967)
13) Annison, E.F., and Linzell, J.L., *J. Physiol.*, **213**, 372, (1964)

14) Ambo, K., Fujita, M., Sugawara, M., and Tsuda, T., *Environ. Control in Biol.*, **14**, 115, (1976) (in Japanese, with English summary)
15) Steel, J.W., Ph. D. Thesis, University of New England, Armidale N.S.W., Australia. (1970)
16) Cassuto, Y., Chayoth, R., and Rabi, T., *Am. J. Physiol.*, **218**, 1287, (1970)
17) Chayoth, R., and Cassuto, Y., *Am. J. Physiol.*, **220**, 1067, (1971)
18) Chayoth, R., and Cassuto, Y., *Am. J. Physiol.*, **220**, 1071, (1971)
19) Chayoth, R., and Cassuto, Y., *Am. J. Physiol.*, **222**, 126, (1972)
20) Cassuto, Y., Chayoth, R., and Zor, U., *Am. J. Physiol.*, **227**, 851, (1974)
21) Chayoth, R., *J. Appl. Physiol.*, **41**, 871, (1976)