# EARTHWORM ADENOSINE TRIPHOSPHATASE: A TEMPERATURE STUDY OF A POIKILOTHERM ENZYME*

By R. L. Hancock†

Much of the behavioural aspects of an organism is based upon its immediate environmental temperature. Homeotherm metabolism usually increases with a decrease in external temperature, whereas poikilotherms will have a decrease in metabolic activities. Metabolism in turn is subject to analysis through the study of temperature effects on enzyme activity. A pertinent enzyme system to study would be

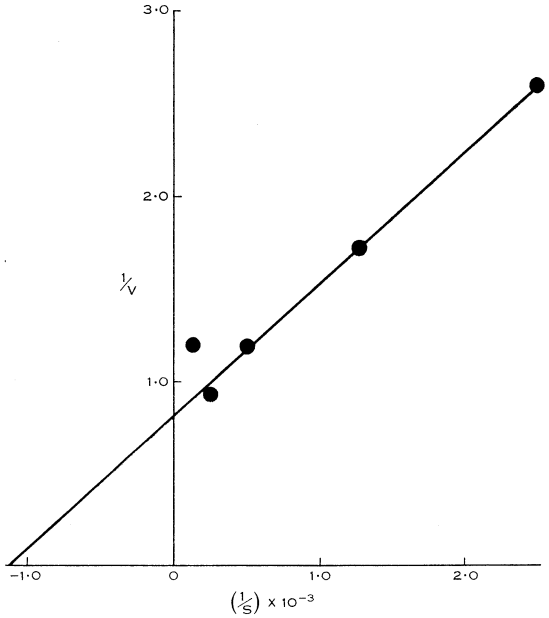


Fig. 1.—A Lineweaver–Burk plot of $K_m$ for *Lumbricus* myosin ATPase activity. Reaction mixture contained 768 $\mu$g enzyme preparation protein, varying amounts of ATP, 200 $\mu$moles glycine–NaOH buffer, pH 8, 20 $\mu$moles $CaCl_2$, in a total volume of 1·5 ml, and was incubated for 10 min at 27°C. $V$, velocity of reaction; $S$, substrate concentration.

that derived from muscle cells, since locomotion is one of the more pronounced changes in response to temperature. The poikilotherm *Lumbricus terrestris*, the earthworm, was chosen as a source of the muscle protein myosin, which can be extracted as a viscous preparation with strong salt solutions. Myosin has the properties of an adenosine triphosphatase (ATPase), although it is not specific for ATP and also hydrolyses other nucleoside triphosphates but not nucleoside mono- or diphosphates (Kielley 1961).

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† Hulls Cove, Maine, U.S.A.; present address: The Jackson Laboratory, Bar Harbor, Maine, U.S.A.

The ATPase was isolated from the muscular walls of the earthworm. This enzyme preparation (through step 2) and assay was essentially as that described by Perry (1955). After optimal substrate concentration was determined, ion-dependent pH studies were undertaken. A magnesium-dependent ATPase activity had a maximum at pH 6 and a calcium-ion-dependent activity at pH 8. Further studies were

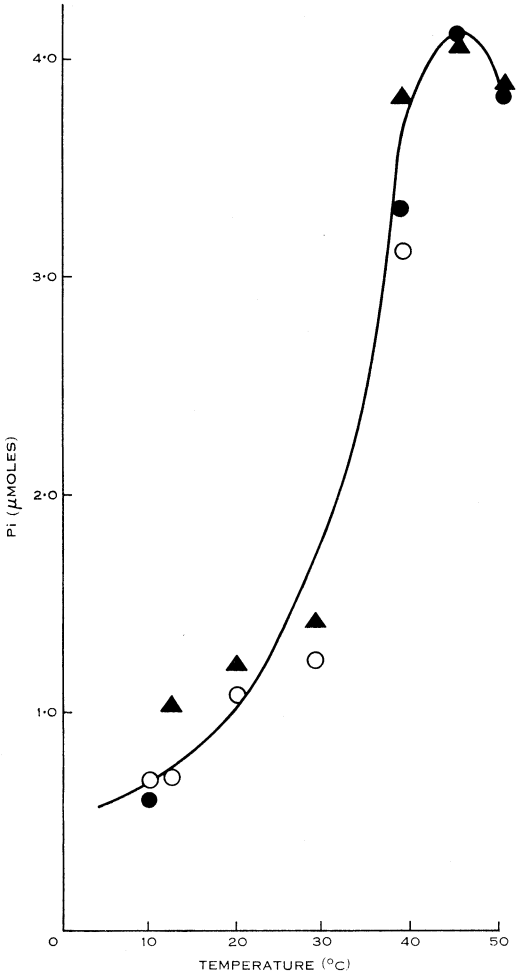


Fig. 2.—Temperature dependency curve for *Lumbricus* myosin ATPase activity. Reaction mixture contained 768 μg enzyme preparation protein, 6 μmoles ATP, 200 μmoles glycine–NaOH buffer, pH 8, 20 μmoles $CaCl_2$, in a total volume of 1·5 ml, and was incubated at varying temperatures for 10 min. Values are corrected for incubated enzyme control and incubated ATP control. *Pi*, inorganic phosphorus released.

done with the calcium-dependent activity. The enzyme was quite labile and lost over 60% of its activity in 24 hr at −4°C. A time course showed linear kinetics for the first 15 min of the reaction and had a $K_m$ with respect to ATP of $9{\cdot}1 \times 10^{-5}$M (Fig. 1). With these parameters known, an attempt was made to learn the optimal temperature of the earthworm ATPase.

Myosin ATPase is presumed to be used in changing the configuration of actomyosin. Actomyosin is a complex of actin and myosin proteins. Globular actin converts to a fibrous form involving an ATP dephosphorylation reaction. The threads

of actomyosin have been made to contract *in vitro* and the myosin ATPase has been implicated in this mechanism. The body temperature at which this enzyme works, varies among the vertebrates: e.g. *Felis* (cat) 40°C, *Ornithorhynchus* (platypus) 35°C, *Echidna* (spiny ant-eater) 37°C, and *Cyclodus* (lizard) 35°C, all with an environmental temperature of 35°C. However, if the external temperature is reduced to 10°C then the lizard's temperature is also 10°C, the spiny ant-eater's is about 29°C, while that of the platypus is 32°C and that of the cat 39°C—thus showing an evolutionary developement of homeothermism (Martin 1903).

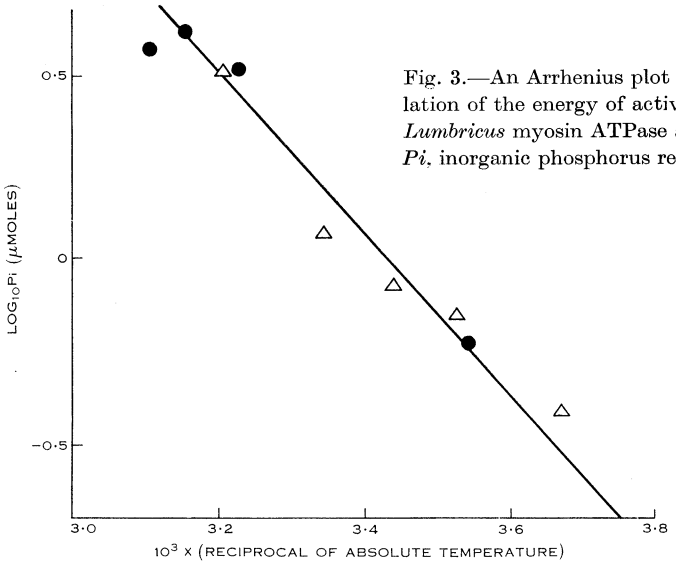


Fig. 3.—An Arrhenius plot for calculation of the energy of activation for *Lumbricus* myosin ATPase activity. *Pi*, inorganic phosphorus released.

Figure 2 shows the results of three separate experiments with the same enzyme preparation. There is an optimum temperature of 45°C for earthworm ATPase and the energy of activation, $E$,* for this enzyme system was calculated to be 9·6 kcal mole$^{-1}$ from two plots of the data (Fig. 3). This value is in the range typical of many enzymes, e.g. yeast invertase action on sucrose, and is similar to that found for rabbit muscle myosin, namely 7·3 kcal mole$^{-1}$ (Lumry 1959). An ATPase has been isolated from insect thoracic muscle. The pH optimum was 7·8–8 and a temperature optimum of 42°C, but the activation energy for hydrolysis of ATP was 29·6 kcal mole$^{-1}$ (Gilmour 1955). However, this ATPase is derived from mitochondria.

Thus an enzyme which, in nature, must be active in a great range of temperatures, was found to have its optimum similar to that of a homologous enzyme derived from a homeotherm. This finding is based upon the assumption that no adaptive changes have occurred during culturing. Furthermore, the activation energy of the earthworm ATPase–ATP system was similar to that of a homeotherm (rabbit). This might suggest an evolutionary significance. The higher homeotherms may have been

* Actually $E$ is $\Delta F_a^{\ddagger}$ because the activation energy of the substrate alone is not known. $\Delta F_a^{\ddagger}$ is the activation energy of the forward reaction for the activated complex.

forced to evolve a regulated internal temperature mechanism (hypothalamic centres, etc.) around 40°C because of the inherited information for enzyme proteins possessing previously developed characteristics achieved in early invertebrate poikilotherms. Prosser and Brown (1961), in discussing optimal temperatures for homeotherms, stress that the body temperatures are usually several degrees below lethal values, but are higher than the usual experienced environmental temperatures. They therefore feel that this is a compromise between maximum metabolic activity and inactivation of the essential proteins.

*References*

GILMOUR, D. (1955).—Insect ATPase. In "Methods of Enzymology". Vol. II. pp. 595–8. (Eds. S. Colowick and N. Kaplan.) (Academic Press: New York.)

KIELLEY, W. (1961).—Myosin adenosine triphosphatase. In "The Enzymes". (Eds. P. Boyer, H. Lardy, and K. Myrback.) Vol. V. pp. 159–68. (Academic Press: New York.)

LUMRY, R. (1959).—Some aspects of the thermodynamics and mechanism of enzyme catalysis. In "The Enzymes". Vol. I. pp. 157–231. (Eds. P. Boyer, H. Lardy, and K. Myrback.) (Academic Press: New York.)

MARTIN, C. (1903).—Thermal adjustment and respiratory exchange in monotremes and marsupials—a study in the development of homeothermism. *Phil. Trans. R. Soc.* B **195**: 1–37.

PERRY, V. (1955).—Myosin adenosine triphosphatase. In "Methods of Enzymology". Vol. II. pp. 582–8. (Eds. S. Colowick and N. Kaplan.) (Academic Press: New York.)

PROSSER, C., and BROWN, F. JR. (1961).—"Comparative Animal Physiology." 2nd Ed. (W. B. Saunders Co.: Philadelphia.)