THE UNIVERSITY OF MANITOBA

METABOLIC ADAPTATION TO TEMPERATURE IN THE RED-SIDED GARTER SNAKE (*THAMNOPHIS SIRTALIS PARIETALIS*)

by

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LIST OF ABBREVIATIONS

$(NH_4)_2SO_4$	Ammonium sulphate
EDTA	Ethylenediamine tetraacetate
HCl	Hydrochloric acid
LDH	Lactate dehydrogenase (L-Lactate: NAD oxidoreductase EC 1.1.1.28)
MDH	Malate dehydrogenase (L-Malate: NAD oxidoreductase EC 1.1.1.37)
NAD	Nicotinamide adenine dinucleotide
KOH	Potassium hydroxide
NaOH	Sodium hydroxide
H_2SO_4	Sulphuric acid

PART I

Effects of Temperature on *In Vitro* Metabolic Rates

of Tissues from a Cold Climate Reptile,

Thamnophis sirtalis parietalis

Abstract 1. Oxygen consumption of liver, heart, and muscle homogenates was measured *in vitro* from 34° to 4°C for *Thamnophis sirtalis parietalis* acclimated to 26°C, 12L12D; 4°C, 12L12D and 4°C, 0L24D in summer and winter.

2. In most cases, the relationship of metabolic rate (μmoles O_2/mg protein/hr) to temperature in a semi-logarithmic plot was biphasic, with a low Q_{10} at high temperatures and a high Q_{10} at low temperatures. All changes in Q_{10} occurred between 24° and 16°C. In some cases, the increase in Q_{10} with decreasing temperature was preceeded by a region of relative metabolic stability.

3. Acclimation to 4°C, 12L12D resulted in a decrease in the metabolic rate of liver homogenates and an increase in the metabolic rate of heart homogenates at low temperatures, relative to the 26°C, 12L12D group. These effects were most pronounced in tissues from snakes acclimated during winter. The metabolic rate of muscle homogenates was increased by cold acclimation during the summer, but was unaffected during the winter.

4. Acclimation to 4°C, 0L24D in winter (simulated hibernating conditions) resulted in an increase in the metabolic rate of liver homogenates and a decrease in the metabolic rate of heart homogenates at low temperatures, relative to the 4°C, 12L12D group. The metabolic

rate of muscle homogenates was not affected at temperatures below 12°C.

5. Seasonal differences in metabolic rates were most apparent in tissues from snakes acclimated to 26°C, 12L12D. Tissue homogenates from snakes acclimated during the winter showed higher metabolic rates relative to those from snakes acclimated during the summer over most of the temperature range studied.

6. Cold acclimation caused a decrease in the metabolic rate of liver and muscle homogenates from Thamnophis sirtalis concinnus, a warm temperate sub-species.

INTRODUCTION

Studies concerning the effects of immediate temperature change on the metabolic rates of reptiles have been mainly directed toward the response of the whole animal. Standard oxygen consumption is a direct exponential function of temperature in a number of reptilian species (Bartholomew et al., 1965; Dawson, 1960; Dawson and Bartholomew, 1958; Dawson and Templeton, 1966; Roberts, 1968; and others). In others, there are specific temperature regions in which the rate of standard oxygen consumption is relatively independent of temperature change (Aleksiuk, 1971a; Buikema and Armitage, 1969; Fitzpatrick et al., 1971; and others). As a result, the organism is said to achieve a degree of metabolic independence from variations in the thermal environment. Aleksiuk (1971a) demonstrated an actual increase in the standard metabolic rate of Thamnophis sirtalis parietalis with decreasing temperature over a limited temperature range. The presence of this effect in in vitro heart rates of T. sirtalis parietalis (Aleksiuk, 1970) suggests its existence in aspects of metabolism at the cellular or sub-cellular level of organization.

The purpose of this study was to determine the effects of temperature change on the oxygen consumption of tissue homogenates from cool temperate representatives of T. sirtalis parietalis, with particular reference to low temperatures. Three types of temporal responses, under two photoperiodic regimes, were investigated: (1) responses of metabolic rate to immediate temperature change; (2) responses of metabolic rate to long-term cold acclimation; and (3)

possible inherent seasonal changes in immediate and long-term responses. The effect of cold acclimation was deemed necessary, since metabolic changes induced by thermal acclimation have been commonly observed in poikilotherms at the whole organism (Dawson and Bartholomew, 1956; Fitzpatrick et al., 1971; Gelineo and Gelineo, 1955; Murrish and Vance, 1968; and others), cellular (Evans et al., 1962; Peiss, 1950) and sub-cellular levels (Ekberg, 1958; Hochachka and Hayes, 1962; Kanungo and Prosser, 1959a,b). Season has rarely been included in thermal adaptation studies, and constitutes an unknown variable. Limited comparative data were obtained for warm temperate representatives of Thamnophis sirtalis concinnus to determine the possible effects of climate on thermal adaptation.

The life history of T. sirtalis parietalis at northern latitudes is outlined by Aleksiuk and Stewart (1971).

MATERIALS AND METHODS

Experimental animals

Adult representatives of Thamnophis sirtalis parietalis, collected from a hibernaculum 50 miles north of Winnipeg, Manitoba, Canada, were divided randomly with respect to body weight and sex into three acclimation groups: 26°C, 12L12D (L = light, D = dark); 4°C, 12L12D; and 4°C, 0L24D. The hibernacula and the environmental temperature conditions for this population of T. sirtalis parietalis have been described by Aleksiuk and Stewart (1971) and by Gregory (1971). Food (ocean perch) and water were provided ad libitum to snakes in the 26°C group until one week prior to experimentation. Neither food nor water was given to either of the groups held at 4°C, as T. sirtalis parietalis does not feed at this temperature. The acclimation period extended from 5 to 6 weeks.

Adult representatives of Thamnophis sirtalis concinnus, a subspecies from a warm temperate climate, were obtained from Oregon in the spring of 1971. They were divided randomly with respect to body weight and sex into two acclimation groups; 26°C, 12L12D and 10°C, 12L12D. The acclimation period and the feeding conditions were similar to those for T. sirtalis parietalis.

Preparation of tissue homogenates

Three to six snakes of similar body weights (25-35 grams) were selected from a given acclimation group for each tissue preparation. Gravid females were excluded. Liver, heart or skeletal muscle was rapidly excised from decapitated snakes and immediately immersed in

ice-cold Kreb's phosphate buffer, pH 7.4. Snakes from the 4°C, 0L24D group were decapitated in the dark prior to tissue removal to avoid any exposure of the intact animal to light. Liver at a concentration of 0.40g/ml of Kreb's phosphate buffer, and heart at a concentration of 0.15g/ml, were homogenized in an ice-jacketed Potter-Elvejhem homogenizer employing a teflon pestle. Skeletal muscle was minced prior to homogenization. The concentration of skeletal muscle varied from 0.20 to 0.35 g/ml of Kreb's phosphate buffer.

Measurement of in vitro tissue oxygen consumption

Oxygen consumption of tissue homogenates was measured at 2°C intervals from 34° to 4°C using a Clark oxygen electrode (Model No. YS1 4004) with a Model KM Gilson oxygraph (Gilson Medical Electronics, Wisconsin, U.S.A.). The reaction vessel contained 0.5 ml of tissue homogenate plus 1.2 ml of air-saturated Kreb's phosphate buffer. The temperature of the reaction mixture was controlled by a water jacket connected to a Neslab circulating temperature bath (Model No. 681 276). Homogenate preparations were maintained at 0°C at all times except during oxygen consumption determinations. A complete metabolic rate-temperature curve was established for each homogenate between 9:00 a.m. and 4:00 p.m. of a given day. At least three replicates of tissue oxygen consumption were obtained over the temperature range indicated above for each acclimation group, and the mean value was used as a measure of the metabolic rate.

Protein content of the tissue homogenates was estimated by the

Biuret method (Layne, 1957). Bovine serum albumin (Sigma Chemical Co., Mo. U.S.A.) was used as a standard. All samples were digested in a 1M KOH solution prior to protein determination. The oxygen consumption data were expressed as μmoles O_2/mg protein/hr.

The above experimental procedure was carried out during winter, January 28 to March 3, 1971, and during summer, July 28 to August 30, 1971. The purpose of performing this experiment in the winter and in the summer was to determine possible seasonal effects on the oxygen consumption of tissue homogenates from T. sirtalis parietalis maintained under constant temperature and photoperiod conditions.

Statistical analyses

Semi-logarithmic plots of mean oxygen consumption versus temperature were constructed. Q_{10} values were determined from regression lines fitted to the data according to the least squares principle. Analysis of variance was used to test differences in the slopes of the regression lines. Differences in mean values at specific temperatures for a given tissue were tested with the Student's t-test.

RESULTS AND DISCUSSION

Effects of immediate temperature change on in vitro tissue oxygen consumption

The *in vitro* oxygen consumption of tissue homogenates from 34° to 4°C was a direct exponential function of temperature in only three situations (Figs. 1 - 6). Q_{10} values, representing the slopes of the metabolic rate-temperature curves, were used as a measure of the sensitivity of oxygen consumption to temperature change. The metabolic rate curves for muscle homogenates from snakes acclimated to 26°C, 12L12D in summer (Fig. 6) and liver homogenate from snakes acclimated to 26°C, 12L12D and 4°C, 0L24D in summer (Fig. 4) possessed Q_{10} values of 3.40, 4.21 and 4.00, respectively, over the entire temperature range studied. Similar constant Q_{10}'s were obtained by Freeman (1950) for brain breis from goldfish (*Carassius auratus*) acclimated to 20° and 27°C and by Peiss (1950) for brain mince and liver slices from the arctic-adapted Polar cod (*Boreogadus saida*). Peiss (1950) suggested that the constant Q_{10}, especially in the low temperature region, was indicative of a metabolic adaptation to arctic temperature conditions.

There was a distinct change in Q_{10} between 24° and 16°C in most of the metabolic rate curves obtained in the present study. The temperature at which the change in Q_{10} occurred was dependent upon the type of tissue examined and the acclimation state of the organism. Statistically significant increases in Q_{10} with decreasing temperature were observed in the metabolic rate curves for the following tissues

(Table 1): muscle from snakes acclimated to 26°C, 12L12D and 4°C, 12L12D in winter (Fig. 5) and 4°C, 12L12D in summer (Fig. 6), heart from snakes acclimated to 4°C, 0L24D in winter (Fig. 1) and 4°C, 12L12D and 26°C, 12L12D in summer (Fig. 2), and liver from snakes acclimated to 4°C, 12L12D and 4°C, 0L24D in winter (Fig. 3) and 4°C, 12L12D in summer (Fig. 4). A similar type of metabolic rate curve has also been demonstrated for the oxygen consumption of brain mince and liver slices from warm-adapted Golden orfe (Idus melanotus) (Peiss, 1950) and for the oxygen consumption of epaxial muscle homogenates from warm and cold acclimated brook trout (Salvelinus fontinalis) (Hochachka and Hayes, 1962).

This temperature-dependent change in Q_{10} suggests the existence of a mechanism which allows a rapid decrease in metabolic rate with decreasing temperature below a certain temperature, or conversely, a rapid increase in metabolic rate to a specific temperature level. A change in the Q_{10} value in a similar temperature region is also characteristic of the standard metabolic rate curve for the intact organism (Aleksiuk, 1971a). A general metabolic depression for the conservation of energy was said to be of adaptive value during the extended hibernation period of T. sirtalis parietalis (Aleksiuk and Stewart, 1971); such a depression is reflected by a high Q_{10} at low temperatures. Furthermore, a high Q_{10} in the low temperature range reflects a rapid increase in metabolic rates during emergence from hibernation or during temperature increases experienced in normal activity periods.

Regions of metabolic stability, as indicated by portions of the metabolic rate curves where the Q_{10} approaches a value of 1.0, were apparent in several of the metabolic rate-temperature curves for tissue homogenates from T. sirtalis parietalis (Table 2; Figs. 1, 3 and 5). Such temperature regions, in which the rate of oxygen consumption varies little or not at all, are not uncommon in the standard metabolic rate-temperature curves of intact poikilotherms (Aleksiuk, 1971a; Buikema and Armitage, 1969; Dawson and Bartholomew, 1956; Jacobson and Whitford, 1970, 1971; Newell and Northcroft, 1967; Prieto and Whitford, 1971; and others). Similar regions have also been observed in the oxygen consumption of cell-free homogenates and mitochondria from the winkle (Littorina littorea) (Newell and Pye, 1971a,b) and in the oxygen consumption of red and white muscle minces from tuna (Katsuwonus perlamis and Thunnus obesus) (Gordon, 1968).

The adaptive value of a region of metabolic stability is believed to be that it represents a degree of physiological independence of the organism from variations in the thermal environment (Bullock, 1955). In the present study, the location of the metabolic plateaus in the metabolic rate-temperature curves, generally in the normal body temperature range for activity, would appear to be in accordance with this type of adaptive value. However, the plateaus are only present in the metabolic rate curves of homogenates from snakes acclimated during the winter. In its natural habitat, T. sirtalis parietalis hibernates from November to April (Aleksiuk and Stewart, 1971) and does not encounter temperatures near the regions of the metabolic plateaus.

Rather than representing a metabolic adaptation to temperature in this population of T. sirtalis parietalis, the regions of metabolic stability may have been of major adaptive value in ancestral forms of T. sirtalis parietalis living in a warm climate where activity is possible throughout the winter.

In this study, a shift in the metabolic rate curve has been used to designate a limited temperature region in which oxygen consumption increased or remained constant with decreasing temperature. Such a region was subsequently followed by a lateral displacement of the metabolic rate curve. A single shift region was present from 28° to 24°C in the metabolic rate curve for heart homogenates from T. sirtalis parietalis acclimated to 4°C, 0L24D in summer (Fig. 2). Two shift regions were evident in the metabolic rate curves for heart homogenates from snakes acclimated to 4°C, 12L12D in winter (from 20° to 16° and 10° to 6°C) and muscle homogenates from snakes acclimated to 4°C, 0L24D in summer (from 20° to 16°C and 14° to 10°C) (Table 3; Figs. 1 and 6). Similar shift regions have been observed in the standard oxygen consumption curves for adult Thamnophis sirtalis parietalis (Aleksiuk, 1971a), hibernating Dipsosaurus dorsalis (Moberly, 1963), and warm acclimated Thamnophis proximus (Jacobson and Whitford, 1970).

It has been suggested that the activation of isoenzymes with different thermal optima is the basis for the stabilization or an increase in metabolic rate with decreasing temperature (Aleksiuk, 1971a,b). According to this suggestion, the activation of specific

isoenzymes with decreasing temperature results in an off-setting of the depressive effects of low temperature on catalysis. The multiple shift regions observed in several of the metabolic rate curves in the present study may reflect the activation of certain isoenzymes better suited for catalysis at low temperatures. In fact, the elevations of the lower portions of the metabolic rate curves following the shift regions, may be indicative of a mechanism which has lessened the depressive effects of decreased temperature on oxygen consumption. The presence of shift regions in the metabolic rate curves of tissues only from cold acclimated snakes suggests that cold acclimation may be involved in the stimulation of specific enzyme adjustments. The possible role of this type of mechanism in the immediate or long-term metabolic adjustment of T. sirtalis parietalis to temperature has been examined (Part II of this thesis).

Effects of temperature acclimation on in vitro tissue oxygen consumption

The effects of long-term photoperiodic and thermal acclimation on tissue homogenate oxygen consumption are complicated by the different responses of the various tissues as well as by the seasonal differences in the response of a given tissue. Since the major interests of this paper are in cold adaptation, the discussion will be oriented towards the effect of cold, rather than warm, acclimation on oxygen consumption.

The effect of cold acclimation on the metabolic rate of heart homogenates from snakes acclimated during the winter was reflected by a slight clock-wise rotation of the $4^{o}C$, 12L12D curve, such that the metabolic rate was decreased in the cold group at 22^{o} and $20^{o}C$ ($P \geq 0.1$)

and increased at 6^{o} and $4^{o}C$ ($P \geq 0.025$) (Fig. 1). In the summer experiments, the only significant effect of cold acclimation on the metabolic rate of heart homogenates was an increase in the oxygen consumption of the $4^{o}C$, 12L12D group at 8^{o} and $4^{o}C$ ($P \geq 0.025$) (Fig. 2). Although the two curves were otherwise similar at all temperatures, the $4^{o}C$, 12L12D curve was translated above the $26^{o}C$, 12L12D curve over most of the temperature range. The significant increase in oxygen consumption at $4^{o}C$ for heart homogenates from snakes acclimated to $4^{o}C$, 12L12D in both winter and summer represents partial compensation of metabolic rates for low temperatures.

Cold acclimation was accompanied by a downward translation of the metabolic rate curve for liver homogenate from snakes acclimated during the winter, in combination with a slight counter-clockwise rotation (Fig. 3). As a result, the oxygen consumption of the $4^{o}C$, 12L12D group was significantly depressed relative to the $26^{o}C$, 12L12D group from 18^{o} to $4^{o}C$ ($P \geq 0.05$). The only significant effect of cold acclimation on the oxygen consumption of liver homogenate from snakes acclimated during the summer was a decrease in the oxygen consumption of the $4^{o}C$, 12L12D group at $34^{o}C$ ($P \geq 0.05$).

Cold acclimation had no significant effect on the metabolic rate of muscle homogenates from snakes acclimated during the winter (Fig. 5). In the summer experiments, however, the entire $4^{o}C$, 12L12D curve was translated above the $26^{o}C$, 12L12D curve (Fig. 6). The differences in metabolic rate between the two groups were significant at all temperatures with the exception of $4^{o}C$ ($P \geq 0.05$). Simple upward translation of the metabolic rate curve with cold acclimation has also

been observed in the *in vitro* oxygen consumption of goldfish (*Carassius auratus*) gills (Ekberg, 1958) and in the rate of protein synthesis in goldfish muscle (Das and Prosser, 1967).

The greatest response to cold acclimation, of all tissues studied, was observed in the metabolic rate of muscle homogenates from snakes acclimated during the summer. This response of muscle tissue to continued cold exposure may represent an important mechanism for the maintenance of the metabolic rate of skeletal muscle, and possibly the actual activity of *T. sirtalis parietalis* during cold periods in summer months.

With the exception of muscle, cold acclimation effects were most pronounced in the metabolic rates of tissues from snakes acclimated during the winter. In the natural environment, however, *T. sirtalis parietalis* hibernates from November to April (Aleksiuk and Stewart, 1971) under conditions of complete darkness. Therefore, it is more appropriate to consider the combined effects of acclimation to cold and complete darkness when assessing the metabolic rates of tissues within *T. sirtalis parietalis* during the winter.

At low temperatures, presumably similar to those experienced by hibernating snakes, cold acclimation caused a decrease in the metabolic rate of liver homogenates and an increase in the metabolic rate of heart homogenates. These effects were most pronounced in tissues from snakes cold acclimated during the winter. The metabolic rate of muscle homogenates was not affected by cold acclimation in the winter, but was increased in summer over most of the temperature range studied. Opposite metabolic responses of tissues to cold

acclimation, of the type observed in heart and liver homogenates especially at low temperatures, may be the reason why the standard metabolic rate of intact T. sirtalis parietalis is unaffected by cold acclimation (Aleksiuk, unpublished results). If the oxygen consumption of some tissues is increased, while that of others is decreased, the net result may be the apparent absence of an effect of cold acclimation on the standard oxygen consumption of the intact animal.

Effects of photoperiodic acclimation on in vitro tissue oxygen consumption

The discussion of the effects of photoperiodic acclimation on tissue oxygen consumption will involve a comparison of the metabolic rate curves for tissues from snakes acclimated to $4^{o}C$, 12L12D with those for tissues from snakes acclimated to $4^{o}C$, 0L24D. The purpose of the comparison is to attempt to assess the role of photoperiod in influencing metabolic changes in snakes existing under simulated hibernating conditions ($4^{o}C$, 0L12D).

Acclimation to complete darkness was accompanied by a significant depression of metabolic rates, from 12^{o} to $4^{o}C$ ($P \geq 0.05$), of heart homogenates from snakes acclimated during the winter, and a simultaneous increase in Q_{10} (Fig. 1). Partial cold compensation of the oxygen consumption of heart homogenates from snakes acclimated to $4^{o}C$, 12L12D was earlier attributed to some form of enzymic alteration; perhaps exposure of the intact animal to light in addition to cold is necessary to induce this enzymic response. The major decrease in metabolic rate at low temperatures for heart tissue from snakes

acclimated to 4°C, 0L24D during the winter may be of adaptive value during the extended period of hibernation, since a metabolic reduction is conducive to energy conservation.

Cardiac muscle responded to acclimation of the intact organism to complete darkness at the tissue as well as at the sub-cellular level. In vitro heart rates of T. sirtalis parietalis acclimated to 5°C, 0L24D were depressed below about 22°C as compared with those of snakes acclimated to 5°C, 8L16D; 32°C, 8L16D; and 32°C, 0L24D (Aleksiuk, 1970). The fact that acclimation of the intact snake to complete darkness and reduced temperature is necessary to induce this depression stresses the importance of considering both thermal and photoperiodic factors in assessing metabolic rate changes in the hibernating snake.

Complete darkness, in conjunction with low temperature, had little effect on the metabolic rate of muscle homogenates from snakes acclimated during the winter. Oxygen consumption of the 4°C, 0L24D group was slightly higher than that of the 4°C, 12L12D group from 34° to 10°C, but only significantly so at 34°, 32°, 28° and 22°C ($P \geq 0.05$). From 10° to 4°C, the curves were similar (Fig. 5). The predominant effect of acclimation of the intact snakes to 4°C, 0L24D in winter on the metabolic rate of liver homogenates was an elevation, relative to the 4°C, 12L12D group, of the metabolic rate at 4°C ($P \geq 0.025$) (Fig. 4). Protein has been shown to be an important energy source for hibernating T. sirtalis parietalis (Aleksiuk and Stewart, 1971). The absence of a metabolic depression in liver tissue from snakes acclimated to 4°C, 0L24D may relate to the important role of the liver in protein catabolism in hibernating snakes.

Acclimation of the intact snakes to 4°C, 0L24D during the summer resulted in a decrease in the oxygen consumption of muscle homogenates from 34° to 14°C ($P \geq 0.05$) and heart homogenates at 30°, 28° and 24°C ($P \geq 0.05$) (Figs. 2 and 6). The presence of a series of small shifts resulted in the elevation of the lower portions of the metabolic rate curves, such that the 4°C, 0L24D and the 4°C, 12L12D curves were identical from 20° to 4°C for heart homogenate oxygen consumption and 8° to 4°C for muscle homogenate oxygen consumption. The presence of these shifts and the absence of a metabolic depression at low temperatures suggests that enzymic cold compensatory mechanisms may be active. Therefore, the induction of normal hibernation responses may not be possible in snakes acclimated during the summer. However, the metabolic rate of liver homogenates from snakes acclimated to 4°C, 0L24D during the summer showed an elevation at 4°C similar to that of liver homogenates from snakes acclimated to 4°C, 0L24D during the winter.

Previous studies of photoperiodic effects in reptiles have been concerned with resistance adaptations at the whole organism level (Licht, 1968; Ballinger, 1969). Vincent (1971) demonstrated that the presence of light reduced cold tolerance in cold acclimated T. sirtalis parietalis. Vincent's results and the observations of this study suggest that both temperature reduction and the absence of light are essential for the activation of cellular responses which may be conducive to survival of T. sirtalis parietalis in prolonged hibernation.

Seasonal variation in vitro tissue oxygen consumption

Few studies with poikilotherms have considered inherent seasonal patterns in the metabolic rates of animals maintained under

constant thermal and photoperiodic conditions. The significant seasonal differences in the metabolic rates of tissue homogenates observed in this study emphasize the importance of considering season as a variable when performing metabolic studies.

Seasonal effects on metabolic rates were observed to be most pronounced in tissues from warm acclimated snakes. The entire metabolic rate curve for muscle homogenate from snakes acclimated during the winter was translated above that for muscle from snakes acclimated during the summer (Fig. 7a). Metabolic rates determined in the summer and in the winter were significantly different at each experimental temperature ($P \geq 0.025$) with the exception of 6° and $4^{\circ}C$. The metabolic rate of liver homogenates from snakes acclimated during the winter was greater than that of the summer group from 20° to $8^{\circ}C$ ($P \geq 0.05$) but was less at $34^{\circ}C$ ($P \geq 0.005$) (Fig. 8a). The metabolic rate of heart homogenates from snakes acclimated during the winter was slightly greater than that of the summer group over the entire temperature range studied, but significantly so only at 22° to 16°, 8° and $4^{\circ}C$ ($P \geq 0.1$) (Fig. 9a).

The most pronounced difference in the metabolic rates of tissues from snakes acclimated to $4^{\circ}C$, 0L24D in winter and summer was an increase in the oxygen consumption of muscle homogenates from snakes acclimated during the winter from 34° to $22^{\circ}C$ ($P \geq 0.05$) (Fig. 7c). Metabolic rate curves established in the winter and the summer for liver homogenates were similar with the exception of a significant elevation in metabolic rates at 34°, 32° ($P \geq 0.01$) 6° and $4^{\circ}C$ ($P \geq 0.05$) in the summer experiments (Fig. 8c). Metabolic rate curves for heart

homogenates were similar from 34° to 22°C, but showed an increase in Q_{10} and a decrease in absolute values from 12° to 4°C ($P \geq 0.01$) in the winter experiments (Fig. 9c).

From these results, it appears that acclimation to 4°C, 0L24D (simulated hibernating conditions) may not have the same effect on the metabolic rates of tissues within animals acclimated during the summer as in those acclimated during the winter. Mayhew (1965) demonstrated that winter dormancy and metabolic depression in the horned lizard (Phrynosoma m'calli) were induced by a combination of reduced photoperiod and decreased temperature. This, however, occurred only if the temperature and photoperiod change were in conjunction with a specific time of year.

In contrast, metabolic rate curves for liver, heart and muscle homogenates from snakes acclimated to 4°C, 12L12D were similar in the winter and summer experiments (Figs. 7b, 8b and 9b). Significant differences in absolute values included a decrease in oxygen consumption at 4°C ($P \geq 0.025$) for liver homogenates from snakes acclimated during the winter and at 6° and 4°C ($P \geq 0.025$) for heart homogenates from snakes acclimated during the summer.

Seasonal differences in metabolic rates at sub-cellular and cellular levels have been observed previously. For example, the temperature region in which the oxygen consumption of the intact winkle (Littorina littorea), as well as that of cell-free homogenates and isolated mitochondria, showed no significant fluctuations, was dependent upon season (Newell and Pye, 1970). Metabolic rates of gill

tissue from warm and cold acclimated goldfish (Carassius auratus) showed seasonal variation with a gradual decrease in oxygen consumption from July to February (Ekberg, 1958). The major difference between seasonal variation in the metabolic rates of tissue homogenates from T. sirtalis parietalis and the limited number of other poikilotherms studied in this respect is that seasonal variation in T. sirtalis parietalis is dependent upon the thermal history of the intact snake. That is, variations in metabolic rates due to season are not constant for tissue homogenates from warm and cold acclimated animals. Rather than attributing seasonal variations in the metabolic rates of tissues from T. sirtalis parietalis strictly to an inherent rhythm, it may be more appropriate to suggest that this organism possesses an inherent capacity to adjust to specific temperature conditions only during the season when these temperature conditions are typical.

In vitro oxygen consumption of tissues from T. sirtalis concinnus, a warm temperate sub-species

Thamnophis sirtalis concinnus, the red-spotted garter snake, is restricted in range to north-west Oregon and is subjected to a warm temperate climate. Although it is generally dormant from October to April, it is not uncommon to find male snakes basking on the mildest days of even the coldest months (Stewart, 1965).

The effects of low temperature on the oxygen consumption of tissue homogenates from T. sirtalis concinnus were examined to determine possible effects of climate on the capability of a given population to adjust to cold.

The forms of the metabolic rate curves for muscle and liver homogenates from T. sirtalis concinnus were similar to those of T. sirtalis parietalis. The slopes of the metabolic rate curves for muscle and liver homogenates from snakes acclimated to 26°C, 12L12D and 10°C, 12L12D increased with decreasing temperature at 24°, 20°, 22° and 24°C respectively (Table 1; Figs. 10 and 11). As in T. sirtalis parietalis, the high Q_{10} values in the low temperature region denote a rapid decrease in metabolic rates with decreasing temperature and a rapid increase in metabolic rates with increasing temperature to a specific temperature level.

Cold acclimation was accompanied by a translation of the metabolic rate curve below that of tissues from warm acclimated individuals for the oxygen consumption of both muscle and liver homogenates (Figs. 10 and 11). Differences in metabolic rates of muscle homogenates from snakes acclimated to 26°C, 12L12D and 10°C, 12L12D were significant ($P \geq 0.025$) at all temperatures with the exception of 34°, 16°, 14° and 10°C. In the case of liver homogenates, the metabolic rates were significantly different ($P \geq 0.05$) at all temperatures with the exception of 34°, 26°, 14°, 12°, and 10°C. Stewart (1965) demonstrated the reverse situation at the level of the whole animal. The metabolic rate of warm acclimated (32°C) T. sirtalis concinnus was significantly lower at 25°C than the metabolic rate of cold acclimated (8°C) T. sirtalis concinnus. This effect may be the result of systemic rather than sub-cellular or cellular control mechanisms.

CONCLUDING REMARKS

The metabolic response of tissue homogenates from T. sirtalis parietalis to immediate temperature change was frequently characterized by an increase in Q_{10} with decreasing temperature. In all cases, the change in Q_{10} occurred between 24° and 16°C. A rapid change in metabolic rates with changing temperature, as indicated by a high Q_{10} in the low temperature range, is clearly of adaptive value in cold climates, where large temperature fluctuations are frequent and irregular. It facilitates a rapid increase in metabolic rate with increasing temperature to a specific temperature level, which is particularly adaptive in a behavioural thermoregulator. In some instances, the increase in Q_{10} with decreasing temperature was preceeded by a region of relative metabolic stability.

The form of the metabolic rate curve for a specific tissue homogenate as well as the actual level of oxygen consumption was dependent upon the thermal and photoperiodic history of the intact snake, and upon season. In general, cold acclimation was accompanied by an increase in the metabolic rate of heart homogenates and a decrease in the metabolic rate of liver homogenates at low temperatures. The effect was most pronounced in tissue homogenates from snakes acclimated to cold during the winter. The metabolic rate of muscle homogenates was unaffected by cold acclimation in winter, but was increased in the summer over most of the temperature range studied.

The importance of photoperiod as an environmental factor which may influence metabolic rates is emphasized by the observations of this

study. Heart and liver homogenates from snakes acclimated to 4°C, 0L24D (simulated hibernating conditions) during the winter and summer, respectively, showed metabolic responses at low temperatures completely opposite to those from snakes acclimated to 4°C, 12L12D. With respect to heart tissue, the cold compensatory effects present in homogenates from snakes acclimated to 4°C, 12L12D were absent in homogenates from snakes acclimated to 4°C, 0L24D. In fact, the metabolic rate of heart homogenates from snakes acclimated to 4°C, 0L24D during the winter was significantly less than that of homogenates from snakes acclimated to 26°C, at low temperatures. The depression of the metabolic rate of liver homogenates from snakes acclimated to 4°C, 0L24D in winter was lesser than that of homogenates from the 4°C, 12L12D group, as compared with 26°C levels. This may be important in relation to protein catabolism during hibernation.

An important variable, season, is often neglected in studies of the effects of thermal and photoperiodic acclimation on metabolic rates. Metabolic adjustments to immediate temperature change were not only most apparent in tissues from cold acclimated snakes, but particularly from those acclimated to cold during winter. Long-term cold acclimation in summer was accompanied by simple translation of the metabolic rate curves. More complex rotational and translational results were apparent in the metabolic rate curves of tissues from snakes cold acclimated during the winter. It is suggested that T. sirtalis parietalis possesses an inherent capacity for physiological adjustments to temperature change during the season for which the temperature

conditions are typical. A more complete analysis of the metabolic basis for the observations in this study can be obtained only through further studies at the sub-cellular level.

The metabolic responses of tissue homogenates from T. sirtalis concinnus to immediate temperature change were similar to those of most of the tissues from T. sirtalis parietalis. That is, the metabolic rate curves were characterized by a low Q_{10} at high temperatures and a high Q_{10} at low temperatures. Cold acclimation was accompanied by a simple decrease in the metabolic rates of tissue homogenates from T. sirtalis concinnus at most of the experimental temperatures. A more complete study involving seasonal and photoperiodic aspects is necessary to allow any major conclusions as to the role of climate in cold adaptation.

Acknowledgements

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Table 1. Statistically significant ($P \geq 0.01$) changes in Q_{10} in the metabolic rate-temperature curves of various tissues from two sub-species of *Thamnophis sirtalis* (p = *parietalis*; c = *concinnus*).

Tissue	Acclimation state	Temperature range (°C)	Q_{10}
Heart (*T.s.p.*)	Winter; 4°C, 0L24D	34-24	2.79
		24-4	6.10
Heart (*T.s.p.*)	Summer; 26°C, 12L12D	34-20	2.17
		20-4	6.04
Heart (*T.s.p.*)	Summer; 4°C, 12L12D	34-24	2.33
		24-4	4.90
Liver (*T.s.p.*)	Winter; 4°C, 12L12D	34-22	3.41
		22-4	6.95
Liver (*T.s.p.*)	Winter; 4°C, 0L24D	34-20	2.74
		20-4	5.27
Liver (*T.s.p.*)	Summer; 4°C, 12L12D	34-20	3.21
		20-4	5.41
Muscle (*T.s.p.*)	Winter; 26°C, 12L12D	34-18	3.38
		18-4	6.08
Muscle (*T.s.p.*)	Winter; 4°C, 0L24D	34-24	2.19
		24-4	6.40
Muscle (*T.s.p.*)	Summer; 4°C, 12L12D	34-18	2.36
		18-4	4.67
Liver (*T.s.c.*)	Summer; 26°C, 12L12D	34-22	2.33
		22-4	4.99
Liver (*T.s.c.*)	Summer; 26°C, 12L12D	34-22	2.69
		22-4	5.63
Muscle (*T.s.c.*)	Summer; 26°C, 12L12D	34-24	2.07
		24-4	5.45
Muscle (*T.s.c.*)	Summer; 10°C, 12L12D	34-20	2.84
		20-4	5.20

Table 2. Temperature ranges and Q_{10} values of stable regions in the metabolic rate-temperature curves for various tissues from Thamnophis sirtalis parietalis. The second figure in each case denotes the Q_{10} at temperatures immediately below the stable region.

Tissue	Acclimation state	Temperature range (°C)	Q_{10}
Liver	Winter; 26°C, 12L12D	32-18	1.49
		18-4	6.50
Heart	Winter; 26°C, 12L12D	32-20	1.19
		20-4	2.80
Muscle	Winter; 4°C, 0L24D	32-22	1.47
		22-4	4.73

Table 3 Temperature ranges of actual increases or shifts in oxygen consumption with decreasing temperature in the metabolic rate-temperature curves of tissues from Thamnophis sirtalis parietalis.

Tissue	Acclimation state	Temperature range (^{o}C)
Heart	Summer; 4^{o}C, 0L24D	28-22
Heart	Winter; 4^{o}C, 12L12D	28-24
		10-6
Muscle	Summer; 4^{o}C, 0L24D	20-16
		14-10

Figure 1. The effect of temperature on the oxygen consumption of heart homogenates from T. sirtalis parietalis acclimated to 26°C, 12L12D (▲); 4°C, 12L12D (●); and 4°C, 0L24D (○) in winter. Each point represents a mean of a least three values.

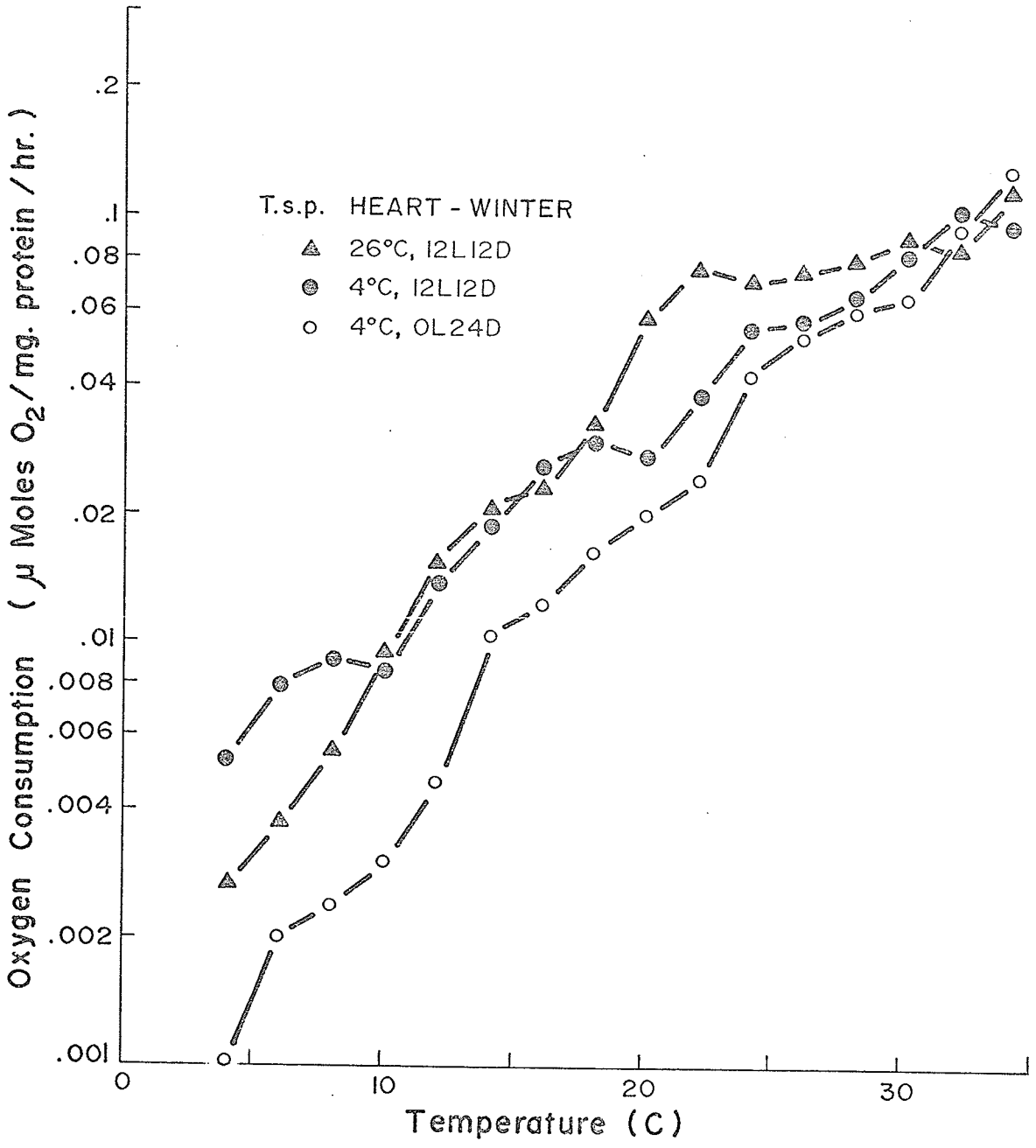

T.s.p. HEART - WINTER
26°C, 12L12D
4°C, 12L12D
4°C, 0L24D
Oxygen Consumption (μ Moles O2 / mg. protein / hr.)
.2
.1
.08
.06
.04
.02
.01
.008
.006
.004
.002
.001
0
10
20
30
Temperature (C)

Figure 2. The effect of temperature on the oxygen consumption of heart homogenates from T. sirtalis parietalis acclimated to 26°C, 12L12D (▲); 4°C, 12L12D (●); and 4°C, 0L24D (○) in summer. Each point represents a mean of at least three values.

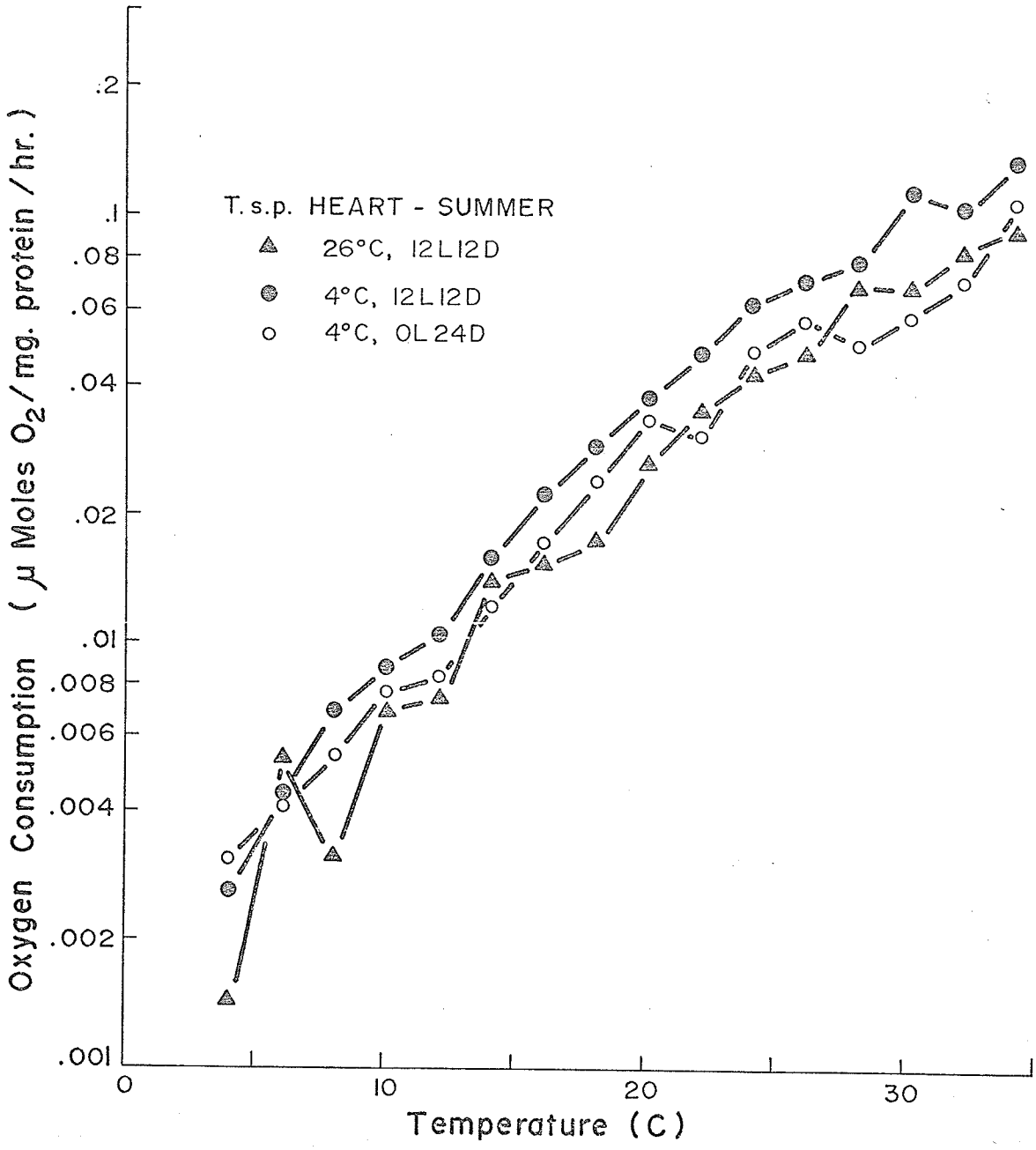

T. s.p. HEART - SUMMER
26°C, 12L12D
4°C, 12L12D
4°C, 0L24D
Oxygen Consumption (μ Moles O2/mg. protein / hr.)
.2
.1
.08
.06
.04
.02
.01
.008
.006
.004
.002
.001
0
10
20
30
Temperature (C)

Figure 3. The effect of temperature on the oxygen consumption of liver homogenates from T. sirtalis parietalis acclimated to 26°C, 12L12D (▲); 4°C, 12L12D (●); and 4°C, 0L24D (○) in winter. Each point represents a mean of at least three values.

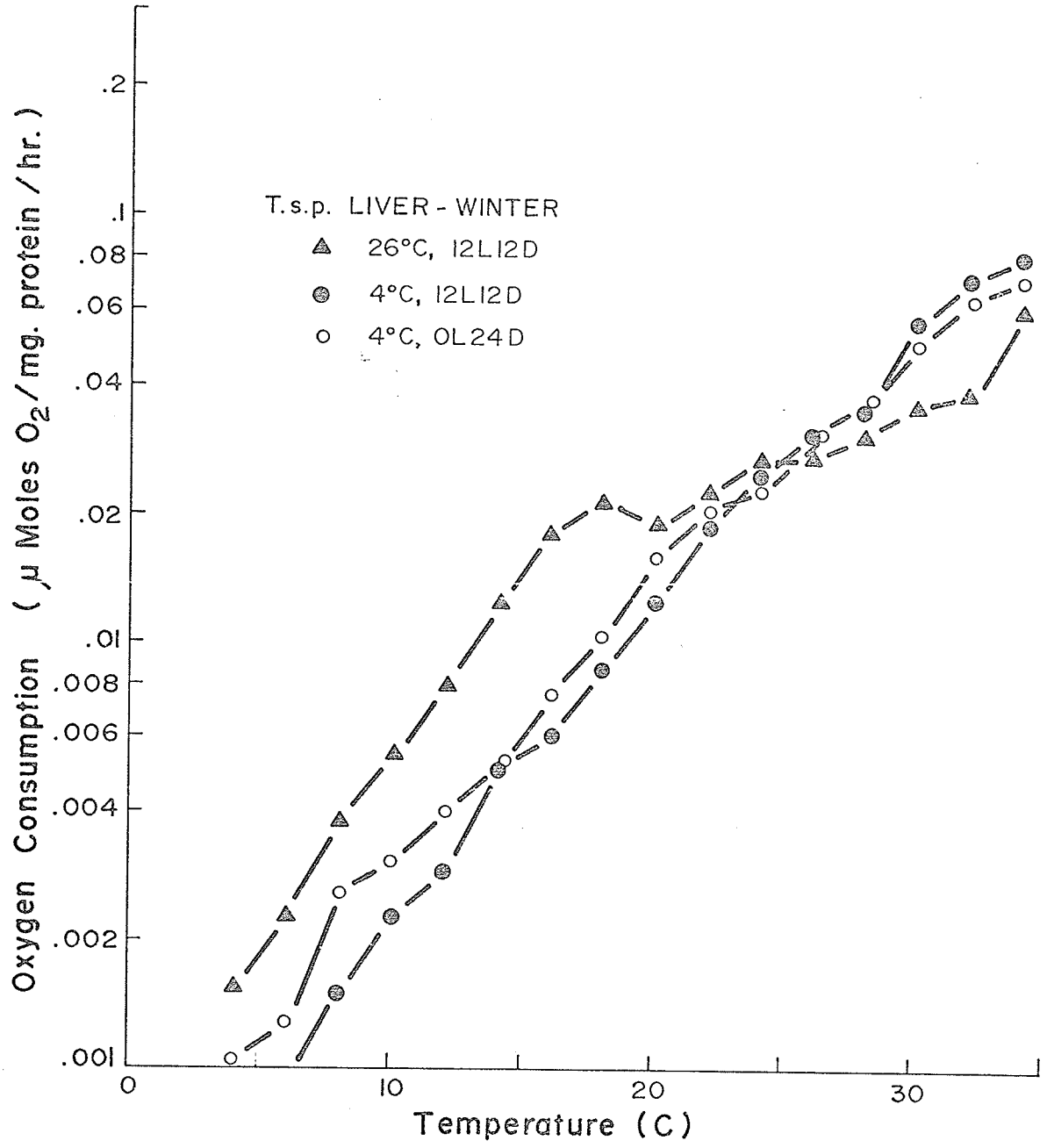

T.s.p. LIVER - WINTER
26°C, 12L12D
4°C, 12L12D
4°C, 0L24D
Oxygen Consumption (μ Moles O2 / mg. protein / hr.)
.2
.1
.08
.06
.04
.02
.01
.008
.006
.004
.002
.001
0
10
20
30
Temperature (C)

Figure 4. The effect of temperature on the oxygen consumption of liver homogenates from T. sirtalis parietalis acclimated to 26°C, 12L12D (▲); 4°C, 12L12D (●); and 4°C, 0L24D (○) in winter. Each point represents a mean of at least three values.

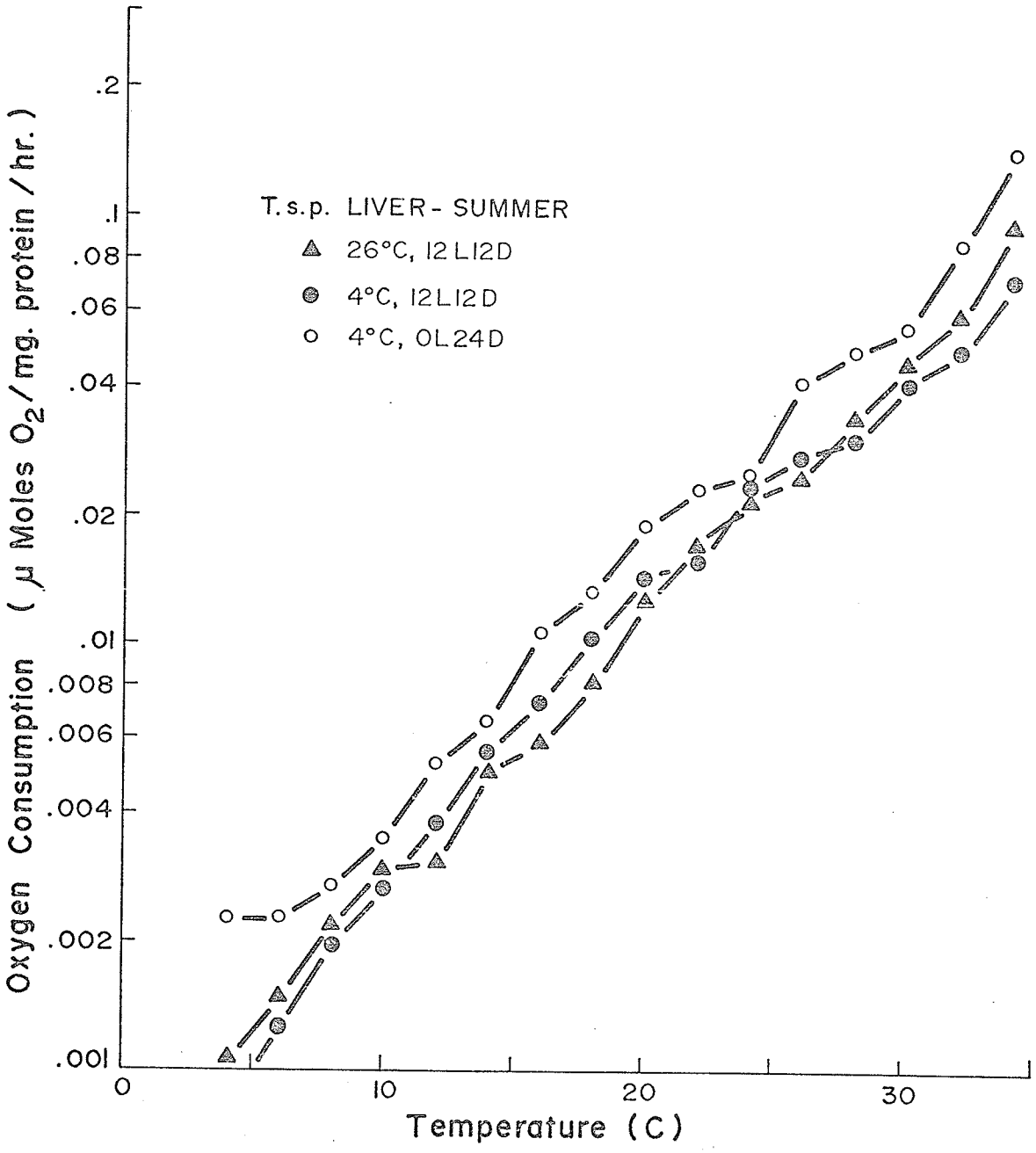


Figure 5. The effect of temperature on the oxygen consumption of muscle homogenates from T. sirtalis parietalis acclimated to 26°C, 12L12D (▲); 4°C, 12L12D (●); and 4°C, 0L24D (○) in winter. Each point represents a mean of at least three values.

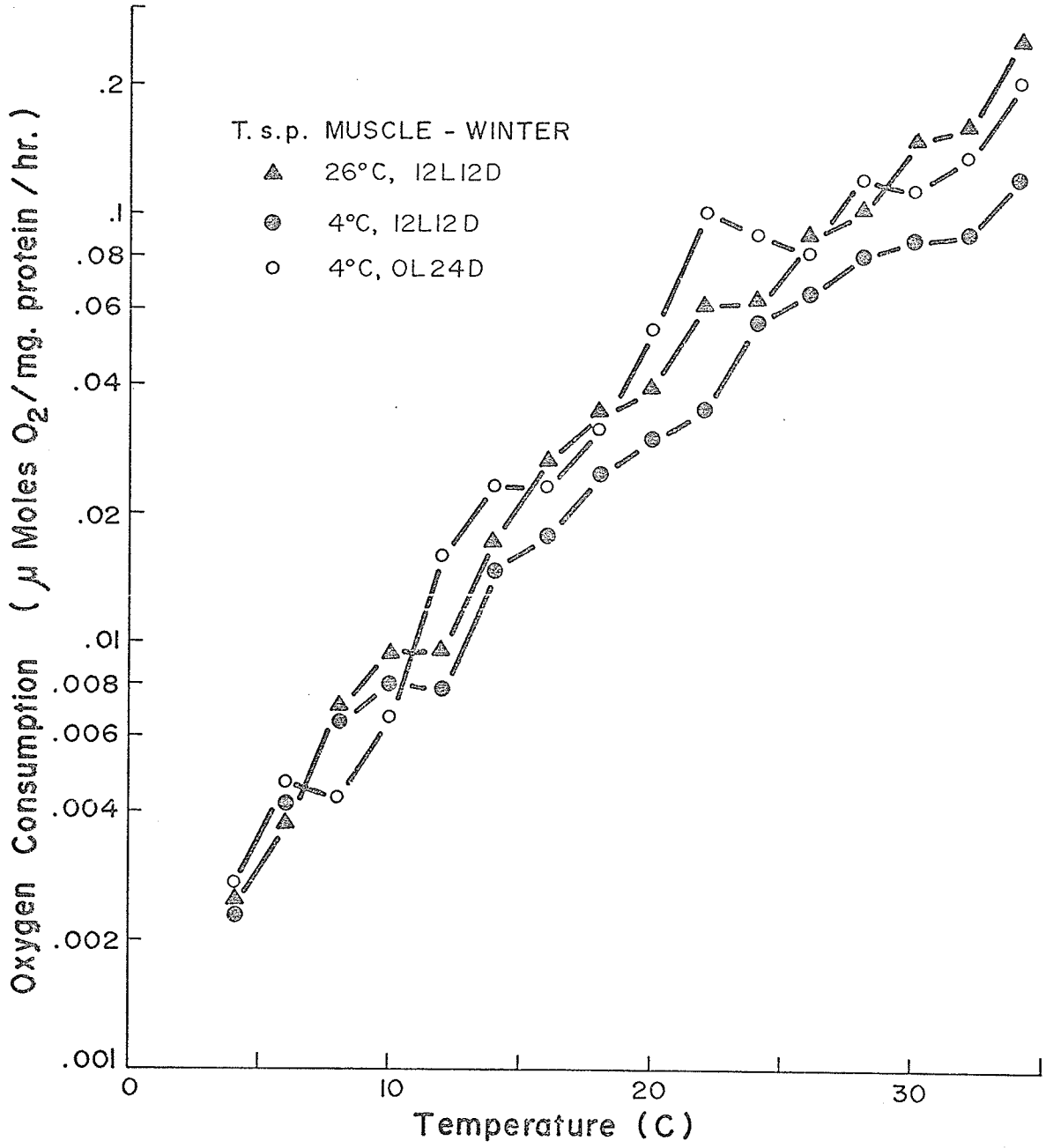

T. s.p. MUSCLE - WINTER
26°C, 12L12D
4°C, 12L12D
4°C, 0L24D
Oxygen Consumption (μ Moles O2 / mg. protein / hr.)
.2
.1
.08
.06
.04
.02
.01
.008
.006
.004
.002
.001
0
10
20
30
Temperature (C)

Figure 6. The effect of temperature on the oxygen consumption of muscle homogenates from T. sirtalis parietalis acclimated to 26°C, 12L12D (▲); 4°C, 12L12D (●); and 4°C, 0L24D (○) in summer. Each point represents a mean of at least three values.

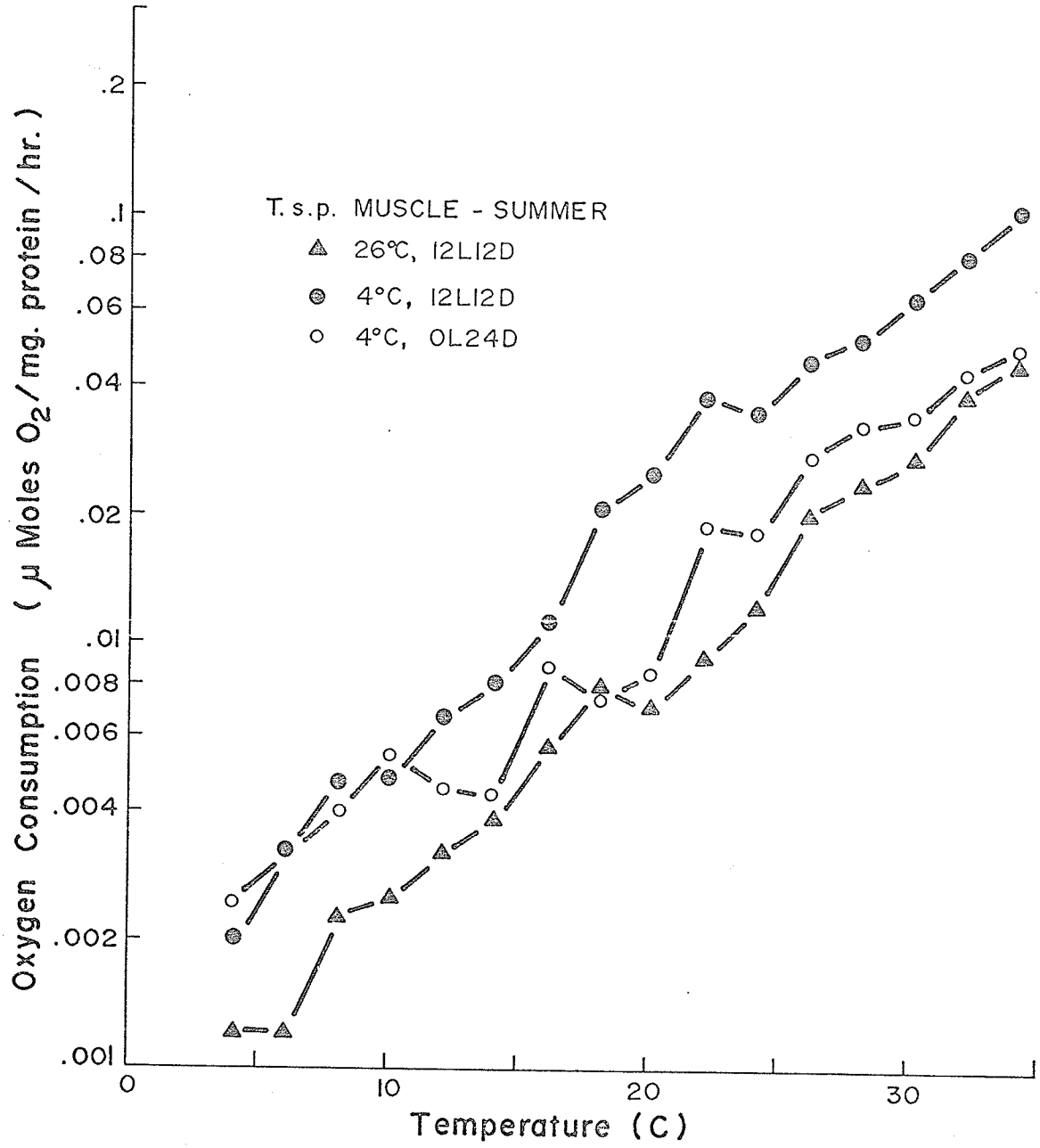

T. s.p. MUSCLE - SUMMER
26°C, 12L12D
4°C, 12L12D
4°C, 0L24D
Oxygen Consumption (μ Moles O_2 / mg. protein / hr.)
.2
.1
.08
.06
.04
.02
.01
.008
.006
.004
.002
.001
0
10
20
30
Temperature (C)

Figure 7. The effect of season on the oxygen consumption of muscle homogenates from T. sirtalis parietalis acclimated to 26°C, 12L12D (a); 4°C, 12L12D (b); and 4°C, 0L24D (c).

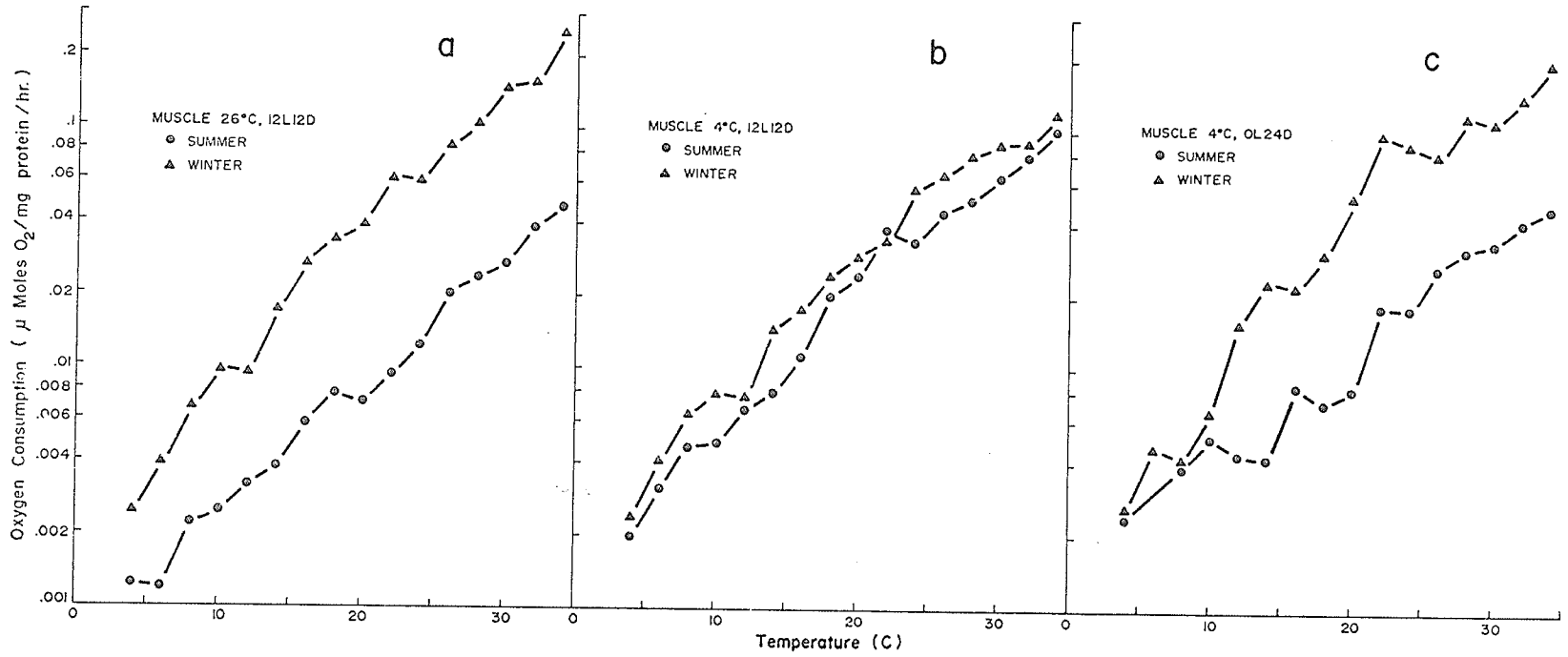

a
b
c
MUSCLE 26°C, 12L12D
SUMMER
WINTER
MUSCLE 4°C, 12L12D
SUMMER
WINTER
MUSCLE 4°C, 0L24D
SUMMER
WINTER
Oxygen Consumption (μ Moles O_2/mg protein/hr.)
.2
.1
.08
.06
.04
.02
.01
.008
.006
.004
.002
.001
0
10
20
30
Temperature (C)

Figure 8. The effect of season on the oxygen consumption of liver homogenates from T. sirtalis parietalis acclimated to 26°C, 12L12D (a); 4°C, 12L12D (b); and 4°C, 0L24D (c).

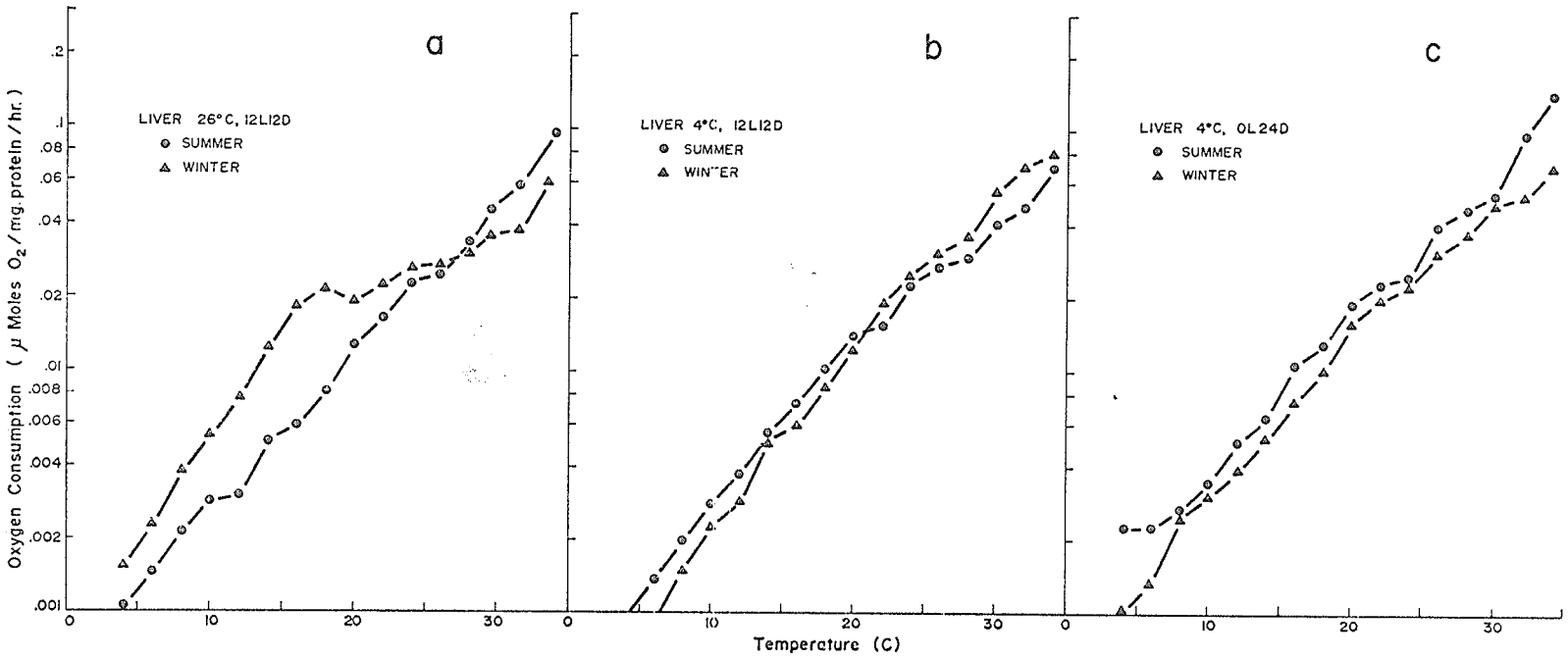

a
b
c
LIVER 26°C, 12L12D
SUMMER
WINTER
LIVER 4°C, 12L12D
SUMMER
WINTER
LIVER 4°C, 0L24D
SUMMER
WINTER
Oxygen Consumption (μ Moles O2 / mg. protein / hr.)
.2
.1
.08
.06
.04
.02
.01
.008
.006
.004
.002
.001
0
10
20
30
Temperature (C)

Figure 9. The effect of season on the oxygen consumption of heart homogenates from T. sirtalis parietalis acclimated to 26°C, 12L12D (a); 4°C, 12L12D (b); and 4°C, 0L24D (c).

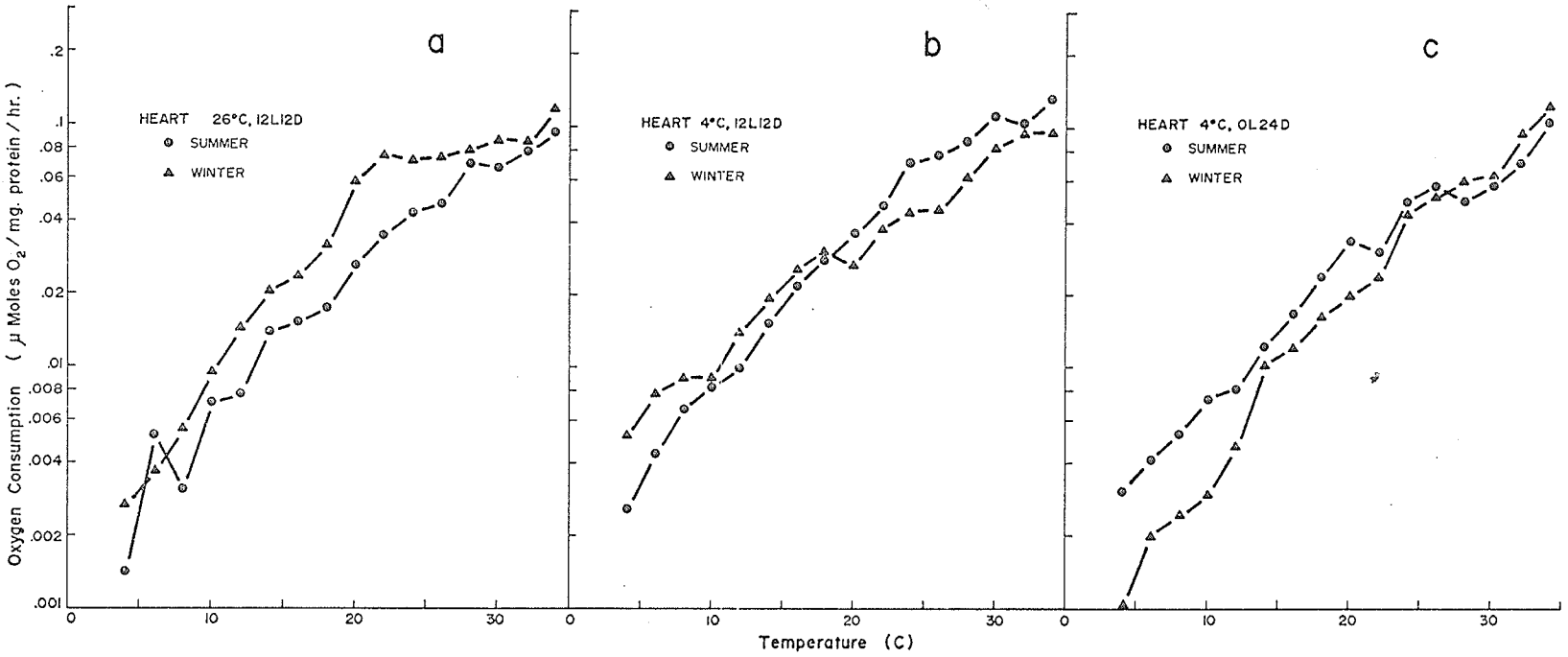

a
b
c
HEART 26°C, 12L12D
SUMMER
WINTER
HEART 4°C, 12L12D
SUMMER
WINTER
HEART 4°C, 0L24D
SUMMER
WINTER
Oxygen Consumption (μ Moles O_2 / mg. protein / hr.)
.2
.1
.08
.06
.04
.02
.01
.008
.006
.004
.002
.001
0
10
20
30
Temperature (C)

Figure 10. The effect of temperature on the oxygen consumption of liver homogenates from T. sirtalis concinnus acclimated to 26°C, 12L12D (●) and 10°C, 12L12D (▲). Each point represents a mean of three values.

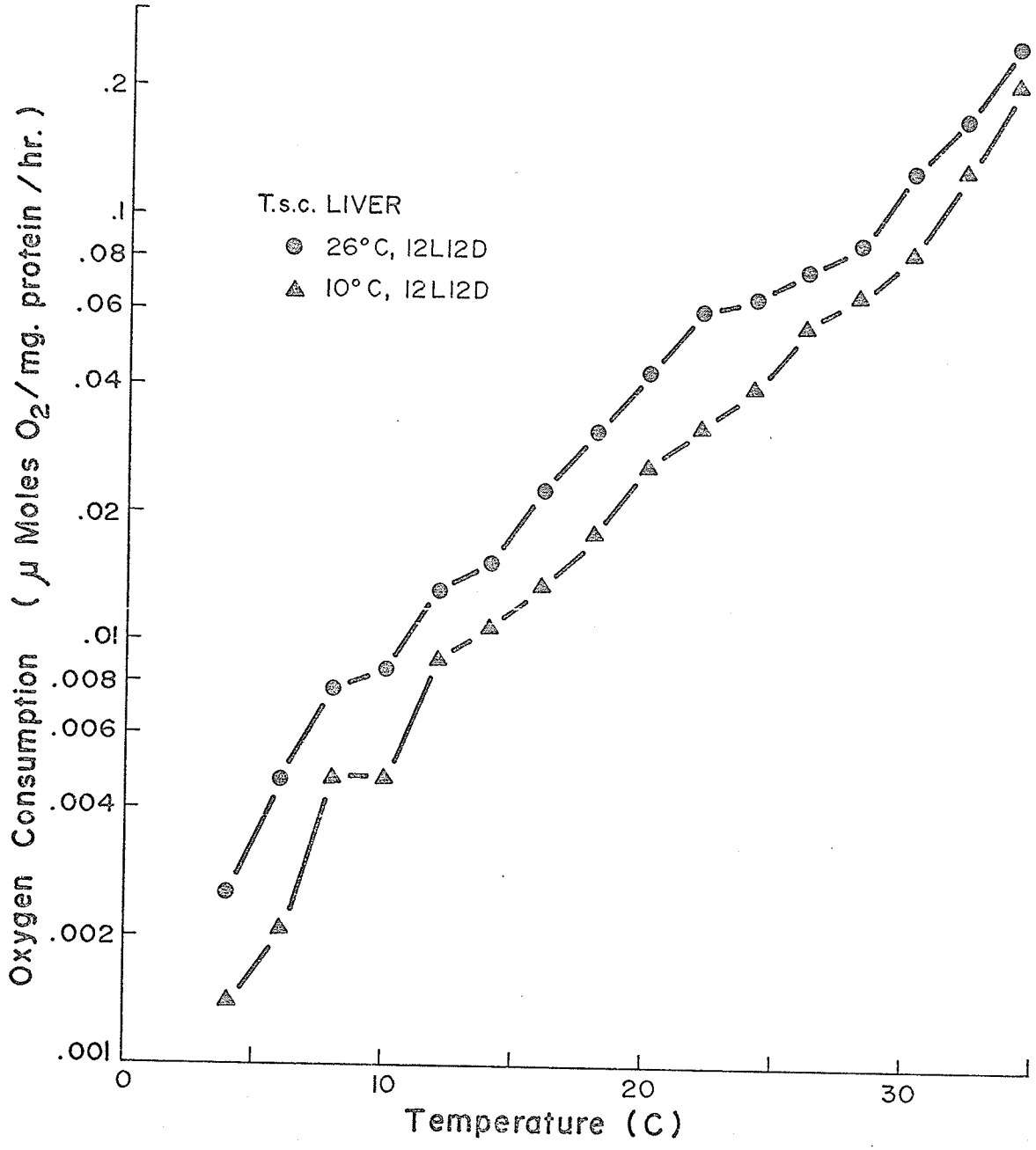
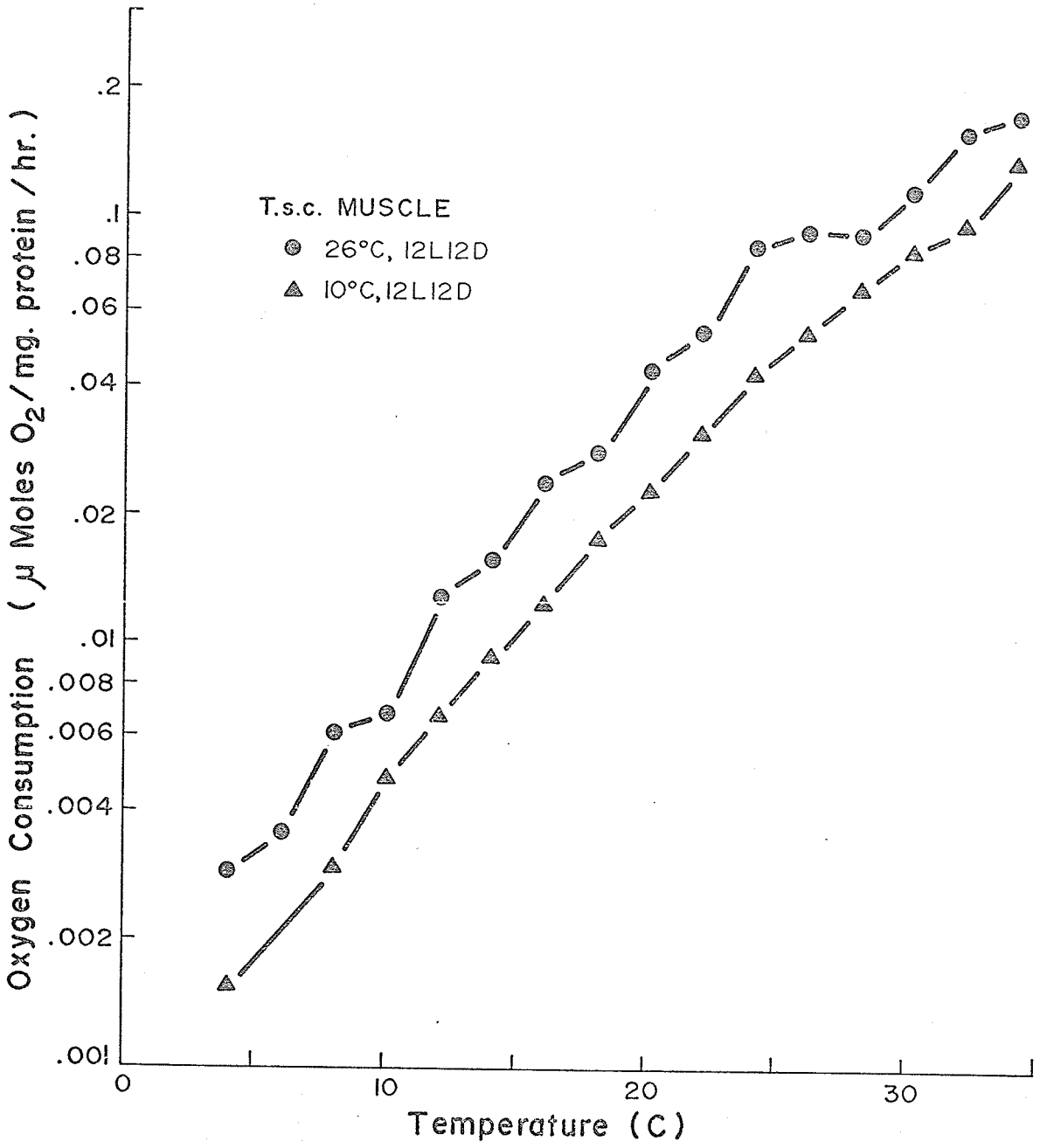

T.s.c. LIVER
26° C, 12L12D
10° C, 12L12D
Oxygen Consumption (μ Moles O_2 / mg. protein / hr.)
.2
.1
.08
.06
.04
.02
.01
.008
.006
.004
.002
.001
0
10
20
30
Temperature (C)

Figure 11. The effect of temperature on the oxygen consumption of muscle homogenates from T. sirtalis concinnus acclimated to 26°C, 12L12D (●) and 10°C, 12L12D (▲). Each point represents a mean of three values.


T.s.c. MUSCLE
26°C, 12L12D
10°C, 12L12D
Oxygen Consumption (μ Moles O2 / mg. protein / hr.)
.2
.1
.08
.06
.04
.02
.01
.008
.006
.004
.002
.001
0
10
20
30
Temperature (C)

PART II

Enzymic Adaptation to Temperature: Malate Dehydrogenase in a Cold Climate Reptile, Thamnophis sirtalis parietalis

Abstract. 1. Liver supernatant malate dehydrogenase (S-MDH) from Thamnophis sirtalis parietalis consists of two electrophoretically distinguishable forms. These isoenzymes were observed to be equally active at 35°C, whereas one was strongly dominant at 5°C.

2. Long-term acclimation of T. sirtalis parietalis to 26°C and 4°C had no effect on the electrophoretic pattern of S-MDH or on several of its temperature-dependent kinetic properties.

3. The Km of NADH for S-MDH was relatively temperature-independent from 40° to 5°C.

4. The Km of oxaloacetate for S-MDH showed a major decrease from 40° to 20°C. Reaction velocities for S-MDH at Km levels of oxaloacetate and NADH possessed Q_{10} values of 0.91 to 1.83 over the same temperature range.

5. The pH optimum of S-MDH was 7.4 and 8.5 at 35° and 10°C respectively. The increase in pH optimum and a high enzyme-substrate affinity at low temperatures were correlated with a decrease in Q_{10} from 10° to 5°C at Km concentrations of substrates.

6. It is hypothesized that different velocity-temperature relationships of the two S-MDH isoenzymes play an important role in the stabilization of enzyme activity during short-term body temperature fluctuations in T. sirtalis parietalis.

INTRODUCTION

In common with certain other poikilothermic species, *in vitro* tissue oxygen consumption in the red-sided garter snake (*Thamnophis sirtalis parietalis*) can be quantitatively altered by thermal and photoperiodic acclimation (Part I of this thesis). Moreover, the metabolic rate curves of certain tissues from *T. sirtalis parietalis* are usually characterized by (1) a biphasic relationship of metabolic rate to immediate temperature change, with Q_{10} values of approximately 1.0 to 3.5 at temperatures above 20°C and 4.5 to 7.0 below 20°C, and less commonly, by (2) temperature regions in which oxygen consumption shows no significant fluctuations. These metabolic responses to immediate temperature change are considered to be an adaptation to a cool temperate climate, where temperature fluctuations are large, frequent and irregular. Aleksiuk (1971) postulated the molecular basis of such responses to be isoenzymic in nature, with various isoenzymes of a given enzyme system possessing different velocity-temperature curves.

The purpose of the present study was to explore further aspects of enzymic adaptation to temperature in cold climate representatives of *T. sirtalis parietalis*. Liver supernatant malate dehydrogenase (L-malate: NAD oxidoreductase EC 1.1.1.37) was chosen because it consists of a two-isoenzyme system in this species (Aleksiuk, unpublished results).

MATERIALS AND METHODS

Experimental animals

Adult Thamnophis sirtalis parietalis were acclimated to 4°C, 12L12D (L = light, D = dark) and 26°C, 12L12D for five to six weeks. Food (ocean perch) and water were provided ad libitum to the 26°C, 12L12D group. Neither food nor water was made available to the 4°C, 12L12D snakes. Liver samples were removed only from males and non-gravid females in the 25-35 gram body weight range.

Preparation of S-MDH

For each enzyme preparation, liver tissue from three snakes was homogenized in four volumes of 0.01M tris-HCl buffer, pH 7.4, containing 2mM EDTA, in an ice-jacketed Potter Elvejhem homogenizer. The homogenate was centrifuged for 20 minutes at 12,000g and 4°C, and the resulting pellet discarded. The supernatant was brought to 40% saturation with solid $(NH_4)_2SO_4$ and stirred for a minimum of 1 hour at 4°C. The suspension was centrifuged for 20 minutes at 12,000g and 4°C, the pellet discarded, and the supernatant brought to 75% saturation with solid $(NH_4)_2SO_4$. Following stirring for 1 hour at 4°C, the precipitate was collected in a minimal volume of 0.01M tris-HCl buffer, pH 7.4. The preparation was dialysed against 500 ml of 0.01M tris-HCl buffer, pH 7.4, for 6-7 hours at 4°C with three changes of buffer. This resulted in a two-fold purification of the enzyme. The final extract was frozen in 0.5 ml aliquots for a two week period. No loss of supernatant malate dehydrogenase (S-MDH) activity occurred during this interval.

Determination of S-MDH activity

Supernatant MDH activity was assayed by following the oxidation of NADH at 340 mμ in an Unicam SP800 recording spectrophotometer fitted with an accessory scale expander. The cuvettes, of 1-cm light path, contained enzyme, varying levels of oxaloacetate and NADH, and sufficient 0.1M phosphate buffer to make a final volume of 3.0 mls. For the determination of pH optima, the pH of the buffer was altered by the addition of HCl or NaOH. The temperature of the reaction mixture was controlled by a Neslab circulating temperature bath (Model No. 681 276). Condensation on the cuvette walls at low temperatures was prevented by a steady flow of dry gaseous nitrogen through the cellular compartment. Initial reaction rates were determined by measuring the rate of NADH oxidation in a 30 to 90 second interval following the addition of enzyme to the reaction mixture. Changes in absorbance were usually linear for more than two minutes.

Electrophoresis

The enzyme preparation was subjected to standard vertical acrylamide gel electrophoresis (Davis, 1964) using a 4.5% stacking gel (prepared in 0.75 M tris-H SO buffer, pH 8.4), a 6% separating gel (prepared as above) and a 0.065 M tris-borate tank buffer pH 8.4. The source of acrylamide was the J. T. Baker Chemical Co., Phillipsburg, New Jersey, U.S.A. Samples containing 30% sucrose were run at 1.0 milliamps per tube for 1 hour followed by 2.0 milliamps per tube for 2 hours. The S-MDH bands were stained with a solution containing 0.05 g NAD, 0.002 g phenazine methsulphate, 0.02 g nitro blue

tetrazolium and 0.115 g L-malate in 50 ml of 0.1 M phosphate buffer, pH 7.4 (a modification of the method used by Goldberg, 1963). Staining was carried out under conditions of complete darkness at incubation temperatures between 5° and 45°C, using two different procedures. One procedure involved the incubation of gels from a given run at 10°C intervals (from 5° to 45°C) for a constant time period (approximately 45 minutes). The relative staining of the isoenzymes was quantified by means of a Gelscan automatic recording and integrating scanner (Model No. 39365; Gelscan Instrument Co., Ann Arbor, Michigan), and used as a measure of relative activity. The other procedure involved incubating one set of gels from a given run at 35°C for 45 minutes, and another set of gels from the same run at 5°C until at least one band was as dark as the darkest band at 35°C.

RESULTS AND DISCUSSION

Isoenzymes of liver supernatant MDH

Liver S-MDH from garter snakes acclimated to 26°C consists of two electrophoretically distinguishable forms, designated 1 and 2 in Fig. 1A. Smaller bands were also discernible but were insignificant relative to the two major bands. The two isoenzymes, MDH-1 and MDH-2, were both prominent at a staining temperature of 35°C (Fig. 1 A); only one major isoenzyme, MDH-1, was apparent at 5°C (Fig. 1 B).

The relative activity of each isoenzyme in relation to total enzyme activity was estimated from densitometer tracings of stained electrophoretic gels and was found to vary with temperature (Fig. 2). From 45° to 25°C, the activities of the two isoenzymes were nearly equal with MDH-2 being slightly dominant. Below 25°C, the relative activity of MDH-2 decreased with decreasing temperature until it was essentially inactive at 5°C. MDH-1 followed an opposite pattern and was strongly dominant at low temperatures.

The effect of temperature on the activity of each isoenzyme relative to maximal activity at 45°C is given in Table 1. The lesser effect of decreasing temperature on MDH-1 activity is clearly apparent. At 15° and 5°C, MDH-1 retained 34% and 16% of its total activity at 45°C, whereas MDH-2 retained only 16% and 2%, respectively.

In all cases, the results obtained for liver S-MDH from T. sirtalis parietalis acclimated to 4°C were similar to those from T. sirtalis parietalis acclimated to 26°C.

The effect of excess substrate on liver S-MDH activity

Mammalian mitochondrial and cytoplasmic forms of MDH differ not only in their specific electrophoretic, chromatographic, immunological and catalytic properties (Englard et al., 1960; Grimm and Doherty, 1961; Siegel and Englard, 1962; Silverstein and Sulebelle, 1969; Thorne, 1960; Thorne and Cooper, 1964; and others), but also in their response to excess substrate. Mitochondrial MDH is inhibited by excess oxaloacetate, whereas cytoplasmic MDH is inhibited by excess malate (Grimm and Doherty, 1961; Siegel and Englard, 1960; Thorne and Cooper, 1964; and others). Oxaloacetate saturation curves for liver S-MDH from T. sirtalis parietalis acclimated to 26°C and 4°C were observed to be hyperbolic at temperatures from 40° to 5°C (unpublished results). There was little substrate inhibition at any temperature to a concentration of 1.5×10^{-3} M oxaloacetate.

Effect of temperature on activation energy

It has been suggested that certain enzymes from cold-adapted organisms characteristically possess lower activation energies relative to those from warm-adapted organisms (Somero and Hochachka, 1968; Vroman and Brown, 1963). The overall result of such an effect would be an increase in catalytic rates at low temperatures in the cold-adapted form. However, there was no apparent correlation between acclimation temperature and activation energy for S-MDH from T. sirtalis parietalis. Arrhenius plots for S-MDH from warm and cold acclimated T. sirtalis parietalis were linear and essentially similar from 40° to 5°C (Fig. 3).

Effect of temperature on the apparent Km of oxaloacetate

There was a major decrease in the apparent Km of oxaloacetate for liver S-MDH from 40° to 20°C (Fig. 4 and Table 2). As temperature decreased from 20° to 5°C, the Km values increased slightly to 15°C then decreased to 5°C. This Km-temperature relationship in the 20° to 5°C region may be the result of different kinetic properties of the two isoenzymes of liver S-MDH (MDH-1 and MDH-2) at low temperatures. From the electrophoretograms (Fig. 1 and 2), it was apparent that MDH-1, the "low temperature isoenzyme", gradually assumed most of the total S-MDH activity at low temperatures. In fact, the activity of MDH-2, the "high temperature isoenzyme", was negligible at 5°C. The apparent increase in Km from 20° to 15°C may represent a transitional stage, where the activity of MDH-2 decreases rapidly and the activity of MDH-1 increases or is maintained. The increased activity of MDH-1, relative to MDH-2, at low temperatures is correlated with a high enzyme-substrate affinity over the same temperature range (Fig. 4).

"Induction" of isoenzymes in conjunction with long-term cold acclimation has been demonstrated for a number of enzymes, including liver isocitrate dehydrogenase (Moon and Hochachka, 1971), brain acetylcholinesterase (Baldwin and Hochachka, 1970), and liver citrate synthetase (Hochachka and Lewis, 1970) from Salmo gairdneri and muscle lactate dehydrogenases from Salmo namaycush and Salvelinus fontinalis (Hochachka and Somero, 1968). However, prolonged cold acclimation of T. sirtalis parietalis had no apparent effect on S-MDH isoenzymes or on the catalytic properties of S-MDH examined. There was no

significant difference between the Km values of oxaloacetate for S-MDH from the two acclimation groups at any of the experimental temperatures (Table 2). This similarity in the Km values of warm and cold acclimated snakes may indicate a purely temperature-dependent (time-independent) mechanism of enzymic adjustment to temperature in T. sirtalis parietalis. Aleksiuk (1971) obtained similar results for skeletal muscle LDH's from this species. Immediate activation of isoenzymes with diverse velocity-temperature curves may represent an important mechanism for the stabilization of enzyme reaction rates in T. sirtalis parietalis during exposure to fluctuating temperatures.

Positive modulation of enzyme activity by decreasing temperature is thought to off-set the depressive effects of low temperature on catalysis (Somero, 1969). This is considered to be highly adaptive in poikilotherms. The major decrease in Km for S-MDH from 40° to 20°C was correlated with a low Q_{10} at Km concentrations of substrates (Table 3). The increase in enzyme-substrate affinity in this temperature range apparently compensates for the reduction in thermal energy such that reaction rates are relatively stable (Fig. 5) and Q_{10} values vary from 0.91 to 1.83 (Table 3). Only at Km levels of oxaloacetate was there a degree of stabilization in reaction rates over a broad temperature range (Table 3).

The Km values of oxaloacetate at 10° and 5°C were nearly equal to the Km value at 20°C. In spite of this, the Q_{10} at Km concentrations of substrates was reduced to a value of 1.57 between 10° and 5°C (Fig. 5 and Table 3). Consequently, the effect of temperature on regulatory factors other than enzyme-substrate affinity must also be

considered.

Effect of temperature on other controlling factors

The possible role of thermally modulated changes in the Km of NADH in controlling S-MDH catalysis were examined. In agreement with the results obtained for monotreme S-MDH by Baldwin and Aleksiuk (in press), the Km of NADH for S-MDH was relatively temperature independent from 40° to 5°C in the present study and was unaffected by temperature acclimation (Table 4). In addition, variations in NADH concentration from Km values to saturating levels had little effect on the velocity-temperature curves for S-MDH, especially at Km levels of oxaloacetate (Fig. 5).

Figure 6 shows pH optima of liver S-MDH at 35° and 10°C. The pH optimum shifted from 7.4 at 35°C to 8.5 at 10°C. This may mean that the two isoenzymes, MDH-1 and MDH-2, have different pH optima. MDH-1 and MDH-2 were about equally active at 35°C, whereas MDH-1 was predominant at 10°C (Fig. 2). Consequently, MDH-1 may possess a higher pH optimum than MDH-2.

This shift in pH optimum with temperature change not only reinforces the suggestion that the two isoenzymes have different temperature-dependent catalytic properties, but also implicates pH in the regulation of reaction rates. An increase in intracellular and blood pH with a decrease in environmental temperature has been observed for a number of poikilothermic species (Howell et al, 1970; Reeves and Wilson, 1969). If the intracellular pH of T. sirtalis parietalis behaves similarly, the increase in pH at low temperatures would favor

MDH-1 activity (assuming that MDH-1 actually does have a higher pH optimum than MDH-2). Although the increase in enzyme-substrate affinity with decreasing temperature is sufficient to account for the stable Q_{10} values in the 40° to $20^{\circ}C$ region, it is not adequate to explain the low Q_{10} from 10° to $5^{\circ}C$. A high enzyme-substrate affinity may act synergistically with an increase in pH optimum, to raise reaction rates and lower Q_{10} values in this temperature region.

CONCLUDING REMARKS

Liver S-MDH from T. sirtalis parietalis exists in two electrophoretically distinguishable forms. Both isoenzymes (MDH-1 and MDH-2) are equally active at 35°C, whereas MDH-1 is strongly dominant at 5°C. The electrophoretic data, in addition to the Km-temperature relationship observed and the absence of an acclimation effect, suggests a temperature-dependent but time-independent transition of activity from one isoenzyme to the other. This apparently represents a mechanism of adaptation to short-term fluctuations of body temperature in T. sirtalis parietalis.

As temperature decreases from 40° to 20°C, an increase in enzyme-substrate affinity compensates for the effect of reduction of thermal energy on MDH reaction rates. Q_{10} values at Km levels of substrates are relatively low, varying from 0.91 to 1.83 over the same temperature range. Regions in the metabolic rate-temperature curves of certain tissues from T. sirtalis parietalis, in which oxygen consumption shows little fluctuation, occur in a similar temperature range (Part I of this thesis). It appears that the molecular basis for stable metabolic rates may be partly due to the positive modulation of enzyme activity by decreasing temperature.

A reduction observed in Q_{10} of S-MDH activity at low temperatures (10°-5°C), in absence of a change in Km of oxaloacetate, may be explained by an increase in pH optimum acting in conjunction with high enzyme-substrate affinity.

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Figure 1. Electrophoretic patterns of liver S-MDH from *T. sirtalis parietalis*. Temperatures (35° and 5°C) refer to staining temperatures. The gel at 5°C was stained until at least one of the bands was as dark as the darkest band at 35°C.

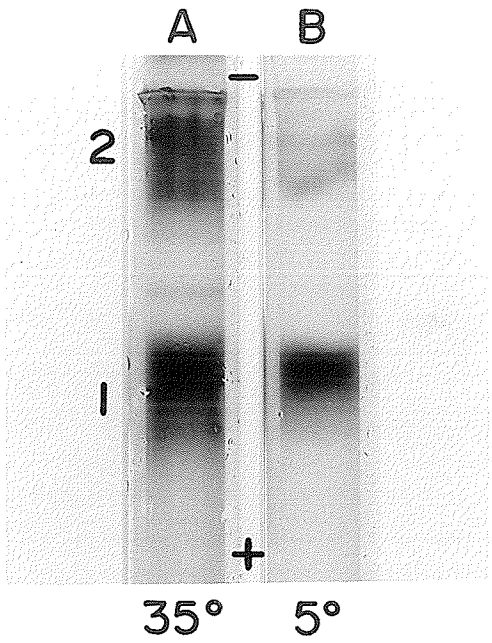
A
B
−
2
1
+
35°
5°

Figure 2. The effect of incubation temperature on the relative activity of the two supernatant isoenzymes, MDH-1 and MDH-2, expressed as a percentage of total MDH activity at each particular temperature. Relative activity was determined from densitometric tracings of electrophoretic gels stained at various temperatures for a constant time interval.

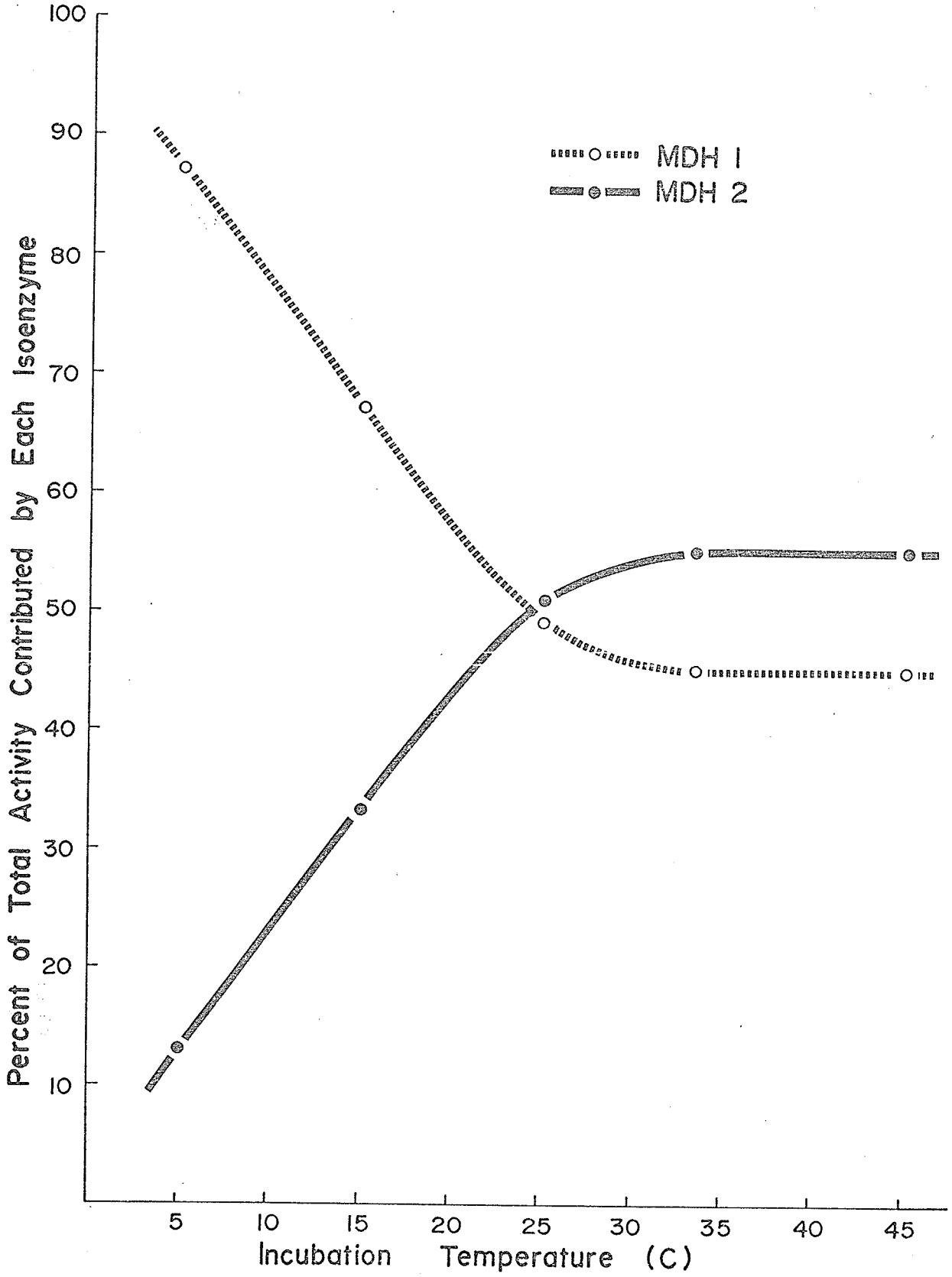
MDH 1
MDH 2
Percent of Total Activity Contributed by Each Isoenzyme
100
90
80
70
60
50
40
30
20
10
5
10
15
20
25
30
35
40
45
Incubation Temperature (C)

Figure 3. Arrhenius plots of liver S-MDH activity from 26°C (●) and 4°C (○) acclimated T. sirtalis parietalis. Regression lines were fitted to the data according to the least squares principle. Vmax values were determined from Lineweaver-Burk plots.

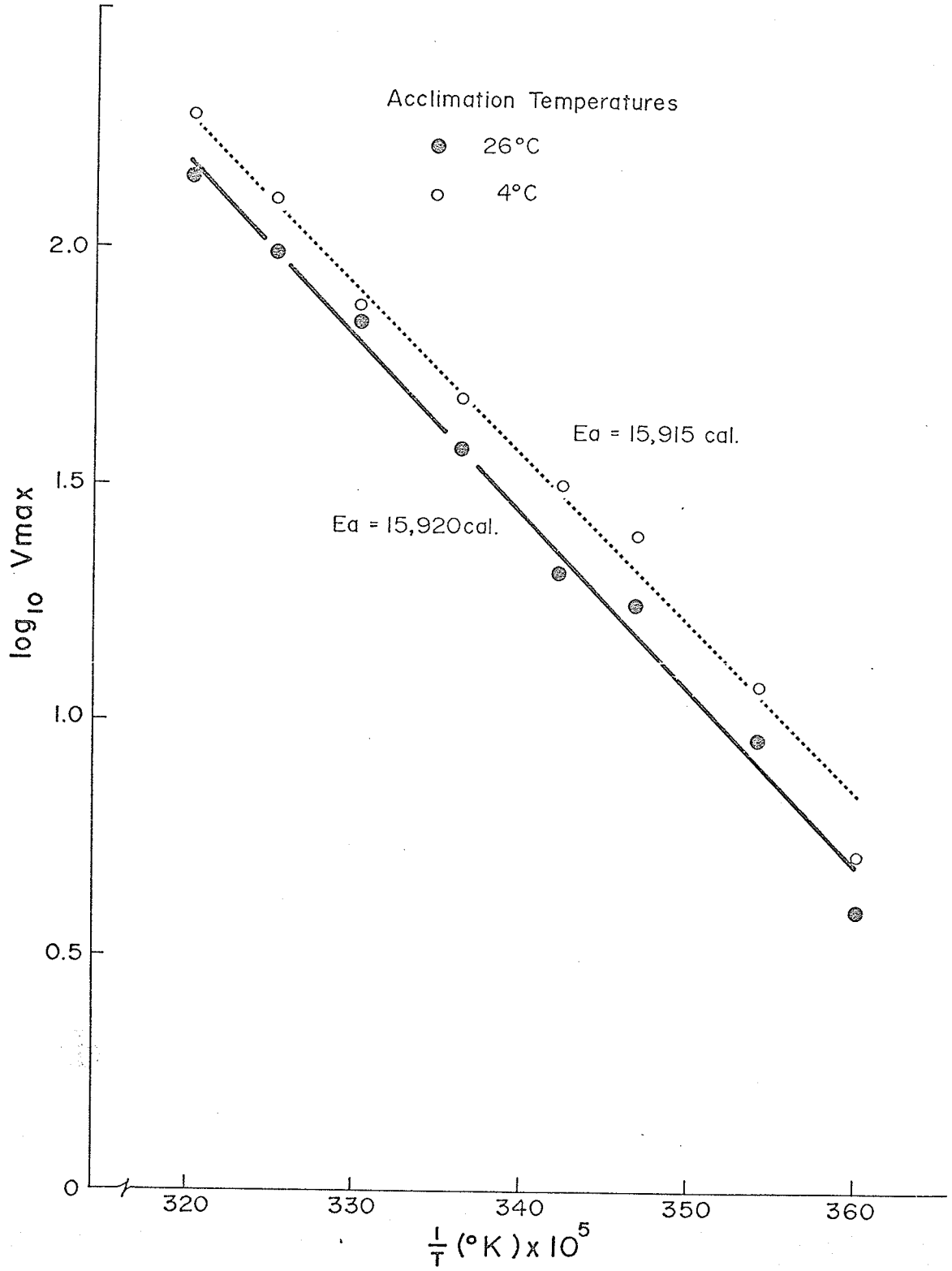
Acclimation Temperatures
26°C
4°C
Ea = 15,915 cal.
Ea = 15,920cal.
log10 Vmax
2.0
1.5
1.0
0.5
0
320
330
340
350
360
1/T (°K) x 10^5

Figure 4. The Michaelis-Menton constant (Km) of oxaloacetate for S-MDH enzymes from 26°C (●) and 4°C (○) acclimated T. sirtalis parietalis as a function of assay temperature. Km values were determined from Lineweaver-Burk plots. Each point represents a mean of three values. The assay mixture contained, in a final volume of 3.0 ml: S-MDH, 0.187 mM NADH, 0.022 to 0.095 mM oxaloacetate and 0.1M phosphate buffer, pH 7.4.

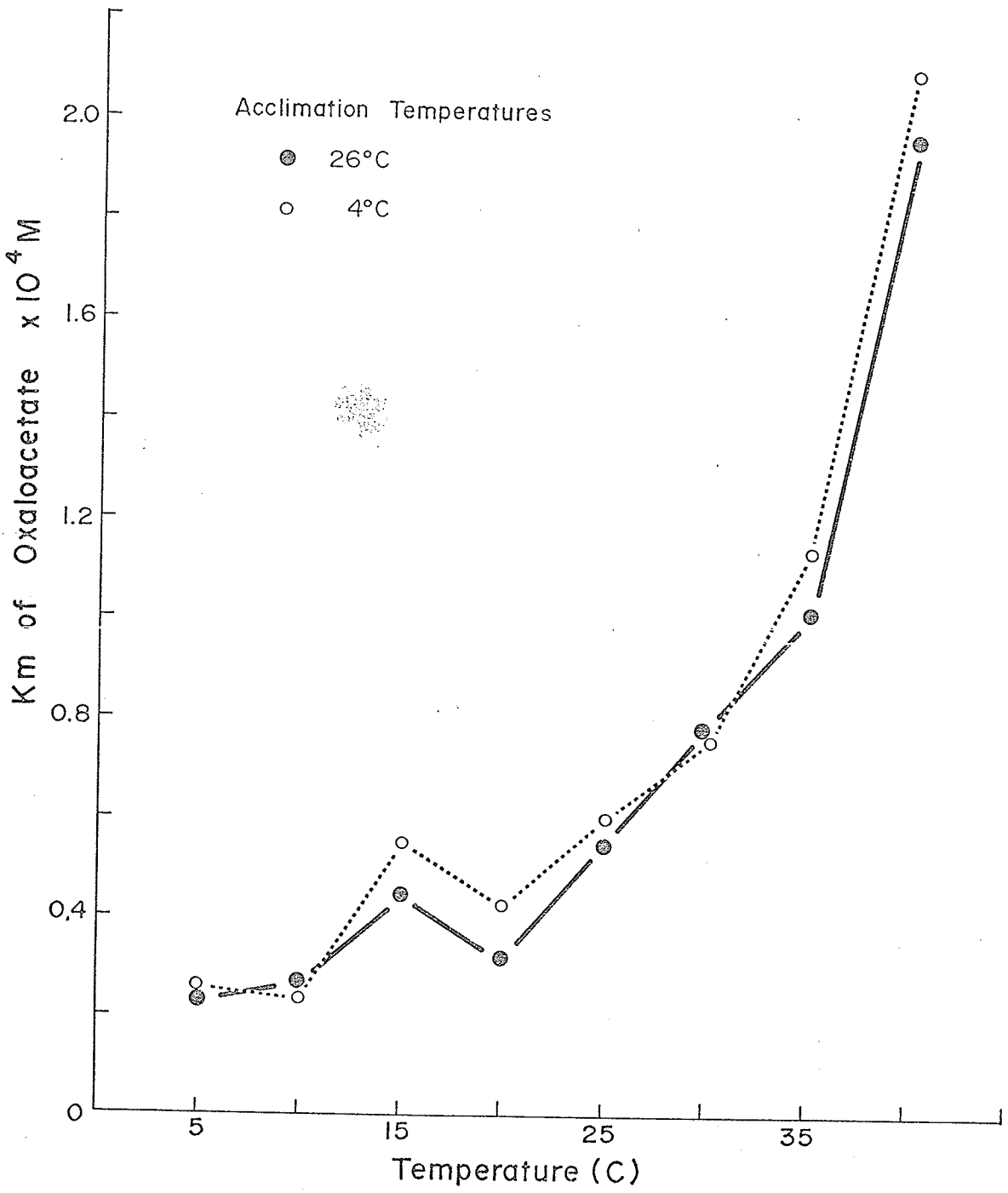
Acclimation Temperatures
26°C
4°C
Km of Oxaloacetate x 10⁴ M
2.0
1.6
1.2
0.8
0.4
0
5
15
25
35
Temperature (C)

Figure 5. The effect of temperature on the reaction velocities for liver S-MDH from 26°C acclimated T. sirtalis parietalis with various combinations of saturating and Km levels of oxaloacetate and NADH. Km and saturating levels of oxaloacetate are 0.0138 mM and 0.379 mM respectively. Comparable figures for NADH are 0.0278 mM and 0.188 mM.

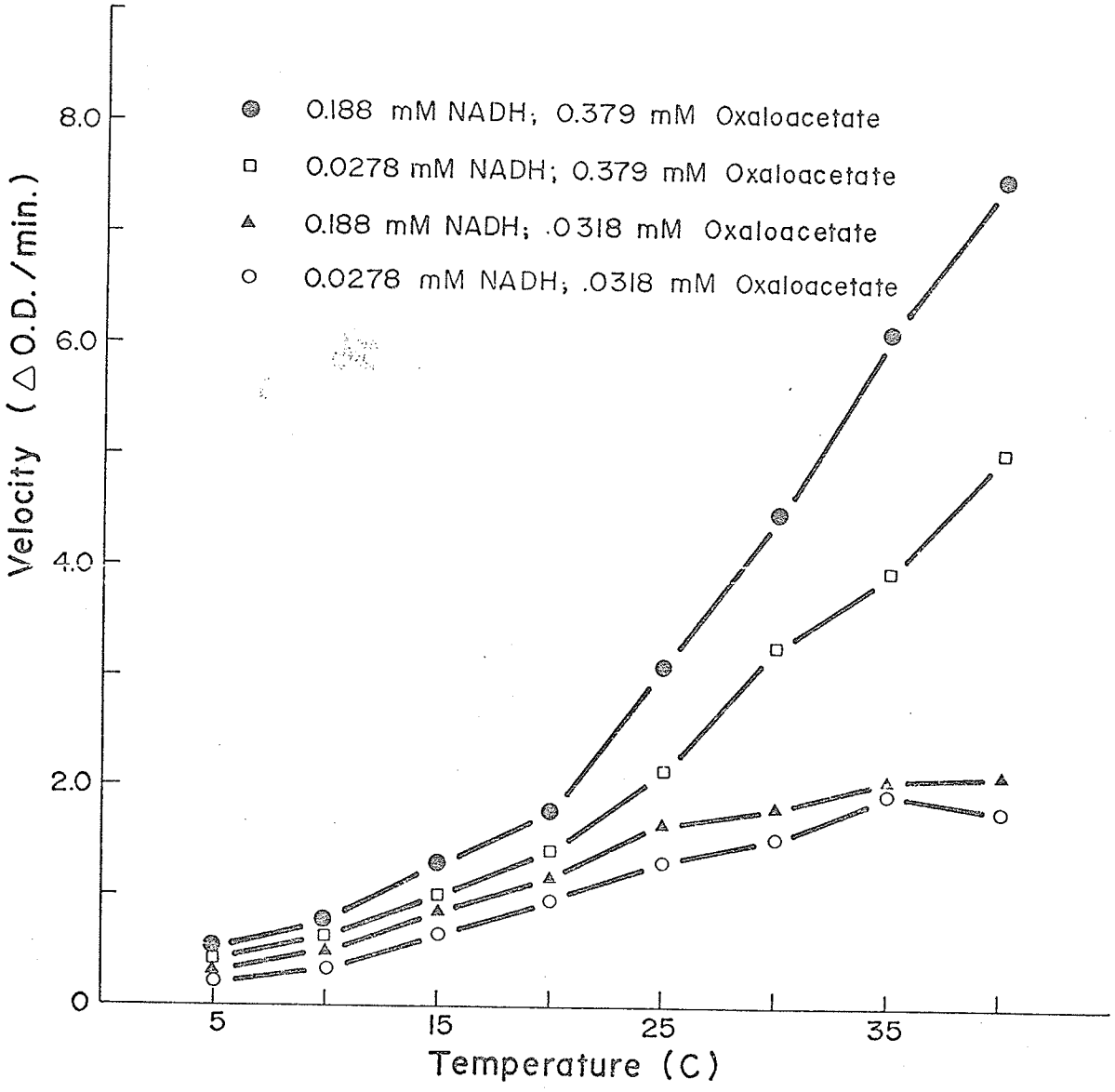
0.188 mM NADH; 0.379 mM Oxaloacetate
0.0278 mM NADH; 0.379 mM Oxaloacetate
0.188 mM NADH; .0318 mM Oxaloacetate
0.0278 mM NADH; .0318 mM Oxaloacetate
Velocity (ΔO.D./min.)
8.0
6.0
4.0
2.0
0
5
15
25
35
Temperature (C)

Figure 6. The effect of pH on liver S-MDH activity from 26°C acclimated T. sirtalis parietalis at 35°C (●) and 10°C (○). The concentrations of oxaloacetate and NADH were 0.187 mM and 0.265 mM, respectively.

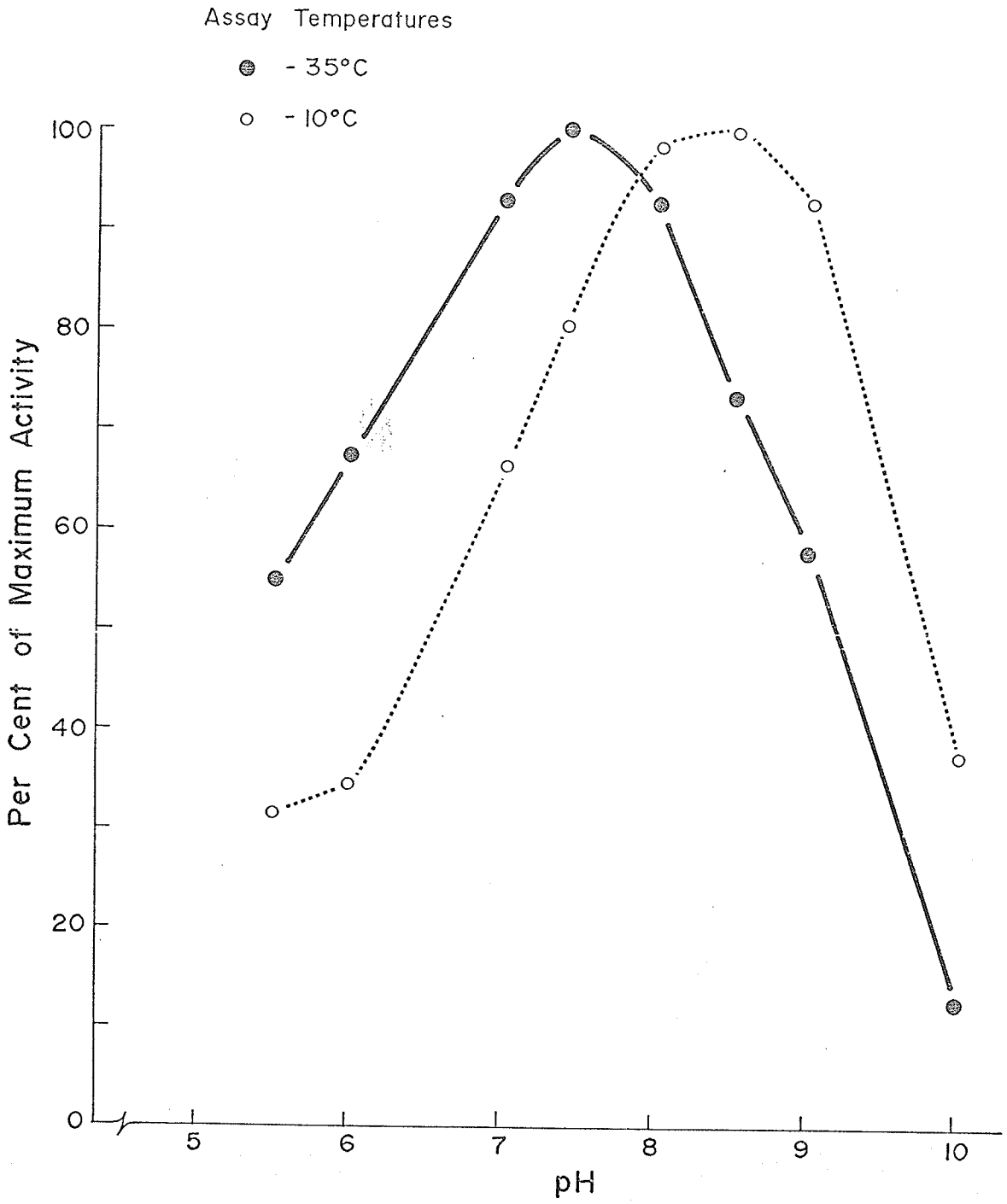
Assay Temperatures
- 35°C
- 10°C
Per Cent of Maximum Activity
100
80
60
40
20
0
5
6
7
8
9
10
pH

Table 1. The effect of temperature on the activity of liver S-MDH isoenzymes from T. sirtalis parietalis relative to their maximum activity at 45°C. This set of data is representative of six obtained.

	% of Activity at 45°C				
	45°C	35°C	25°C	15°C	5°C
MDH-1	100	79	51	34	15
MDH-2	100	78	44	16	2

Table 3. Temperature coefficients (Q_{10}'s) of the reaction velocities of liver S-MDH from T. sirtalis parietalis at saturating and Km levels of oxaloacetate and NADH. Km and saturating levels of oxaloacetate are 0.0318 mM and 0.379 mM respectively; comparable figures for NADH are 0.0278 mM and 0.188 mM.

Assay Temperature (°C)	Q_{10}			
	Sat. NADH Sat. Ox.	Sat. NADH Km Ox.	Km NADH Sat. Ox.	Km NADH Km Ox.
40-35	1.51	1.02	1.60	0.91
35-30	1.87	1.26	1.49	1.69
30-25	2.06	1.25	2.32	1.29
25-20	3.04	1.97	2.37	1.83
20-15	1.81	1.59	2.01	2.12
15-10	2.77	3.10	2.69	2.32
10-5	2.39	1.73	2.00	1.57

Table 4. The effect of temperature and temperature acclimation on the Km of NADH for liver S-MDH.

Assay Temperature	Km x 10^{-2} mM	
(^{o}C)	Warm (26^{o}C) Acclimated	Cold (4^{o}C) Acclimated
40	2.21	2.47
35	1.55	2.03
30	2.24	2.16
25	2.40	2.70
20	1.38	1.26
15	1.78	2.17
10	1.59	3.02
5	2.00	3.74

APPENDIX

APPENDIX Table 1. *In vitro* metabolic rates (μM O_2/mg protein/hr) of heart homogenates from *T. sirtalis parietalis* acclimated to 26°C, 12L12D during the winter.

Temperature (°C)	Metabolic Rates				$\bar{x}$ ± S.D.
34	0.2420	0.1200	0.0710	0.0563	0.1220 ± 0.0843
32	0.1440	0.1022	0.0530	0.0382	0.0843 ± 0.0482
30	0.1580	0.1180	0.0364	0.0315	0.0860 ± 0.0623
28	0.1320	0.1141	0.0390	0.0285	0.0784 ± 0.0522
26	0.1070	0.1070	0.0324	0.0540	0.0751 ± 0.0379
24	0.1260	0.0814	0.0314	0.0465	0.0713 ± 0.0420
22	0.1070	0.1090	0.0300	0.0520	0.0745 ± 0.0397
20	0.0720	0.0870	0.0200	0.0330	0.0530 ± 0.0317
18	0.0575	0.0141	0.0346	0.0336	0.0350 ± 0.0178
16	0.0322	0.0185	0.0157	0.0284	0.0237 ± 0.0079
14	0.0322	0.0141	0.0116	0.0250	0.0207 ± 0.0096
12	0.0197	0.0123	0.0117	0.0150	0.0147 ± 0.0036
10	0.0124	0.0079	0.0077	0.0099	0.0095 ± 0.0022
8	0.0079	0.0058	0.0062	0.0023	0.0055 ± 0.0024
6	0.0054	0.0022	0.0037	0.0034	0.0037 ± 0.0016
4	0.0036	0.0013	0.0027	0.0030	0.0026 ± 0.0019

APPENDIX Table 2. *In vitro* metabolic rates (μM O_2/mg protein/hr) of heart homogenates from *T. sirtalis parietalis* acclimated to 4°C, 12L12D during the winter.

Temperature (°C)	Metabolic Rates				$\bar{x}$ ± S.D.
34	0.1610	0.0620	0.0820	0.0802	0.0963 ± 0.0441
32	0.1820	0.0650	0.0812	0.0680	0.0990 ± 0.0558
30	0.1300	0.0680	0.0820	0.0560	0.0840 ± 0.0325
28	0.0610	0.0562	0.0820	0.0440	0.0608 ± 0.0159
26	0.0745	0.0500	0.0812	0.0160	0.0554 ± 0.0295
24	0.0710	0.0393	0.0840	0.0260	0.0550 ± 0.027
22	0.0348	0.0350	0.0520	0.0300	0.0380 ± 0.0097
20	0.0340	0.0150	0.0420	0.0180	0.0273 ± 0.0129
18	0.0278	0.0275	0.0480	0.0170	0.0301 ± 0.0130
16	0.0202	0.0280	0.0440	0.0125	0.0261 ± 0.0132
14	0.0094	0.0224	0.0380	0.0096	0.0199 ± 0.0135
12	0.0130	0.0146	0.0200	0.0078	0.0139 ± 0.0050
10	0.0090	0.0075	0.0150	0.0043	0.0090 ± 0.0045
8	0.0092	0.0084	0.0140	0.0048	0.0091 ± 0.0033
6	0.0091	0.0084	0.0040	0.0049	0.0081 ± 0.0020
4	0.0052	0.0057	0.0047	0.0049	0.0051 ± 0.00053

APPENDIX Table 3. *In vitro* metabolic rates (μM O_2/mg protein/hr) of heart homogenates from *T. sirtalis parietalis* acclimated to 4°C, 0L24D.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.1190	0.1510	0.1150	0.1280 ± 0.0197
32	0.1011	0.0944	0.0902	0.0952 ± 0.0054
30	0.0900	0.0451	0.0560	0.0637 ± 0.0235
28	0.0770	0.0600	0.0450	0.0607 ± 0.0160
26	0.0572	0.0456	0.0526	0.0517 ± 0.0052
24	0.0520	0.0360	0.0446	0.0442 ± 0.0080
22	0.0378	0.0112	0.024	0.0243 ± 0.0133
20	0.0305	0.0150	0.0148	0.0201 ± 0.0090
18	0.0226	0.0119	0.0150	0.0165 ± 0.0055
16	0.0186	0.0084	0.0091	0.0120 ± 0.0057
14	0.0132	0.0077	0.0097	0.0102 ± 0.0028
12	0.0041	0.0058	0.0043	0.0047 ± 0.0009
10	0.0026	0.0038	0.0025	0.0030 ± 0.0008
8	0.0028	0.0032	0.0012	0.0024 ± 0.0010
6	0.0016	0.0017	0.0027	0.0020 ± 0.0006
4	0.0009	0.0010	0.0010	0.0010 ± 0.0001

APPENDIX Table 4. *In vitro* metabolic rates (μM O_2/mg protein/hr) of heart homogenates from *T. sirtalis parietalis* acclimated to 26°C, 12L12D during the summer.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.1020	0.1070	0.0808	0.0959 ± 0.0139
32	0.0950	0.0820	0.0790	0.0853 ± 0.0084
30	0.0852	0.0716	0.0605	0.0724 ± 0.0125
28	0.1151	0.0501	0.0470	0.0707 ± 0.0384
26	0.0640	0.0314	0.0465	0.0473 ± 0.0163
24	0.0610	0.0340	0.0360	0.0437 ± 0.0150
22	0.0480	0.0210	0.0360	0.0350 ± 0.0135
20	0.0350	0.0238	0.0202	0.0263 ± 0.0077
18	0.0210	0.0167	0.0145	0.0174 ± 0.0033
16	0.0150	0.0094	0.0220	0.0155 ± 0.0063
14	0.0180	0.0083	0.0160	0.0141 ± 0.0051
12	0.0100	0.0051	0.0078	0.0076 ± 0.0025
10	0.0088	0.0040	0.0090	0.0073 ± 0.0028
8	0.0025	0.0026	0.0042	0.0031 ± 0.0010
6	0.0049	0.0032	0.0077	0.0053 ± 0.0023
4	0.0015	0.0015	0.0013	0.0014 ± 0.0001

APPENDIX Table 5. *In vitro* metabolic rates (μM O_2/mg protein/hr) of heart homogenates from *T. sirtalis parietalis* acclimated to 4°C, 12L12D during the summer.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.1310	0.1000	0.1620	0.1310 ± 0.0310
32	0.0750	0.0920	0.1412	0.1030 ± 0.0343
30	0.0830	0.0980	0.1430	0.1080 ± 0.0312
28	0.0644	0.0620	0.1100	0.0788 ± 0.0270
26	0.0560	0.0550	0.0941	0.0683 ± 0.0222
24	0.0520	0.0530	0.0780	0.0610 ± 0.0147
22	0.0380	0.0515	0.0510	0.0468 ± 0.0077
20	0.0210	0.0400	0.0475	0.0362 ± 0.0137
18	0.0200	0.0330	0.0325	0.0285 ± 0.0074
16	0.0170	0.0260	0.0230	0.0220 ± 0.0046
14	0.0150	0.0130	0.0185	0.0155 ± 0.0028
12	0.0091	0.0078	0.0137	0.0102 ± 0.0031
10	0.0065	0.0078	0.0116	0.0086 ± 0.0027
8	0.0046	0.0078	0.0085	0.0068 ± 0.0020
6	0.0033	0.0051	0.0047	0.0044 ± 0.0010
4	0.0028	0.0017	0.0034	0.0026 ± 0.0008

APPENDIX Table 6. *In vitro* metabolic rates (μM O_2/mg protein/hr) of heart homogenates from *T. sirtalis parietalis* acclimated to 4°C, 0L24D during the summer.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.1500	0.0810	0.0885	0.1070 ± 0.0379
32	0.0790	0.0765	0.0600	0.0718 ± 0.0103
30	0.0655	0.0550	0.0560	0.0588 ± 0.0058
28	0.0600	0.0575	0.0322	0.0498 ± 0.0155
26	0.0600	0.0700	0.0426	0.0575 ± 0.0139
24	0.0485	0.0620	0.0370	0.0492 ± 0.0125
22	0.0380	0.0370	0.0173	0.0308 ± 0.0117
20	0.0400	0.0440	0.0173	0.0338 ± 0.0144
18	0.0350	0.0202	0.0168	0.0239 ± 0.0097
16	0.0210	0.0180	0.0128	0.0173 ± 0.0042
14	0.0162	0.0132	0.0080	0.0125 ± 0.0042
12	0.0085	0.0084	0.0080	0.0083 ± 0.0003
10	0.0096	0.0071	0.0063	0.0077 ± 0.0017
8	0.0065	0.0060	0.0037	0.0054 ± 0.0015
6	0.0055	0.0043	0.0028	0.0042 ± 0.0014
4	0.0024	0.0042	0.0026	0.0031 ± 0.0010

APPENDIX Table 7. *In vitro* metabolic rates (μM O_2/mg protein/hr) of liver homogenates from *T. sirtalis parietalis* acclimated to 26°C, 12L12D during the winter.

Temperature (°C)	Metabolic Rates				$\bar{x}$ ± S.D.
34	0.0669	0.0604	0.0649	0.0485	0.0602 ± 0.0082
32	0.0449	0.0460	0.0351	0.0257	0.0380 ± 0.0097
30	0.0410	0.0504	0.0365	0.0168	0.0362 ± 0.0142
28	0.0351	0.0357	0.0289	0.0248	0.0311 ± 0.0052
26	0.0334	0.0333	0.0216	0.0234	0.0279 ± 0.0063
24	0.0291	0.0358	0.0196	0.0214	0.0265 ± 0.0075
22	0.0294	0.0346	0.0111	0.0160	0.0228 ± 0.0110
20	0.0230	0.0280	0.0114	0.0153	0.0194 ± 0.0075
18	0.0398	0.0182	0.0150	0.0197	0.0232 ± 0.0113
16	0.0332	0.0152	0.0133	0.0118	0.0184 ± 0.0100
14	0.0218	0.0092	0.0074	0.0105	0.0122 ± 0.0065
12	0.0149	0.0058	0.0051	0.0056	0.0078 ± 0.0047
10	0.0083	0.0045	0.0045	0.0044	0.0054 ± 0.0019
8	0.0068	0.0025	0.0025	0.0036	0.0038 ± 0.0020
6	0.0039	0.0017	0.0022	0.0013	0.0023 ± 0.0012
4	0.0027	0.0012	0.0016	0.0007	0.0016 ± 0.0009

APPENDIX Table 8. *In vitro* metabolic rates (μM O_2/mg protein/hr) of liver homogenates from *T. sirtalis parietalis* acclimated to 4°C, 12L12D during the winter.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.0755	0.0840	0.0830	0.0808 ± 0.0047
32	0.086	0.0570	0.0661	0.0697 ± 0.0148
30	0.0696	0.0470	0.0516	0.0559 ± 0.0121
28	0.0404	0.0375	0.0302	0.0360 ± 0.0053
26	0.0320	0.0380	0.0234	0.03110± 0.0073
24	0.0305	0.0261	0.0192	0.0253 ± 0.0057
22	0.0197	0.0252	0.0132	0.0194 ± 0.0060
20	0.0156	0.0123	0.0092	0.0124 ± 0.0032
18	0.0078	0.0108	0.0076	0.0087 ± 0.0018
16	0.0068	0.0060	0.0051	0.0059 ± 0.0009
14	0.0059	0.0064	0.0035	0.0053 ± 0.0016
12	0.0028	0.0031	0.0028	0.0029 ± 0.0002
10	0.0022	0.0021	0.0025	0.0023 ± 0.0002
8	0.0014	0.0017	0.0014	0.0015 ± 0.0002
6	0.0010	0.0012	0.0006	0.0092 ± 0.0003
4	0.0007	0.0005	0.0003	0.0005 ± 0.0002

APPENDIX Table 9. *In vitro* metabolic rates (μM O_2/mg protein/hr) of liver homogenates from *T. sirtalis parietalis* acclimated to 4°C, 0L24D during the winter.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.0680	0.0781	0.0634	0.0698 ± 0.0075
32	0.0602	0.0744	0.0572	0.0639 ± 0.0092
30	0.0461	0.0534	0.0493	0.0496 ± 0.0037
28	0.0364	0.0370	0.0362	0.0365 ± 0.0004
26	0.0244	0.0414	0.0268	0.0309 ± 0.0092
24	0.0222	0.0311	0.0192	0.0242 ± 0.0062
22	0.0172	0.0229	0.0201	0.0201 ± 0.0029
20	0.0119	0.0197	0.0162	0.0159 ± 0.0039
18	0.0072	0.0140	0.0099	0.0104 ± 0.0034
16	0.0047	0.0095	0.0091	0.0077 ± 0.0027
14	0.0035	0.0072	0.0048	0.0052 ± 0.0019
12	0.0027	0.0048	0.0044	0.0040 ± 0.0011
10	0.0022	0.0044	0.0027	0.0031 ± 0.0012
8	0.0019	0.0042	0.0016	0.0026 ± 010014
6	0.0012	0.0014	0.0013	0.0013 ± 0.0001
4	0.0009	0.0013	0.0010	0.0010 ± 0.0002

APPENDIX Table 10. *In vitro* metabolic rates (μM O_2/mg protein/hr) of liver homogenates from *T. sirtalis parietalis* acclimated to 26°C, 12L12D during the summer.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.0985	0.0964	0.0962	0.0970 ± 0.0012
32	0.0640	0.0508	0.055	0.0566 ± 0.0067
30	0.0495	0.042	0.047	0.0462 ± 0.0038
28	0.0394	0.0314	0.0295	0.0334 ± 0.0053
26	0.0220	0.0307	0.0235	0.0254 ± 0.0047
24	0.0180	0.0283	0.022	0.0228 ± 0.0052
22	0.0141	0.0182	0.0174	0.0167 ± 0.0022
20	0.0150	0.0108	0.0142	0.0133 ± 0.0022
18	0.0083	0.0087	0.0081	0.0084 ± 0.0003
16	0.0055	0.0063	0.0057	0.0059 ± 0.0004
14	0.0047	0.0056	0.0057	0.0054 ± 0.0006
12	0.0030	0.0030	0.0033	0.0031 ± 0.0002
10	0.0028	0.0030	0.0027	0.0029 ± 0.0001
8	0.0022	0.0021	0.0021	0.0022 ± 0.0001
6	0.0013	0.0016	0.0017	0.0016 ± 0.0002
4	0.0011	0.0010	0.0010	0.0011 ± 0.0001

APPENDIX Table 11. *In vitro* metabolic rates (μM O_2/mg protein/hr) of liver homogenates from *T. sirtalis parietalis* acclimated to 4°C, 12L12D during the summer.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.0639	0.0540	0.0945	0.0708 ± 0.0211
32	0.0347	0.0380	0.0722	0.0483 ± 0.0208
30	0.0314	0.0328	0.0590	0.0411 ± 0.0156
28	0.0190	0.0252	0.0476	0.0306 ± 0.0151
26	0.0188	0.0234	0.0396	0.0273 ± 0.0109
24	0.0171	0.0182	0.0272	0.0210 ± 0.0055
22	0.0113	0.0115	0.0275	0.0168 ± 0.0093
20	0.0082	0.0110	0.0233	0.0142 ± 0.0080
18	0.0072	0.0074	0.0159	0.0102 ± 0.0050
16	0.0045	0.0060	0.0115	0.0073 ± 0.0037
14	0.0032	0.0049	0.0085	0.0055 ± 0.0027
12	0.0023	0.0027	0.0063	0.0038 ± 0.0022
10	0.0017	0.0021	0.0046	0.0028 ± 0.0016
8	0.0011	0.0019	0.0030	0.0020 ± 0.0010
6	0.0010	0.0011	0.0020	0.0014 ± 0.0006
4	0.0007	0.0009	0.0011	0.0009 ± 0.0002

APPENDIX Table 12. *In vitro* metabolic rates (μM O_2/mg protein/hr) of liver homogenates from *T. sirtalis parietalis* acclimated to 4°C, 0L24D during the summer.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.1280	0.1370	0.1510	0.1390 ± 0.0116
32	0.0905	0.0860	0.0872	0.0879 ± 0.0023
30	0.0473	0.0580	0.0555	0.0536 ± 0.0056
28	0.0314	0.0541	0.0560	0.0472 ± 0.0137
26	0.0309	0.0500	0.0415	0.0408 ± 0.0096
24	0.0192	0.0221	0.0311	0.0241 ± 0.0062
22	0.0161	0.0254	0.0272	0.0229 ± 0.0060
20	0.0125	0.0198	0.0254	0.0192 ± 0.0065
18	0.0062	0.0140	0.0193	0.0132 ± 0.0066
16	0.0073	0.0125	0.0125	0.0108 ± 0.0030
14	0.0057	0.0053	0.0087	0.0066 ± 0.0019
12	0.0033	0.0049	0.0075	0.0052 ± 0.0021
10	0.0030	0.0033	0.0044	0.0035 ± 0.0007
8	0.0024	0.0023	0.0035	0.0027 ± 0.0007
6	0.0018	0.0018	0.0034	0.0023 ± 0.0009
4	0.0024	0.0019	0.0026	0.0023 ± 0.0004

APPENDIX Table 13. *In vitro* metabolic rates (μM O_2/mg protein/hr) of muscle homogenates from *T. sirtalis parietalis* acclimated to 26°C, 12L12D during the winter.

Temperature (°C)	Metabolic Rates				$\bar{x}$ ± S.D.
34	0.4800	0.1840	0.1990	0.1560	0.2550 ± 0.1510
32	0.2080	0.1640	0.1380	0.1190	0.1570 ± 0.0385
30	0.2200	0.1650	0.1340	0.1160	0.1590 ± 0.0456
28	0.1510	0.1390	0.0700	0.0540	0.1040 ± 0.0486
26	0.1310	0.1120	0.0580	0.0495	0.0876 ± 0.0400
24	0.0920	0.0710	0.0388	0.0425	0.0611 ± 0.0251
22	0.0930	0.0690	0.0490	0.0360	0.0618 ± 0.0249
20	0.0515	0.0478	0.0297	0.0284	0.0394 ± 0.0120
18	0.0420	0.0390	0.0282	0.0230	0.0331 ± 0.0089
16	0.0425	0.0278	0.0225	0.0151	0.0270 ± 0.0116
14	0.0225	0.0250	0.0102	0.0114	0.0173 ± 0.0076
12	0.0120	0.0140	0.0062	0.0051	0.0093 ± 0.0044
10	0.0131	0.0142	0.0060	0.0046	0.0095 ± 0.0049
8	0.0107	0.0074	0.0051	0.0031	0.0066 ± 0.0033
6	0.0071	0.0056	0.0017	0.0014	0.0040 ± 0.0028
4	0.0055	0.0028	0.0006	0.0009	0.0025 ± 0.0022

APPENDIX Table 14. *In vitro* metabolic rates (μM O_2/mg protein/hr) of muscle homogenates from *T. sirtalis parietalis* acclimated to 4°C, 12L12D during the winter.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.1410	0.1390	0.0810	0.1200 ± 0.0341
32	0.0992	0.0874	0.0859	0.0908 ± 0.0073
30	0.1140	0.0815	0.0716	0.0890 ± 0.0222
28	0.0865	0.0780	0.0743	0.0796 ± 0.0063
26	0.0870	0.0540	0.0570	0.0660 ± 0.0183
24	0.0870	0.0500	0.0390	0.0587 ± 0.0252
22	0.0445	0.0392	0.0225	0.0354 ± 0.0115
20	0.0410	0.0210	0.0283	0.0301 ± 0.0101
18	0.0340	0.0300	0.0107	0.0249 ± 0.0125
16	0.0260	0.0200	0.0089	0.0183 ± 0.0087
14	0.0220	0.0131	0.0106	0.0152 ± 0.0060
12	0.0083	0.0079	0.0069	0.0077 ± 0.0007
10	0.0115	0.0068	0.0056	0.0080 ± 0.0031
8	0.0068	0.0082	0.0045	0.0065 ± 0.0019
6	0.0040	0.0031	0.0048	0.0040 ± 0.0009
4	0.0027	0.0024	0.0021	0.0024 ± 0.0003

APPENDIX Table 15. In vitro metabolic rates (μM O_2/mg protein/hr) of muscle homogenates from T. sirtalis parietalis acclimated to 4°C, 0L24D during the winter.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.2340	0.1830	0.1800	0.1990 ± 0.0303
32	0.1670	0.1240	0.1172	0.1360 ± 0.0271
30	0.1540	0.1061	0.0826	0.1140 ± 0.0364
28	0.1500	0.1090	0.1088	0.1230 ± 0.0237
26	0.1310	0.0640	0.0500	0.0817 ± 0.0433
24	0.1540	0.0547	0.0568	0.0885 ± 0.0749
22	0.1500	0.0766	0.0695	0.0987 ± 0.0446
20	0.1170	0.0275	0.0209	0.0551 ± 0.0537
18	0.0582	0.0210	0.0181	0.0324 ± 0.0224
16	0.0407	0.0146	0.0149	0.0234 ± 0.0150
14	0.0430	0.0131	0.0149	0.0237 ± 0.0168
12	0.0320	0.0091	0.0065	0.0159 ± 0.0140
10	0.0099	0.0064	0.0041	0.0068 ± 0.0029
8	0.0052	0.0044	0.0033	0.0043 ± 0.0010
6	0.0081	0.0035	0.0023	0.0046 ± 0.0031
4	0.0055	0.0014	0.0012	0.0027 ± 0.0024

APPENDIX Table 16. *In vitro* metabolic rates (μM O_2/mg protein/hr) of muscle homogenates from *T. sirtalis parietalis* acclimated to 26°C, 12L12D during the summer.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.0643	0.0423	0.0348	0.0471 ± 0.0153
32	0.0506	0.0358	0.0308	0.0391 ± 0.0103
30	0.0345	0.0246	0.0247	0.0279 ± 0.0057
28	0.0329	0.0206	0.0190	0.0242 ± 0.0076
26	0.0314	0.0173	0.0130	0.0206 ± 0.0096
24	0.0180	0.0093	0.0090	0.0121 ± 0.0051
22	0.0150	0.0061	0.0071	0.0094 ± 0.0049
20	0.0110	0.0062	0.0044	0.0072 ± 0.0034
18	0.0127	0.0058	0.0048	0.0078 ± 0.0043
16	0.0092	0.0054	0.0029	0.0058 ± 0.0032
14	0.0083	0.0029	0.0024	0.0045 ± 0.0033
12	0.0048	0.0017	0.0031	0.0032 ± 0.0015
10	0.0038	0.0014	0.0023	0.0025 ± 0.0012
8	0.0032	0.0020	0.0016	0.0023 ± 0.0009
6	0.0012	0.0014	0.0012	0.0013 ± 0.0001
4	0.0006	0.0010	0.0020	0.0012 ± 0.0008

APPENDIX Table 17. *In vitro* metabolic rates (μM O_2/mg protein/hr) of muscle homogenates from *T. sirtalis parietalis* acclimated to 4°C, 12L12D during the summer.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.1270	0.1160	0.0700	0.1040 ± 0.0302
32	0.0900	0.0844	0.0680	0.0808 ± 0.0114
30	0.0803	0.0640	0.0512	0.0652 ± 0.0146
28	0.0554	0.0632	0.0350	0.0512 ± 0.0147
26	0.0582	0.0500	0.0312	0.0465 ± 0.0138
24	0.0452	0.0365	0.0286	0.0368 ± 0.0083
22	0.0500	0.0364	0.0281	0.0382 ± 0.0111
20	0.0423	0.0211	0.0116	0.0250 ± 0.0157
18	0.0280	0.0250	0.0103	0.0211 ± 0.0095
16	0.0130	0.0140	0.0071	0.0114 ± 0.0037
14	0.0092	0.0089	0.0062	0.0081 ± 0.0017
12	0.0090	0.0063	0.0051	0.0068 ± 0.0020
10	0.0076	0.0041	0.0032	0.0050 ± 0.0023
8	0.0077	0.0039	0.0026	0.0047 ± 0.0027
6	0.0038	0.0033	0.0022	0.0031 ± 0.0008
4	0.0033	0.0015	0.0016	0.0021 ± 0.0010

APPENDIX Table 18. *In vitro* metabolic rates (μM O_2/mg protein/hr) of muscle homogenates from *T. sirtalis parietalis* acclimated to 4°C, 0L24D during the summer.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.0489	0.0480	0.0502	0.0490 ± 0.0011
32	0.0437	0.0426	0.0441	0.0435 ± 0.0008
30	0.0326	0.0369	0.0354	0.0350 ± 0.0022
28	0.0307	0.0341	0.0330	0.0326 ± 0.0017
26	0.0250	0.0299	0.0275	0.0275 ± 0.0025
24	0.0173	0.0190	0.0184	0.0182 ± 0.0009
22	0.0187	0.0190	0.0181	0.0186 ± 0.0005
20	0.0104	0.0071	0.0088	0.0088 ± 0.0017
18	0.0075	0.0077	0.0073	0.0075 ± 0.0011
16	0.0090	0.0087	0.0085	0.0087 ± 0.0002
14	0.0045	0.0043	0.0048	0.0045 ± 0.0011
12	0.0045	0.0045	0.0046	0.0045 ± 0.0005
10	0.0065	0.0041	0.0056	0.0054 ± 0.0012
8	0.0046	0.0036	0.0039	0.0040 ± 0.0005
6	-	-	-	- -
4	0.0030	0.0020	0.0022	0.0024 ± 0.0005

APPENDIX Table 19. In vitro metabolic rates (μM O_2/mg protein/hr) of liver homogenates from T. sirtalis concinnus acclimated to 26°C, 12L12D, during the summer.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.1820	0.2700	0.1910	0.2140 ± 0.0484
32	0.1700	0.1532	0.1870	0.1700 ± 0.0170
30	0.1233	0.1330	0.1450	0.1341 ± 0.0110
28	0.0850	0.0840	0.0924	0.0871 ± 0.0046
26	0.0573	0.0752	0.0913	0.0746 ± 0.0170
24	0.0550	0.0643	0.0754	0.0649 ± 0.0102
22	0.0631	0.0591	0.0670	0.0631 ± 0.0040
20	0.0513	0.0337	0.0448	0.0433 ± 0.0089
18	0.0244	0.0430	0.0274	0.0316 ± 0.0100
16	0.0215	0.0275	0.0195	0.0228 ± 0.0042
14	0.0146	0.0163	0.0118	0.0142 ± 0.0023
12	0.0119	0.0163	0.0118	0.0133 ± 0.0003
10	0.0068	0.0115	0.0071	0.0085 ± 0.0026
8	0.0073	0.0096	0.0066	0.0078 ± 0.0016
6	0.0041	0.0068	0.0033	0.0047 ± 0.0018
4	0.0028	0.0026	0.0023	0.0026 ± 0.0003

APPENDIX Table 20. *In vitro* metabolic rates (μM O_2/mg protein/hr) of muscle homogenates from *T. sirtalis parietalis* acclimated to 26°C, 12L12D during the summer.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.1350	0.1900	0.1880	0.1710 ± 0.0312
32	0.1542	0.1700	0.1421	0.1550 ± 0.0141
30	0.1030	0.1320	0.1090	0.1150 ± 0.0153
28	0.0885	0.0980	0.0870	0.0912 ± 0.0060
26	0.0850	0.0960	0.0980	0.0930 ± 0.0070
24	0.0724	0.1040	0.0872	0.0878 ± 0.0158
22	0.0476	0.0564	0.0533	0.0524 ± 0.0045
20	0.0397	0.0490	0.0444	0.0444 ± 0.0047
18	0.0264	0.0306	0.0276	0.0282 ± 0.0022
16	0.0125	0.0363	0.0240	0.0243 ± 0.0119
14	0.0096	0.0218	0.0150	0.0155 ± 0.0061
12	0.0130	0.0140	0.0118	0.0129 ± 0.0011
10	0.0046	0.0083	0.0076	0.0068 ± 0.0020
8	0.0071	0.0066	0.0051	0.0063 ± 0.0010
6	0.0029	0.0044	0.0034	0.0036 ± 0.0008
4	0.0026	0.0039	0.0020	0.0028 ± 0.0010

APPENDIX Table 21. *In vitro* metabolic rates (μM O_2/mg protein/hr) of liver homogenates from *T. sirtalis concinnus* acclimated to 10°C, 12L12D during the summer.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.1390	0.1570	0.1760	0.1570 ± 0.0185
32	0.1230	0.1200	0.1530	0.1320 ± 0.0183
30	0.0875	0.0864	0.0790	0.0843 ± 0.0046
28	0.0717	0.0601	0.0620	0.0646 ± 0.0062
26	0.0544	0.0605	0.0516	0.0555 ± 0.0046
24	0.0455	0.0440	0.0278	0.0391 ± 0.0098
22	0.0320	0.0320	0.0316	0.0319 ± 0.0002
20	0.0270	0.0260	0.0240	0.0257 ± 0.0015
18	0.0164	0.0190	0.0185	0.0180 ± 0.0014
16	0.0108	0.0138	0.0013	0.0125 ± 0.0015
14	0.0078	0.0138	0.0012	0.0111 ± 0.0030
12	0.0064	0.0127	0.0082	0.0091 ± 0.0033
10	0.0036	0.0066	0.0040	0.0047 ± 0.0017
8	0.0038	0.0052	0.0054	0.0048 ± 0.0009
6	0.0020	0.0017	0.0021	0.0019 ± 0.0002
4	0.0011	0.0015	0.0016	0.0014 ± 0.0002

APPENDIX Table 22. *In vitro* metabolic rates (μM O_2/mg protein/hr) of muscle homogenates from *T. sirtalis concinnus* acclimated to 10°C, 12L12D during the summer.

Temperature (°C)	Metabolic Rates			$\bar{x}$ ± S.D.
34	0.0950	0.1760	0.1250	0.1320 ± 0.0410
32	0.0950	0.1050	0.0850	0.0950 ± 0.0100
30	0.0755	0.0864	0.0820	0.0813 ± 0.0055
28	0.0730	0.0755	0.0535	0.0673 ± 0.0121
26	0.0460	0.0670	0.0481	0.0537 ± 0.0116
24	0.0410	0.0460	0.0430	0.0433 ± 0.0025
22	0.0324	0.0275	0.0330	0.0310 ± 0.0030
20	0.0195	0.0240	0.0240	0.0225 ± 0.0026
18	0.0130	0.0155	0.0220	0.0168 ± 0.0047
16	0.0095	0.0106	0.0155	0.0119 ± 0.0032
14	0.0079	0.0067	0.0126	0.0091 ± 0.0031
12	0.0067	0.0056	0.0072	0.0065 ± 0.0008
10	0.0040	0.0053	0.0051	0.0048 ± 0.0007
8	0.0030	0.0028	0.0028	0.0029 ± 0.0001
6	-	-	-	- -
4	0.0014	0.0016	0.0016	0.0015 ± 0.0001