Proc. Indian natn. Sci. Acad. **B48** No. 1 pp 44-54 (1982)

Patterns of Enzymatic Compensations for Temperature in the Fat Body of Male and Female *Periplaneta americana* (Linn.)

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(Received 6 July 1981; after revision 27 January 1982)

SDH in male and LDH in female cockroaches depict the most common "translational-cum-rotational" patterns of compensation in their activities during acclimation to high (32°C) and low (16°C) temperatures. While aldolase activity exhibits a "translational" change, LDH activity shows a "rotational" change in the male insects. An "inverse" compensation is exemplified by the specific activity of SDH in females, but recalculation per unit of wet weight of the tissue reveals a "translational-cum-rotational" pattern. The results do not support the proposition of an adaptive diminution of 'Q_{10}' values during cold habituation. The LDH and SDH activities are characterized by unusually high thermo-sensitivity, and 32°C seems to be the upper critical temperature for these enzymes. Previous thermal history and the temperature of assay determine the sex-specificity of the enzymatic activities.

Key Words: **SDH, LDH, Translocational-cum-rotationnl patterns,** ***Periplaneta americana***

Introduction

Adaptive alterations in catalytic activities of different enzymes have been exhibited by various poikilotherms against the effects of a change in the environmental temperature (Hazel & Prosser 1974, Shaklee et al. 1977 and Somero 1978). Differential patterns of these compensatory responses have been classified separately by Precht (1958) and Prosser (1958). However, only a few investigations seem to have been conducted in order to record the enzymatic changes in insects during thermal acclimation (Mutchmor 1967 and Precht 1967). Marzusch (1952), Anders et al. (1964), Burr and Hunter (1970), Hunter and Cediel (1970) and Somme (1972) reported thermal acclimation of the catalytic rates of certain enzymes associated with HMP shunt, Krebs' cycle, electron transport and transamination in insects like *Leptinotarsa*, *Drosophila* and *Ephestia*. As far as the common household pest, *Periplaneta americana* is concerned, no investigation

has been made on the enzymatic compensation to thermal alteration, except the isolated reports of Mutchmor and Richards (1961), Thiessen and Mutchmor (1967) on the muscle apyrase, and of Piccione and Baust (1977) on the neural ATPase, although compensatory alteration of respiratory metabolism has been reported both at the organismal level as well as the cellular level in this hexapod by Dehnel and Segal (1956), Das and Singh (1974), and Singh and Das (1977).

Hence in the present investigation two glycolytic enzymes (aldolase and LDH) and one TCA cycle enzyme (SDH) have been assayed in the fat body of the American cockroach (male and female) in order to reveal the acclimatory response, if any, during habituation to low and high ambient temperatures simulating the prevailing temperatures in summer and winter in this locality.

Materials and Methods

Periplaneta americana (Linn.) of both the sexes, weighing 1–2g, were collected from the grocery stores and maintained at 16°±1°C and 32°±1°C for a period of three weeks for acclimation as described earlier (Das & Singh 1974). The maximal catalytic rate of enzymes, 'V_{max}' was determined at the saturating substrate concentration, at optimal *p*H and at different temperatures (10°, 16°, 24°, 32° and 37°C), after checking for the linearity with respect to the enzyme concentration (dilution of tissue extract) and the time-period of incubation. The van't Hoff's thermal coefficient, 'Q_{10}', was also calculated for the rate of enzymatic catalysis. In certain enzymes, where the activity decreased due to increasing temperature, 'Q_{10}' was calculated for the rate of decrease within that range (instead of an increase).

Fructose 1, 6-diphosphate aldolase (EC 4.1.2.13)

Colorimetric method of Bruns and Bergmeyer (1963) with suitable modifications was followed. The reaction mixture had 1.0ml of 0.4% tissue extract (in buffer), 1.9ml of collidine hydrazine buffer (*p*H 8.0) and 0.1ml of 0.3M fructose 1, 6-diphosphate solution, incubated up to 2 hrs.

Lactate dehydrogenase (EC 1.1.1.27)

The enzyme was assayed employing the method of Srikantan and Krishnamurti (1955). A total volume of 3.0 ml of reaction mixture contained 1.0ml of 0.066M potassium phosphate buffer (at *p*H 7.0) with 0.4mg of NAD, 1.0 ml of 3% tissue extract (in buffer), 0.5 ml of 0.1M sodium lactate and 0.5ml of 0.5% aqueous triphenyl tetrazolium chloride. The colour of the formazan formed after incubation for 12 hrs was extracted by adding 6.0 ml of acetone and subsequently keeping it for overnight in cold.

Succinate dehydrogenase (EC 1.3.99.1)

The same method was followed for the assay of SDH, as described for LDH. However, the homogenate (4g%) was prepared with the potassium phosphate buffer of *p*H 6.0, and incubation was followed up to 24 hrs.

Specific activities of all the enzymes were expressed per unit protein content of the tissue extracts. The concentration of protein was determined by the method of Lowry et al. (1951). However, in case of aldolase the protein content had to be estimated in separate distilled water homogenates of the tissue because of the interference by collidine hydrazine buffer, The statistical significance of difference, if any, between the two sexes of insects and between insects of the two thermal

regimes was determined by '*t*' test (Croxton 1959).

Results

The figures 1–3B, drawn as modified Arrhenius plots (activity in log scale versus increasing temperature of assay) of the mean values of 6–8 replicates (±S.E.), as suggested by Prosser (1958), depict differential patterns of thermal acclimatory responses of the three enzymes studied in this insect. The van't Hoff's thermal coefficient ('Q_{10}') at different thermal ranges of measurement are recorded in table 1.

Fructose 1, 6-diphosphate aldolase

Male insects

It is observed from the figure 1A that aldolase activity is increased during cold adaptation in males. The percentages of increase over the warm-adapted insects were 170%, 200%, 178%, 190% and 158% at 10°, 16°, 24°, 32° and 37°C respectively, and these changes were statistically significant ($P<0.001$).

The thermal coefficient ('Q_{10}') remained apparently unchanged by adaptation to high and low temperatures (table 1). But the value showed a gradual decrease with increasing temperature of measurement

Table 1 *Alteration of values of 'Q_{10}' of enzymatic activities in fat body of cockroach due to thermal acclimation*

Enzyme	Insect	Value of 'Q_{10}'			
		10°–16°C	16°–24°C	24°–32°C	32°3–7°C
Aldolase	Cold male	5.79	2.58	1.37	1.38
	Warm male	4.78	2.81	1.33	1.66
	Cold female	6·02	2.11	1.45	1.44
		(6.50)*	(2.17)	(1.44)	(1.44)
	Warm female	4.78	2.89	1.49	1.31
		(6.02)	(2.98)	(1.49)	(1.31)
LDH	Cold male	—	—	11.22	19.95**
	Warm male	—	2.17	10.00	165.96**
	Cold female	4.97	5.78	7.94	4.57**
		(5.17)	(5.78)	(7.49)	(4.36)
	Warm female	—	74.98	5.15	5.00**
			(84.14)	(4.73)	(4.00)
SDH	Cold male	—	—	2.66	2.50**
	Warm male	—	—	1.77	3.80**
	Cold female	—	—	7.94	1.26
				(6.68)	(1.26)
	Warm female	—	—	8.65	2·63
				(7.94)	(2.88)

* Values in parenthesis indicate 'Q_{10}' of the activities calculated per mg tissue

** 'Q_{10}' of the rate of decrease as a function of temperature

(remaining more or less constant at 24°–37°C), irrespective of the thermal groups of insects.

Female insects

The female roaches, however, depicted a different picture (figure 1A). No difference could be observed between the insects of two thermal regimes in the aldolase activity of their fat body.

No alteration of 'Q_{10}'-value was observed due to cold adaptation, except for a marginal increase noticed within the lower thermal range of measurement (table 1). Increasing temperature had identical effect on the 'Q_{10}' value as found in male insects.

When the enzymatic activity was expressed per mg tissue instead of per mg protein(figure 1B), the female cockroaches exhibited an augmentation due to cold adaptation to the extents of 125%, 133%, 79%, 75% and 85% at the five temperatures of measurement and these were all statistically significant ($P<0.01$—0.001).

Lactate dehydrogenase

Male insect

Though the LDH activity could not be detected at 10°C (figure 2), warm adapted males exhibited 20-times greater activity than cold adapted insects at 16°C ($P<0.05$). But at higher temperatures of measurement (24°, 32° and 37°C) cold acclimated males had 3-, 4- and 11-fold greater activities over warm adapted individuals ($P<0.01$—0.005). It is thus observed that the "R-T"curve rotated in an anticlockwise direction due to cold acclimation and the two curves for the two thermal categories of insects intersected at an intermediate temperature.

The exact nature of the alteration of 'Q_{10}' (table 1) during thermal acclimation could not be understood, because the values could not be determined at lower

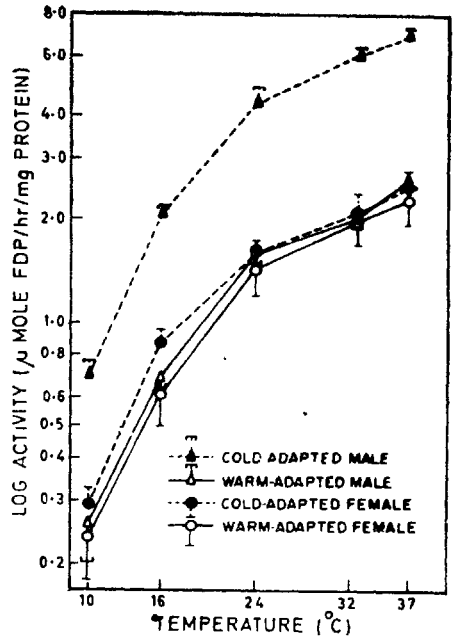

Fig 1A Alteration of aldolase activity of cockroach due to thermal acclimation

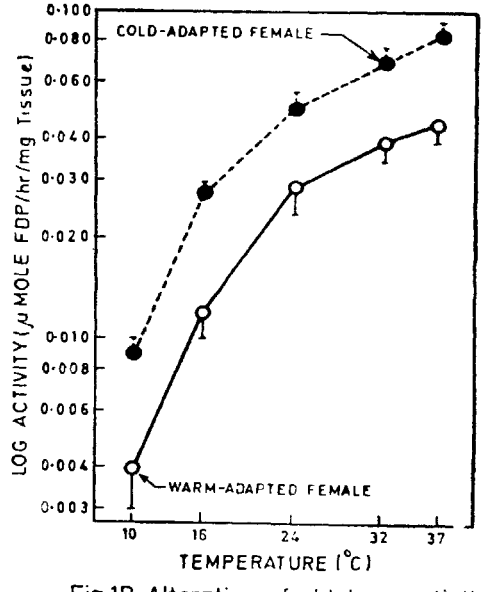

Fig 1B Alteration of aldolase activity (per mg tissue) of female cockroach due to thermal acclimation

temperatures (10°–24°C). However, the thermal acclimation of the insect apparently caused no change of the value of this constant in the range 24°–32°C. The high temperature of 32°C was found to be the critical temperature for the enzyme in male roaches of both the thermal groups. The unusually high 'Q_{10}' value in warm adapted male at 32°–37°C is noteworthy.

Female insect

The degree of increase during acclimation to low temperature was 6-times, 2-times and 2.5-times at 16°, 24°, and 32°–37°C respectively (figure 2). 10°C proved to be too low a temperature for detection of the enzymatic activity in warm-adapted females in contrast to cold-adapted ones. The differences between the two thermal categories of insects were statistically significant at 16°C only ($P<0.05$), but not at higher temperatures of measurement. After 32°C the activity showed a decline, irrespective of the thermal regime of the insects.

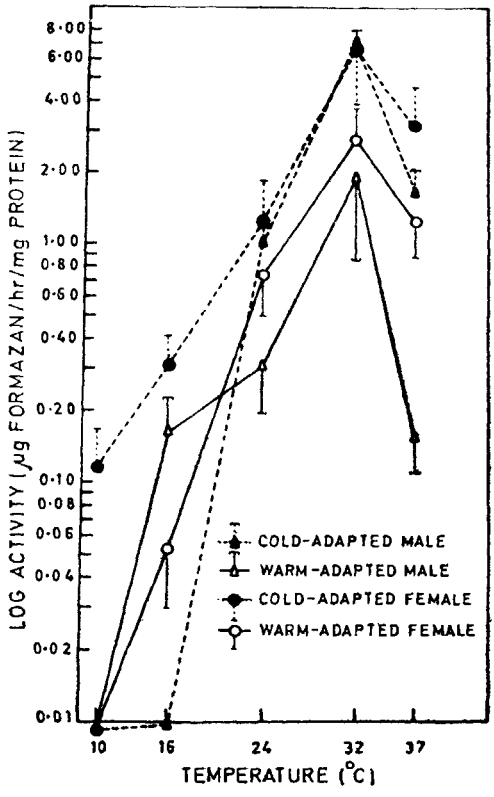

Figure 2 Alteration of lactate dehydrogenase activity of cockroach due to thermal acclimation

The enzyme, exhibited a remarkable diminution (14-fold) of its thermal coefficient at 16°–24°C, but a comparatively lesser degree of increase (60%) at 24°–32°C, due to adaptation to cold (table 1). Although the increasing temperature of assay (10°C to 32°C) had a gradual increasing effect on the value of 'Q_{10}' (from 5 to 8) in case of cold acclimated females, the enzyme was apparently extremely sensitive to variation of temperature in the range of 16°–24°C in case of warm-adapted insects.

Succinate dehydrogenase

Male insect

The enzyme was not sufficiently active at 10°–16°C in both the thermal groups of insects. The cold adapted group possessed 2.0–2.5 times more activity than the warm adapted individuals at 24° and 32°C ($P<0.005$–0.001). But at 37°C the difference was minimal and insignificant.

The 'Q_{10}' value at 24°–32°C was increased (50%) due to the cold adaptation of male insects. While 32°C proved to be the apparent "thermal maxima" for the enzyme in cold-adapted insects, the 'Q_{10}' value for the enzyme in warm acclimated males was found to be increased in the higher thermal range (32°–37°C) of measurement!

Female insect

The specific activity of SDH showed a 50% rise of its activity in warm-adapted

insects over that of the cold adapted ones at 24° and 32°C, though the difference was significant only at the latter temperature ($P<0.02$).

The 'Q_{10}' values for the enzyme remained almost unaltered during thermal acclimation. In the higher thermal range (32°–37°C), however, the value exhibited a diminution (32°C being the apparent "thermal maxima").

It was interesting to observe that the activity of SDH, when recalculated per mg tissue (figure 3B) instead of per mg protein (specific activity), presented an augmentation of the activity in cold adapted females over warm adapted ones (the difference being 40–100%). At 32° and 37°C these differences were statistically significant ($P<0.05$—0.001). However, the recalculated valves of 'Q_{10}' (table 1) were not much different from the original values. The higher thermal range of assay (32°–37°C) apparently reduced the 'Q_{10}' of the enzyme in cold acclimated insects and diminished the enzyme activity in warm adapted individuals.

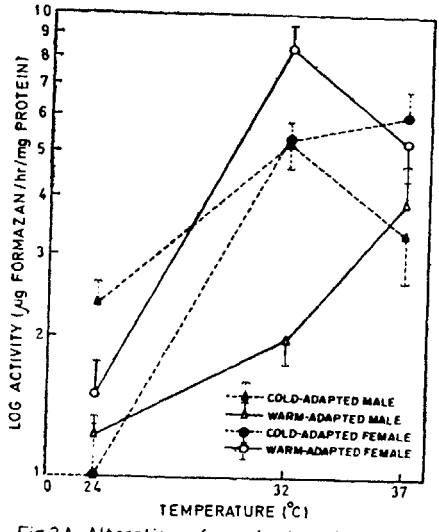

Fig 3A Alteration of succinate dehydrogenase activity of cockroach due to thermal acclimation

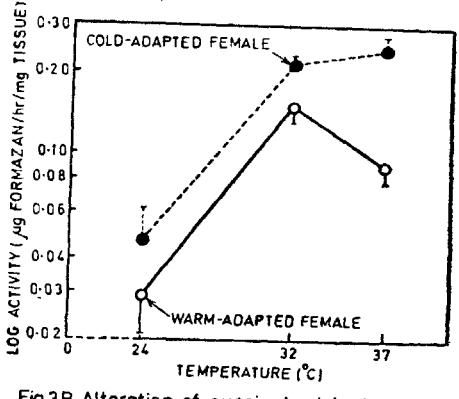

Fig 3B Alteration of succinate dehydrogenase activity (per mg tissue) of female cockroach due to thermal acclimation

Discussion

A review of the available literature reveals a capacity in many insects for organismal and cellular adaptation to varying temperature (Clarke 1967, Mutchmor 1967, Precht 1967, Somme 1966, 1968a, b & c, Das & Singh 1972 and Bursell 1974), in spite of a generalized assumption regarding the lack of thermal metabolic acclimation in these behavioural thermoregulators (Edwards 1953, Bullock 1955 and Keister & Buck 1965). The present investigation reveals the compensatory modulation of maximal catalytic rates ('V_{max}') of three enzymes associated with the energy metabolism in an insect for the first time, during acclimation to high and low temperatures.

Different patterns of acclimation

The enzymes aldolase, LDH and SDH exhibit almost all the patterns of thermal acclimation, classified by Precht (1958) and Prosser (1958), in the fat body of both the sexes of *P. americana*. A "translational" pattern (IIA) of Prosser (1958) with a "partial" degree (type 3) of compensation (Precht 1958) characterizes the adaptive alteration of aldolase specific activity in fat body of male cockroaches, although the females exhibit a "non-compensation" (Prosser's

pattern I and Precht's type 4). However, a recalculation of the catalytic rate per unit wet weight of the tissue reveals a "translational" compensation in female insects quite similar to males, plausibly because the increase in the enzyme activity due to cold adaptation is masked by a similar increase of the protein content of the tissue as reported already by Singh and Das (1980). A remarkable warm acclimation of male roaches and cold acclimation of females in the LDH specific activity are indicated by the "rotational" pattern (III of Prosser) of compensation in male insects and the "translational-cum-rotational" pattern (IVA of Prosser) in females. Thus the male cockroaches exhibit a "partial" (type 3 of Precht) adaptation of the enzymatic activity at higher temperatures (24°–32°C) but an "inverse" compensation (type 5 of Precht) at lower temperatures (10–16°C), while the females show a "partial" degree of compensation to cold. The catalytic rate of SDH in male fat body reveals a "translational-cum-rotational" thermal acclimation, with a clockwise rotation of the "R–T curve" during cold adaptation and indicating an intersection of these curves for the two thermal categories of insects at a higher temperature (Prosser's IVA pattern). While this enzyme, in its specific activity, shows an "inverse" compensation to temperature, the maximal catalytic rate expressed per unit tissue weight reveals "translational-cum-rotational" pattern (Prosser's IVC) of thermal acclimation, with a tendency of intersection of the "R–T curves" at lower temperature, in female cockroaches. Thus an overview of the results focusses on the "translational-cum-rotational" pattern (Prosser's IV) as being the most common type of enzymatic thermal acclimation (dehydrogenases like SDH in both the sexes and LDH in females only) in male and female cockroaches. This pattern also characterizes the thermal respiratory acclimation of entire insects (Singh & Das 1977). Besides, the degree of metabolic adaptation of this insect ("partial" or type 3 of Precht) at both organismal and cellular levels (Singh & Das 1977 and Das & Singh 1974) can be explained on the basis of compensatory regulation of the maximal catalytic rates of enzymes like aldolase, LDH and SDH in a tissue like fat body of this insect. However, different sex-specific and tissue-specific thermal responses of the enzymes in this insect resemble the findings of Shaklee et al. (1977) on the green sunfish.

Relative roles played by glycolytic and TCA cycle enzymes during thermal metabolic acclimation

Wilson (1973) suggested an increased glycolytic capacity during warm adaptation and its reduction during cold adaptation in goldfish. This view was later supported by studies of Hazel and Prosser (1974), using metabolic inhibitors indicating "a predominance of the glycolytic pathway at higher temperature and utilisation of alternate pathways of carbohydrate metabolism (hexose monophosphate shunt) in the cold". They also reported that the extent of compensation of glycolytic enzymes is far less than the electron transport or TCA cycle enzymes.

In order to find out the comparative utilisation of the two pathways, the ratios of the mean specific activities of SDH to LDH and of SDH to aldolase have been calculated and shown in table 2. The data indicate that in the fat body of *P. americana* (both sexes) both aerobic and anaerobic energy production occur during thermal acclimation. However, acclimation to 32°C makes the tissue seemingly more dependent on anaerobic

metabolism and thus the aldolase activity in warm-adapted roaches is significantly more than the SDH activity (particularly in the male cockroaches). While the warm-adapted male insects exhibit a lesser activity of SDH than that of LDH (particularly at lower temperatures of assay), the female ones demonstrate a reverse relationship. The possibility of a "metabolic reorganisation" (Somero & Hochachka 1976) during thermal acclimation of *P. americana* "in terms of the contribution of a given pathway to the total metabolism" (Hazel & Prosser 1974) is thus indicated by the results of the present work.

Sex-specificity in enzymatic activities

Thermal history of the insect and the assay-temperature seem to be the limiting factors in determining the sex-specificity of the enzymatic activities in this insect (table 3). The males exhibit a higher aldolase activity of fat body than

Table 2 *Enzyme activity ratios in fat body of male and female cockroaches acclimated to high and low temperatures*

Temperature of assay (°C)	16°C-adapted				32°C-adapted			
	Male		Female		Male		Female	
	SDH LDH	*SDH* Aldolase	*SDH* LDH	*SDH* Aldolase	*SDH* LDH	*SDH* Aldolase	*SDH* LDH	*SDH* Aldolase
10	**	*	*	*	**	*	*	*
16	*	*	*	*	*	*	*	*
24	2.29	0.53	0.79	0.63	4.07	0.76	2.00	1.04
32	0.74	0.90	0.79	2.46	1.03	0.97	3.05	4.24
37	2.06	0.48	1.90	2.30	25.83	1.47	4.25	2.30

* SDH activity below the level of detection
** No activity of either enzyme

Table 3 *Sexual dimorphism in the enzymatic activity of cockroach*

Enzyme	Temperature of assay (°C)	Cold-adapted insects		Warm-adapted insects	
		Comparison of sex	The level of significance	Comparison of sex	The level of significance
Aldolase	10	M > F*	$P<0.001$	M = F	N.S.
	16	M > F	$P<0.001$	M = F	N.S.
	24	M > F	$P<0.001$	M = F	N.S.
	32	M > F	$P<0.001$	M = F	N.S.
	37	M > F	$P<0.001$	M = F	N.S.
LDH	10	No activity in male	—	No activity in either sex	—
	16	M < F	$P<0.02$	M = F	N.S.
	24	M = F	N.S.	M = F	N.S.
	32	M = F	N.S.	M = F	N.S.
	37	M = F	N.S.	M < F	$P< 0.002$
SDH	24	M > F	$P<0.005$	M = F	N.S.
	32	M = F	N.S.	M < F	$P< 0.001$
	37	M < F	$P<0.05$	M = F	N.S.

**M*=Male and *F*=Female

the females amongst cold-adapted insects. But no difference is detectable between the two sexes with respect to this enzymatic activity in fat body of 32°C-acclimated insects. However, no clearly defined sex-specific activities can be observed for LDH and SDH, irrespective of the thermal regime of the insects.

Considering the sex-specific thermal acclimatory responses, the enzymes seem to behave identically in the fat body and coxal muscle (to be published elsewhere). A male cockroach seems to have a greater capacity of cold acclimation at the level of the activities of aldolase and SDH (figures 1A & 3A) and of warm acclimation with respect to LDH activity (figure 2), when compared to a female insect. A female roach, on the other hand, possesses a higher capacity of cold acclimation of its LDH activity (figure 2).

Speculations for strategies underlying the enzymatic compensation

While a "translational" change in enzyme activity implies a "quantitative" strategy, the "rotational" alteration signifies more of a "qualitative" strategy during thermal acclimation of an ectotherm (Prosser 1958, 1962). The "quantitative" strategy involves change in the enzyme concentration, but the "qualitative" one indicates either a conformational change in the enzyme-protein, or a change in the enzyme, substrate affinity or a change in the thermal characteristic like the energy of activation etc. The results of the present work support an involvement of both the strategies in the thermal adaptation of cockroach. While the "translational" pattern of compensation in the aldolase activity (figures 1A & 1B) apparently speaks of an alteration in the total enzyme concentration, the other two enzymes perhaps change in their thermal characteristics like 'Q_{10}' (table 1). In case of LDH in female insects cold acclimation is seen to be accompanied by a diminution of the 'Q_{10}' value. Rao and Bullock (1954) recognised such a change to be advantageous to a poikilothermal organism in order to maintain its relative constancy in enzymatic activity during fluctuation of ambient temperature.

However, as the present determinations have been conducted by colorimetric fixed-time assays in the crude tissue extracts, the exact molecular mechanism underlying the enzymatic compensations are difficult to be conjectured in absence of information regarding change in the catalytic micro-environment (*p*H, ion etc.) of the cell and change of the actual net synthesis of the enzymatic protein or its subunits during thermal acclimation. But an alteration of the 'V_{max}', as investigated in the present work, indicates a variation in the total enzymatic concentration in the tissue, because the maximal catalytic rate is a kinetic index of the enzyme concentration ('V_{max}'='K_{cat}' X enzyme concentration). As a matter of fact an increase of the total protein and RNA concentrations have been reported in fat body of cockroach due to cold acclimation (Singh & Das 1980). Hence, an augmentation of 'V_{max}', probably due to an increase in enzyme-protein concentration, seems to be the likely mechanism of enzymatic compensations of *P. americana* during thermal adaptation.

A "resistance acclimation" (Precht 1958) to higher temperature is lacking in the activities of LDH and SDH, 32°C being the upper critical temperature in both the thermal groups of insects for these enzymes (except in case of SDH of male, where the activity increases beyond 32°C).

The data of the present work certainly call for a more comprehensive investigation on the kinetic and regulatory properties of the enzymes associated with

different metabolic pathways for a better understanding of the molecular mechanisms of temperature adaptation of this insect.

Acknowledgement

The financial support from the University Grants Commission, New Delhi, India (sanctioned research scheme No. F. 23-77/75/SR-II/75-76) is acknowledged herewith.

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