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Effect of Temperature Acclimation on Activities of Serum Alkaline and Acid Phosphatases of the Rat

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溫度順應이 흰쥐의 血淸알칼리 및 酸性 Phosphatase 活性에 미치는 影響

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摘 要

Sprague-Dawley系인 成熟한 雄性흰쥐를 對照群과 實驗群으로 나누어 對照群은 23°C에서 保存하였으며 實驗群은 30°C 및 33°C에서 各各 240時間 및 64時間동안 繼續하여 溫熱順應시켰다. 溫熱順應의 各 時間區마다 血淸알칼리性 및 酸性 Phosphatase의 活性을 測定하였다. 30°C에서는 上記 酵素活性의 變化相은 33°C에 比하여 一般的으로 顯著하였다. 다시 말하면 30°C에서의 溫熱順應에 있어서 血淸알칼리性 및 酸性 Phosphatase는 對照群에 比하여 一般的으로 높은 活性의 恒定持續性을 나타내었다. 以上의 結果로 미루어 보아 上記 酵素는 30°C의 溫熱順應에 있어서 生化學的으로 直接 또는 間接으로 溫度調節에 關與하고 있는 것으로 思料된다.

INTRODUCTION

The net result of the summed chemical and physical reactions to temperature stress is the maintenance of internal temperature constancy despite varying thermal gradients between body and environment. This may be contrasted with poikilotherms where, aside from behavioral regulation of body temperature, the best regulated response to temperature stress is a tendency to maintain constancy of metabolic rates despite varying body temperature.

Because of its effect on life processes and because most cells have only a small range of adaptation to thermal changes, temperature is an important factor in the distribution of living thing. Most organisms when in a dormant state can withstand greater temperature variation than when they are in an active state (Bélehrádek, 1957).

Thermal acclimation has been shown to influence the levels of a number of enzymes in a variety of animals. Moreover, alterations in activity of serum enzymes during heat acclimation have been reported in man and mammals(Squibb *et al.*, 1954 ; Freeman and Polis, 1959 ; Bedrak *et al.*, 1963, 1964 ; Bedrak, 1965 a, b; Posen *et al.*, 1965 ; Robert *et al.*, 1966 ; Bedrak *et al.*, 1967 ; Sverre, 1969; Choi and Nam, 1974 ; Kim and Nam, 1975).

The occurrence and distribution of phosphatases in the tissues of many animals have been studied histo- and cytochemically or biochemically by many authors (Bradfield, 1950 ; Chévremont and Firket, 1953 ; Doyle, 1953 ; Usuki, 1959 ; Ashworth *et al.*, 1963 ; Jonek *et al.*, 1963 ; and Berg and Chapman, 1965.)

In the present study, the alteration in activity of serum alkaline and acid phosphatases during heat acclimation has been investigated in rats to clarify the effect of heat acclimation on these enzyme activities.

MATERIALS AND METHODS

Laboratory-conditioned adult male Sprague-Dawley rats, weighing 200 — 250 *g*, were employed. Both controls and experimentals were watered and fed *ad libitum* with a standard rat chow.

Animals were assigned to experimental groups at random on the basis of age, and each group was placed in a wire cage. Control groups were kept in a room that had maintanied a temperature of 23±1°C and a humidity of 50 to 70% ; experimental groups were kept for at least 240 hours or 64 hours in a converted incubator, where a ventilating fan and thermostatic control maintained a temperature of 30±1°C or 33±1°C and a humidity of 50 to 70%.

Blood samples were obtained with tubes from severed left saphenous vein. Serum was separated by centrifugation at 3,000 rpm for 30 minutes and kept in a refrigerator until determination of enzyme activities. Haemolysed sera were rejected.

Serum alkaline phosphatase (SALP) and serum acid phosphatase (SACP) activities were determined at 410 *nm* and 420 *nm*, respectively, with a Coleman Model 295E Spectrophotometer according to the procedure outlined by Bessey *et al.* (1946). The chemicals were supplied mainly from Sigma Chemical Co., St. Louis, U.S.A. The SALP and SACP activities were expressed as units/*l*. The enzyme activities were determined at various time intervals during acclimation to heat.

Both rats acclimated to 30°C and 33°C were kept under the above mentioned conditions up to the time of sacrifice; 4th, 8th, 16th, 30th, 48th, 64th, 72th, 144th, and 240th hour. The experimental data obtained were analyzed statistically (Snedecor and Cochran, 1967).

RESULTS

Table 1 and Fig. 1 show the serum alkaline phosphatase (SALP) levels and Table 2 the serum acid phosphatase (SACP) levels. Figs. 1 and 2 show the SALP and SACP activities of rats acclimated to 30°C for 240 hours and to 33°C for 64 hours, respectively. Average control values (23°C) were 5.56 SALP units/*l* and 0.95 SACP units/*l*, respectively. With higher environmental temperature (33±1°C), heat acclimated rats were dead at about 64th hour.

The activities of SALP and SACP were altered by consecutive heat acclimation in the different environmental temperature. As is evident from the tables, SALP activity of the 30°C acclimated rats rose from 5.56 units/*l* to 7.10 at 16th hour and to 6.47 at 64th hour and thereafter showed a persistently high level in comparison with that of the control by 24th hour. On the other hand, the SALP activity of the 33°C acclimated rats increased from 5.56 units/*l* to 8.39 at 8th hour and decreased to 3.91 at 48th hour, and thereafter increased gradually (Table 1 and Fig. 1).

Table 1. Alkaline phosphatase activity in serum of rats acclimated to 30° and 33°C

Temperature °C	Time (hour) during heat acclimation	No. of rats	Serum alkaline phosphatase activity level (units/*l*) Mean±S.E.
23±1°C (Control)	0	12	5.56±0.67
	144	12	5.58±0.64
	240	12	5.57±0.69
30±1°C	0	12	5.56±0.67
	4	7	6.53±0.87
	8	7	6.78±0.69
	16	7	7.10±0.28*
	30	7	6.67±1.37
	48	7	5.19±0.56
	64	7	6.47±0.69
	72	7	6.39±0.83
	144	7	6.24±1.20
	240	7	5.91±1.01
33±1°C	0	12	5.56±0.67
	4	7	7.84±0.85**
	8	7	8.39±1.20**
	16	7	8.02±0.72**
	30	7	8.01±0.65**
	48	7	3.91±0.53*
	64	7	6.31±0.94

*$p<0.05$, **$p<0.01$

As is shown in Table 2 and Fig. 2, the SACP activity of the 30°C acclimated rats rose from 0.95 units/l to 1.30 at 8th hour, to 1.20 at 30th hour, and to 1.09 at 72th hour and thereafter decreased slowly by 240th hour, while the 33°C acclimated group from 0.95 units/l to 1.46 at 8th hour and 1.30 at 30th hour.

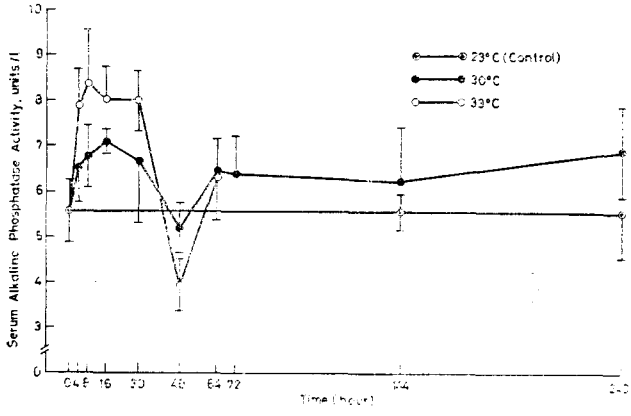

Fig. 1. Effect of environmental temperature(30° and 33°C) on activity of serum alkaline phosphatase of rats at various time intervals during heat acclimation. Each point represents average for the number of rats given in Table 1 and the vertical bars indicate the standard error of the mean.

Table 2. Acid phosphatase activity in serum of rats acclimated to 30° and 33°C

Temperature (°C)	Time (hour) during heat acclimation	No. of rats	Serum acid phosphatase activity level(units/l) Mean±S.E.
23±1°C (Control)	0	12	0.95±0.07
	144	12	0.93±0.09
	240	12	0.95±0.96
30±1°C	0	12	0.95±0.07
	4	7	1.09±0.10
	8	7	1.30±0.15*
	16	7	1.02±0.13
	30	7	1.20±0.09
	48	7	1.08±0.07
	64	7	0.97±0.06
	72	7	1.09±0.09
	144	7	0.99±0.04
	240	7	0.98±0.05
33±1°C	0	12	0.95±0.07
	4	7	1.26±0.23*
	8	7	1.46±0.12**
	16	7	1.13±0.08
	30	7	1.30±0.04*
	48	7	1.22±0.16
	64	7	0.93±0.04

*$p<0.05$, **$p<0.01$

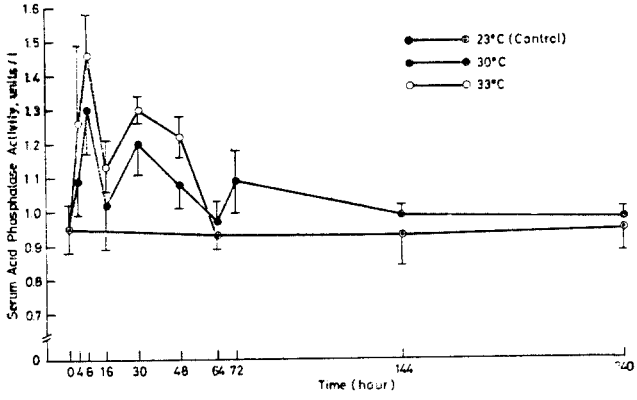

Fig. 2. Effect of environmental temperature (30° and 33°C) on activity of serum acid phosphatase of rats at various time intervals during heat acclimation. Each point represents average value for the number of rats given in Table 2 and the vertical bars indicate the standard error of the mean.

In these data, it should be noted that acclimation to a hot environment (33°C) results in a significant rise in the values of SALP and SACP within a relatively early period and an insignificant rise in the values of SALP and SACP of the 30°C acclimated rats except the early period.

The heat-acclimated elevation of SALP and SACP levels may be attributed mainly to the release of the enzymes from the cells following prolonged heat exposure. Thus, the fluctuation of the rise of SALP and SACP activities observed in the rat acclimated to 30°C could be attributed to the difference in the state of acclimation. The data suggested that the SALP and SACP level did not maintain constant over a wide range of temperature (30°C), and that they may be involved directly or indirectly in thermoregulation.

DISCUSSION

In this investigation it was observed that the activities of SALP and SACP increased significantly at relatively early period in both experimental groups during heat acclimation.

The mechanisms of acclimation by which the environment induces adaptive change are multiple integrative responses as by nervous, hormonal, behavioral, metabolic, and enzymatic responses (Prosser, 1964).

According to Hoffman (1952), *Streptococcus cremoris* growth at high temperature favors production of more acetone and less butylene glycol; low-temperature cultures produce more lactic acid even when tested at the same temperature as high temperature cultures. This suggests a shift in metabolic pathways in the acclimation process. It is possible, therefore, that thermal acclimation at the cellular level may result from a type of enzyme induction.

It has been already reported that the phosphatase activity varies with some physiological changes, such as regeneration, carcinogenesis, growth, embryonic

development, etc. (Chévremont and Firket, 1953 ; Taguchi *et al.*, 1956). In spite of the accumulation of many studies, little is known of the mechanism controlling the concentration and activity of this enzyme *in vivo*.

With *ad libitum* feeding, heat-treated rats at 32°C for 72 hours lost weight, and the levels of riboflavin, vitamin A, ascorbic acid and alkaline phosphatase in their blood sera were significantly depressed, whereas the levels of total protein were significantly elevated (Squibb *et al.*, 1954). Also they found that with restricted feeding, both control and heat-treated rats lost weight, and in the heat-treated rats only the vitamin A and ascorbic acid levels were significantly depressed. However, the present data on SALP activity are not in agreement with the result of Squibb *et al.* (1954).

The foregoing data demonstrated that the heat acclimation in the present study increased the level of all enzymes in the serum of rats. In most cases, the magnitude of change was higher in rat acclimated to higher environmental temperature.

The effect of high temperature has produced great increase in permeability and the changes in semipermeability, at temperatures above the optimum (Ponder, 1949). On the other hand, one of the explanation suggested for the elevated level of enzymes in the blood serum of animals exposed to physical stress, heat and cold is a general increase in cellular permeability (Zierler, 1956 ; Blair *et al.*, 1961 ; Halonen and Konttinen, 1962 ; Highman and Altland, 1962).

According to Brock (1967), there is an increasing evidence of disruption of cell membranes as the possible cause of heat death. On the other hand, the liberation of a coagulating enzyme by heat is suggested as a possible explanation for heat injury. Heilbrunn (1954) had reported that heat is found to release calcium from the other portion of the cytoplasm of some cells, and this calcium is said to liberate a clotting enzyme which gets the cell. However, according to Allen(1960) working with bacteria, bacterial spores which are highly resistant to heat may have ten times as much calcium as the vegetative stages. A temperature which may appear to be favorable for a given function during brief exposure of a cell may prove harmful on longer consective exposures. Therfore, heat death of a cell may conceivably result from thermal inactivation of its enzymes. It is well known that many enzymes which are reversibly inactivated by mild temperature irreversibly inactivated by high temperature.

From the results obtained in the present experiments, thermal acclimation to 30°C and 33°C seems to affect the SALP and SACP activities during heat acclimation suggesting that the above enzyme levels did not maintain constant over a wide range of temperature. Therefore, it seems probable that the SALP and SACP may be involved directly or indirectly in thermoregulation during heat acclimation at 30°C.

SUMMARY

Activities of serum alkaline phosphatase (SALP) and serum acid phosphatase (SACP) have been assayed in adult male Sprague-Dawley rats acclimated to 30°C and 33°C for 240 hours and 64 hours, respectively. The alteration in the activities of SALP and SACP in rats acclimated to 33°C was generally greater than those acclimated to 30°C. The SACP and SALP activities of the rats acclimated to 30°C showed generally a persistently high levels in comparison to the control.

It may be inferred that the SALP and SACP positibly be involved directly or indirectly in thermoregulation during acclimation to 30°C.

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