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THE UNIVERSITY OF ALBERTA

THE EFFECTS OF THERMAL ENVIRONMENT ON THE METABOLISM OF MATURE SHEEP

by

WRAY THOMAS WHITMORE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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(SIGNED) W Whitmore

PERMANENT ADDRESS:

204 7000 - 113 St
Edmonton, Alberta Canada
T6H 5T6

DATED April 24 1988

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled THE EFFECTS OF THERMAL ENVIRONMENT ON THE METABOLISM OF MATURE SHEEP submitted by WRAY THOMAS WHITMORE in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL PHYSIOLOGY.

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Supervisor

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Date.. April 21 1988

Abstract

Studies were conducted to elucidate some of the effects of thermal enviroment on the energy metabolism in mature, closely shorn sheep fed at a near maintenance levels. Mature sheep lack brown adipose tissue, therefore any change in metabolism related to thermal exposure will be likely to occur through mechanisms different from those often described in small mammals and associated with brown adipose tissue. Metabolism was measured by open-circuit respiration calorimetry while the sheep were confined in a temperature controlled, water immersion system. Resting metabolism was 4.5, 3.8 and 3.1 ± 0.1 $W/kg^{.75}$ for the 0°, 18° and 36° C acclimation temperatures with the respective summit metabolisms being 24.1, 20.4 and 17.0 ± 1.0 $W/kg^{.75}$.

In vitro measurements made on oxygen uptake by external intercostal muscle and transmembrane Na^+/K^+ ATPase activity showed that thermal acclimation shifts in metabolic intensity occured in non-shivering muscle tissue. The whole body O_2 uptake of the sheep from which the muscle samples were obtained was 257 ± 0.35, 241 ± 3.25 and 204 ± 3.25 ml O_2/h per kg for the 0°, 18° and 36° C acclimation temperatures and the external intercostal muscle O_2 values were respectively 1.11 ± 0.04, 0.83 ± 0.05 and 0.48 ± 0.05 μl O_2/h per mg of tissue DM. The proportion of O_2 uptake in the external intercostal muscle inhibited by ouabain, a specific inhibitor of Na^+/K^+ ATPase, remained relatively constant across thermal environments and averaged 19%.

The estimated upper critical temperatures of sheep acclimated to 0°, 18° and 36° C were 41.1, 41.4 and 40.8 ± 0.1 ° C respectively and the lower critical temperature values were 35.3, 34.7 and 33.8 ± 0.5° C. The upper and lower critical temperatures were measured in thermally acclimated sheep while partially immersed in water, and are not the same as still air temperature values.

Results reported in this thesis show that mature sheep undergo thermal acclimation which is independent of feed intake and external thermal insulation. This acclimation is accompanied by changes in resting and cold-induced summit metabolism. The whole animal metabolism changes are accompanied by changes in oxygen uptake by nonshivering skeletal

muscle which is associated with Na^+/K^+ ATPase activity in the muscle. There was shifts in the upper and lower critical temperatures of sheep as a consequence of thermal acclimation.

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1. INTRODUCTION

Animals must respond to their thermal environment when the ambient temperature exceeds their upper and lower critical temperatures as physiological mechanisms of heat loss or production are needed to maintain a constant body temperature. Exposure of a homeotherm to a hot or cold environment results in physiological responses (Thompson 1977; Alexander 1979; Sasaki and Weekes 1986; Hales 1974; Webster 1976; Rousset et al. 1984) that enable the animal to cope better with the unfavorable conditions. When the environmental temperature changes from above to below the lower critical temperature, food intake usually increases (Hamilton 1963). This higher energy intake increases resting metabolic rate thereby reducing the demand for additional heat production in the cold. The opposite to the increase in food intake associated with cold exposure is seen in exposure to an environment above the upper critical temperature (Montsma et al. 1985; Luiting et al. 1985; Curtis 1981). When exposed to heat stress, animals reduce their metabolic energy intake which results in a lower level of metabolic heat production (Webster 1976).

Thermal exposures, and the accompanying changes in intake, lead to alterations in endocrine status (Sasaki and Weekes 1986; Kennedy et al. 1986; Mount 1979; Rousset et al. 1984). These endocrine alterations result in both catabolic and anabolic hormone secretion changes which cause metabolism to either increase or decrease. This change in metabolism may allow the animal to be more comfortable in it's thermal environment.

During cold adaptation or seasonal acclimatization small mammals improve their cold tolerance mainly by increasing their capacity for nonshivering thermogenesis (Bruck et al. 1979; Jansky 1973; Heldmaier et al. 1981). This increase in nonshivering thermogenesis is associated with hypertrophy of brown adipose tissue (B.A.T.) (Cameron and Smith 1964) and morphological changes in skeletal muscle mitochondria (Behrens and Himms-Hagen 1977). B.A.T. thermogenesis is a result of norepinephrine release from sympathetic nerve endings within the tissue activating lipolysis and the proton conductance pathway.(Sasaki and Weekes 1986). Catecholamine induced increases in B.A.T. thermogenesis contribute to an increase in resting and cold induced summit metabolism in rodents and neonate sheep (Pasquis et al.

1970; Rosenmann and Morrison 1974; Alexander et al. 1970).

Adult ruminants contain no B.A.T., even after acclimation to cold (Sasaki and Weekes 1986). However apparent acclimation to cold is characterized by an elevated resting metabolic rate (Webster et al. 1969a; Alexander et al. 1970; Young and Degen 1981) and possible replacement of shivering thermogenesis with nonshivering thermogenesis (Schaefer et al. 1982; Young 1975). Earlier work suggested that norepinephrine had no direct calorigenic action in either warm or cold acclimated adult sheep (Webster et al. 1969b). However more recent work has demonstrated a small calorigenic response to norepinephrine infusion and a response to epinephrine which is potentiated by chronic cold exposure (Graham and Christopherson 1981). The relative potency of epinephrine clearly differs from the classical pattern of norepinephrine stimulated thermogenesis associated with B.A.T. (Sasaki and Weekes 1986).

Few reports exist describing the effect of prolonged thermal exposure on the metabolism of adult ruminants that are not confounded by alterations in food intake, or changes in insulation and body size. Serious questions arise as to the appropriateness of applied correction factors for the confounding issues.

Reports in the literature exist linking changes in resting metabolism with changes in cold induced summit metabolism in small mammals (Depocas et al. 1957 and Heroux 1963). Webster et al. (1969b) reported that cold acclimated and acclimatized sheep showed an enhanced capacity to increase metabolic heat production during severe cold stress. With different feed intakes, animals exposed to the cold had higher levels of heat production compared to controls. Although cold induced summit metabolism values are reported, it is doubtful if summit metabolism was achieved. Bennett (1972) suggested that a fall in rectal temperature of 0.5 to 2.0° C per hour is needed to prove summit metabolism in mature sheep. This rate of decline in rectal temperature was not achieved in the cold stress experiments of Webster et al. (1969b).

In the present series of experiments, mature sheep receiving the same type and level of feed were exposed to three thermal environments to see the effect on their energy metabolism.

Resting and cold induced summit metabolism were estimated after a thermal acclimation period in a water immersion system similar to one described by Eales and Small (1980) and Therminarias et al. (1979). The sheep in the trials underwent a period of acclimation to a near constant ambient temperature in a controlled temperature chamber and did not undergo acclimatization to a complex environment as occurs naturally. Along with measured changes in resting metabolism and summit metabolism, the upper and lower critical temperatures and a nonshivering component of muscle tissue metabolism were measured. Two techniques for measuring resting metabolism allow for the calculation of an energy cost for maintaining a standing position. The objectives of the studies were to elucidate the effect of thermal acclimation on the metabolism of mature sheep.

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II. THE EFFECT OF THERMAL ENVIRONMENT ON RESTING AND SUMMIT METABOLISM IN MATURE SHEEP

A. Introduction

When faced with a different thermal environment, a homeotherm will adjust it's rate of heat production and/or heat loss. Prolonged thermal exposure may result in acclimation. Bligh and Johnson (1973) defined thermal acclimation as "a physiological change, occurring within the lifetime of an organism, that reduces the strain caused by experimentally induced stressful changes in particular climatic factors". Acclimation results from exposure to one single climatic factor such as ambient temperature in a controlled environment. Acclimatization is defined as a physiological change, occuring within the lifetime of an organism which reduces the strain caused by stressful changes in the natural climate (Bligh and Johnson 1973). Acclimatization refers to adaptive changes incurred due to exposure to natural climatic conditions.

Wickler (1980) has shown the effects of cold acclimatization on various rodent species. Winter-acclimatized animals had higher resting metabolic rates and were able to increase their metabolic heat production during severe cold stress to higher levels than could summer acclimatized animals. The ability of rodents and small mammals (<5.0 kg.) to increase metabolic heat production during cold stress, is largely due to norepinephrine-sensitive metabolism (Wickler 1980), which is associated with brown adipose tissue (Alexander 1979). Brown adipose tissue has a marked capacity for producing heat (Alexander 1979). Present in most mammalian species at some time of life, it is considered a major site of heat generation in arousing hibernators, most cold-exposed newborns, and cold-exposed adults of several non-hibernating species (Horwitz 1979). Brown adipose tissue in neonate lambs is converted to white adipose tissue at a rate dependent on the degree of cold stress experienced (Alexander 1979) and is not present in adult sheep (Sasaki and Weekes 1986).

Despite the absence of brown adipose tissue in adult ruminants, cold-induced physiological acclimation has been shown to increase resting metabolism in mature sheep and cattle (Webster et al. 1969; Alexander et al. 1970; Young and Degen 1981), though this occurs in a different manner than in rodents with brown adipose tissue. Although a calorigenic response to norepinephrine infusion has been demonstrated in adult sheep (Graham and Christopherson 1981), the relative magnitude of the response differs from the norepinephrine-stimulated brown adipose tissue heat production seen in rodents.

Winter acclimatized sheep showed increased cold hardiness in the experiments of Webster et al.(1969) and Slee (1974); however, feed intake varied with thermal treatment. The increased feed intake associated with cold exposure would have had a confounding effect on resting heat production measurements. As feed intake increases, metabolic heat production increases (Mount 1979).

Sheep exposed to hot environments significantly reduce their voluntary feed intake (Thwaites 1968). Thermal exposure also results in decreases in thyroid activity which may reduce the animal's metabolic heat production (Webster 1976).

The effects of thermal exposure on level of summit metabolism in adult sheep are unknown. Exposure to a cold environment may enhance summit metabolism, while exposure to a hot environment may inhibit it.

The conditions of measurement described in this paper are not necessarily the same as those used in other laboratories. The following definitions are therefore provided.

Resting Metabolism (Watts and Watts/$kg^{.75}$): The metabolic rate of a physically inactive animal, 15-22 h postprandial and submerged to the neck in warm (38° C) water; calculated from the average rate of whole-body O_2 consumption during a 10 to 20 minute period.

Cold-Induced Summit Metabolism (Watts and Watts/$kg^{.75}$): The highest metabolic rate achieved by a physically inactive animal submerged to the neck in 22- 18° C water; calculated from the highest rate of whole-body O_2 consumption sustained for 20 minutes.

This experiment was undertaken to determine the effects of prolonged thermal exposure to 36, 18 and 0° C on resting and summit metabolism in mature sheep at a constant

level of feed intake.

B. Materials and Methods

Animals and Management

The experiment was conducted with six mature, suffolk crossbred ewes, (initial body weight of 54±0.9 kg; mean±SEM). They were maintained in individual metabolism crates in temperature-controlled rooms at 36, 18 or 0° C and exposed to constant lighting throughout the study. Prior to the trial, the ewes were closely shorn (5 mm) and drenched with Thiobenzol for control of internal parasites. The sheep were given an intermuscularl A,D and E injection containing retinol (75 mg), cholecalciferol (.94 mg) and α-tocopherol (16 mg). The sheep were subsequently reshorn every 14 to 18 d while on trial.

Regardless of temperature treatment, the ewes were fed once daily at 1500 h. They received a diet of 1 kg alfalfa hay pellets containing 15.0% crude protein on a dry matter basis. Water and cobaltized iodized salt were available free chpice.

Experimental Design

The study was a double 3x3 Latin Square design. Animals were initially paired on the basis of body weight and one of each pair was allocated at random to one of the two Latin Squares.

The experimental schedule for each animal comprised three thermal acclimation periods of 24 d, each followed by a 3-d measuring period. The 24 d acclimation period was used after consultation, (Young, personal communication). The measuring period allowed for measuring resting and summit metabolism of two sheep per day, selected at random, with the measurement taking approximately 4 h. The animals were not fed for 17 to 23 h prior to these measurements.

For metabolic measurements, the ewe was strapped into a tubular aluminum frame which was then placed into, and attached to, a wooden water bath (dimensions 46.3 cm wide,

102 cm long, 96 cm deep). An electric water pump circulated water (rate = 133 l/min) to ensure an even temperature throughout the bath. Resting metabolism was measured using indirect calorimetry with the ewe stabilized in 38±0.2° C water for a minimum of 20 min. Water bath temperature was then lowered using cold tap water over 2.5 h to between 22 and 18° C until maximum metabolism was achieved. Metabolic rate was then recorded for 20 min.

After summit metabolism was measured, the cold water in the bath was replaced with water at approximately 39° C and the ewe was rewarmed for 45-60 min. The ewe was removed from the waterbath, towel-dried, placed in a room at 20° C for at least 16 h, and fed before returning to its particular thermal environment. The ewes were not moved to a new thermal environment as a pair, the pair was split up. For example, in period 2, one of the ewes assigned to the 18° C environment had previously been in the 36° C environment, and the other in the 0° C environment.

Measurements

Metabolic rate (M) was measured using an open-circuit respiratory analyzer (Young et al. 1975) connected to a ventilated face mask to which the sheep had been previously accustomed. Ventilation rate of the mask was read from a flowmeter (Rotameter, Fischer and Porter, Warminster,Pa.), and the oxygen concentration difference between incoming and outgoing air, from a paramagnetic oxygen analyzer (Taylor Servomex OA184, Sussex, England). The open-circuit respiratory analyzer was calibrated using the procedures of Young et al. (1984); M was calculated using the equation of McLean (1972).

Water and rectal temperatures were measured using 40 s.w.g. Cu/Con wire. The thermo-junction for measuring water temperature was placed close to the H_2O pump. The thermocouple for measuring rectal temperature was sheathed in a flexible plastic tube and inserted 10 cm into the rectum. All temperatures were measured using the same measuring instrument (Bailey BAT8, Saddle Brook, N.J.) which was calibrated electronically between experimental periods. Temperatures were recorded by means of a Electronic-19 potentiometric recorder (Honeywell, Inc., Denver, Co.) to provide a hard copy.

Blood sampling and analysis

Approximately 20 ml of blood was collected using a heparinized veni-puncture (Vacutainer, Becton Dickinson, Mississauga, Ont.) after 27 d of thermal exposure. A packed-cell volume was measured using heparinized micro hematocrit tubes. After centrifugation, plasma samples were stored at -25° C until their analyses for free fatty acids (FFA), glucose, and T_3 and T_4. Free fatty acid levels were analyzed according to the enzymatic method of Shimizu et al. (1979), which was based on Acyl CoA Synthetase. Glucose levels were analyzed using the Technicon Method (No. SE4-0002FF4, Technicon Instruments Corporation, Tarrytown N.Y.). The Technicon method was based on the reaction of a cupric neocuproine chelate with glucose. Total triiodothyronine and thyroxine concentrations were measured using radioimmunoassay (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA.).

Statistical Procedures

Data were analyzed using least-squares analysis of variance and where significant differences existed, means were compared using the Student-Newman-Keuls Multiple Range Test (Steel and Torrie 1980).

C. Results

Because of health problems, one animal was removed from the study; the reported results are based on five animals.

The thermal exposure temperatures were 0±0.2° C, 18.3±0.4° C and 36.2± 0.2° C, (mean±SEM) respectively. The relative humidities for the 0, 18 and 36° C environments were 81, 49 and 39% respectively, calculated using wet and dry bulb temperatures.

Resting Metabolism

To account for possible effects of differences in body weight during each thermal exposure period, metabolic heat production has been expressed in both watts (W) and in

$W/kg^{.75}$ (Table II.1). Resting metabolism was significantly altered ($P<0.05$) by thermal exposure when expressed in either W or $W/kg^{.75}$. Resting metabolism measured in 38° C water was highest in animals after exposure to the 0° C environment and lowest after exposure to the 36° C. Changes in body weight during the 24 d thermal exposure periods resulted in differences when resting metabolism was expressed in W or $W/kg^{.75}$. When resting metabolism was measured in W, the 0° C thermal exposure temperature was no longer significantly different from the 18° C exposure temperature, Table II.1. It appears that changes in body weight are confounding the estimates of resting metabolism.

When the increase or decrease in resting metabolism that accompanied thermal exposure was expressed as a percentage of the 18° C value, differences could be seen. Resting metabolism expressed in $W/kg^{.75}$ resulted in exactly the same percentage increase or decrease, 18.4%. The same is not true for resting metabolism expressed in W. Thermal exposure results in a 4.5% increase and a 23.9% decrease in resting metabolism. The differences in correlation coefficients (see below) for resting metabolism expressed in W or $W/kg^{.75}$ is a reflection of this unequal changes in magnitude.

The regression of acclimation temperature on resting metabolism (R.M.) with 5 observations per acclimation temperature can be expressed as:

$$R.M.(W) = -0.49x(\pm 0.22) + 78.86(\pm 5.02),\ (\text{mean} \pm \text{SEM}),\ \text{with } r = -.531 \text{ or}$$

$$R.M.(W/kg^{.75}) = -0.04x(\pm 0.01) + 4.51(\pm 0.24),\ (\text{mean} \pm \text{SEM}),\ \text{with } r = -.706.$$

See the lower half of Figure II.1 for the effect of thermal acclimation on the resting metabolism of mature sheep. The change in resting metabolism associated with thermal exposure is linear.

Summit Metabolism

Summit metabolism was significantly ($P<0.05$) affected by thermal exposure (Table II.1). Summit metabolism measurements made after exposure to 0° C and to 36° C showed the same trend as with resting metabolism. However, unlike resting metabolism, when summit metabolism is expressed in either W or $W/kg^{.75}$, the significance of differences between treatment means remains the same, Table II.1.

The effect of thermal exposure on summit metabolism results in consistent increases or decreases when expressed as a percentage of the 18° C value. Thermal exposure resulted in a 8.2% increase and a 11.8% decrease in summit metabolism when expressed in W. When summit metabolism is expressed in $W/kg^{.75}$, the increase was 17.6% and the decrease is 16.2%.

The regression of acclimation temperature on summit metabolism (S.M.) with 5 observations per acclimation temperature is:

$$S.M.(W) = -2.48x(\pm 0.68) + 423.94(\pm 15.72),\ (\text{mean} \pm \text{SEM}),\ \text{with } r = -.712 \text{ or}$$

$$S.M.(W/kg^{.75}) = -0.19x(\pm 0.04) + 23.88(\pm 1.04),\ (\text{mean} \pm \text{SEM}),\ \text{with } r = -.760.$$

See the upper half of Figure II.1 for the effect of thermal acclimation on summit metabolism. Summit metabolism in either W or $W/kg^{.75}$ is affected linearly by thermal exposure.

Ratio of Summit Metabolism to Resting Metabolism

The ratio of summit metabolism to resting metabolism was not significantly affected by thermal exposure. The ratio was similar for all temperature treatments and averaged 5.5. The ratio was not changed by expressing resting or summit metabolism in either W or $W/kg^{.75}$. The significance of the constant ratio is unknown at this time.

Effect of Thermal Exposure on Rectal Temperature

The rectal temperature recorded during the first five minutes in the water bath during the measurement of resting metabolism was significantly ($P<0.01$) affected by thermal exposure of the sheep. The rectal temperature values were 38.8°, 38.8° and 39.5° ± 0.1 ° C (mean ± SEM) for the 0°, 18° and 36° C thermal exposure temperatures, respectively. The 36° C exposure temperature differs significantly ($P<0.05$) from the other two.

Neither the effect of thermal exposure on the rectal temperature of the animal at summit metabolism or the time needed for the animals to reach summit metabolism were significantly different between treatments, $P=0.24$ and $P=0.22$ respectively.

Effect of Thermal Exposure on Body Weight

Table II.1. gives the average body weight of the animals after 24-26 days of thermal exposure. The effect of thermal exposure on body weight was significant ($P<0.05$). The difference in weight between the 0° C and 36° C temperatures was significant ($P<0.05$).

The regression of acclimation temperature on body weight (B.W.) after 24-26 days of exposure with 5 observations per acclimation temperature is:

B.W.(kg) = 0.14x(±0.04) + 46.62(±1.13), (mean ± SEM), with r = .614.

The linear change in body weight occurred despite the fact that each pair of sheep was from a different thermal environment.

Effect of Thermal Exposure on Plasma Endocrine and Metabolite Concentrations

Table II.2. shows the effect of thermal exposure on various endocrine and metabolite concentrations. The effect of thermal exposure was significant ($P<0.05$) in all cases. Exposure to 0° C resulted in increases in all plasma concentrations of hormones and metabolites measured and exposure to 36° C lead to decreases. The increase in plasma concentrations of triiodothyronine; thyroxine, free fatty acids and glucose associated with

exposure to 0° C indicates a higher, or more intense, level of metabolism due to thermal exposure. The effects of hyperthyroidism on metabolism are known, and when accompanied by increased substrates, one would expect metabolism to increase.

D. Discussion

The feeding routine and level used in this experiment did not result in any feed refusals by the animals in the 36° C environment; thus the reduced resting metabolic rates observed were not a result of different feeding level. The same can be said for the increased resting metabolism seen in the animals in the 0° C environment.

Physiological adaptation to cold is well documented in small mammals (Smith et al. 1972), and similar changes apparently occur in ruminant animals (Sykes and Slee 1969; Webster et al. 1970; Young 1975a, b). Adaptation to cold in ruminants involves increases in thermal insulation, appetite and basal metabolic intensity (Young 1980; Young and Degen 1981). Resting metabolism, which reflects metabolic intensity, was significantly increased in the present study, despite constraints put on external insulation and feed intake.

Cold thermogenesis can be divided into two types: (1) shivering thermogenesis: cold thermogenesis in striated muscle ultimately derived entirely from the increased rate of hydrolysis of ATP and creatine phosphate releasing energy for muscle contraction, the energy all being dispensed as heat (Webster 1974), and (2) nonshivering thermogenesis: mechanisms for producing heat by means other than shivering whose sole function is to maintain homeothermy in a cold environment (Webster, 1974). Shivering intensity may decrease during prolonged mild cold exposure of sheep (Schaefer et al. 1982) and cattle (Young 1975b). The findings of this experiment are in agreement with Schaefer and Young, when the ewes were placed into the cold, shivering was observed for the first few days, but not after 7 to 10 d. Thus the majority of the increase in resting metabolism is likely due to an increase in nonshivering thermogenesis.

Chronic cold exposure of adult sheep results in elevated plasma concentrations of adrenaline and noradrenaline (Christopherson et al. 1978) and urinary catecholamine excretion

(Sasaki and Takahashi 1980). Webster (1974) has reported that catecholamines stimulate lipolysis, induce hyperglycemia and increase permeability of cell membranes to cations. Responses to catecholamines in the cold may be potentiated by elevated thyroid hormone levels (Fregly et al. 1979). Prolonged cold exposure of cattle and sheep elevates their plasma concentrations of thyroxine and the more-active triiodothyronine (Christopherson and Thompson 1983; Kennedy et al. 1986). Elevated thyroid hormone levels may increase the thermogenic capacity of skeletal muscle by influencing mitochondrial structure and membrane Na^+/K^+ ATPase activity (Sasaki and Weekes 1986). Sasaki and Takahashi (1980) have demonstrated an effect of cold exposure on the progressive inhibition of the insulin secretory response to intravenous glucose injection. The reduced level of insulin secretion in a cold environment would allow enhanced mobilization of glucose and free fatty acids in response to elevated sympathoadrenomedullar activity (Sasaki and Weekes 1986). These endocrine changes are reflected in an increased resting metabolism in mature sheep as the thermal environment becomes colder. Animals which are acclimated to cold temperatures should be able to withstand colder temperatures before increasing thermoregulatory heat production compared to animals acclimated to a warm thermal environment.

Chronic heat stress in growing or lactating cattle leads to a reduction in appetite when animals are fed above maintenance (Webster 1976). This decrease in intake reduces the thermoregulatory burden on the animal by reducing metabolic rate. Webster (1976) reported that, if food intake is forcibly maintained by putting any refused food directly into the rumen through a fistula, metabolic rate does not alter significantly, even though marked hormonal changes may occur. Our findings do not agree. One possible explanation is that sheep offered a high quality diet as in the present study during exposure to heat stress would show a smaller reduction in intake than if high fibre diets were given, due to the heat increment of the ration. It appears that the alfalfa pellets were a high quality feedstuff, as no feed refusals occurred. Although feed intake did not change throughout the study, metabolic rate has changed through other physiological responses.

In cattle, thyroid secretion rate fell during exposure to heat even when the normal reduction in feed intake was prevented by force feeding through a rumen fistula (Yousef et al. 1968). Chronic heat exposure results in depression of plasma hydrocortisone concentration and turnover rate (Christison and Johnson 1972). Similar depressions were observed in the turnover rates of insulin (Kamal et al. 1970) and growth hormone (Mitra et al. 1972). Hyperthermic steers on controlled food intake showed increased urinary output of both urea and creatinine, suggesting increased protein catabolism in muscle (Colditz and Kellaway 1972; Vercoe 1969; Vercoe and Frisch 1970). During heat stress both catabolic and anabolic hormone secretions are depressed, and as a result, the whole process of metabolism slows down (Webster 1976). This is reflected in the lower resting metabolism seen in this trial. The lower resting metabolism should lower the thermal burden on the animal, thereby allowing it to be more comfortable in the hot environment.

Few summit metabolism trials have been conducted on mature sheep. Bennett (1972) reported the effects of rectal temperature, shearing, body posture and body weight on summit metabolism in sheep. No consistent relationship between summit metabolism and rectal temperature exists above 37° C, but a direct relationship exists between 30° and 37° C. Shearing or fasting for 19-21 h prior to the measurement did not influence summit metabolism. Summit metabolism values for sheep lying down were 27% lower than for those standing and in Bennett's study summit metabolism was proportional to fleece free body weight raised to the power 0.9.

The sheep used in Bennett's (1972) trial were all held in a warm or thermoneutral environment before the measurement of summit metabolism. An average value for summit metabolism of 25 $W/kg^{.75}$ was reported compared to an average of 20.4 $W/kg^{.75}$ for the sheep held in the 18° C environment in the present study. The differences in the two values, 18.4% may be partially explained by the use of different measurement techniques. Bennett's (1972) sheep were standing in a wind tunnel while summit metabolism was measured. In the present study the animal was in the water bath and bouyed up, and although the sheep's legs are dangling down, as if standing, the amount of muscle tone is less than if the sheep had to

support their body weight. This may have resulted in a reduced heat production. Further work in comparing resting metabolism in a water bath to resting metabolism while standing is required.

The increase in summit metabolism due to cold acclimation agrees in principle with that of Pasquis et al. (1970) and Alexander et al. (1970), who reported increases in rodents and lambs with cold acclimation. Webster et al. (1969) reported that the cold acclimated sheep showed an enhanced capacity to increased metabolism during severe cold stress but was unable to measure summit metabolism.

Summit metabolism appears to increase as the temperature of the environment of acclimation is reduced. When homeothermy is challenged by exposure to cold, the increased level of summit metabolism observed in the cold-acclimated ewes should prove useful in preventing hypothermia. A ewe acclimated to 35° C may have a lower tolerance to the cold, and may be more susceptible to hypothermia when cold stressed.

Both resting metabolism and summit metabolism have changed as a result of exposure to different ambient temperatures. The changes in metabolism have occurred without different feed intakes and, therefore, reflect a state of acclimation.

Table II.1 Effect of thermal environment (°C) on resting metabolism (W; $W/kg^{.75}$), summit metabolism (W; $W/kg^{.75}$) and the ratio of summit metabolism to resting metabolism and body weight (kg)

	Environmental temperature			
	0 (5)*	18 (5)*	36 (5)*	SEM
Resting metabolism	78.3^{a}	74.9^{a}	57.0^{b}	±4.0
	4.5^{a}	3.8^{b}	3.1^{c}	±.1
Summit metabolism	420.2^{a}	388.2^{ab}	342.3^{b}	±19.8
	24.0^{a}	20.4^{ab}	17.1^{b}	±1.0
Ratio of summit to resting	5.54^{a}	5.48^{a}	5.46^{a}	±.23
Body weight	46.3^{a}	49.8^{ab}	51.1^{b}	±1.0

*Animals per treatment.

^{a,b,c} Row means are significantly different if followed by different letters ($P<0.05$).

Table II.2 The effect of thermal environment (°C) on plasma triiodothyronine [T_3] (nmol/l), plasma thyroxine [T_4](nmol/l), and packed cell volume [P.C.V.] (%), plasma glucose (mg/100ml) and plasma free fatty acids (mM) concentrations

	Environmental Temperature			
	0 (5)*	18 (5)*	36 (5)*	SEM
T_3	2.55[a]	1.41[b]	1.31[b]	±0.15
T_4	158.61[a]	115.70[b]	111.61[b]	±5.51
P.C.V.	41.77[a]	36.29[a]	33.12[b]	±1.59
Glucose	70.3[a]	51.6[b]	52.0[b]	±3.16
Free fatty acids	.40[a]	.13[b]	.01[b]	±0.05

*Animals per treatment.

[a,b,c] Row means are significantly different if followed by different letters ($P<0.05$).

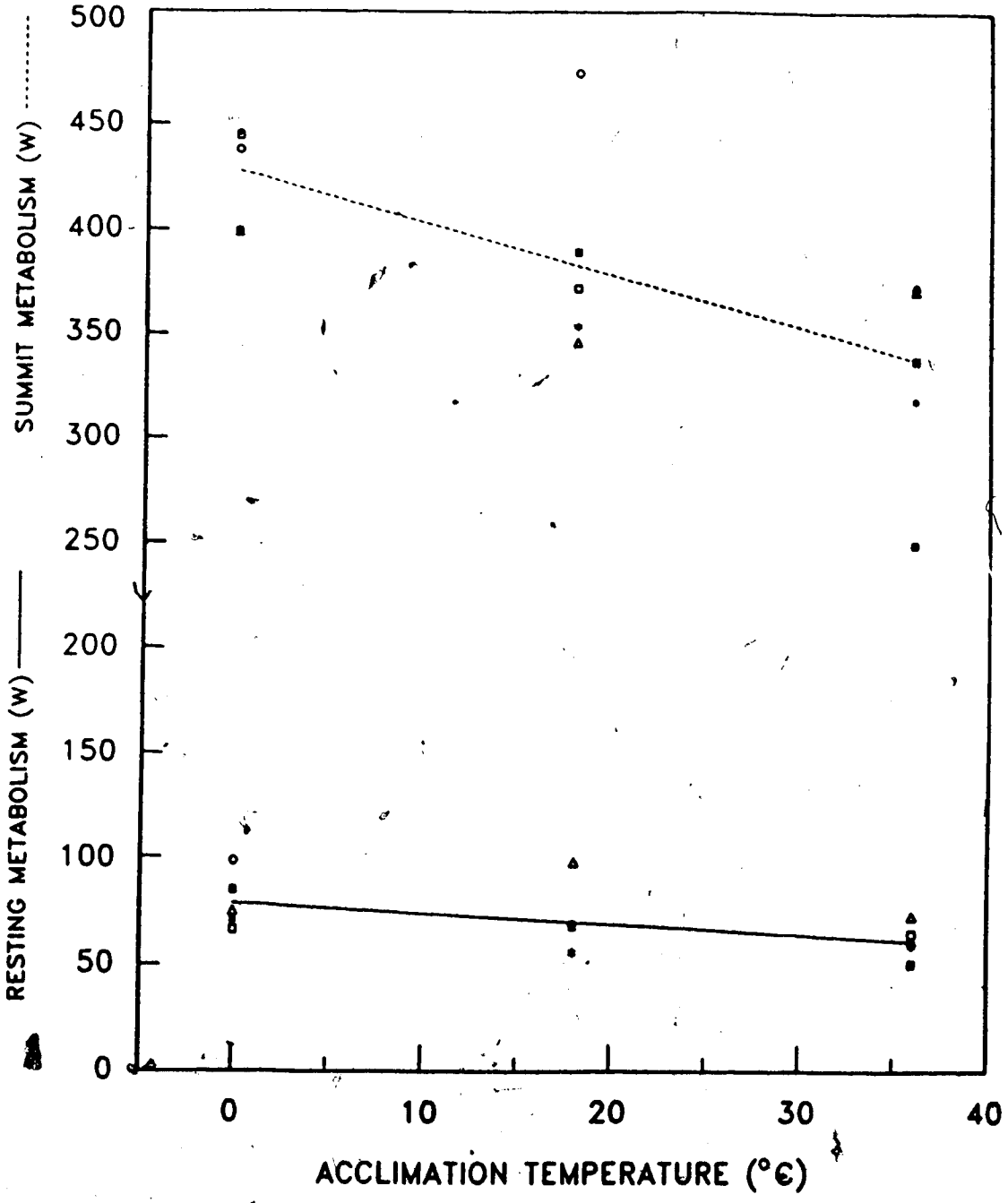

Figure II.1 The effect of thermal acclimation on resting and summit metabolism in mature sheep

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III. RESTING METABOLISM MEASUREMENTS IN A WATER IMMERSION SYSTEM COMPARED TO STANDING IN A HEAD-HOOD SYSTEM

A. Introduction

Acute cold stress measurements on animals are difficult because these measurements can result in peripheral cold injury (frost bite) or lethal hypothermia (Jansky 1966). Studies of cold induced summit metabolism require a decreasing rectal temperature which results from severe cold exposure in a temperature controlled chamber. An alternative to a cold chamber, used with small homeotherms, is a 80% He, 20% O_2 environment. The increased thermal conductance of the helium mixture results in maximum metabolism measurements being made at relatively mild cold exposure (Rosenmann and Morrison 1974). A third method for applying an acute cold stress, is a temperature controlled water immersion system similar to those described by Eales and Small (1980). The high thermal conductance of water will allow severe cold stress measurements without injury, after which warm water could be used to rewarm the subject. Whittow (1976) reports a convective constant for water that is 167 times that of air, this constant is given by density x specific heat x thermal conductivity/ viscosity.

A water immersion system may also be applied in the study of acute heat stress. It would be easy to slowly increase the water temperature and measure the effect on a subject's metabolism. Any injury risks would again be minimal.

Bligh and Johnson (1973) defined resting metabolism "as the metabolic rate of an animal which is resting in a thermoneutral environment but not in a post absorptive state". In our laboratory, resting metabolism is measured with the animal in a temperature controlled water immersion system or while standing in a head-hood system.

The position or posture of the animal is the same in each system. In both systems the animal has its legs hanging beneath it. In the water immersion system, the animal is bouyed up by the water. If differences in resting metabolism rates exist between the two systems, one possible cause may be due to the amount of energy expended in order to maintain a standing position.

The study reported here was conducted to determine if differences exist between resting metabolism values measured in either the water immersion or the head-hood system. The study arose as part of a larger experiment concerned with the effects of thermal environment on the metabolism of mature sheep.

B. Materials and Methods

Animals and Management

Six suffolk crossbred ewes with an initial body weight of 54±0.1 kg (mean ±SEM) were randomly assigned to one of three environmental temperatures (0° C, 18° C, 36° C). The ewes were closely shorn at intervals of 14-18 days and were held in individual metabolism crates in animal rooms at 18° or 36° C or in a controlled environment chamber at 0° C. The ewes were maintained in their respective thermal environments for 42 days. Animals were fed 1.1 kg of pelleted alfalfa hay per day, water and cobaltized iodized salt available free choice.

Measurement Systems

Resting metabolism was estimated by oxygen consumption using open-circuit respiration calorimetry while the ewes were in either a water immersion system stabilized at 38±0.01° C or when standing in a head-hood system at an ambient air temperature of 24.0±1.0° C, (mean±SEM). Respiration rates of 16-18 per minute and the absence of shivering were used as indicators of a thermoneutral environment. The ventilation rate of the mask or the hood around the sheep's head was read from a flowmeter (Rotameter, Fischer and Porter, Westminster, Pa.). The oxygen concentration difference between incoming and outgoing air was measured using paramagnetic analyser, (Taylor Servomex OA184, Sussex England) for the water immersion system and (Beckman F3M, Fullerton, Ca.) for the head-hood system. Both calorimetric systems were calibrated using the procedure of Young et al. (1984) and resting metabolism was calculated using the equation of McLean (1972). Metabolic rate measurements for each system were made on the animals at least 17 hrs. after

feeding

Statistical Procedures

Results were analyzed using least square analysis of variance, and differences between means were compared using the Student Newman-Keuls test (Steel and Torrie 1980).

C. Results

The Effect of Measurement System on Resting Metabolism

Table III.1 presents mean resting metabolism values for each treatment group using either the water immersion or head-hood system. Respiration rate averaged 16 ± 2 breaths/min (mean ± SEM) in both systems and shivering was not observed, which indicates a thermoneutral environment in both cases. Resting metabolism measured with the head-hood system 4.52 ± 0.30 $W/kg^{.75}$, (mean ± SEM) was significantly greater than measured in the water immersion system 3.61 ± 0.16, ($P=0.035$). The average differences between the head-hood system and the water immersion system were 1.22, 0.66 and 0.87 $W/kg^{.75}$ respectively for the 0°, 18° and 36° C thermal treatments. The mean difference in resting metabolism expressed as a percent of the water immersion value was 25.4 ± 2.7% (mean ± SEM). In both the water immersion and head-hood system, resting metabolism was highest in the 0° C acclimated sheep and lowest in the 36° C acclimated sheep, confirming the results of chapter II. The resting metabolism means, measured using the head-hood system, were not significantly different ($P>0.05$) across temperature treatments, but resting metabolism means were significantly different when measured using the water immersion system ($P<0.05$).

The Effect of Measurment System on the Coefficient of Variation in Resting Metabolism

The coefficient of variation for each measuring system is presented in Table III.2. The six ewes had their resting metabolism measured in $W/kg^{.75}$ using both the water immersion

system and the standing in a head-hood system on consecutive days. There were two ewes in each acclimation temperature. A mean of the two resting metabolism values for each system was calculated, and the means and the standard deviation of the means used to calculate the coeffient of variation for each measurment system. The coefficient of variation was lower for the water immersion system. The increase in variation progressing from 0° to 18° to 36° C in the water immersion system is opposite to that seen in the head-hood system and the reasons for this have not, as yet been established.

D. Discussion

The difference between resting metabolism measured in the water immersion system and that in the head-hood system (Table III.1) is similar in magnitude to changes in metabolic heat production found when an animal moves from a lying to a standing position. Resting (or basal) metabolic rate in humans and cattle is 7-25% greater in the standing subject than in a lying position, (Vercoe, 1973; Bandyopadhyay et al. 1980 and Geissler et al. 1985).

In the present experiment the animal's posture or position was the same whether in the water immersion or head-hood system. Some of the difference in resting metabolic heat production measured by the two systems could be a result of the energy expenditure required to maintain a standing position. Factors such as age, breed and plane of nutrition have been standardized in the trial. Results of this trial indicated that energy expenditure required to maintain a standing posture may be 25.4% greater in air than in water. Thus there may be a considerable energy cost for maintenance of muscle tone and posture, which was not included by Baldwin et al. (1980) in their consideration of basal energy expenditure.

Different heat production values have been observed using a water immersion system and a standing with a face mask system. Summit metabolism values measured in adult sheep in a water immersion system, chapter II., were 18.4% lower than similar values reported by Bennett (1972). Bennett's (1972) values were derived from shorn sheep standing in a wind tunnel, and if the animal layed down summit metabolism was reduced by 27.0%. It has been

hypothesized that when it lies down, certain muscles used for shivering are no longer available and thus heat production was compromised (Alexander 1979). It is also possible that a reduction in surface area as a result of lying down leads to the reduced heat production.

Preliminary work in this laboratory seems to indicate that the differences observed between resting metabolism measured in a water immersion and in a head-hood system is linear. For the water immersion, resting metabolic rate was 17.8% lower than for the head-hood system for sheep acclimated to 18° C (Table III.1). This 17.8% difference is similar to the 18.4% difference in summit metabolism between standing and the water immersion system for sheep acclimated to 18° C. The difference between the water immersion system and standing in a head-hood system was significant and should be considered when comparing results from one laboratory to another.

The high thermal conductivity of water allows one to make fast and humane summit metabolism measurements, after which the water temperature may be raised and thus the animal is rapidly rewarmed. Since skin temperature is the same as water temperature, tissue insulation can be calculated using the method described by Webster (1974):

$$\text{Insulation}_{(\text{tissue})} = \text{Temperature}_{(\text{rectal})} - \text{Temperature}_{(\text{skin})} / \text{ heat loss.}$$

Once a subject became habituated to the water immersion system, it appeared to enter a sleep like state (Okamoto et al. 1986 and Eales and Small 1980) and this relaxed state resulted in rapid resting metabolism measurement. The relaxed state may be responsible for the reduced coefficient of variation in the water immersion system compared to the head-hood (Table III.2). One can calculate the energy expenditure for maintaining a standing posture when using a water immersion system in parallel with a conventional head-hood system. Thus the water immersion system will allow thermal physiologists the latitude to make accurate and varied measurements in an easier fashion than previously available.

A comparison of resting metabolism measured by either a water immersion system or a head-hood system has been described. The water immersion system resulted in a

significantly lower resting metabolism. It is thought that some of the difference may be due to muscle tone needed to maintain a standing posture.

Table III.1 The effect of thermal acclimation (°C) on resting metabolism ($W/kg^{.75}$) measured in a water immersion system or a head-hood system

	Acclimation Temperature		
	0°	[redacted]	36°
Water immersion (2)*	3.96 ± 0.01[a]	3.71 ± 0.05[b]	3.14 ± 0.05[c]
Head-hood (2)*	5.18 ± 0.69[a]	4.37 ± 0.30[a]	4.01 ± 0.21[a]

†Values represent means ± SEM.

*Number of animals per acclimation temperature in parentheses.

[a-c] Row means are significantly different if followed by different letters ($P<0.05$).

Table III.2 The effect of thermal acclimation (°C) on the coefficient of variation for resting metabolism in a water immersion system vs a head-hood system

	Acclimation Temperature		
	0°	18°	36°
Water immersion	0.2%	1.9%	2.3%
Head-hood	18.8%	9.7%	7.4%

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IV. OUABAIN-SENSITIVE AND -INSENSITIVE RESPIRATION IN THE EXTERNAL INTERCOSTAL MUSCLE OF THERMALLY ACCLIMATED SHEEP

A. Introduction

Cold induced physiological acclimation increases non-shivering thermogenesis, as reflected by increased resting metabolism in sheep and cattle (Webster et al. 1969; Alexander et al. 1970; Young and Degen 1981). This increase in non-shivering themogenesis results from changes in thyroid and catecholamine status (Christopherson et al. 1978; Graham and Christopherson 1981; Kennedy et al. 1985). The thermogenic effect of catecholamines increases in states of hyperthyroidism and decreases in hypothyroidism (Himms-Hagen 1983). Three basic mechanisms are believed to underlie the thermogenic action of thyroid hormones: (1) a change in the properties of mitochondria, such that respiration increases even in a coupled state, (2) increases in the total mitochondrial content of tissues, and 3) an increase in Na^+/K^+ ATPase activity in tissue, which is related to an increased activity and number of pump sites in the tissue (Himms-Hagen 1983).

Isolated tissue oxygen uptake (QO_2) and active transmembrane transport change markedly with the physiological state of an animal (McBride 1984; McBride and Milligan 1984; 1985a; 1985b; Milligan and McBride 1985). In sheep, exposure to cold increased QO_2 and Na^+ pump-dependent oxygen uptake [$QO_2(t)$] of skeletal muscle, (Gregg and Milligan 1982). The work of Gregg and Milligan (1982) on ion transport in tissue obtained from cold-exposed sheep used tied fibre bundles prepared from the sternomandibularis muscle. Recently, a superior muscle preparation using the external intercostal muscle (E.I.C.) has been established for in vitro studies with large ruminants (Wijasinghe et al. 1984). The present study used this muscle preparation to verify earlier observations on the effects of cold acclimation on QO_2 and active Na^+ transport and to determine the effects of acclimation to hot conditions on these parameters.

The conditions of measurement described in this paper follows the convention of Guernsey and Eddlman, 1983. The following definitions are provided:

QO_2(μlO_2/h per mg dry matter): Initial oxygen consumption of tissue biopsy before any chemical treatment is applied, measured for 20 minutes.

QO_2'(μlO_2/h per mg dry matter): Oxygen consumption in the presence of ouabain, a specific inhibitor of Na^+/K^+ ATPase activity, measured for a 20 minute period. This is the ouabain insensitive component.

$QO_2(t)$(μlO_2/h per mg dry matter): The difference between initial O_2 consumption and the ouabain insensitive component. This is the ouabain sensitive component.

B. Materials and Methods

Animals and Management

Nine mature, non-lactating, non pregnant Suffolk crossbred ewes (52.1±3.5 kg, mean±SEM) were randomly assigned to one of three ambient temperature treatments (36°, 18° and 0° C) They were placed in individual metabolism crates (0.61 x 1.52 m) in either temperature controlled rooms or temperature controlled chambers under constant lighting and held for at least 6 weeks prior to measurement. They were closely shorn at the beginning of the trial and every two weeks thereafter to reduce external thermal insulation.

Each ewe was offered 1100 g of pelleted alfalfa hay daily, between 1500-1530 h regardless of acclimation temperature and there were no refusals. Water and cobaltized iodized salt were available free choice.

Whole-Animal O_2 Consumption

Whole animal resting O_2 consumption was measured after at least 42 d of thermal acclimation and 17 h after feeding. Each animal was strapped into a simple restraining frame and immersed in a water bath controlled at 38±0.2° C similar to that used by Eales and Small (1980) and Therminarias et al. (1979). After the metabolic heat production of the sheep had stabilized in the water bath, rate of respiratory consumption of O_2 was measured over a minimum of 20 minutes using an open-circuit respiratory analyzer, (Taylor Servomex OA

184, Sussex England), connected to a ventilated face mask to which the sheep had been previously accustomed, Young et al. (1975). The system was calibrated to within 2% by the Fe-burner method of Young et al (1984). Water temperature was measured using 40 s.w.g. Cu/Con thermocouples and a Bailey (BAT8, Saddle Brook, N.J.) instrument, which was regularly electronically calibrated.

Muscle O_2 Uptake and Ouabain Sensitivity

Within a week of measuring whole-animal O_2 consumption, samples of external intercostal muscle were obtained from each sheep using the method of Wijasinghe et al. (1984). Ambient temperature in the surgery was maintained at 20-25° C throughout the sampling process. Gregg and Milligan (1982) have previously showed that ambient temperature in the surgery at time of sampling had no effect on either QO_2 or ouabain sensitivity of sternomandibularis muscle isolated from cold-acclimated sheep. Biopsy fibre bundles were mounted at resting length onto plastic supports using adhesive (Krazy Glue Inc., Chicago, Ill) and pre-incubated for a minimum of 10 minutes in a modified Krebs-Ringer bicarbonate buffer (Wijasinghe et al. 1984) maintained at 37° C and saturated with air.

Oxygen uptake measurements were made in four or five viable biopsies per sheep using a YSI Model 53 (Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio) O_2 electrode system. Initial O_2 uptake (QO_2) was measured for 20 minutes. The sample chamber was then injected with 60 μl of a 10^{-2} M ouabain stock solution to achieve a final ouabain concentration of 10^{-4} M, which inhibits Na^+ / K^+ ATPase activity in the EIC muscle of adult sheep (Wijasinghe and Milligan unpublished results). Oxygen uptake in the presence of ouabain (QO_2') representing the ouabain insensitive component, was measured for a further 20 min, and by difference, the ouabain sensitive component was calculated (Guernsey and Edelman 1983). After the oxygen uptake measurements, the fibre bundles were dried overnight at 60° C for determination of dry matter content.

Blood Sampling and Analysis

Approximately 20 ml of blood was taken from each sheep using venipucture, (Vacutainer, Becton Dickinson, Mississauga, Ont.), again 17 h postfeeding and after 42 d of thermal acclimation. Plasma was stored at -25° C until analyzed for total triiodothyronine (T_3) and thyroxine (T_4) levels using the radioimmunoassay method of Coat-A-Count (Diagnostic Products Corporation, Los Angeles, Ca.).

Statistical Procedures

The tissue O_2 data were adjusted to a 10 mg dry weight using covariate analysis. Data were analyzed using least squares analysis of variance, and where significant differences existed, treatment means were compared using the Student-Newman-Keuls multiple range test (Steele and Torrie 1980).

C. Results

Plasma Thyroid Hormone Concentrations

Thermal acclimation to 0° C resulted in the highest plasma concentrations of T_3 and T_4 while acclimation to 36° C resulted in the lowest plasma concentrations (Table IV.1). Thermal acclimation to 36° C resulted in a 5.4% reduction in T_3 concentration, compared to 18° C and a 29% reduction in T_4 concentration, relative to the 18° C acclimation temperature value. Acclimation to the 0° C, resulted in a 130% increase in T_3 concentration and a 51% increase in T_4 concentration relative to the 18° C acclimation temperature values.

Whole-Animal O_2 Consumption

The thermal environment to which the sheep were acclimated significantly ($P<0.05$) affected whole-animal resting O_2 uptake across all treatments (Table IV.2). Sheep acclimated to 36° C had markedly reduced resting metabolisms, whereas those acclimated to 0° C had increased resting metabolisms. This confirms the earlier results of chapter II.

Thermal acclimation to 0° C resulted in a 6.8% increase in resting metabolism, while thermal acclimation to 36° C resulted in a 15.4% decrease in resting metabolism, compared to the 18° C value.

Muscle O_2 Uptake and Ouabain Sensitivity

Because four or five muscle biopsies were used, approximately 2.5 h elapsed between starting measurement of the first sample and completing the final measurement. No difference in tissue O_2 uptake was observed over this period; indeed, Wijasinghe et al. (1984) found that the resting membrane potentials, rates of protein synthesis, and rates of O_2 uptake in intact E.I.C. muscle fibre bundles were relatively stable over an incubation period of more than 3 h.

The QO_2 was parallel with the whole-animal resting O_2 consumption, (Figure IV.1), with both increasing with cold acclimation and decreasing with acclimation to the hot environment. Changes were also observed in the $QO_2(t)$ and the QO_2' components of the muscle O_2 uptake. The increase ($P<0.05$) in QO_2 with cold acclimation was explained almost entirely (86%) by an elevation ($P<0.05$) in QO_2' with only a small ($P>0.05$) rise in $QO_2(t)$ (Figure IV.1). Acclimation to 36° C lead to a significant ($P<0.05$) lowering of QO_2, which was the result of reductions ($P<0.05$) in both $QO_2(t)$ and QO_2'. Seventy-three percent of the drop in QO_2 was due to alterations in QO_2'. It should noted that the change in QO_2 is more marked with acclimation to 36° C than to acclimation to 0° C. The former treatment leading to a 43% decrease in QO_2 whereas the latter treatment resulted in a 33% increase in QO_2.

The percentage inhibition of O_2 uptake by ouabain was unaffected by thermal environment (Table IV.2) with an average of 19% of QO_2 being attributable to $QO_2(t)$.

D. Discussion

The three acclimation temperatures chosen in this experiment provide an opportunity to compare chronic exposure of mature sheep in a hot temperature and a cold temperature to a control or comfortable temperature. Chronic exposure of the ewes to the three temperatures resulted in changes to whole-animal resting O_2 consumption and to E.I.C. muscle O_2 uptake.

Cold acclimation increased whole-animal resting O_2 consumption (Table IV.1) probably explainable, at least in part, by an altered thyroid status in cold acclimated animals (Westra and Christopherson, 1976; Christopherson and Thompson 1983; Kennedy et al. 1986). Cold acclimation can result in changes in plasma concentrations of adrenaline and noradrenaline (Christopherson et al. 1978), and responses to these catecholamines in the cold may be potentiated by elevated thyroid hormone levels (Fregly et al. 1979). Much of the metabolic response to thyroid hormones appeared to be accounted for by the energy requirements for Na^+ pump activity (Guernsey and Edelman 1983). From the elevated plasma T_3 and T_4 levels in cold-exposed sheep (Table IV.1), one can predict an increase in QO_2 of the E.I.C. muscle. This was evident, but QO_2 uptake also changed markedly with cold exposure. The ouabain sensitive and insensitive fractions of the E.I.C. both increased, but the proportion of each in total O_2 uptake remains relatively constant. Other components of maintenance energy expenditure known to be influenced by thyroid status are Ca^{+2} ATPase activity (Van Hardeveld and Clausen 1984), phospholipid turnover (Vladimirov et al. 1984) and protein synthesis (Brown and Millward 1983). The elevation of QO_2 may have arisen from an increase in one or more of these components. If tissue sensitivity to thyroid hormones in the 0° C acclimated sheep is unchanged, then other mechanisms of cold-induced thermogenesis, such as the action of catacholamines (Guernsey and Edelman 1983; Graham and Christopherson 1981), may be acting. Previous work in this laboratory, (chapter II), has shown increased plasma free fatty acid levels and plasma blood glucose levels that could act as substrates for increased maintenance energy requirements.

The whole-animal O_2 consumption and QO_2 of the 0° C acclimated animals agree with those in previous studies (Gregg and Milligan, 1982). However, in the present

experiment, cold-induced thermogenesis in the E.I.C. muscle was largely accounted for by an increase in QO_2 uptake, whereas Gregg and Milligan (1982) found a rise in $QO_2(t)$ uptake constituted most (79%) of the higher QO_2 in the sternomandibularis muscle of cold-adapted sheep fed at the same level of intake as warm-adapted controls. No plausible explanation for these contradictory findings is apparent. The energy expenditure of Na^+/K^+ transport varies with the physiological state of the animal. Change occurs with age, lactation, level of energy intake, and perhaps health status and such change may be quite specifically restricted to Na^+/K^+ transport or may be an overall constituent of a changed O_2 consumption (McBride and Milligan, 1984).

Gregg and Milligan (1982) used cut and tied fibre bundles incubated free; the present experiment uses intact fibre bundles incubated at resting length. The sex and genetic background of the animals used in the two experiments were the same, but their weights differed considerably (35 kg vs 52), and feed intake ($g/kg^{.75}$) was thus 35% greater for the animals of Gregg and Milligan (1982). It is known that the Na^+/K^+ pump plays its greatest quantitative role in highly productive states (Milligan and McBride 1985). Animals used in the current experiment were mature and on a near maintenance level of intake so Na^+/K^+ pump activity likely contributed less to maintenance energy expenditure than in the earlier study. The influence of age on Na^+/K^+ ATPase dependent respiration has been documented by Gregg and Milligan (1982 a,b) in sheep and cattle. The percentage of total aerobic energy in support of Na^+/K^+ transport was higher in the younger calves and lambs. McBride (1984) has reported that hepatocytes from mature sheep expended less cellular energy on Na^+/K^+ transport than did those from 1 to 8 week old lambs.

Functionally, the sternomandibularis and the E.I.C. muscles differ; the former is a postural muscle, the latter a respiratory. Schaeffer et al. (1982) reported that in chronic cold-exposure, blood flow to nonrespiratory skeletal muscle increases markedly, whereas blood flow to respiratory muscle is little changed, which is consistent with a reduced respiration rate. Cellular processes such as ion transport may be differentially altered in the two muscle groups; this is however, somewhat unlikely since the QO_2 of both muscle groups increases

with cold exposure.

Little information is available on the effects of heat acclimation at the cellular level. The 36° C acclimated sheep in the current study had $QO_2(t)$ and QO_2' values 55% and 39% less, respectively, than did the 18° C acclimated animals. Although in absolute terms the change in QO_2' constitutes most of the difference in QO_2 between the 36° and 18° C acclimated animals, the two components of maintenance energy expenditure studied here changed disproportionately, with the effect being most marked for $QO_2(t)$ uptake. This implicates a thyroid hormone response since, as mentioned earlier, T_3 and T_4 are mediated through Na^+/K^+ ATPase (Guernsey and Edelman 1983). Plasma T_3 and T_4 levels were decreased by acclimation to 36° C (Table IV.1.) The decrease may result in a thyroid hormone response that was seen as the decrease in $QO_2(t)$ uptake. The decrease in thyroid hormone concentrations was not due to a decreased feed intake which has been reported by Rousset et al. (1984).

The change due to thermal acclimation in energy expended on Na^+/K^+ transport is reflected in a changed whole animal O_2 uptake values. The changed plasma T_3 and T_4 concentrations and accompanying changes in catecholamine concentrations were expressed through Na^+/K^+ ATPase. A change in the nonshivering component of animal metabolism due to thermal acclimation has been described.

Table IV.1 Effect of thermal acclimation (°C) on plasma triiodothyronine (T_3) and plasma thyroxine (T_4) levels

	Acclimation Temperature		
	0	18	36
Plasma Concentration† (nmol/l)			
Triiodothyronine (3)•	2.53 ± .06[a]	1.10 ± .13[b]	1.04 ± .13[b]
Thyroxinine (3)	152.64 ± 4.62[a]	100.94 ± 4.12[b]	71.56 ± 5.57[c]

†Values represent means ± SEM.

•Number of animals per acclimation temperature treatment in parenthesis

[a-c]Means within a row followed by a different letter are significantly different ($P<0.05$).

Table IV.2 Effect of thermal acclimation (°C) on whole body O_2 uptake (ml O_2/h per kg) and external intercostal muscle tissue O_2 uptake (μl O_2/h per mg dry matter)

	Acclimation Temperature		
	0	18	36
Whole animal	257.45 ± .35[a]	240.95 ± 3.25[b]	203.85 ± 3.25[c]
Total muscle	1.11 ± .04[a]	.83 ± .05[b]	.48 ± .05[c]
Ouabain sensitive	.21 ± .01[a]	.17 ± .02[b]	.08 ± .01[b]
Ouabain insensitive	.90 ± .04[a]	.66 ± .05[b]	.40 ± .05[c]
Percent inhibition	19.21 ± .79[a]	20.79 ± 1.88[a]	16.71 ± 2.41[a]

Values are means ± SEM.

[a-c]Means within a row followed by a different letter are significantly different ($P<0.05$).

Three animals per acclimation temperature treatment.

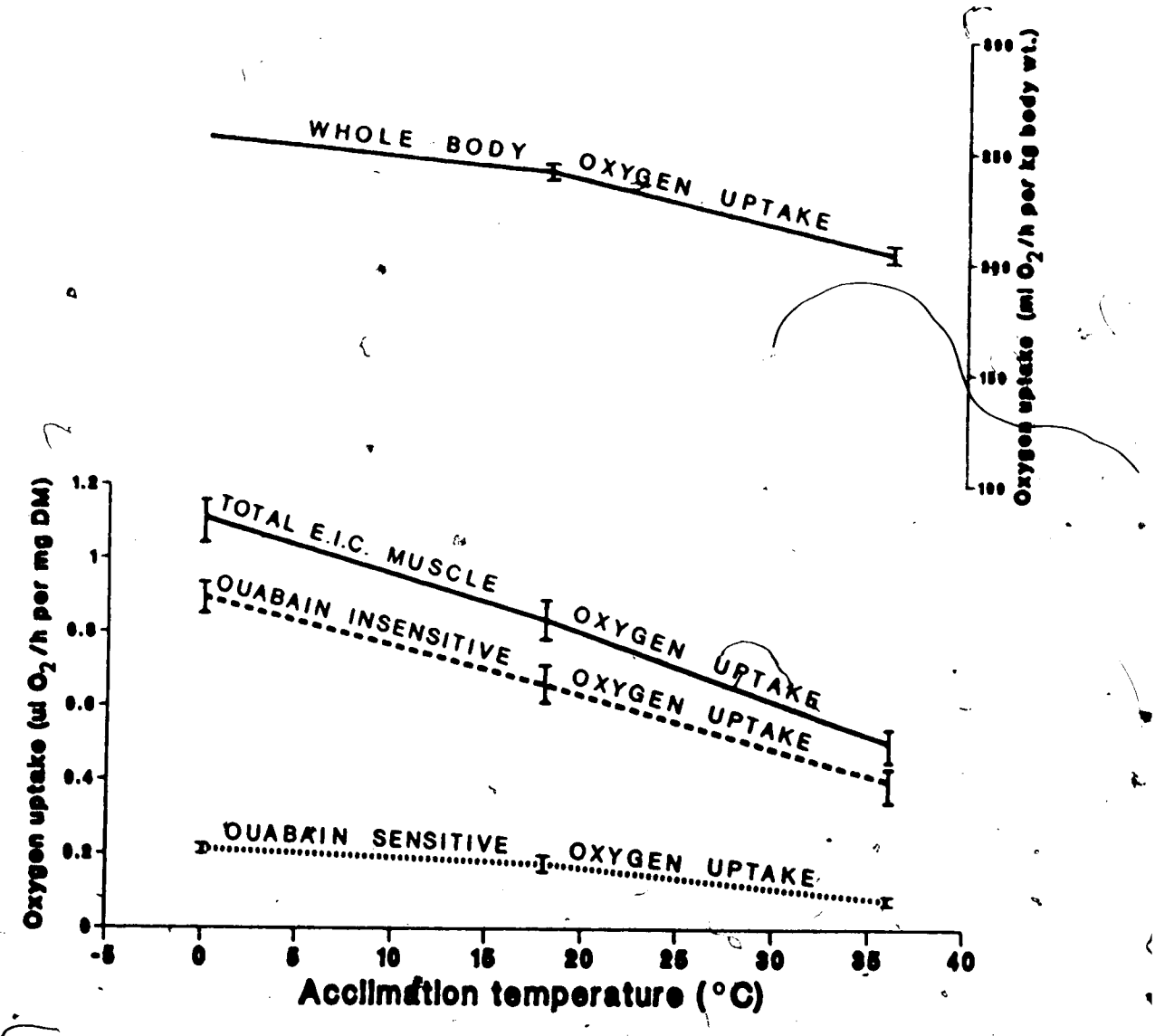

Figure IV.1 The effect of thermal acclimation on whole body oxygen uptake and external intercostal muscle tissue oxygen uptake

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V. THE EFFECTS OF THERMAL ACCLIMATION ON THE UPPER AND LOWER CRITICAL TEMPERATURES OF MATURE SHEEP

A. Introduction

Previous work in this laboratory has shown that thermal acclimation alters the heat production of sheep and cattle (Young 1975; chapter II; and chapter III). In one study (chapter II) the resting heat production of mature sheep in ($W/kg^{.75}$) acclimated to 36° C was 18.4% lower than that of sheep acclimated to 18° while acclimation to 0° C increased resting heat production by 18.4%, despite food intake being held constant for all temperature treatments.

Mount (1979) defines the zone of thermal neutrality as "the range of environmental temperatures in which the animal's metabolic rate is at a minimum, constant and independent of the environmental temperature". Another more specific term relating to the influence of temperature on metabolism is the zone of least thermoregulatory effort, which is defined as the range of environmental temperatures for a given level of feeding, in which the metabolic rate of an individual resting animal is at a minimum and evaporative heat loss is not increased as the result of sweating or increased respiratory ventilation (C-D in Figure V.1) (Mount 1974).

The zone of least thermoregulatory effort is bordered by two points, the lower critical temperature (L.C.T.) and the upper critical temperature (U.C.T.). At environmental temperatures below the L.C.T., an animal must increase its rate of heat production roughly in proportion to the fall in effective environmental temperature to maintain body temperature (Curtis 1981; Mount 1979). The rate of increase in heat production depends on the species, age, effective thermal insulation and adaptation history of the animal and enviromental factors (Mount 1979). The L.C.T. is different for different animals and depends on three things: the animal's body-core temperature, its heat production rate at thermoneutrality, and its maximal thermal insulation (Curtis 1981). Thermal insulation is determined by the integrity of the boundary layer, cover type and depth, subcutaneous fat thickness, effective

surface area, and state of vasoconstriction or vasodilation of peripheral vessels (Curtis 1981; Blaxter 1977).

As environmental temperatures increase above the U.C.T., animals increase their rate of heat loss above basal levels to avoid hyperthermia. Evaporative cooling from the respiratory tract (panting) is a major factor in facilitating heat loss from adult sheep (Brockway et al. 1965; Hofmeyr et al. 1969). Animals on a high plane of nutrition have lower U.C.T.'s than animals on lower planes. Pregnancy, lactation, and exercise also lower the U.C.T. (Mount 1979). Summer acclimatization results in downward shifts in the U.C.T. in dogs (Sugano 1981), sheep (Bern[illegible]n 1976), and penguins (Barre 1984).

If changes in resting heat production due to thermal acclimation are not accompanied by changes in other factors such as changes in thermal insulation, then an animal's U.C.T. and L.C.T. should change with thermal acclimation. Many animals increase their pelage in the cold and increase their metabolic heat production to meet the thermal demands of their environment (Sugano 1981). Nevertheless, acclimatization to winter has been shown to alter the L.C.T. in dogs (Sugano 1981), hamsters (Heldmaier et al. 1981), voles (Rosenmann et al. 1974), and penguins (Barre 1984). In all of these studies, food was offered ad libitum or intake increased with cold exposure or external insulation increased. Changes occurring in feed intake, external insulation, and body size will confound the effect of thermal exposure on an animal's L.C.T..

The L.C.T. is estimated as the intersection of extrapolations of the slopes of increasing heat production of animals exposed to cold temperatures below their L.C.T. and of the constant heat production of thermal neutrality (Mount 1979). This method of estimation has been used by Erikson et al. (1956) and Sato et al. (1985). Similarly, the U.C.T. can be estimated by the intersection of the slope associated with increasing respiration rate with increases in heat exposure, with the mean or basal respiration rate associated with a thermal neutral temperature zone.

A study was conducted to determine the effect of thermal acclimation on (1) the zone of least thermoregulatory effort in mature sheep and (2) the animal's U.C.T. and L.C.T..

B. Material and Methods

Design

The experiment was conducted as a replicated 3x3 latin square with three thermal acclimation treatments 0°, 18° and 36° C with two sheep per treatment. Treatment periods were 42 d. No two sheep were exposed to the three temperature treatments in the same order, so as to eliminate any influence of previous environment.

Animals

This study used six mature, non pregnant Suffolk crossbred ewes with an initial body weight of 49.3±4.8 kg (mean ± SEM). The ewes were maintained in indiyidual metabolism crates (0.61 mx 1.52 m) in controlled-temperature rooms under constant lighting. The animals were fed 1100 g of pelleted alfalfa hay once daily (1430-1500 h); water and cobalt-iodized salt were available free choice. To minimize and standardize external insulation, the sheep were shorn to a fleece depth of .5 to .75 cm at the beginning of every second week during the study.

Measurements

Body weight was recorded weekly except when animals were on a digestibility study. Metabolism measurements were made on days 39 to 41 of each treatment period using a water-immersion system as described by Young et al.(1988). This system provides a stable thermal environment that can be altered quickly and accurately.

Metabolism was measured by indirect calorimetry with the ewe stabilized in 38±0.2° C water (mean±SEM). Water temperature was increased in 1° C increments to 40° C, then it was increased in 0.5° C increments. The animal was maintained at each water temperature for 15 minutes. The trial was stopped when the animal's respiration rate reached 150 breaths per minute. The rate of 150 breaths per minute is approximately half the maximum respiration rate for panting sheep (Alexander, 1974). The water temperature was then reduced to 38° C

and the animal was held at this water temperature until a stable respiration rate and metabolic rate were obtained. The water temperature was lowered in increments of 1° C until 35° C was reached. After this it was lowered in 0.5° C increments until the oxygen consumption level was at least twice the level recorded in 38° C water.

Water and rectal temperatures were monitored continuously with thermocouples. The water temperature thermojunction was made of 40 s.w.g. Cu/Con wires, and the end was placed in the return flow from the circulating water pump. The thermocouple for rectal temperature was sheathed in a flexible plastic tube and inserted 10 cm into the rectum. All temperatures were measured using the same instrument (Bailey BAT8, Saddle Brook, N.J.), which was calibrated during each period. During experiments a spot check on the calibration was made against a Hg-in-glass thermometer to ± .05° C.

Metabolic rate (M) was measured using an open circuit procedure employing a mask over the animal's muzzle. The animals were accustomed to the mask and other measurement procedures before this trial began. Ventilation rate of the mask was read from a flowmeter (Rotameter, Fischer and Porter, Warminster, Pa.); the oxygen concentration difference between incoming and outgoing air, from a paramagnetic analyzer (Taylor Servomex 0A184, Sussex England). The calorimeteric system was calibrated using the procedures of Young et al. (1984), and M was calculated using the equation of McLean (1972).

Respiratory frequency was determined from pressure changes in a pneumograph that was fixed on the left flank of the animal after it was strapped into the frame and before it was placed in the waterbath. Gain control on the physiological recorder (Carolina Instruments) was adjusted until a satisfactory tracing of respiration rate was obtained.

Animal weights were recorded using a balance (Berkel Products Co. Ltd., Toronto, Ont.). Animals were weighed to within 0.5 kg in the morning between 0830 - 1000 h.

On days 28-33 of each period, screens were installed in each metabolism crate to allow for separation of urine and feces. The feces from each animal were dried at 60° C in a forced air oven for 24 h and used to estimate dry matter digestibility.

Calculations and Statistical Procedures

The data for each animal included respiration rate and rate of metabolic heat production for all the different water bath temperatures. The U.C.T. was estimated by the linear regression of respiration rate on water bath temperature. The regression used the three highest respiration rate water temperature points which always occurred at water temperatures above 40° C. The point of intersection between the regression and the mean respiration rate when water temperature was less than 40° C was used to estimate the U.C.T..

A similar method was used to calculate the L.C.T. based on heat production (W/kg) and water bath temperature. Since baseline regressions of heat production on water temperature resulted in no or low significance, mean heat production values were used for water temperatures greater than 37° C. The three highest heat production rates and corresponding water bath temperature points were used to generate a linear regression. The intersection of the linear regression with the mean heat production values at water bath temperatures above 37° C was used to calculate the L.C.T. The method described for measurement of U.C.T. and L.C.T. is similar to that used by Erikson et al. (1956), for studies on humans and by Sato et al. (1985), also for humans.

Treatment means were analyzed using least-squares analysis of variance and, where significant differences existed, means were compared using the Student-Newman-Keuls-Multiple Range test (Steele and Torrie 1980).

C. Results

One animal became sick while in the 0° C environment during period two and had to be removed. It recovered and was returned to the 0° C for period 3. The critical temperatures reported have been adjusted accordingly.

The Effect of Thermal Acclimation on the U.C.T. and L.C.T.

Table V.1 shows the effect of thermal acclimation on U.C.T. and L.C.T.. For animals acclimated to 0°, 18° and 36° C, U.C.T.s were 41.1, 41.4 and 40.8±0.1° C.(mean±

SEM) respectively. Mean L.C.T.s were 35.3°, 34.7° and 33.8±0.5° C respectively, (mean± SEM).

The Effect of Thermal Acclimation on Rectal Temperatures at the U.C.T. and L.C.T.

Table V.1 gives the average rectal temperature at L.C.T. and U.C.T. while in water for each acclimation temperature. The average rectal temperature at the L.C.T. was lowest for the 36° C acclimation temperature and highest for the 0° C acclimation temperature. The same was true for the U.C.T.. The difference between the rectal temperatures at the L.C.T. and U.C.T. are the same for the 0° C and 36° C acclimation temperatures, with both greater than the 18° C acclimation temperature.

The Effect of Thermal Acclimation on the Range of the Zone of Least Thermoregulatory Effort

The zone of least thermoregulatory effort or the difference between the L.C.T. and U.C.T., for the 0°, 18° and 36° C acclimated animals was 5.8°, 6.7 ° and 7.2±0.5° C respectively (difference between means±SEM). The range in the zone of least thermoregulatory effort apparently widened as acclimation temperature increased.

The Effect of Thermal Acclimation on Rectal Temperature

Initial rectal temperatures, measured during the first 5 minutes in 38° C water, for animals from the 0°, 18° and 36° C environments averaged 38.9°, 39.1° and 39.6±0.2° C (mean±SEM). It appears that the animals were allowing their body temperature to fluctuate with acclimation temperature, and thus conserving energy that would be expended on mechanisms of heat loss or heat production.

The Effect of Thermal Acclimation on Tissue Insulation

The tissue insulation values were calculated for 17 complete tests according to the method described by Webster (1974), where:

$$\text{Insulation}_{(\text{tissue})} \quad \text{Temperature}_{(\text{rectal})} \quad \text{Temperature}_{(\text{skin})} \ / \text{ heat loss.}$$

with rectal temperature and skin temperature measured in °C, and with heat loss measured in W/m^2. Therefore, tissue insulation has units of $°Cm^2/W$. Skin temperature and water temperature were assumed to be the same.

Heat loss was estimated from heat production with a correction factor for change in body heat storage. Fluctuations in rectal temperature during the measurement of heat production, result in changes to body heat storage. Changes in body heat storage result because:

Heat loss = Heat production ± Storage, as a result of this:

if T_{rectal} decreases, Storage + Heat production = Heat loss.

if T_{rectal} increases, Heat production - Storage = Heat loss.

Heat loss was corrected for the evaporative component which was 7.5 W/m^2.

Change in heat storage was calculated using the equation:

$$\text{Storage} = (\text{Trectal}_{\text{start}} - \text{Trectal}_{\text{end}}) \times \text{specific heat of the animal} \times \text{weight} \times \text{time} \times 1.163 / \text{surface area}.$$

Rectal temperature was measured in °C, specific heat measured in kcal/kg, time in hours and surface area in m^2. The specific heat constant used was 0.83 and the 1.163 factor is used to change kcal/h to W. Heat loss was estimated using calculated changes in body heat storage and heat production. Tissue insulation values were then calculated.

Analysis of variance showed thermal acclimation to significantly ($P<0.05$) affect tissue insulation, with the 36° C acclimation temperature being significantly greater than the 18° C or 0° C. The greater tissue insulation after acclimation to 36° C was .076 ± .006 compared to .049 ± .005 and .044 ± .005 °C m^2/W (mean ± SEM) for 18° C and 0° C respectively (Table V.2).

The Effect of Thermal Acclimation on Body Weight and Dry Matter Digestibility

Table V.3 illustrates the effect of thermal environment on body weight. The average weight loss over each 42-d period at 0° and 18° C was 6.3 ± 1.6 and 2.8 ± 1.6 kg. The average increase at 36° C is 0.3 ± 1.9 kg (mean ± SEM).

The dry matter digestibility was highest for animals in 36° C and lowest for animals in 0° C.

D. Discussion

Figure V.1 shows the classic model of heat production on heat loss versus environmental temperature (Monteith and Mount 1974). The increase in heat production associated with the decrease in effective ambient temperature and the increase in evaporative heat loss associated with increasing ambient temperature in which the animal's metabolic rate is at a minimum, constant and independent of the environmental temperature is classically known as the zone of least thermoregulatory effect (C-D).

Thermal acclimation results in change to resting metabolic rates (chapter II, chapter III). Variation in the rate of minimal metabolism, C-E, would move C relative to the temperature scale and thus change the L.C.T. (Fig V.1). This type of adaptation is seen in cats (Hensel 1981) and dogs (Suganp 1981). In both cases changes in resting heat production were accompanied by changes in insulation. The change in metabolic intensity was coupled with the changes in insulation, with both exerting influence on the animal's critical temperature.

Depocas et al. (1957) showed that the effect of thermal acclimation to 30° C and 6° C on the resting metabolism of rats. The interesting point shown here is that the L.C.T. value for each group of rats appears to be equal. An increase in heat production was seen at approximately 20° C and the two heat production curves appear parallel. This indicates equal insulation between the two groups. This is evidence of thermal acclimation changing resting heat production without changing the critical temperature of the animal.

The results of the current experiment seem contradictory to the classic theory of the influence of ambient temperature on metabolism. If resting heat production is shifted by thermal acclimation, then according to theory (Curtis 1981; Young 1983; Mount 1979), the critical temperature of the animal should shift accordingly. Changes in the critical temperatures did not occur in the current experiment. It was thought that thermal acclimation of the sheep to 36° C would result in a raising of the U.C.T. similar to that described by Sugano (1981) for dogs and Barre (1984) for penguins, and that acclimation to 0° C would lower the U.C.T. relative to 18° C. The U.C.T. was highest for the acclimation temperature of 18° C. Acclimation to 36° C resulted in the lowest U.C.T. and the U.C.T. after acclimation to 0° C was between the other two temperatures (Table V.1). The reasons for the lowering of the U.C.T. with acclimation to 36° C are unclear and an explanation is not readily available.

Acclimation to a cold environment should lower the L.C.T. while acclimation to a hot environment should raise the U.C.T. (Hensel 1981; Young 1983; Curtis 1981). The L.C.T. was lowest for the 36° C acclimation temperature and highest for the 0° C acclimation temperature. Initially the results appear to contradict the theory of a shift in the U.C.T. or L.C.T. associated with changes in resting metabolism.

Non-evaporative heat loss is predominant in the homeotherm in a cold environment and thermal insulation impedes heat flow (Mount 1979). Therefore any changes in insulation will have a dramatic effect on rate of heat loss. An animal's thermal insulation consists of two components in series; the tissue insulation deep to the skin surface and the external insulation superficial to the skin (Mount 1979). The ewes in this experiment were closely shorn to remove any differences in external insulation. However if tissue insulation changed, heat loss would be altered and metabolism measurements to determine the influence of thermal enviroment on an animal would be affected.

Vasoconstriction or vasodilatation of peripheral blood vessels is important in determining a tissue's insulation. Blaxter (1977) reported tissue thermal insulation values for Down Sheep of .08 and .03 °C m^2/W under vasoconstriction and vasodilatation respectively. Sykes and Slee (1968) showed that cold acclimation lowers the critical temperature of

Blackface sheep and Slee (1973) concluded that the presence of vasoconstriction which occurs near the critical temperature and affects cold-induced inhibition of thermal panting were inter-connected. The perception of a cold stimulus by the nervous system may be necessary for the occurrence of both vasoconstriction and the block of panting (Slee 1973).

The tissue insulation values seen in Table V.2 may explain how the 36° C acclimated animal could have a lower L.C.T. This acclimation temperature results in a decreased heat flow from the animal to the water. An increase in tissue insulation should be accompanied by a change in the slope of the heat production versus ambient temperature line (C-B, Fig V.1). Although the heat production versus ambient temperature slope is not as precise as the tissue insulation values, as there is no correction for evaporative heat loss or change in body heat storage, a trend appears in the data with the slope being lowest for the 36° C acclimation temperature. This change in the slope helps to confirm the change in tissue insulation. This state of vasodilatation does not agree with the findings of Blaxter (1964) who stated that once an animal is below its critical temperature, tissue insulation is virtually constant.

Water temperatures necessary to elicit an increase in resting heat production ranged from 36 to 32° C. The similar tissue insulation values for the 18° and 0° C temperatures implies that these animals were in the same state of vasoconstriction or vasodilation. Their skin temperature is fixed by the water bath (as described by Jessen et al. 1986) probably at a temperature above what it would be in their acclimation environment. This could result in these animals being in a state of vasodilatation while undergoing a cold stress which would explain their lower tissue insulation values. The animals from the 36° C acclimation temperature have their skin temperature clamped at temperatures which are likely below what they would be in the acclimation environment. The heat stress of the 36° C acclimation temperature would result in an increase in cardiac output passing through peripheral ateriovenous anastomoses (Hales, 1973) which results in a state of vasodilation. When exposed to water in the 32-36° C range, these animals will likely vasoconstrict with the changes in tissue insulation accompanying. This increase in tissue insulation as a consequence of vasoconstriction will result in the lowering of the L.C.T..

The heat flow between the animal and the water in the bath decreased, not due to changes in resting metabolism per se but rather because of differences in states of peripheral vasoconstriction or vasodilatation. The change in vasoconstriction or vasodilation resulted in different tissue insulation values which influenced the L.C.T. measurements. The change in insulation may have occured because of the water bath procedure. In the water bath, skin temperature is fixed by the water temperature, but in a normal air environment, skin temperature differs greatly over the body (McLean et al. 1974). The fixing of skin temperature in a water bath results in certain physiological responses. The normal integration by the central nervous system of the afferent impulses from thermal receptors must be considerably interferred with in a bath experiment (Burton and Edholm 1955). This interference resulted in the changes in tissue insulation seen in the L.C.T. and may be involved in the contradictions seen in the U.C.T..

The biological significance of the apparent increase in the width of the zone of least thermoregulatory effort as acclimation temperature increases is unknown. It is likely due to the techniques of measurement used in this experiment.

The increase in rectal temperature associated with increasing acclimation temperature is in agreement with the results of Montsma et al. (1985). By allowing their body temperature to fluctuate in response to the thermal environments the animals may be conserving energy that would be expended in increasing the rate of heat loss or heat production (McLean 1983a; b).

Degen and Young (1980 and 1981) have reported the effect of cold exposure and feed intake on liveweight and body fluid compartments in sheep. They reported that of the total weight lost during a 10 day exposure to 0° C, 66% was due to loss of body water which came entirely from extracellular compartments. Morris et al. (1962) reported similar body fluid shifts in poorly and well nourished sheep during cold exposure. In cold exposure, vasoconstriciton and consequently the reduced vascular volume was probably involved in the loss of plasma value, which is supported by an increased hematocrit (chapter II, Table II.2). Although the 0° and 18° C acclimation temperatures resulted in decreases in liveweight over the

42 day period, a large component of this weight loss, especially in the 0° C temperature, was probably body water and not body solids. One could however expect the 0° C acclimated animal to lose weight because of the fixed food intake coupled with the animal's increased resting metabolic rate (chapter II).

Kennedy et al. (1986) reported on the relationship between the effect of cold exposure on digestion of roughage based diets by shorn and unshorn sheep. The results of (Kennedy et al. 1986) indicated a pronounced influence of temperature on digestion in the shorn sheep indicating that the response is related to the degree of thermal stress imposes upon the animal. There was a steady decline in digestibility as environmental temperature decreased over the range of 35° to -10° C. The dry matter digestibilities of the pelleted alfalfa hay follow the trends shown by Kennedy et al (1986).

The average rectal temperature of the three acclimation temperatures at the L.C.T. is seen in Table V.1. The rectal temperature for the 36° C acclimation group was lower than the other two acclimation temperatures which were equal. This lower rectal temperature may indicate that the animals were cold, and thus they would have vasoconstricted which resulted in the observed changes in tissue insulation. The 0° C and 18° C acclimation groups were not yet as cold so they could differ in their state of vaso-constriction or dilation. The average rectal temperatures for the three acclimation temperatures at the U.C.T. is the same for the 36° C and I8° C groups while it is highest for the 0° C acclimated animals. The increase in rectal temperature in the 0° C acclimated animals before panting is achieved is similar to the findings of Bligh (1963) and Slee (1974). The lower initial rectal temperature of the 0° C acclimation temperature may have indicated a heat debt which should be repaid before the panting response (Table V.1). Acclimation to 36° C may result in a heat load, which was seen as an increased rectal temperature, then this acclimation group should have the lowest rectal temperature at its U.C.T. While this was the case, the 18° C acclimation temperature had the same rectal temperature at the U.C.T. While the heat debt and load may explain the 0° C acclimation temperature, the same result for the 18° and 0° C acclimation temperature was unclear and an explanation is not readily available.

The change in the zone of least thermoregulatory effort as a result of thermal acclimation has been described. The original premise and assumptions upon which the hypothesis of the study was based and the estimation of U.C.T. and L.C.T. associated with changes in resting metabolism had to be reevaluated after examination of the data. The technique used in this experiment apparently had a major influence on the final results. While measurements of the L.C.T. may have been compromised by using the water bath system, changes in tissue insulation can be measured with precision as skin temperature is clamped at a known value. The L.C.T. data reveals the importance of understanding the biology of thermal regulation. The core receptors have played a major role in determining the final results. Also the possible changes in heat debt or load must be considered in the U.C.T. data.

Table V.1 The effect of prolonged thermal exposure (°C) on the upper critical temperature (U.C.T.) (°C), the rectal temperature at the U.C.T. (°C), on the lower critical temperature (L.C.T.) (°C), the rectal temperature at the L.C.T. (°C), the width of the zone of least thermoregulatory effort (Z.L.T.E.) (°C) and the rectal temperature (°C) of mature sheep at rest

	Acclimation Temperature		
	0(6)†	18(6)†	36(5)†
U.C.T.*	41.1 ± 0.1^{a}	41.4 ± 0.1^{b}	40.8 ± 0.1^{c}
T. rect. U.C.T.*	41.2 ± 0.1^{a}	40.6 ± 0.1^{b}	40.6 ± 0.1^{b}
L.C.T.*	35.3 ± 0.5^{a}	34.7 ± 0.5^{ab}	33.8 ± 0.5^{b}
T. rect. L.C.T.*	38.9 ± 0.1^{a}	38.8 ± 0.1^{a}	38.2 ± 0.2^{b}
Z.L.T.E.**	5.8 ± 0.5^{a}	6.7 ± 0.5^{a}	7.2 ± 0.5^{a}
Rectal temperature*	38.9 ± 0.2^{a}	39.1 ± 0.2^{a}	39.6 ± 0.2^{b}

*Values are means ± SEM.

**Values are mean differences ± SE of the difference.

†Indicates number of animals per mean value.

$^{a-c}$ Row means followed by different letters are significantly different ($P<0.05$).

Table V.2 The effect of thermal acclimation (°C) on tissue insulation (°Cm^2/W) and on the slopes of heat production [h.p.] vs ambient temperature ($Wxkg^{-.75}$)/°C in mature sheep

	Acclimation Temperature		
	0 (6)	18 (6)	36 (5)
Tissue Insulation	.044 ± .005[a]	.049 ± .005[a]	.076 ± .006[b]
h.p. slope	1.07 ± .20	1.05 ± .20	.44 ± .23

Values are means ± SEM.

[a,b] Row means followed by different letters are significantly different ($P<0.05$).

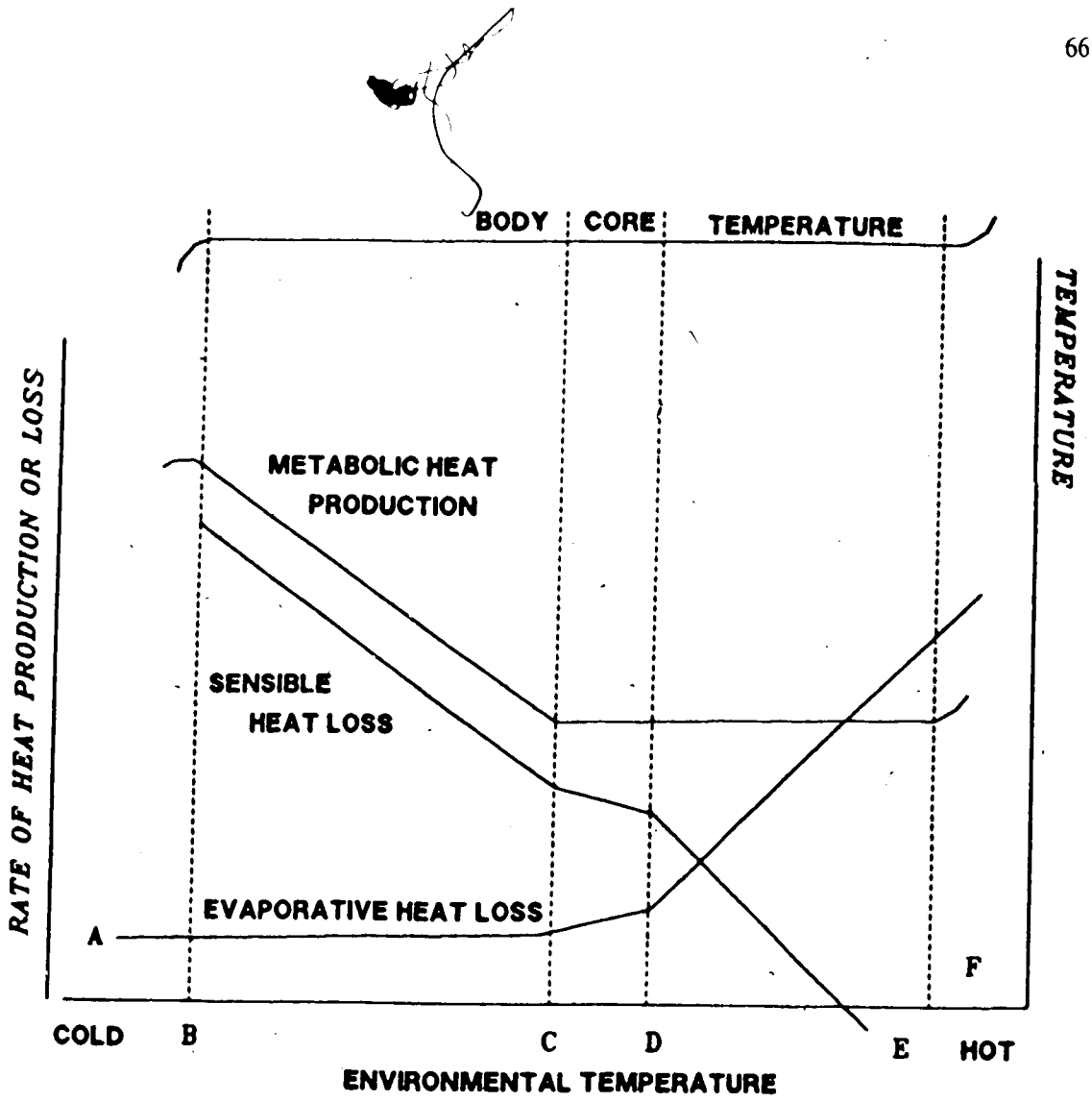
Table V.3 The effect of thermal acclimation (°C) on dry matter digestibility (%) and body weight loss (kg) over a 42 day period

	Acclimation Temperature		
	0 (6)†	18 (6)†	36 (5)†
Dry matter digestibility	51.3 ± 0.4[a]	54.3 ± 0.5[ab]	57.6 ± 2.2[b]
Weight change	-6.3 ± 1.6[a]	-2.8 ± 1.6[b]	.4 ± 1.9[b]

Values are means ± SEM.

†Indicates number of animals per mean value.

[a,b]Means followed by different letters are significantly different ($P<0.05$).



A: zone of hypothermia; B: temperature of summit metabolism and incipient hypothermia; C: lower critical temperature; D: temperature of marked increase in evaporative loss; E: temperature of incipient hyperthermal rise; F: zone of hyperthermia; CD: zone of least thermoregulatory effect; CE: zone of minimal metabolism; BE: thermoregulatory range.

Adapted from Monteith and Mount (1974)

Figure V.1 The effect of environmental temperature on rate of heat production, evaporative and nonevaporative heat loss and core temperature in a homeothermic animal

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VI. CONCLUSIONS

Four experiments have been conducted on mature sheep in order to elucidate the effect of thermal environment on metabolism. In all experiments, the sheep underwent a process of acclimation and not acclimatization, with thermal environment the treatment applied.

The work of Rosenmann and Morrison (1974) and Pasquis et al. (1970) with small mammals, indicates a connection between resting metabolism and cold induced summit metabolism where increases in resting metabolism due to cold acclimation were associated with brown adipose tissue. This increase in nonshivering thermogenesis is additive to shivering thermogenesis and is part of the increase in summit metabolism due to cold acclimation. Most, if not all reports on cold acclimation or acclimatization in small rodents describe changes in insulation, food intake and body size. This tends to make interpretations of the basic physiological mechanisms causing the changes in metabolism due to thermal acclimation much more difficult to elucidate.

Adult ruminants do not contain brown adipose tissue (Sasaki and Weekes 1986). The nonshivering metabolism component of resting metabolism in mature ruminants apparently differs from that in a small mammal. The nonshivering metabolism component is not noradrenaline sensitive in the adult ruminant. Certain endocrine changes occur with acclimation which enhance or reduce metabolism through various physiological avenues. Cold exposure increases plasma concentrations of triiodothyronine, thyroxine, free fatty acids and glucose which is associated with increased or more intense metabolism. Conversely, heat exposure results in decreases in triiodothyronine, thyroxine, free fatty acids and glucose. An elevated resting metabolism has been reported after cold exposure and a decrease after exposure to heat. With most of the small mammal work, food intake and insulation usually changed. Most work with mature sheep is also confounded by differences in food intake and sometimes changes in external insulation.

In the first experiment of this thesis, the effects of thermal environment on whole animal metabolic heat production was studied. The constant level of feed intake forced the

animals to mobilize body energy reserves but removed any critisisms that differences in feed intakes could be responsible for changes in metabolism. External insulation was removed regularly throughout the trial. The experiment showed significant ($P<0.05$) differences in resting metabolism across all three acclimation temperatures which clearly indicates that the animals have undergone thermal acclimation. There was also a significant ($P<0.05$) difference in summit metabolism between the 36° C and 0° C acclimation temperature treatments. Various endocrine concentrations were measured and they changed with acclimation temperature. These endocrine changes agree with other reported data in the literature concerning chronic heat and cold exposure. Cold acclimation generally results in an increase in hormone and substrate concentrations, which may be important in the elevation of resting and summit metabolism. Acclimation to 36° C resulted in a reduction in substrate and hormone concentrations which correspondingly reduced resting and summit metabolism.

Once acclimation was described, it became necessary to identify the possible causes. The increase and decrease in resting metabolism associated with thermal acclimation is due to a combination of factors. One of these is the activity of Na^+ / K^+ ATPase which is known to be influenced by the thyroid status of the animal. Thyroid status of the animals changes with thermal acclimation; it is increased by cold exposure and decreased by heat exposure. A trial was conducted to see if the in vitro metabolism of the external intercostal (E.I.C.) muscle from sheep followed the same trends as the whole animal metabolism and to see the influence of Na^+ / K^+ ATPase on resting heat production. Ouabain, a specific inhibitor of Na^+/K^+ ATPase, was used to measure Na^+, K^+ energy expenditure. The total E.I.C. muscle O_2 uptake paralleled the whole animal metabolism. Contrary to other reports, most of the change was in the ouabain insensitive fraction of muscle metabolism. The percent of muscle metabolism inhibited by ouabain remained constant (19%). Although the amount of stressor from 18° to 36° C and from 18° to 0° C is equal in magnitude, the change in E.I.C. muscle metabolism was larger for the 36° C acclimation (43%) than for the 0° C acclimation (33%). It can be concluded that some of the difference in resting metabolism due to thermal acclimation is a result of changes in Na^+ / K^+ ATPase energy expenditure.

Small changes in body weight associated with constant levels of external insulation should result in shifts in an animal's upper critical temperature (U.C.T.) and lower critical temperature (L.C.T.) when it has undergone thermal acclimation. The water immersion system used for measuring resting and summit metabolism measurements was used to apply acute cold and heat stress in the animals after a period of thermal acclimation. Changes in heat production and respiration rate associated with bath temperature were used to estimate the U.C.T. and L.C.T. The initial results for U.C.T. and L.C.T. were confusing. The U.C.T. was highest for animals from the 18° C acclimation temperature and lowest for the 36° C acclimation temperature. The reason for this was not apparent, but the 0° C acclimation temperature animals may involve a thermal buffering similar to that described by Slee (1973). The L.C.T. results were unclear also. The animals acclimated to 36° C had the lowest L.C.T. relative to 18° C or 0° C. The reasons for this became apparent after tissue insulation values were calculated for all the animals in each acclimation temperature. Once this was done it became obvious that the result was due to differences in the state of vasoconstriction or dilation. The animals acclimated to 0° or 18° C have a skin temperature below the waterbath temperatures needed to achieve the increase in heat production associated with the L.C.T. These animals were in a state of vasodilatation which is seen as their lower insulation values. The animals acclimated to 36° C, have a warmer skin temperature and therefore they vasoconstrict at the bath temperatures used to reach their L.C.T. The biology of thermoregulation is important in this experiment. The input from peripheral and core receptors is being artifically controlled by immersing the animal and clamping skin temperature.

The water immersion system allows a researcher to measure resting metabolism while the animal is bouyed up by water. Another method of measuring resting metabolism is by enclosing the standing animals head in a hood and measuring oxygen consumption. The animals are in the same posture or postion, with the only difference being that one is standing and the other bouyed. This difference allows one to calculate an energy cost for the standing posture. Resting metabolism was calculated using the two methods on one group of sheep and

the results compared. The water bath measurements were significantly lower ($P<.05$) than the head hood measurements. Also a lower level of variation was encountered with the waterbath measurements. This may explain some of the differences between summit metabolism measurements made by Bennett (1972) and those obtained by the water bath procedure in the present studies.

The studies made indicate that there is thermally induced metabolic acclimation in adult sheep which is reflected in the whole animal and in non shivering muscle tissue. These thermally induced metabolic changes could be important to the well being of ruminants in cold climates and in determining their dietary energy requirements.

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APPENDIX

In order to allow the reader an opportunity for further data manipulation, all the raw data used to generate the results used in this thesis are in the following appendix. To simplify matters, data matrices were pulled out of individual computer files and these were then strung together. The appendix begins with the data of chapter II and then chapters III, IV and V follow.

Each matrix consists of several rows of numbers, with each row of numbers defined below the matrix. In all cases, the temperature treatment numbers are in °C. In chapter IV, the muscle biopsy oxygen uptake units are μl O_2/h per mg DM. The whole animal oxygen uptake results are reported in units of ml O_2/h per kg. The results for U.C.T., L.C.T., rectal temperature at the U.C.T., rectal temperature at the L.C.T. and rectal temperature during initial immersion in the water bath are all in units of °C. Finally the results for tissue insulation in chapter V are in units of °C cm^2/W. It is not possible to generate the above symbols for the units when the data is presented in the fashion used in this appendix.

II. The effect of thermal environment on plasma glucose concentration.

1	1	69.01
1	2	51.92
1	3	51.76
2	1	79.77
2	2	59.16
2	3	65.69
3	1	79.77
3	2	55.54
3	3	53.93
4	1	60.86
4	2	48.56
4	3	52.12
5	1	79.87
5	2	42.67
5	3	36.23

-rows are animal#, temperature treatments (1=0 C, 2=18 C and 3=36 C) and plasma glucose concentration (mg/100ml).

II. The effect of thermal environment on the ratio of summit/resting metabolism, summit metabolism and resting metabolism in mature sheep.

1	1	3	4.95	13.60	2.74
1	2	1	4.67	22.40	4.80
1	3	2	5.70	20.86	3.66
2	1	2	6.22	19.22	3.09
2	2	1	4.40	25.21	5.72
2	3	3	6.87	25.81	3.76
3	1	1	5.22	17.42	3.34
3	2	2	6.67	26.49	3.97
3	3	3	5.89	20.73	3.52
4	1	1	5.24	16.19	3.09
4	2	3	6.29	23.90	3.80
4	3	2	6.33	18.81	2.97
5	1	3	5.10	18.85	3.69
5	2	2	5.34	21.14	4.45
5	3	1	3.52	16.85	4.79

-rows are animal#, experimental period, temperature treatment (1=36 C, 2=0 C and 3=18 C), ratio of summit metabolism to resting metabolism, summit metabolism (Wxkg-0.75), resting metabolism (Wxkg-0.75).

II. The effect of thermal exposure on resting metabolism, summit metabolism, body weight, rectal temperature at resting metabolism, rectal temperature at summit metabolism and the time required to reach metabolism.

1	1	3	60.25	315.70	52.5	39.8	36.4	245
1	2	2	55.35	351.04	49.5	38.4	35.7	150
1	3	1	70.32	442.56	49.0	39.0	36.9	170
2	1	1	98.63	434.34	44.5	38.9	36.7	235
2	2	3	59.43	369.46	51.5	39.4	36.9	177
2	3	2	68.55	470.66	48.0	38.9	37.5	197
3	1	3	64.18	334.86	51.5	39.3	36.5	145
3	2	1	66.06	441.00	42.5	38.3	36.6	185
3	3	2	62.68	369.16	46.5	38.7	37.3	145
4	1	3	72.03	367.63	52.5	39.4	37.2	175
4	2	1	73.94	394.62	49.5	39.0	38.0	265
4	3	2	97.32	342.61	55.5	39.2	36.7	192
5	1	1	84.74	395.74	46.0	38.8	36.0	185
5	2	2	67.73	386.31	49.0	39.0	35.0	140
5	3	3	50.06	247.92	48.0	39.7	36.2	88

-rows are animal#, experiment period, temperature treatment(1=0 C, 2=18 C and 3=36 C), resting metabolism (W), summit metabolism (W), body weight after 24-26 days of thermal exposure (kg), rectal temperature at resting metabolism (C), rectal temperature at summit metabolism (C) and the time required to reach summit metabolism (min).

II. The effect of thermal environment on plasma triiodothyronine concentrations.

1	71.18	81.63	175.17
2	95.45	103.00	187.56
3	79.03	117.94	145.51
4	63.81	87.40	128.76
5	116.33	70.07	192.66

-rows are animal#, plasma triiodothyronine (nmol/l) concentrations at 36, 18 and 0 C.

II. The effect of thermal environment on plasma thyroxine concentrations.

1	7.58	7.92	12.63
2	10.28	8.34	12.54
3	7.92	9.33	11.89
4	8.02	8.81	12.75
5	9.56	10.55	11.81

-rows are animal# and plasma thyroxine concentrations (nmol/l) at 36 , 18 and 0 C.

II. The effect of thermal environment on plasma free fatty acids.

1	1	.4506
1	2	.0422
1	3	.0000
2	3	.0199
2	2	.1936
2	1	.2860
3	2	.0415
3	1	.3380
3	3	.0000
4	1	.4318
4	3	.0007
4	2	.3716
5	3	.0439
5	1	.5125
5	2	.0128

-rows are animal#, temperature treatment (1= 0, 2= 18 and 3=36 C) and plasma free fatty acid concentrations.

III. The effect of thermal environment on resting metabolism in air or water.

1	1	4.07	3.66	49.0
2	1	4.67	3.76	41.0
3	2	4.49	3.96	45.5
4	2	5.87	3.97	40.0
5	3	3.80	3.09	51.0
6	3	4.22	3.19	51.0

-characters are animal#, temperature treatments (1=18 C, 2=0 C and 3=36 C), resting metabolism in air (WxKg-0.75), resting metabolism (WxKg-0.75) in water, and body weight (kg).

IV. The effect of thermal environment on E.I.C. muscle biopsy oxygen uptake and on Na/K dependent oxygen uptake.

1	3	1.1344	.2725	.8619	24.02
1	3	.8587	.1616	.6971	18.82
1	3	.9986	.1716	.8270	17.18
1	3	1.1081	.2176	.8905	19.64
2	3	1.0547	.2008	.8539	19.04
2	3	1.2424	.2495	.9929	20.08
2	3	1.0737	.1598	.9139	14.88
2	3	1.1105	.3197	.7908	28.79
2	3	1.3355	.2356	1.0999	17.64
2	3	1.1048	.2224	.8824	20.13
3	3	1.0801	.1429	.9372	13.23
3	3	1.2531	.2811	.9720	22.43
3	3	1.1192	.1931	.9261	17.25
4	2	.9908	.2520	.7388	25.43
4	2	.9413	.2551	.6862	27.10
4	2	.7543	.1451	.6092	19.23
4	2	.8374	.1905	.6469	22.75
5	2	.7814	.1645	.6169	21.05
5	2	.5145	.1255	.3890	23.82
5	2	.8773	.1632	.7141	18.60
5	2	.8225	.1644	.6581	19.99
5	2	.7281	.1780	.5501	24.45
5	2	.6969	.1682	.5287	24.13
5	2	.6814	.1268	.5546	18.61
6	2	.7804	.1609	.6195	20.62
6	2	.9852	.1330	.8522	13.50
6	2	1.0594	.2572	.8022	24.28
6	2	.7430	.0783	.6647	10.54
7	1	.4075	.1050	.3025	25.77
7	1	.4361	.0918	.3443	21.05
7	1	.3409	.0746	.2663	21.88
7	1	.3401	.0535	.2868	15.67
8	1	.4243	.0471	.3772	11.10
8	1	.5680	.0631	.5049	11.11
8	1	.3960	.0423	.3537	10.68
8	1	.5150	.0710	.4440	13.79
8	1	.5322	.0906	.4416	17.02
9	1	.5635	.0640	.4995	11.36
9	1	.5897	.1061	.4836	17.99
9	1	.5486	.0965	.4521	19.59

-rows are animal#, temperature treatment(1=0 C, 2=18 C and 3=36 C), total E.I.C. muscle biopsy oxygen uptake, ouabain sensitive oxygen uptake, ouabain insensitive oxygen uptake and percent inhibition.

IV. The effect of thermal environment on plasma triiodothyronine and thyroxine concentrations.

1	1	12.54	163.75
1	2	11.72	172.30
1	3	11.32	158.99
2	4	7.29	57.87
2	5	7.84	69.90
2	6	8.40	87.02
3	7	5.04	57.82
3	8	6.42	60.66
3	9	5.22	84.68

-rows are temperature treatments (1=0 C, 2=18 C and 3=36 C), animal#, triiodothyronine concentration (nmol/l) and thyroxine concentration(nmol/l).

IV. The effect of thermal environment on whole animal oxygen uptake.

1	1	237.7	49.0
2	1	244.2	41.0
3	2	257.1	45.5
4	2	257.8	40.0
5	3	200.6	51.0
6	3	207.1	51.0

-rows are animal#, temperature treatment (1=18 C, 2=0 C and 3=35 C), whole animal oxygen uptake, and body weight (kg).

V. The effect of thermal environment on tissue insulation and on the slope of the increase in heat production associated with decreases in temperature.

1	1	1	1	.057	.76
1	1	3	2	.078	.53
1	1	2	3	.040	1.14
1	2	2	1	.082	.22
1	2	3	2	.064	1.16
1	2	1	3	.035	.72
1	3	3	1	.076	.88
1	3	1	2	.039	.95
1	3	2	3	.036	1.22
2	4	3	1	.085	.08
2	4	2	2	.044	1.07
2	4	1	3	.031	1.64
2	5	1	1	.085	.75
2	5	3	2	.054	1.60
2	5	2	3	.053	.81
2	6	1	2	.031	1.00
2	6	3	3	.052	.87

-rows are block#, animal#, period# and temperature treatments(1=0 , 2=18 and 3=36 C),and tissue insulation and the slope of heat production.

V. The effect of thermal environment on the upper critical temperature, the lower critical temperature and rectal temperature.

1	1	1	1	40.03	39.62	39.8
1	2	1	3	39.40	37.83	39.5
1	3	1	2	40.79	00.00	40.1
1	1	2	3	40.43	36.69	38.5
1	2	2	1	40.78	40.78	39.1
1	3	2	3	39.84	38.59	38.9
1	1	3	2	39.92	38.79	39.3
1	2	3	2	39.47	38.15	39.0
1	3	3	1	40.60	39.18	40.0
2	4	1	3	40.14	33.77	39.0
2	5	1	1	40.48	39.41	39.6
2	6	1	2	41.18	39.44	39.3
2	4	2	2	39.78	38.93	38.3
2	5	2	2	39.58	34.26	38.5
2	4	3	1	41.09	38.37	40.0
2	5	3	3	41.14	39.94	38.4
2	6	3	3	40.55	38.80	39.3

-rows are block#, animal#, period#, temperature treatment (1=36 , 2=18 and 3=0 C), upper critical temperature, lower critical temperature and rectal temperature.

V. The effect of thermal exposure on weight change, rectal temperature at LCT and rectal temperature at UCT.

1	1	3	1	3.5	39.5	40.9
1	4	1	1	-5.0	39.4	42.0
1	6	2	1	-0.5	39.1	41.3
1	1	1	2	-13.0	39.1	40.8
1	4	2	2	-8.0	38.4	40.4
1	1	2	3	0.5	38.5	40.4
1	4	3	3	7.0	37.6	40.6
1	6	1	3	-8.5	38.0	41.5
2	2	1	1	-6.5	39.6	41.2
2	3	2	1	1.0	39.7	41.4
2	5	3	1	0.0	38.5	40.8
2	2	3	2	1.5	37.5	40.2
2	3	1	2	-2.5	38.5	40.9
2	5	2	2	-7.5	39.0	40.4
2	2	2	3	-2.5	38.1	40.0
2	3	3	3	-0.5	38.3	40.4
2	5	1	3	-2.5	38.7	41.0

-rows are experiment block, animal#, temperature treatment (1=0 C, 2=18 C and 3=36 C), period#, body weight(kg), rectal temperature at LCT and rectal temperature at UCT.

PUBLICATIONS RESULTING FROM THIS THESIS AND WORK DURING GRADUATE STUDIES PROGRAM.

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