

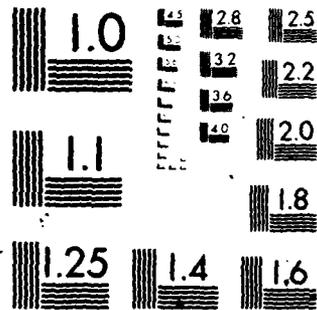
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**PHYSIOLOGICAL AND HEMATOLOGICAL RESPONSES TO SUMMER
AND WINTER DRY-HEAT ACCLIMATION**

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Running Head: Summer and winter acclimation to dry heat

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Abstract

Differences between acclimation to heat at the end of winter (W) and at the end of summer (S) were studied on the same 8 male volunteers. Subjects were exposed to 40°C , 30% rh for 10 days on two separate occasions approximately 5 months apart (S and W). Daily exposures lasted 120 min: 10 min rest, 50 min walking $1.34 \text{ m} \cdot \text{s}^{-1}$ on the level, 10 min rest, 50 min walking. During W acclimation, rectal temperature (T_{re}^{r}) and heart rate (HR) decreased, sweat rate (m_{sw}^{r}) remained unchanged, and plasma and red cell volume of the blood expanded. During S acclimation, HR decreased while T_{re}^{r} and m_{sw}^{r} remained unchanged, and plasma volume increased. The T_{re}^{r} of the acclimated subjects remained higher in W and m_{sw}^{r} lower than in S. It was concluded that acclimation does not totally eliminate the seasonal differences in thermoregulatory set-point and sweat sensitivity. Further, acclimation to a more severe heat did not improve the thermoregulatory set-point that was achieved by natural acclimatization to a milder heat, but affected the cardiovascular adjustment and caused greater plasma volume expansion. W acclimation caused both plasma and blood cell volume expansion while S acclimation affected only plasma volume. ✕

Index terms: acclimatization; blood volume; plasma volume; seasonal acclimation differences; rectal temperature; heart rate; sweat rate; plasma protein; plasma osmolality; hematocrit; mean corpuscular hemoglobin concentration; hemodilution; thermoregulatory set-point; sweat sensitivity.

INTRODUCTION

Acclimatization is defined as: "a physiological change occurring within the lifetime of an organism which reduces the strain caused by stressful changes in the natural climate", while acclimation is defined as: "a physiological change occurring within the lifetime of an organism, which reduces the strain caused by experimentally induced stressful changes in particular climatic factors" (3). As one can see, the difference between the two definitions involves whether the changes are induced by natural or artificial climatic stimuli. In both acclimation and acclimatization, the major physiological changes are: a heightened sweating response, lowered heart rate and lowered internal body temperature during exercise in the heat (17, 19, 27, 28). Both acclimatization and acclimation therefore reduce the risk of heat injury (15). The main physiological mechanism of these two processes is an enhanced sweating responsiveness, via both peripheral and central pathways (17).

In addition to the classical physiological indices of acclimatization and acclimation as mentioned above, several recent studies have shown dynamic changes occurring in body fluid distribution with heat acclimation. Foremost of these changes is a rapid expansion of the plasma volume found to occur in the early days of heat acclimation (2, 25, 28). In that an expansion of the plasma volume is also typical with exercise, this hemodilution is found to be more enhanced and stabilized during acclimation induced by exercise in the heat (4, 20, 22, 24, 25). Several hypotheses exist as to the physiological mechanism(s) responsible for this hemodilution during acclimation. Senay attributes the expansion of the plasma volume to an influx of protein into the vascular volume (20), although others attribute it to an electrolyte shift (12). It has also been shown that if dehydration is allowed to occur during exercise in the heat, this

expansion of the plasma volume would not occur (7), unless the subjects were rehydrated following the exposure (6). Senay has also noted that a lack of plasma volume expansion is related to heat intolerance (23). Although the mechanisms involved remain speculative, it appears that an expansion of the plasma volume is a crucial hemodynamic change associated with cardiovascular adjustment to heat.

There are very few studies comparing the natural acclimatization and the artificial acclimation processes, with the majority of these studies suggesting that natural acclimatization is preferred over the artificial process (11, 13, 14, 31). Wyndham et al. (31), however, have made the assumption that during artificial acclimation, a higher level of adaptation to heat can be achieved than during natural acclimatization. All the above studies have dealt with different groups for the acclimation and the acclimatization experiments, and moreover, the acclimation usually took place in one country and the acclimatization in a foreign country. None of the above studies describe or compare the hemodynamic changes induced by acclimation in different seasons when the subjects are non-acclimatized (winter) or partially acclimatized (summer).

The purpose of this study was to compare both the physiological and hemodynamic acclimation responses of the same subjects to the same hot-dry environment, once in a state of the lowest natural acclimatization (end of the winter), and once in the highest state of natural acclimatization (end of the summer).

METHODS

Subjects.

Eight male soldiers served as volunteer subjects. All subjects were totally informed with regard to experimental risk and gave their written informed

consent. The physical characteristics of the subjects (mean \pm SE) were: age, 22.5 ± 2.0 yr; height, 175.4 ± 2.9 cm; weight, 68.6 ± 2.8 kg; body surface area, 1.83 ± 0.05 m²; and percentage of body fat, $17.0 \pm 1.6\%$.

Procedures.

The first phase of the study, the end of the summer acclimation (S), was conducted early in September and the second phase, the end of the winter acclimation (W), was conducted during the end of February and the beginning of March of the following year. The same subjects were used for both phases of the study. Prior to the heat exposures, all subjects underwent medical examination to determine their fitness for the study. The subjects, dressed in T-shirts, shorts, socks and indoor shoes, were concurrently acclimated for 10 consecutive days ($A_1 - A_{10}$) during both phases (S and W) by walking on a level motor-driven treadmill at 1.34 m \cdot s⁻¹ for two 50-min periods with a preceding and intervening 10-min rest period, at 40°C, 30% rh, 1 m \cdot s⁻¹ wind speed. A day before and a day after this acclimation period, the subjects were exposed to a comfortable environment of 20°C, 50% rh (control days C_1 & C_2) at the same treadmill walking speed and wind velocity.

During all heat exposures, rectal temperature (T_{re}) was recorded from a Y.S.I. rectal thermistor probe inserted ~ 10 cm beyond the anal sphincter. Skin temperatures were monitored with a three-point thermocouple skin harness (chest, calf and forearm) and mean weighted skin temperature (T_{sk}) was calculated according to Burton (5). Using a Hewlett Packard 9825A Calculator and 9862A Plotter on-line during experimentation, both T_{sk} and T_{re} were plotted for each subject at approximately 2-min intervals. Heart rate was measured by radial artery palpation during the rest periods and after each 25 min of walking. Ad lib drinking was encouraged. At the end of the first rest period and at the

end of each walking period, two-min expired air samples were collected in Douglas bags. The volume was measured in a Collins Spirometer and converted to standard environmental conditions (STPD), and the O₂ and CO₂ concentrations were measured with an Applied Electrochemistry Model S-3A O₂ analyzer and Beckman LB-2 infrared CO₂ analyzer. A time weighted average metabolic rate (M) was calculated as 0.17 of the resting value plus 0.83 of the mean of the two level walking values. Total body weight losses were determined from pre- and post-walk measurements on a K-120 Sauter precision electronic balance (accuracy of ± 10 g) for calculation of sweat rate. Sweat rate (\dot{m}_{sw}) was determined from weight loss, adjusted for water intake, urine output, and respiratory and metabolic weight losses. The metabolic weight loss (\dot{m}_r) and the respiratory water loss (\dot{m}_e) were calculated according to Mitchell et al. (16) as: $\dot{m}_r = 0.53 \dot{V}O_2$ in g \cdot min⁻¹ and $\dot{m}_e = 0.019 \dot{V}O_2 (44 - P_a)$ in g \cdot min⁻¹, where P_a is the ambient water vapor pressure (mm Hg) and $\dot{V}O_2$ is the O₂ consumption (l \cdot min⁻¹). The net sweat rate was normalized per m² surface area.

Criteria for terminating any heat exposure were a heart rate of 180 b \cdot min⁻¹ during exercise or 140 b \cdot min⁻¹ during rest, and/or a T_{re} above 39.5°C, dizziness, nausea, or dry skin.

Blood Analysis.

Venous blood samples (8-10 ml each) were drawn from each subject during both control days and on the 1st, 2nd, 3rd, 4th, 6th, 8th and 10th days of acclimation. Two samples were taken on these days: one several min before entering the climatic chamber (pre-exposure), and the other between min 100 and 110 of exposure (post-exposure). The pre-exposure sample was taken after 20 min of resting in the erect position and the post-exposure sample was taken within one min of stepping off the treadmill. All blood samples were taken without stasis, using pre-heparinized Vacutainers.

The blood samples were analyzed for: hematocrit (HCT) in triplicate by the microhematocrit method (reported uncorrected); hemoglobin (Hb) in duplicate by the cyanmethemoglobin method; total protein (TP) in duplicate by an American Optical Refractometer; albumin (ALB) by the bromcresol green method (10), using a Gilford Automated 3400 spectrophotometer; and osmolality by a Precision System automatic osmometer. Plasma volume changes were calculated using the Dill and Costill method (9).

Assuming that the total amount of hemoglobin remained constant during each period of the study, changes in blood volume were calculated on each acclimation day when blood was drawn from the changes in hemoglobin concentration as:

$$\Delta BV_{C-X} (\%) = 100(Hb_C - Hb_X)/Hb_C, \text{ where}$$

Hb_C represents the pre-exposure hemoglobin concentration of the first acclimation day (A_1), Hb_X the hemoglobin concentration of the measured day, and ΔBV_{C-X} the blood volume change between the pre-exposure value of the first acclimation day and the particular measured day. Changes in the volume of the cellular phase of the blood were calculated by multiplying the blood volume changes for the measured day by the hematocrit. Another method (28) of estimating the changes occurring in the volume of the cellular phase of the blood was employed by calculating the mean corpuscular hemoglobin concentration (MCHC).

Statistical Treatment.

Most variables were evaluated by use of a mixed design of several factors, with one factor being the two phases (summer and winter), the second being the day of the acclimation, and the third the time of the day (pre- and post-exposure). If a significant F-value was found ($P < 0.05$), critical differences were analyzed by Tukey's procedure to locate the significant mean differences.

RESULTS

Physiological Parameters.

The metabolic rate varied between 164 and 174 W · m⁻² throughout both phases of the acclimation without any significant differences between the summer and winter experiments, or between the days of acclimation of each season.

INSERT FIGURE 1 ABOUT HERE

INSERT TABLE 1 ABOUT HERE

The rectal temperature (T_{re}) remained unchanged through the 10 days of acclimation at the end of summer, whereas during the winter acclimation, a moderate decrease in T_{re} was observed (average of 0.22°C). However, this difference was not significant. On the other hand, the winter T_{re} was consistently and significantly ($P < 0.01$) higher (0.15 - 0.35°C) than that which was found during the summer experiments (see Fig. 1 and Table 1). The changes observed in the mean weighted skin temperature (T_{sk}) were similar between the two phases, with a significant decrease ($P < 0.05$) in T_{sk} found between the first and the last days of acclimation in both phases (see Table 1). Heart rate (HR) significantly decreased ($P < 0.05$) from the first to the sixth day of acclimation, both in S (15.3 b · min⁻¹) and W (18.2 b · min⁻¹). During the later days of the acclimation, HR remained unchanged (Fig. 1, Table 1). Between the seasons, a significant difference in HR was found only on the first day of acclimation (9.2 b · min⁻¹ higher in W). Sweat rate (\dot{m}_{sw}) remained unchanged during acclimation

in both phases. However, the summer \dot{m}_{sw} was significantly higher (5-14%) than the winter \dot{m}_{sw} during each day of exposure to heat (see Fig. 1, Table 1). No significant differences were found in any of the above parameters between the two control days.

During the summer experiments, the subjects' mean weight varied between 68.2 ± 3.2 kg (values that were observed on C₁, A₁, A₂ and C₂) and 69.1 ± 3.2 kg (on the sixth day of acclimation). During the winter exposures the mean body weight ranged between 69.8 ± 3.3 kg (on C₁) and 70.5 ± 3.5 kg (on A₆).

The level of dehydration during the summer exposures (difference between initial and final weight) varied between 0.2% (on A₂) and 0.8% (on A₆) of the total body weight. During the winter experiment, the values ranged between 0.0% (on A₉) and 0.8% (on A₈).

Hematological Parameters.

The initial values (pre-exposure values for the 1st control day) of hemoglobin (Hb), albumin (ALB), total protein (TP) and MCHC were similar for both phases ($P > 0.05$). However, the hematocrit (HCT) was found to be significantly lower ($P < 0.05$) for W, although the absolute difference (0.7%) was very small (see Table 2).

INSERT FIGURES 2, 3, and TABLE 2 ABOUT HERE

The hemoglobin (see Fig. 2, Table 2) showed a similar pattern in both phases (W and S), as it decreased significantly ($P < 0.05$) to its minimal value on the sixth day of acclimation, and then increased significantly ($P < 0.05$) by A₁₀. The winter values for hemoglobin were lower, but not significantly lower ($P > 0.05$) as compared to the summer values. In both acclimation phases, the daily pre-

exposure levels of Hb were significantly higher ($P < 0.05$) than at the end of the exposure, indicative of hemodilution.

The hematocrit (see Table 2) showed a similar pattern to that of Hb during the summer exposures (decreasing in the first half of the acclimation period and then increasing). However, in the winter exposures the changes were not significant. In both phases of these experiments, the pre-exposure values were significantly higher than the post-exposure values (indicating plasma volume expansion). While the summer hematocrit values for the first and last days of acclimation were significantly higher in comparison to the winter, no significant difference was found on A_G between S and W (see Table 2).

The MCHC, which is a combination of the two previous parameters (Hb and HCT) showed a pattern consistent with a hemodilution process: significantly higher post-exposure than pre-exposure values; significantly lower values on A_G for both S and W; significantly higher S values than W at the middle of the acclimation period, but significantly higher W than S values at the beginning and at the end of the acclimation (Fig. 3, Table 2).

INSERT FIGURE 4 ABOUT HERE

In each season, the highest blood volume value was found on the sixth day of acclimation, and the lowest on the first day. No significant differences were found between S and W values for this parameter. In both seasons the post-exposure values were significantly higher than the pre-exposure values, representing a 1.1 - 2.8% blood volume expansion during exercise (see Figure 4).

A similar pattern was observed in the cellular phase of the blood (Figure 4), except that the winter values for the first and the last days of acclimation were

significantly lower than the summer values (44.4 vs 47.2% for the first day and 47.6 vs 49.3% for the last day). No significant difference was found during the sixth day (48.7% for the summer and 49.5% for the winter) of acclimation.

The differences (post-exposure minus pre-exposure) in plasma volume values (Δ PV) calculated daily using Dill and Costill's method showed 5.7 - 6.7% plasma volume expansion during the summer exposures and 2.2 - 6.2% expansion during the winter experiments (see Figure 5, Table 2); all of these differences were found to be significant ($P < 0.05$). Throughout the seasonal acclimation, no significant differences were found between days during the winter, whereas in the summer, A_6 values were significantly higher than A_1 or A_{10} values for both pre- and post-exposures. Between seasons, the only significant differences were found on A_6 (summer values higher than winter values, $P < 0.05$).

INSERT FIGURE 5 ABOUT HERE

During both summer and winter exposures, the albumin (ALB) concentration was found to be significantly lower at the end of the daily exposure as compared to the pre-exposure values (Fig. 6, Table 2). During the summer acclimation, a significantly lower ALB concentration was found during the middle of the acclimation period (A_6), in comparison to the beginning or the end of the acclimation period. During the winter, such differences could not be shown. The only seasonally significant difference was found on A_6 (S lower than W).

INSERT FIGURE 6 ABOUT HERE

Analyzing the total protein (TP) blood levels (see Fig. 7) yielded significant changes within both phases, but no differences between the two seasons. Significantly lower ($P < 0.05$) values were found in the middle of each period (A_6), as compared to A_1 or A_{10} . As with ALB concentration, lower values were recorded daily for post-exposure as compared to the pre-exposure values.

INSERT FIGURE 7 ABOUT HERE

The osmolality values ranged between 268 ± 3 (summer, A_6 post-exposure) and 290 ± 2 $\text{mOsm} \cdot \text{l}^{-1}$ (summer, A_1 pre-exposure). No significant differences were found either between seasons or between pre- and post-exposure values. In the summer experiments the values for the first day of acclimation (A_1) were found to be higher than A_6 or A_{10} (see Table 2).

DISCUSSION

Since the same test subjects served in both S and W phases and spent the whole year encompassed by the study in the same environment while performing similar activities, these subjects can be considered a homogeneous test group. The experiments were conducted in the northeast region of the USA, with its typical cold winter (below freezing temperatures) and warm summer (daily maximum ambient temperature of $30 - 32^\circ\text{C}$). One therefore could have expected that the subjects would be unacclimated to heat during the winter experiments and at least partially acclimatized during the summer exposures. Analyzing the T_{re} results supports this assumption. The T_{re} remained unchanged during the summer exposures, giving an indication that in terms of internal thermoregulatory set-point, the acclimatization procedure had already been completed naturally. However, during the winter experiments, the drop in T_{re} indicates that the acclimation had to be achieved artificially.

The significant difference in T_{re} between summer and winter phases that remained even after 10 days of acclimation suggests a different thermoregulatory set-point for the different seasons. This difference in set-point cannot be totally eliminated by acclimation. Consequently, it is almost impossible not to compare this difference to the differences in T_{re} during the circadian rhythm (1). The seasonal differences in T_{re} parallel the differences in sweat rate, where the summer sweat rate was consistently and significantly higher than in the winter, regardless of the stage of acclimation. The higher T_{re} in the winter, combined with the lower sweat rate in comparison to the summer, suggests a lower sweat sensitivity in the winter than in the summer (26), both before and after acclimation.

The HR on the other hand showed a different manifestation: it dropped significantly both during the winter and during the summer experiments, suggesting that in terms of cardiovascular adjustment, the subjects were not fully acclimatized either in the winter or in the summer. Obviously, the changes in HR were of a *greater magnitude* during the winter because of a lower natural stage of acclimatization. At the end of the acclimation, the subjects showed similar ($P > 0.05$) HR for both phases (W and S), which is an indication of reaching a similar state of cardiovascular adjustment. In both seasons, these findings are in agreement with those of Wyndham et al. (29). In their study, the investigators defined days two and three in the heat as phase two of acclimation. These authors attributed the decrease in HR during this phase to associated increases in stroke volume. The rapid improvement in the central circulation is explained by these authors as a result of rapid expansion of plasma volume. The expansion of plasma volume continued throughout their third phase of acclimation (days 4 to 6), which is characterized by increases in cardiac output. These authors

further suggest that the plasma volume shrinks in the fourth phase (days 6 to 8) of acclimation.

The changes in blood and plasma volume seen in the present study should be discussed at three different levels: (1) the longitudinal changes during the acclimation process, (2) the daily changes caused by the exposure and (3) the differences between the summer and winter phases. As was expected from the previously published literature (8, 18, 21, 22), the daily differences between the pre- and post-exposure values in all the hematological parameters, except osmolality, were found to be significant. During the actual exposures, hemodilution was observed, as is evident by decreases in Hb, TP and ALB values, and increases in Δ PV and Δ BV. The blood volume increased due to plasma volume expansion and red cell shrinkage. Since changes in mean corpuscular volume can be estimated from changes in MCHC (7), the decrease in HCT and increase in MCHC values indicate that the cellular phase of the blood shrank during this same time period. The unchanged osmolality with decreased ALB and TP suggests that the expansion was due more to water and electrolytes shifting into the intravascular space rather than to protein shifts. These findings were also observed during the control days, both in summer and in winter. It can therefore be assumed that these changes were mainly exercise induced.

During both heat acclimation phases (W and S), the most extreme changes in hematological responses were observed in the middle of the acclimation period (sixth to eighth days). On these days, the highest hemodilution was observed (lowest Hb, HCT, MCHC, TP and highest Δ PV and Δ BV). In comparison to exercise induced hemodilution, the blood volume expansion of the winter acclimation occurred both in the plasma volume and in the volume of the cellular phase. In the latter type of hemodilution, particularly in winter experiments,

most probably albumin was shifted into the vascular space. This suggestion is supported by the unchanged albumin level during the winter exposures, and by the smaller changes in albumin levels than the changes in plasma volume, or in total protein during the summer.

At the seasonal variation level, it seems that basically the same mechanism of blood volume expansion was involved both in summer and in winter. The main difference between the two seasons was the difference in the volume expansion between the two compartments of the blood, i.e., the plasma volume and the cellular phase. The following seasonal findings were observed: higher HCT values during the sixth day as compared to the first and the last day of acclimation in the winter and the opposite trend during the summer; greater changes in MCHC in the winter (significantly higher values in winter for the first and last days of acclimation contrasted to the lower values for the sixth day); no differences in Δ BV and Hb between the two seasons; smaller changes in Δ PV and ALB in the winter than in the summer; and no differences in TP between seasons. This suggests that during the summer the main source of blood volume expansion is plasma volume expansion, while during the winter the cellular phase expanded more. Because this assumption is based on four independent variables (HB, HCT, ALB, TP), it can be assumed that these differences observed in the present study are more than coincidence.

In conclusion, after the summer season, the subjects were partially acclimatized to heat. This acclimatization was manifested by an unchanged T_{re} through a further period of acclimation to a more severe heat load. On the other hand, the cardiovascular adjustment to the heat, represented by HR, was not completed and further adaptation was achieved by acclimation. The mechanism involved in this added acclimation was mainly plasma volume expansion. In spite

of 10 days of acclimation, the winter thermoregulatory set-point remained higher than in the summer. The latter, together with lower sweat rates after acclimation in the winter, seemed to represent the lower sweat sensitivity of the acclimated man in the winter season than in the summer. During winter acclimation the blood volume expansion was due to expansion both in the plasma and cellular phases, while in the summer the acclimation resulted in only plasma volume expansion.

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1. The views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other official documentation.

2. Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Regulation 70-25 on Use of Volunteers in Research.

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Figure Legends

FIG. 1. Final sweat rate (\dot{m}_{sw}), rectal temperature (T_{re}) and heart rate (HR) values during each day of the summer (solid lines) and the winter (dashed lines) exposures are displayed. The C_1 and C_2 are the two control days and 1-10 are the acclimation days.

FIG. 2. Final hemoglobin concentration during summer (solid lines) and winter (dashed lines) exposures with the pre- (open symbols) and post-exposure (closed symbols) values for the two control days (C_1 and C_2) and the ten acclimation days (1-10) are presented.

FIG. 3. MCHC during summer (solid lines) and winter (dashed lines) exposures are illustrated. Pre- (open symbols) and post-exposure (closed symbols) values for the two control days (C_1 and C_2) and the ten acclimation days (1-10) are shown.

FIG. 4. The changes in blood volume (whole blood and the cellular phase of the blood) during the summer (solid lines) and winter (dashed lines) exposures are given; pre-(open symbols) and post-exposure values (closed symbols) for the last control day (C_2) and the ten acclimation days (1-10) are shown. Pre-exposure values of the first acclimation day of each season are defined as 100% for the whole blood. The cellular phase values are the whole blood values multiplied by the hematocrit.

FIG.5. Changes in plasma volume (ΔPV) during summer (solid lines) and winter (dashed lines) exposures are displayed. Pre- (open symbols) and post-exposures (closed symbols) values for the last control day (C_2) and the ten acclimation days (1-10) are shown. The pre-exposure values of the first day of both periods of acclimation are defined as $\Delta PV = 0$ for that season.

FIG. 6. Plasma albumin concentration during summer (solid lines) and winter (dashed lines) exposures with pre- (open symbols) and post-exposure (closed

symbols) values for the two control days (C_1 and C_2) and the ten acclimation days (1-10) are illustrated.

FIG. 7. Plasma total protein concentration during summer (solid lines) and winter (dashed lines) exposures with pre- (open symbols) and post-exposure (closed symbols) values for the two control days (C_1 and C_2) and the ten acclimation days (1-10) are illustrated.

TABLE I. Comparison of rectal temperature (T_{re}), mean weighted skin temperature (\bar{T}_{sk}), heart rate (HR) and sweat rate (\dot{m}_{sw}) (mean \pm SE), during the two control days (C_1 and C_2), and the first, sixth and tenth days of acclimation (A_1 , A_6 , A_{10}) at the end of the summer (S) and the end of the winter (W).

		significance of differences									
		C_1	A_1	A_6	A_{10}	C_2	C_1-C_2	A_1-A_6	A_6-A_{10}	S - W	
T_{re}	$^{\circ}C$	S	37.40 \pm 0.11	37.63 \pm 0.11	37.58 \pm 0.11	37.61 \pm 0.10	37.50 \pm 0.06	NS	NS	NS	
		W	37.61 \pm 0.07	37.98 \pm 0.14	37.87 \pm 0.06	37.76 \pm 0.10	37.50 \pm 0.06	NS	NS	NS	*
\bar{T}_{sk}	$^{\circ}C$	S	30.3 \pm 0.4	34.6 \pm 0.4	34.4 \pm 0.2	33.8 \pm 0.2	30.8 \pm 0.4	NS	NS	NS [†]	
		W	31.2 \pm 0.4	35.4 \pm 0.5	34.7 \pm 0.3	34.3 \pm 0.3	31.0 \pm 0.4	NS	NS	NS [†]	NS
HR	$b \cdot \text{min}^{-1}$	S	93.5 \pm 3.5	123.9 \pm 7.1	108.6 \pm 3.1	117.1 \pm 4.9	94.5 \pm 2.9	NS	*	NS	
		W	95.4 \pm 3.3	133.1 \pm 8.0	114.9 \pm 5.0	113.6 \pm 5.6	90.0 \pm 2.6	NS	*	NS	only on A_1
\dot{m}_{sw}	$g \cdot m^{-2} \cdot h^{-1}$	S	113.5 \pm 18.6	334.8 \pm 15.1	353.7 \pm 21.8	350.0 \pm 11.8	86.4 \pm 10.3	NS	NS	NS	
		W	94.3 \pm 11.6	318.0 \pm 16.8	310.7 \pm 9.5	326.7 \pm 16.3	86.4 \pm 10.8	NS	NS	NS	*

* $P < 0.05$

[†] a significant difference was found between A_1 and A_{10}

TABLE 2. Comparison of hematological data (mean \pm SE) for the first control day (C), and first, sixth and tenth days of the acclimation period (A_1 , A_6 , A_{10}) at the end of summer (S) and end of winter (W) both pre and post-exposures.

	C ₁		A ₁		A ₆		A ₁₀		significance of difference:			
	pre	post	pre	post	pre	post	pre	post	S-W	acclimation	pre-post	
Hb g%	S	17.0 \pm 0.4	17.7 \pm 0.4	17.2 \pm 0.3	16.7 \pm 0.3	15.9 \pm 0.2	15.5 \pm 0.2	16.4 \pm 0.3	16.1 \pm 0.3	NS	$A_1 > A_{10} > A_6$	*
	W	17.2 \pm 0.6	16.2 \pm 0.4	17.0 \pm 0.4	16.8 \pm 0.3	15.6 \pm 0.4	15.2 \pm 0.3	15.8 \pm 0.4	15.6 \pm 0.3			
HCT %	S	46.9 \pm 1.0	45.4 \pm 0.8	47.8 \pm 0.7	45.4 \pm 0.8	45.7 \pm 0.6	43.7 \pm 0.6	47.9 \pm 0.8	45.4 \pm 0.6	*EA ₆	$A_6 < A_1 & A_{10}$	*
	W	46.2 \pm 1.1	44.0 \pm 1.0	45.2 \pm 0.9	43.0 \pm 0.9	46.3 \pm 1.0	44.2 \pm 1.3	45.1 \pm 1.1	43.3 \pm 0.9			
ALB g%	S	4.67 \pm 0.08	4.18 \pm 0.10	4.56 \pm 0.06	4.37 \pm 0.07	4.24 \pm 0.06	3.88 \pm 0.07	4.50 \pm 0.09	4.44 \pm 0.09	*OA ₆	$A_6 < A_1 & A_{10}$	*
	W	4.90 \pm 0.07	4.66 \pm 0.13	4.85 \pm 0.07	4.66 \pm 0.10	4.87 \pm 0.08	4.62 \pm 0.10	4.83 \pm 0.12	4.60 \pm 0.12			
TP g%	S	7.86 \pm 0.08	7.69 \pm 0.09	7.78 \pm 0.07	7.62 \pm 0.08	7.36 \pm 0.10	7.33 \pm 0.06	7.74 \pm 0.09	7.47 \pm 0.05	NS	$A_6 < A_1 & A_{10}$	*
	W	7.76 \pm 0.16	7.38 \pm 0.09	7.75 \pm 0.06	7.49 \pm 0.05	7.53 \pm 0.13	7.29 \pm 0.10	7.61 \pm 0.15	7.49 \pm 0.10			
Δ PV %	S			base line	6.7 \pm 1.6	3.1 \pm 1.0	8.9 \pm 0.9	-0.2 \pm 0.7	5.5 \pm 1.1	*OA ₆	$A_6 > A_1 & A_{10}$	*
	W			base line	2.2 \pm 0.9	-1.7 \pm 0.7	4.5 \pm 1.2	0.2 \pm 0.9	4.3 \pm 1.5			
Δ BV %	S			base line	2.8 \pm 0.9	7.6 \pm 1.1	10.0 \pm 0.8	4.7 \pm 1.1	6.6 \pm 1.0	NS	$A_1 < A_{10} < A_6$	*
	W			base line	1.1 \pm 1.9	7.9 \pm 1.3	10.7 \pm 0.9	7.0 \pm 0.6	8.3 \pm 1.1			
MCHC%	S	36.3 \pm 0.5	39.0 \pm 0.5	36.0 \pm 0.4	36.9 \pm 0.4	34.8 \pm 0.4	35.4 \pm 0.4	34.2 \pm 0.4	35.4 \pm 0.3	*	$A_1 > A_6 & A_{10}$	*
	W	37.2 \pm 0.8	36.9 \pm 0.7	37.7 \pm 0.6	39.1 \pm 0.6	33.8 \pm 0.3	34.4 \pm 0.5	35.1 \pm 0.3	36.0 \pm 0.4			
Osmolality mOsm \cdot l ⁻¹	S	289 \pm 3	287 \pm 4	290 \pm 2	289 \pm 1	274 \pm 2	268 \pm 3	270 \pm 2	273 \pm 2	NS	$A_1 > A_6 & A_{10}$	NS
	W	273 \pm 4	280 \pm 4	276 \pm 2	286 \pm 2	283 \pm 2	279 \pm 1	287 \pm 3	271 \pm 5			

In the significance of differences columns: * denotes $P < 0.05$, E = except, O = only, $A_x > A_y$ = values of A_x are significantly ($P < 0.05$) higher than those of A_y , S-W = difference between summer and winter, NS = not significant. Symbols in the middle space apply to both summer and winter.

Fig. 1

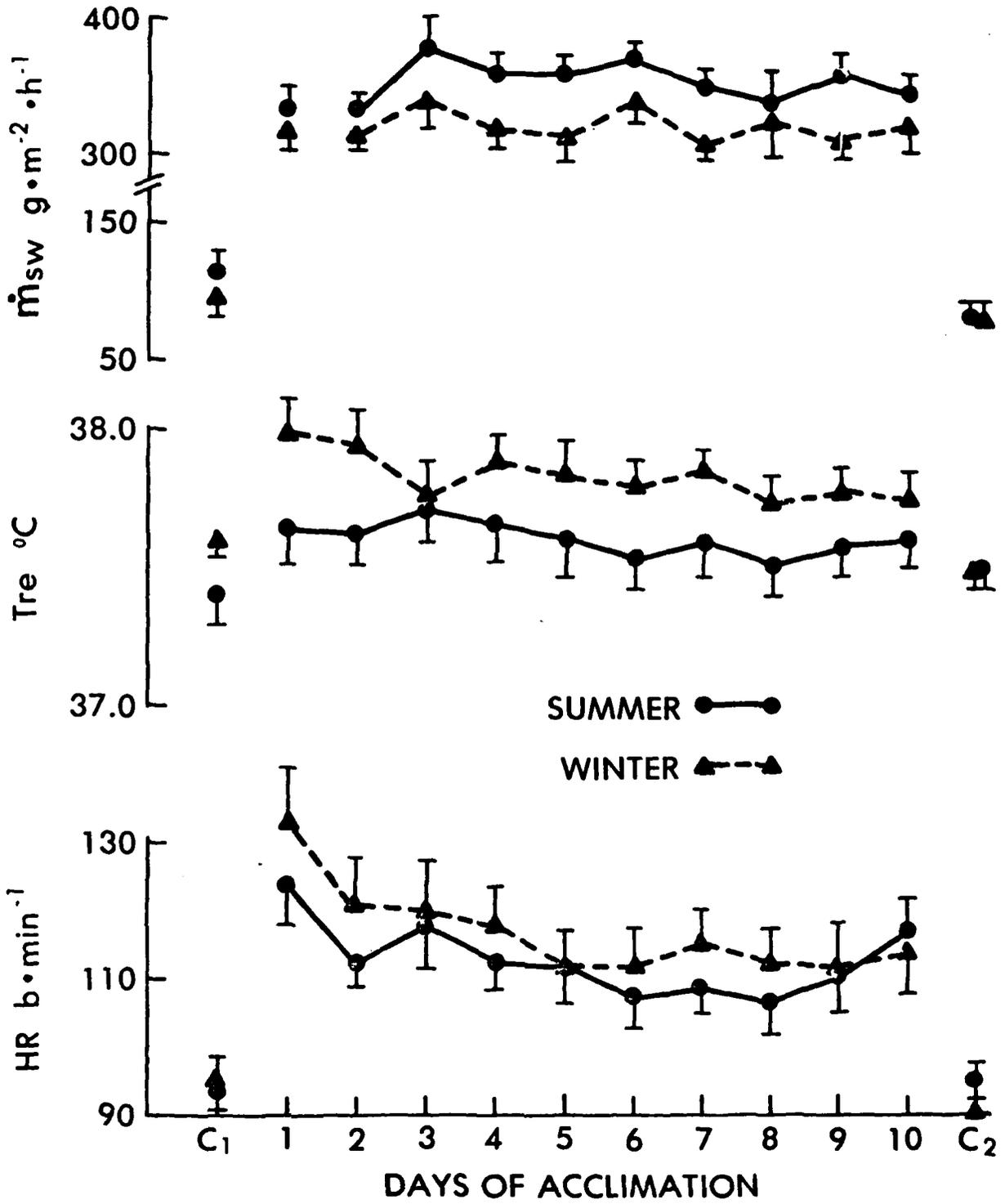


fig 2

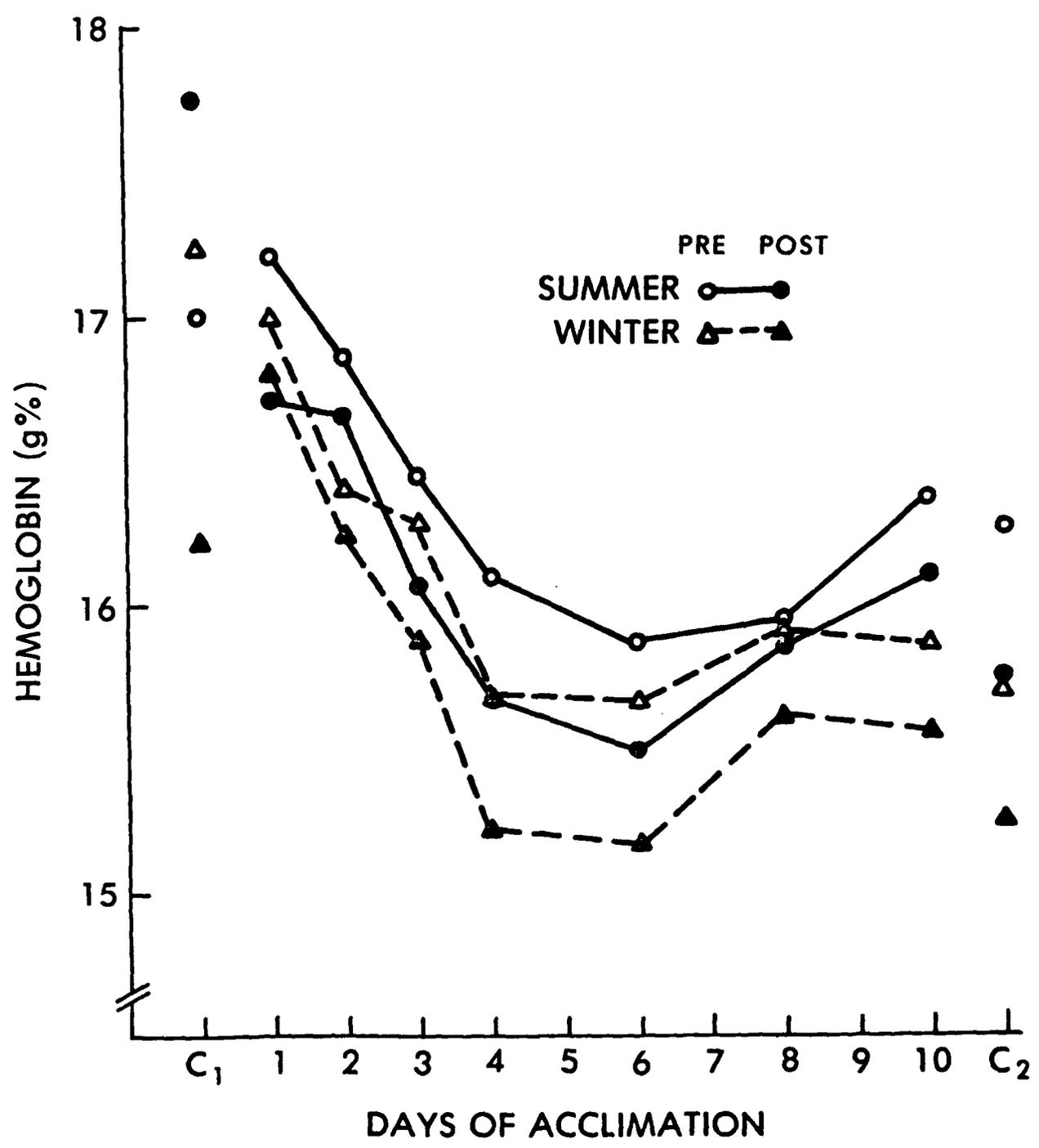


fig 3

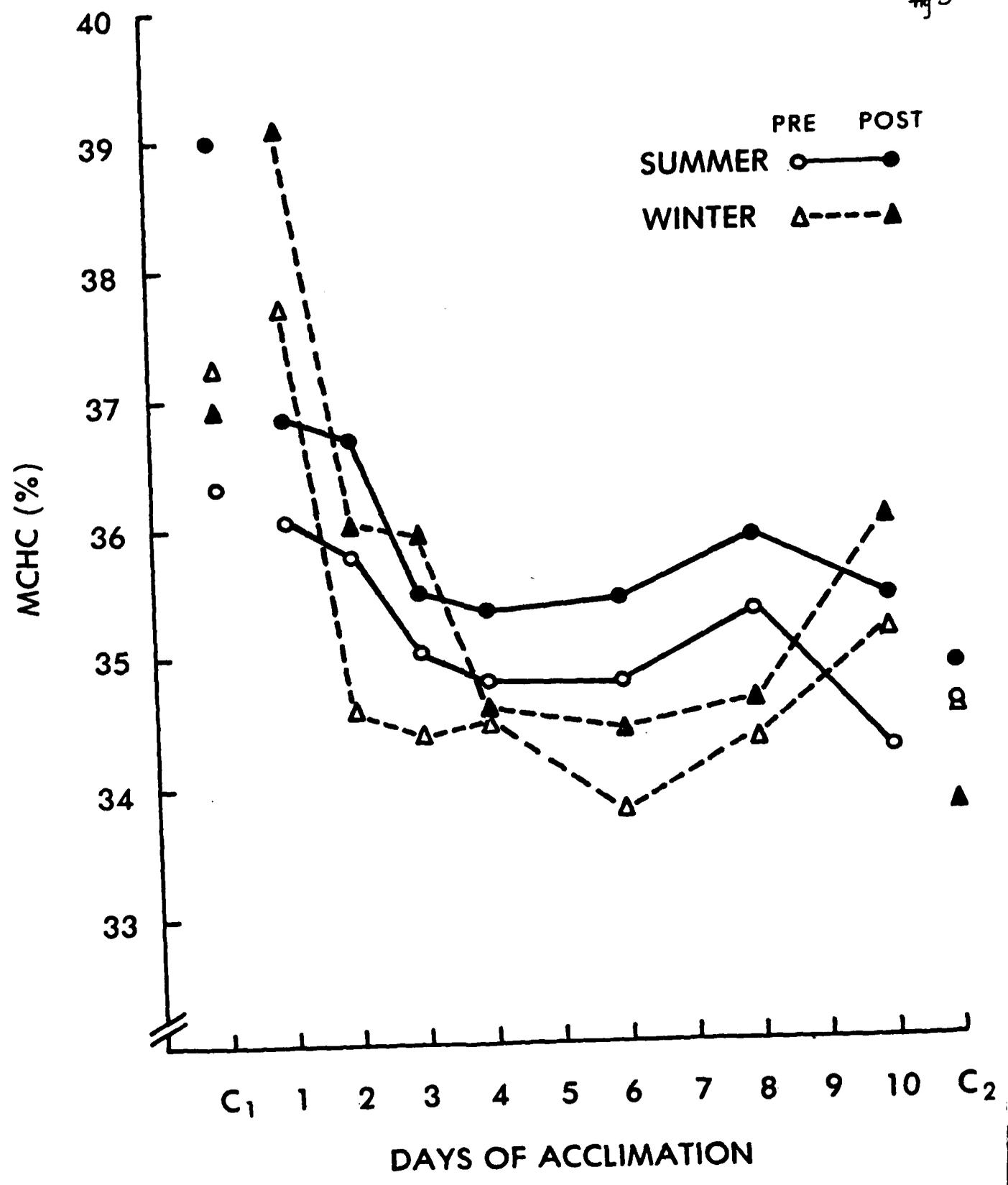


fig. 4

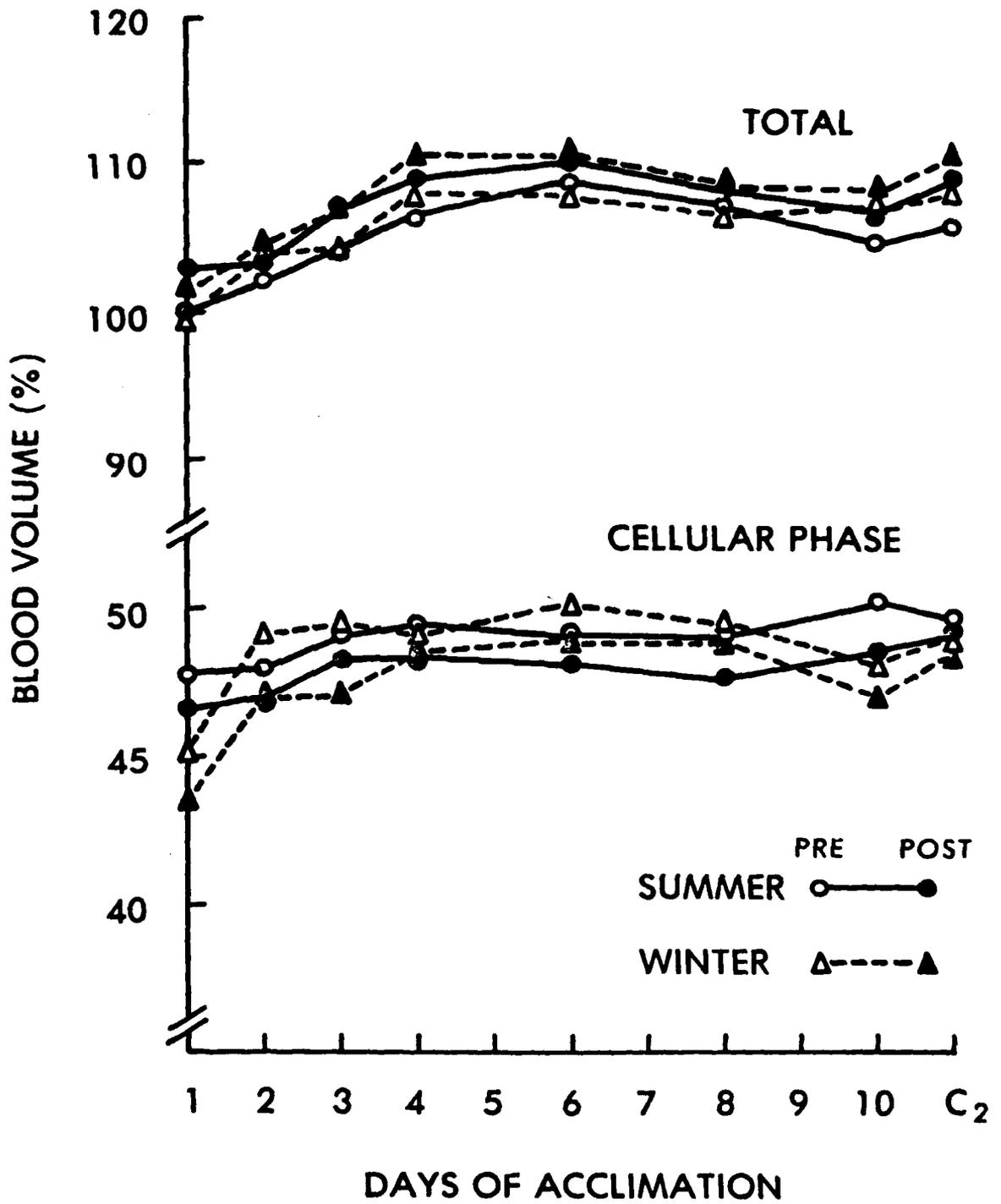


fig 5

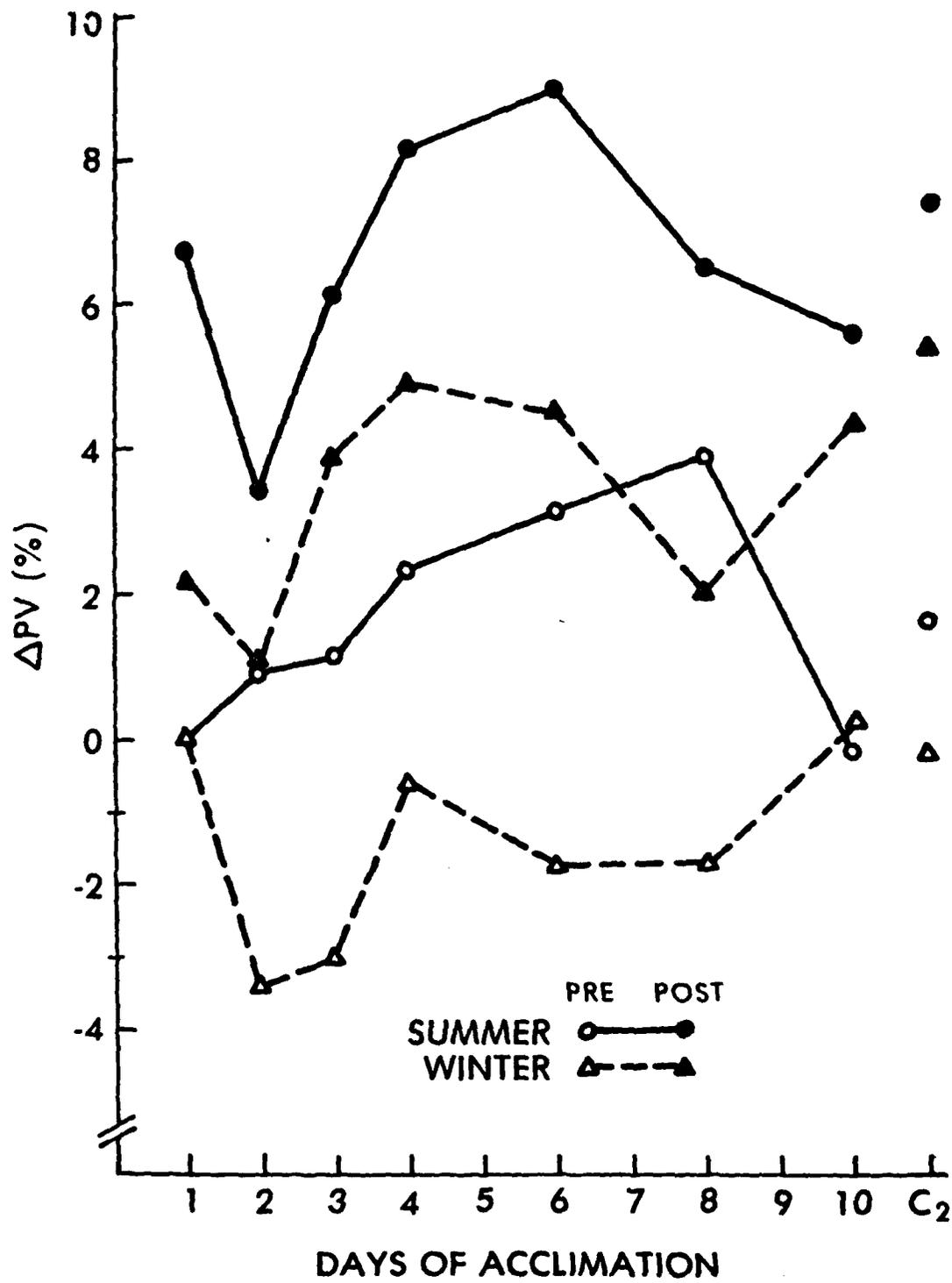


Fig. 6

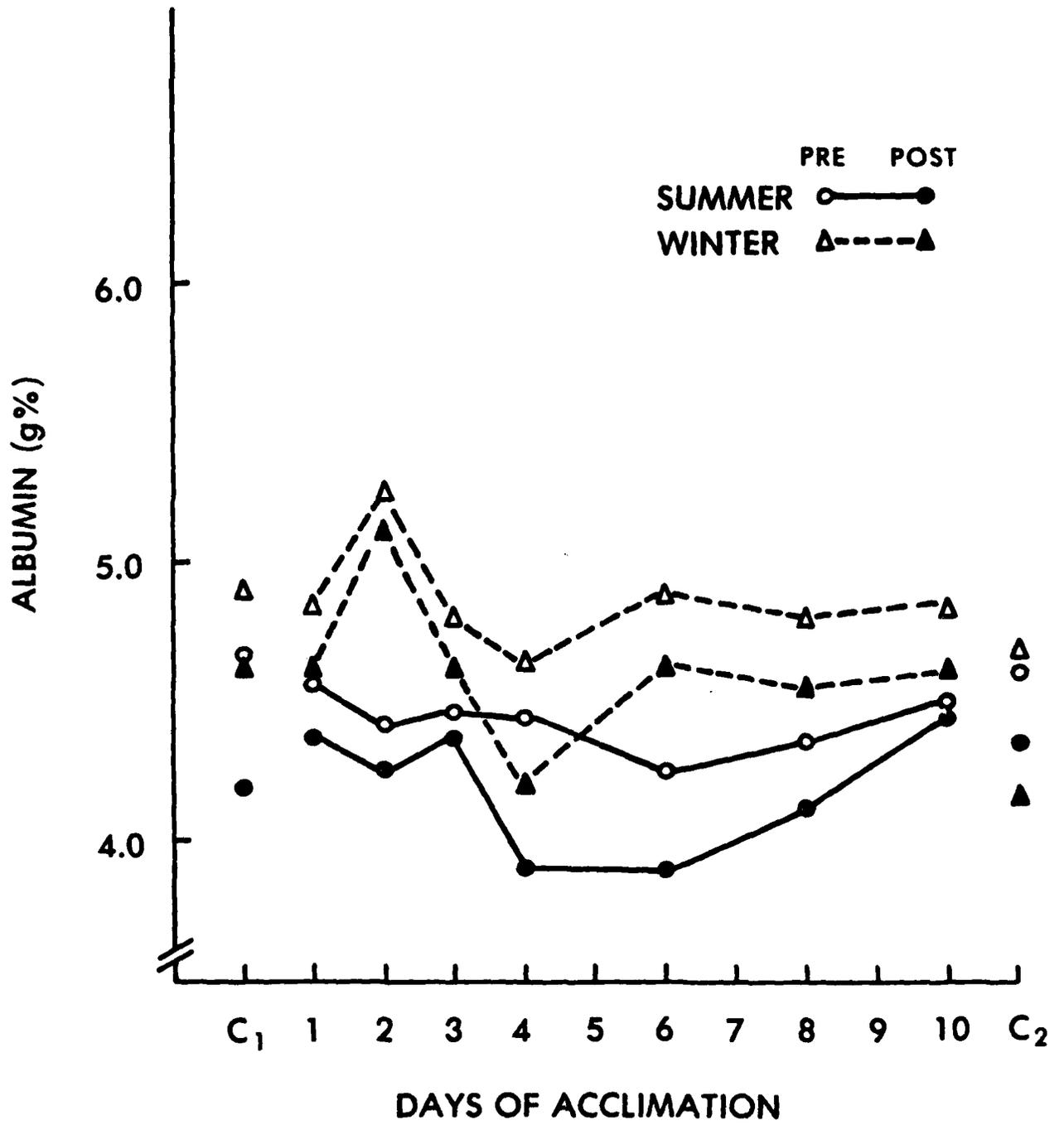
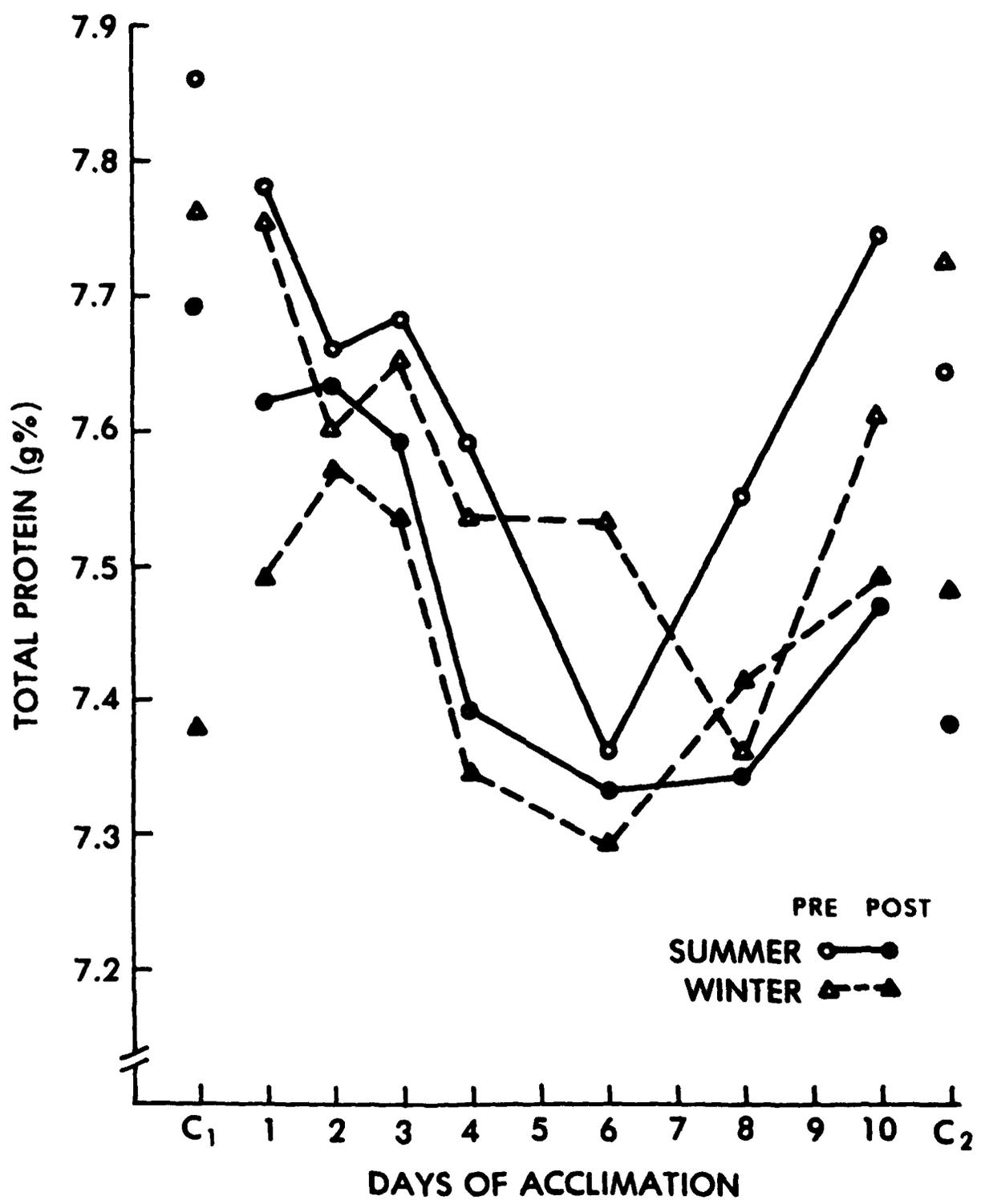


fig 7



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