

## Possible Altered Trophic Influences on Frog Heart During Cold Acclimation

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Most poikilotherms exhibit biochemical and physiological adaptations which tend to maintain vital functions at a relatively constant level against external temperature. The central nervous system possibly mediates many of the compensatory changes observed during thermal acclimation, which include chemical changes in neurons, altered nervous conduction and cable properties of the axon which in turn are related to axonal membrane composition and possibly membrane-bound enzymes involved in active transport of cations. A second adaptation is found at the synapses, both neural and neuromuscular. In vertebrate thermoconformers the metabolism of synaptic transmitter substances and their receptor molecules seems to contribute relatively more to thermal adaptation [1]. However, the possibility of altered trophic influences during temperature acclimations is yet to be demonstrated.

The trophic influences of the innervating nerve regulate many muscle enzyme activities since they act as the fulcrum for functional demands of the muscle [2]. Lysosomal enzymes are known to be controlled by such trophic influences [3], hence their changed activity during physiological and pathological stress may reflect altered trophic functioning. Enhanced acid hydrolase activity during disuse atrophy of rat gastrocnemius muscle [4] may reflect reduced or lack of functional demands of muscle. Employing changes in acid phosphatase activity as trophic influence-mediated index of functional demands, the effect of cold acclimation on the heart of frog, *Rana cyanophlictis* was studied, in addition to the estimation of alkaline phosphatase activity.

Frogs were acclimated to  $12 \pm 1^\circ\text{C}$  while those maintained at room temperature ( $25 \pm 3^\circ\text{C}$ ) constituted controls. The whole

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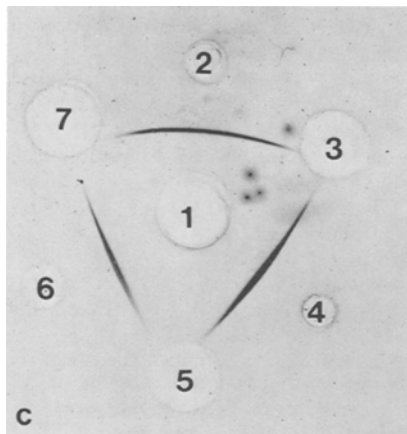
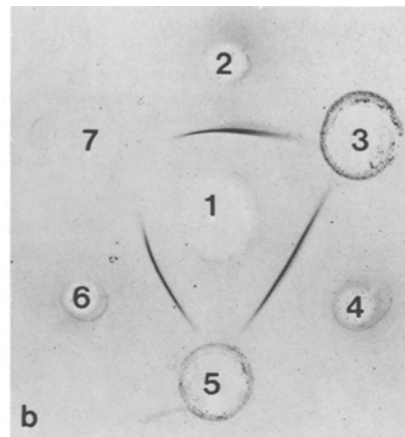
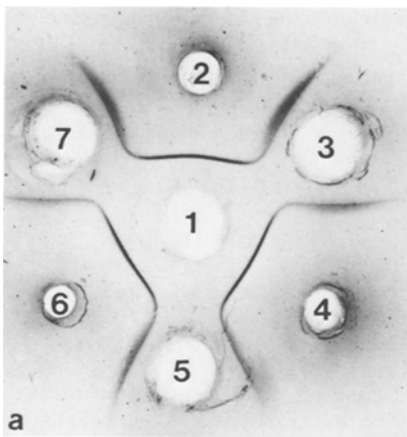


Fig. 1. Immunodiffusion of antibody against *Nereis virens* vitellin (wells 2, 4, 6) and samples of coelomic fluid from *N. virens* (wells 3, 5, 7) to be tested for a vitellin-like antigen. Central well 1: *N. virens* vitellin. (a) Body fluid from a female with oocytes ranging from 57 to 119  $\mu\text{m}$  in diameter (median 99  $\mu\text{m}$ ;  $n=25$ ). Note precipitation lines between the peripheral wells. These lines are confluent with the precipitation lines of vitellin plus antivitelin, thus indicating antigenic identity between both, vitellin and a component from female body fluid. (b) Body fluid from a male in early spermatogenesis. No precipitation between the peripheral wells. (c) Body fluid from a worm without gametocytes in the coelom. No precipitation lines between the peripheral wells

had antigen against antivitelin in the body fluid. This is demonstrated by precipitation lines confluent with those resulting from vitellin-antivitellin precipitation (Fig. 1a). Precipitation was absent in samples from male worms (Fig. 1b) as well as in body fluid from specimens with only small oocytes ( $\leq 35\text{--}45\ \mu\text{m}$  in diameter) or with no oocytes detectable (Fig. 1c).

The present results indicate that body fluids and oocytes in vitellogenic females of *N. virens* both share an antigen which is not detected in the body fluid of either males or females in earlier stages of oogenesis or worms without detectable oocytes. The occurrence of a vitellin-like antigen in the coelomic fluid of vitellogenic females may be interpreted in two different ways. Vitellin-like protein might originate from degenerating oocytes. Such protein is not likely to be found in the body fluid, since it is known that degenerating cells in the nereid coelom are digested by numerous phagocytes. On the other hand, coelomic vitellin-like protein may be regarded as vitellogenin being carried from a somatic

production site to the oocytes. Thus, it may turn out that even in nereid annelids, with a seemingly primitive type of oogenesis, oocyte differentiation will depend upon the incorporation of a vitellogenin. Further experiments will show whether or not a vitellin-like protein is synthesized by nereid somatic tissues and whether or not a vitellin-like protein can enter nereid oocytes.

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Table 1. Cardiac acid and alkaline phosphatase activities in cold acclimated *R. cyanophilictis*. Values are  $\mu\text{g Pi liberated/mg protein/h}$  and are  $\bar{X}$  of 6 observations  $\pm$  S.E.

	At habitat temperature			At optimum temperate		
	control (23 $\pm$ 3 °C)	accli. (10 $\pm$ 2 °C)	<i>p</i>	control (30 °C)	accli. (30 °C)	<i>p</i>
Acid phosphatase	16 $\pm$ 2.3	8 $\pm$ 1.4	>0.05	21 $\pm$ 1.8 (30 °C)	16 $\pm$ 0.5 (30 °C)	>0.05
Alkaline phosphatase	15 $\pm$ 1.5	9 $\pm$ 0.8	>0.05	20 $\pm$ 2.2 (38 °C)	18 $\pm$ 3.1 (30 °C)	<0.2

heart, removed from pithed frogs was immediately homogenized in 15 ml of ice cold 0.25 M sucrose. After adding a few drops of Triton X-100 to the homogenate it was kept in the cold for 1 h and later centrifuged at 600 g. The acid and alkaline phosphatases of the supernatant were assayed according to [5] at 4, 12, 18, 24, 30, 38, and 45 °C. Protein of the homogenate was determined by microbiuret method [6].

Rate-temperature curves illustrated rotational and translational compensation. The optimum enzyme activity was observed at 38 °C for the alkaline phosphatase activity of control while acid phosphatase was highly active at 30 °C for both the thermal categories. Cold acclimation reduced the enzyme activities except alkaline phosphatase at optimum temperature (Table 1).

During temperature adaptation energy-yielding enzymes exhibited translational compensation while degradative enzymes like peroxisomal and lysosomal enzymes, Mg-ATPase, acetylcholine esterase showed no or inverse acclimation [7]. Acid phosphatase activity decreased during winter but increased in spring in the heart of *Rana tigrina* contrarily to unchanged activity of alkaline phosphatase except for a spring decline [8]. The unaltered acid phosphatase activity at habitat and optimum temperatures reflects a relatively constant metabolic rate as it is implicated in membrane transport activities.

The increased lysosomal activity including acid phosphatase activity observed during aging, vitamin E deficiency and cardiac injury represents a nonspecific response to stress [9]. Interstitial cells of the heart have higher concentration of acid phosphatases while myocytes are richer in cathepsins [9]. The increase of cathepsins in the heart during starvation is suggested to reflect a spe-

cific stimulus for synthesis of myocytic proteases [10]. Earlier Sridhara [11] demonstrated increased cathepsin, autolytic and Ca<sup>2+</sup>-activated proteolytic degradation and possible enhanced turnover of proteins in the skeletal and cardiac muscles of frogs during cold adaptation. The reduced acid and alkaline phosphatases reported here may probably imply increased functional demands as well as differential stimulation of myocytes and interstitial cells resulting in altered lysosomal activities, suggesting altered trophic influences of the cardiac innervation during low-temperature acclimation.

## Cycloheximide Suppresses a Behavioral Modification in *Drosophila*

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In *Drosophila* intense blue light converts the photopigment in the peripheral retinula cells into the photostable metarhodopsin and induces a prolonged depolarizing afterpotential (PDA) in these cells [1]. It was shown that the PDA is a necessary but not sufficient prerequisite for the induction of a long-lasting modification of a phototactic behavior [2, 3]. In addition to the PDA in R1-R6, the process requires further illumination of the compound eye for more than 1 h. Based on the spectral sensitivity of this process in the *wildtype* and in *sev* a mutant defective in the visual

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subsystem of the central retinula cells, the behavioral modification is supposed to result from a plasticity of a neural interaction between the visual subsystems R1-R6 and R8 [4]. We have found that the induction of the behavioral modification does *not* take place, if the illumination with short-wavelength light for 1.5 h is carried out at 0 °C. On the other hand, the PDA in R1-R6 is induced at 0 °C, as judged by the elimination of the turning reaction of *Drosophila* [3] (measured after the flies were brought back up to 22 °C in the dark) and observed directly by recording the