ALKALINE PHOSPHATASE IN THE DIGESTIVE SYSTEM OF THE DESERT LOCUST, *SCHISTOCERCA GREGARIA* (FORSKAL)[1, 2]

SHAHID H. ASHRAFI,[3] S. N. H. NAQVI,[3] AND M. A. H. QADRI[4]

ABSTRACT

Alkaline phosphatase activity was determined in the digestive system of the desert locust, *Schistocerca gregaria* (Forskål), using *p*-nitrophenylphosphate disodium as the substrate. The enzyme has an optimum pH of 7.4 and a linear relationship between its concentration and activity. The Michaelis constant was found to be 1.4×10^{-4}M. The enzyme showed a zero order of kinetics, with an incubation period of up to 25 minutes at an optimum temperature of 40°C. The temperature coefficient (Q_{10}) was 1.960 between 5–15°C and 10–20°C, 1.900 between 20–30°C and 25–35°C, and 1.850 between 30 and 40°C. Salt solutions of $MnCl_2$, $MgCl_2$, $CuCl_2$, and $CoCl_2$ activated the enzyme, while Na_2HAsO_4, Na_2HPO_4, $CuSO_4$, $FeCl_2$, and $CdCl_2$ inhibited the activity. Maximum activation was produced by 0.01 M $MnCl_2$.

INTRODUCTION

The alkaline phosphatase (ortho-phosphoric monoester hydrolase [3.1.3.1]) has been characterized in holometabolous insects by many workers, including Denuce (1952), Rockstein (1956), Barker and Alexander (1958), Ashrafi (1960), Hodgson (1963), Sridhara and Bhat (1963), Lambremont and Schrader (1964), and Raychaudhri and Butz (1965). The present investigation was undertaken to identify and determine the kinetics of alkaline phosphatase present in the digestive system of the desert locust, *Schistocerca gregaria* (Forskål), which is a hemimetabolous insect.

MATERIALS AND METHODS

Substrate and Colorimetric Standard

In the present investigation, the modified method of Ashrafi (1960) was followed, because it has a shorter incubation period and a higher sensitivity to the substrate than the method of Bessey, Lowry and Brock (1946). A stock solution (0.0143 M) of *p*-nitrophenylphosphate disodium was prepared by dissolving 100 mg of substrate in 25 ml of double-distilled and demineralized cold water (Anonymous, 1963). Different concentrations were prepared by further dilution of the stock solution. The working standard solution (0.00005 M) of *p*-nitrophenol was prepared as described by Ashrafi and Fisk (1961).

Alkaline buffer Solutions

Tris-maleate buffer (0.2 M) was prepared by dissolving 24.2 g of tris (hydroxymethyl) aminomethane and 23.2 g of maleic acid in 1000 ml of double distilled and demineralized water. Fifty ml of this solution were mixed with a known quantity of 0.2 M NaOH and diluted to 200 ml (Gomori, 1955). The required pH, 6.0 to 8.5, was adjusted using a Beckman Zeromatic pH meter, and a few drops of chloroform were added as a preservative.

Activating and Inhibiting Solutions

Solutions of 22 compounds were prepared in double-distilled and demineralized water. Solutions of $C_6H_5(CH_3COO)_2$, $AlCl_3$, and $ZnSO_4$ were prepared at 0.001 M (final) concentration, $MgCl_2$, KCl, $Mg(CH_3COO)_2$, $CaCl_2$, NaCl, $BaCl_2$, $FeCl_2$, $CuCl_2$, $CoCl_2$, $Ca(CH_3COO)_2$, $MnCl_2$, $HgCl_2$, $Hg(CH_3COO)_2$, and $CdCl_2$ were pre-

[1]Part of Ph.D. dissertation of 2nd author.
[2]Manuscript received July 14, 1967.
[3]Pesticide Research Section, PCSIR., Karachi-32.
[4]Chairman, Department of Zoology, University of Karachi, Karachi-32, PAKISTAN.

pared at 0.01 M, while solutions of NaCN, NaF, Na_2HPO_4, Na_2HAsO_4, and $CuSO_4$ were prepared at 0.1 M. One half-ml portion of each of these solutions was used per reaction tube and the 5-ml total volume was maintained by adding 1.5 ml of water.

Enzyme Source

Twenty adult locusts, eight days following emergence, were fed a 6 percent glucose solution for 24 hours before the experiment. Each locust was frozen and then dissected in chilled, double-distilled and demineralized water. The alimentary canal was immediately transferred to a test tube containing 5 ml of water. It was ground for 3 minutes in a "Teflon Pyrex" tissue grinder. The homogenate was filtered through a 2-mm glass fiber layer in a Gooch crucible and the filtrate was collected in a graduated centrifuge tube under moderate suction pressure. The filtrate was diluted to 10 ml so that each ml contained 0.1 part of the ground alimentary canal.

Enzyme Assay Procedure

For enzyme assay, 2 ml of double-distilled and demineralized water, 2 ml of tris-maleate buffer (pH 7.4, 0.2 M) and 0.5 ml of substrate (0.00143 M final concentration) were placed in five test tubes in a tray of crushed ice. After a few minutes. freshly prepared homogenate (0.5 ml) was added to each tube and shaken. The rest of the method followed that described by Naqvi (1966). The spectral-transmittance and concentration-absorbance curves for measuring the activity were prepared as described by Naqvi, Ashrafi, and Qadri (1967).

RESULTS

pH Optimum

The standard assay procedure was followed, using buffers of different pH and with all other factors constant. The optimum pH for alkaline phosphatase present in the digestive system of the desert locust was found to be 7.4 (fig. 1).

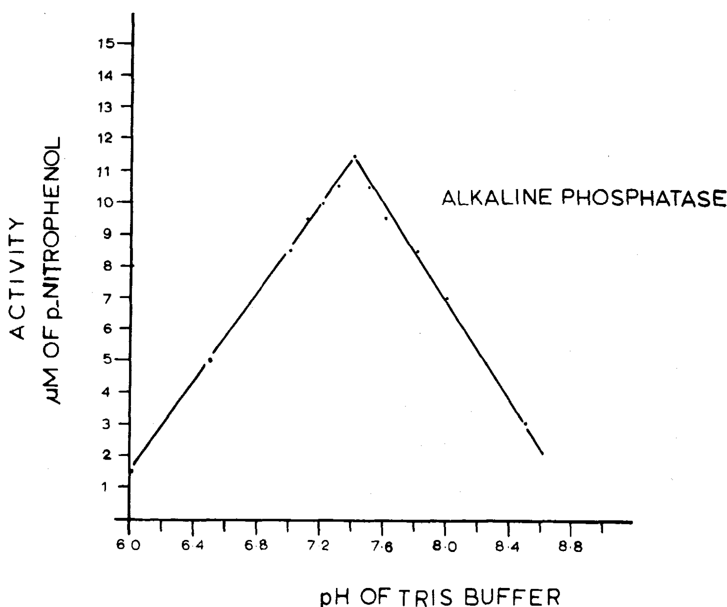

FIGURE 1. Optimum pH for alkaline-phosphatase activity.

Enzyme Concentration

The quantity of the homogenate was varied (0 to 2.50 ml), but the 5-ml volume of the reaction mixture was maintained by adding appropriate amounts of water. The results (fig. 2) showed that a zero-order reaction was maintained throughout the increase of the enzyme concentration.

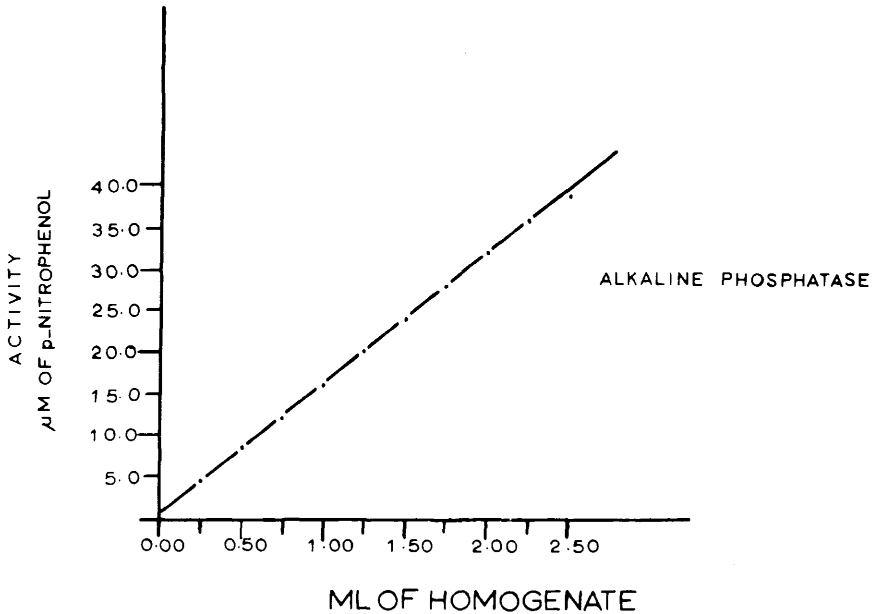

FIGURE 2. Effect of varying enzyme concentrations on alkaline-phosphatase activity.

Substrate Concentration

Different concentrations (0.0001 M to 0.0024 M) of the substrate were used to study the kinetics of enzymatic reaction. The enzyme activity showed a linear relationship up to 0.0006 M substrate concentration, beyond which it entered a zero-order reaction (fig. 3). The Michaelis-Menten constant (Km) was

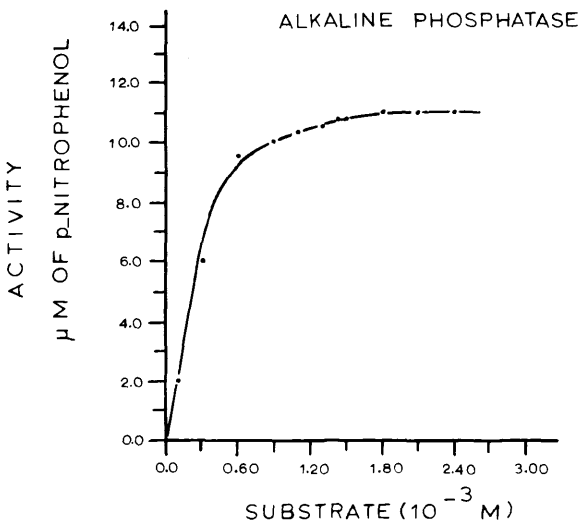

FIGURE 3. Effect of different substrate concentrations on the rate of alkaline-phosphatase activity.

calculated according to Lineweaver and Burk (1934) and was found to be 1.4 x 10^{-4} M for an incubation period of 30 minutes (fig. 4).

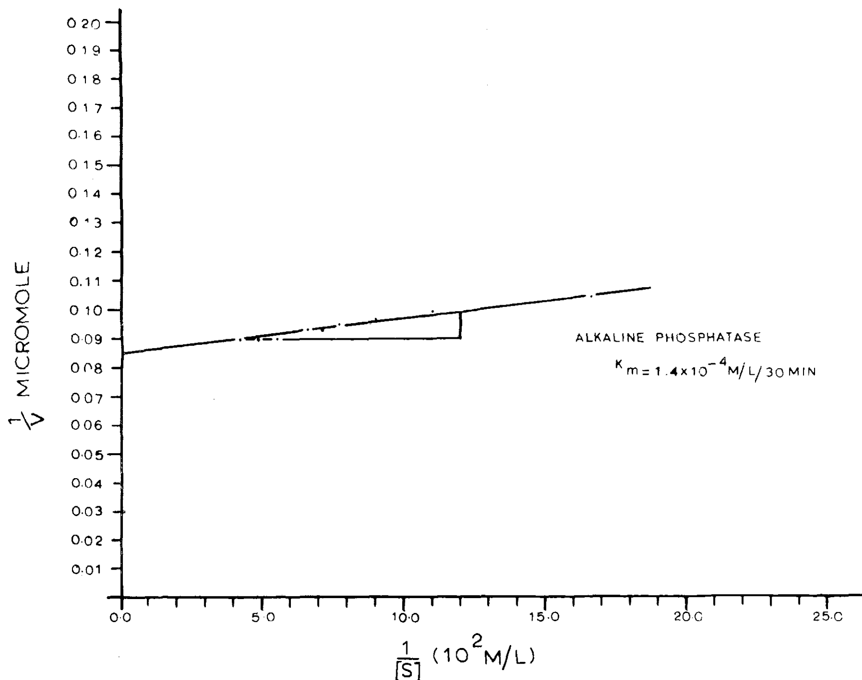

FIGURE 4. Lineweaver and Burk graph for Michaelis constant-value calculation.

Kinetics of Enzyme Action

In order to find a suitable incubation period, 1-ml aliquots from the reaction mixture were taken out after different intervals (10–70 minutes), as shown in Figure 5. A zero-order reaction was maintained for 25 minutes, after which the enzyme entered into a first-order reaction.

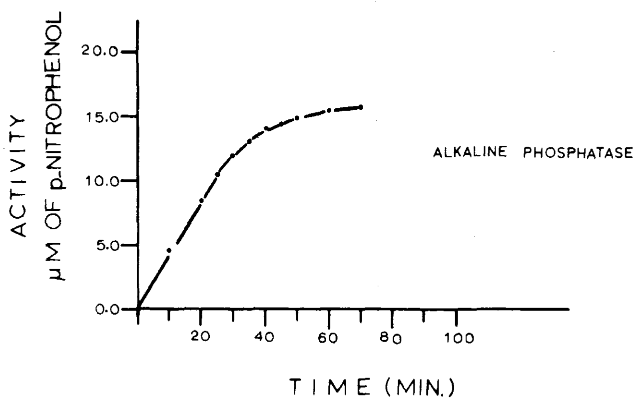

FIGURE 5. Optimum incubation period for alkaline-phosphatase activity.

Temperature Effects

The reaction mixtures were incubated at different temperatures, as shown in Figure 6. A sharp increase in the enzyme activity was found from 15 to 40°C, with a sharp decrease after 40°C. The optimum temperature was found to be 40°C. Temperature-coefficient (Q_{10}) values were found to be 1.960 between 5–15°C and 10–20°C, 1.900 between 20–30°C and 25–35°C and 1.850 between 30 and 40°C for an incubation period of 30 minutes (fig. 7).

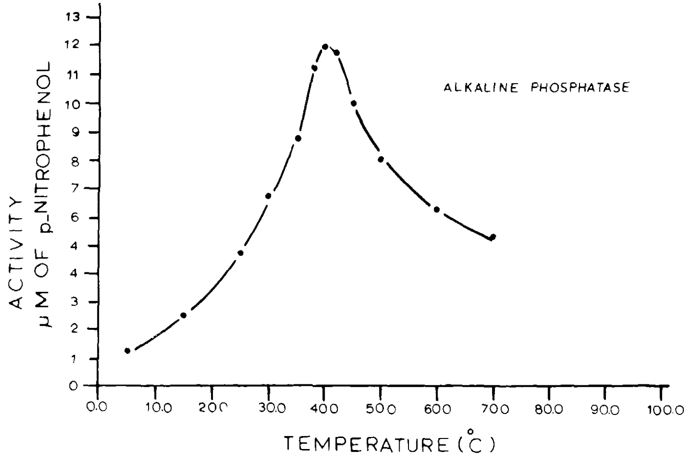

FIGURE 6. Temperature effects on alkaline-phosphatase activity.

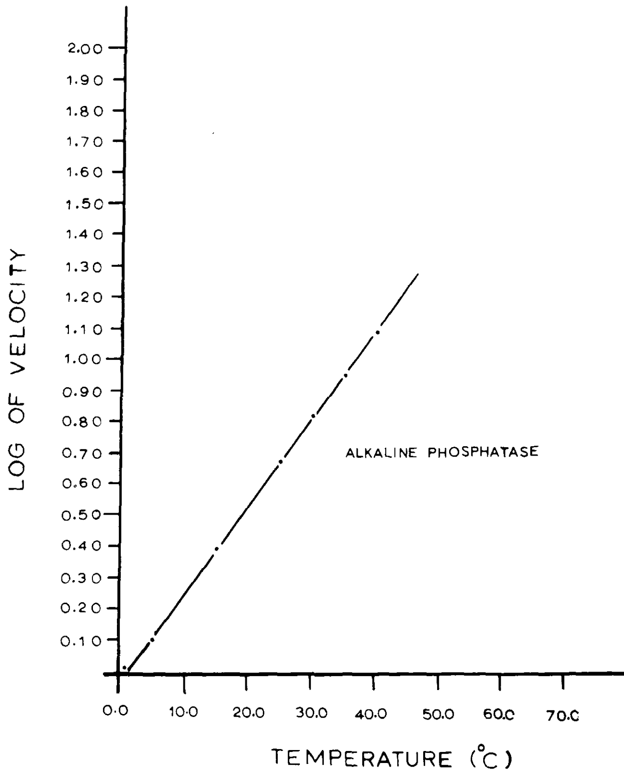

FIGURE 7. Temperature coefficient graph for alkaline phosphatase.

Enzyme Activation and Inhibition

Alkaline phosphatase activity was greatly enhanced by the addition of $MnCl_2$, $CuCl_2$, $CoCl_2$, $MgCl_2$, and $BaCl_2$, and slightly enhanced by the addition of $HgCl_2$ and $Mg(CH_3COO)_2$. It was greatly inhibited by the addition of Na_2HAsO_4, $NaHPO_4$, $CuSO_4$, and $FeCl_2$, and moderately inhibited by the addition of $CdCl_2$, NaCN, and $Hg(CH_3COO)_2$. Among the latter, Na_2HAsO_4 produced the maximum inhibition, while $C_6H_5Hg(CH_3COO_2$, NaCl, KCl, NaF, $ZnSO_4$, $CaCl_2$, $AlCl_3$, and $Ca(CH_3COO)_2$ had a negligible effect (fig. 8). Maximum activation was produced by a 0.01–M $MnCl_2$ solution (fig. 9).

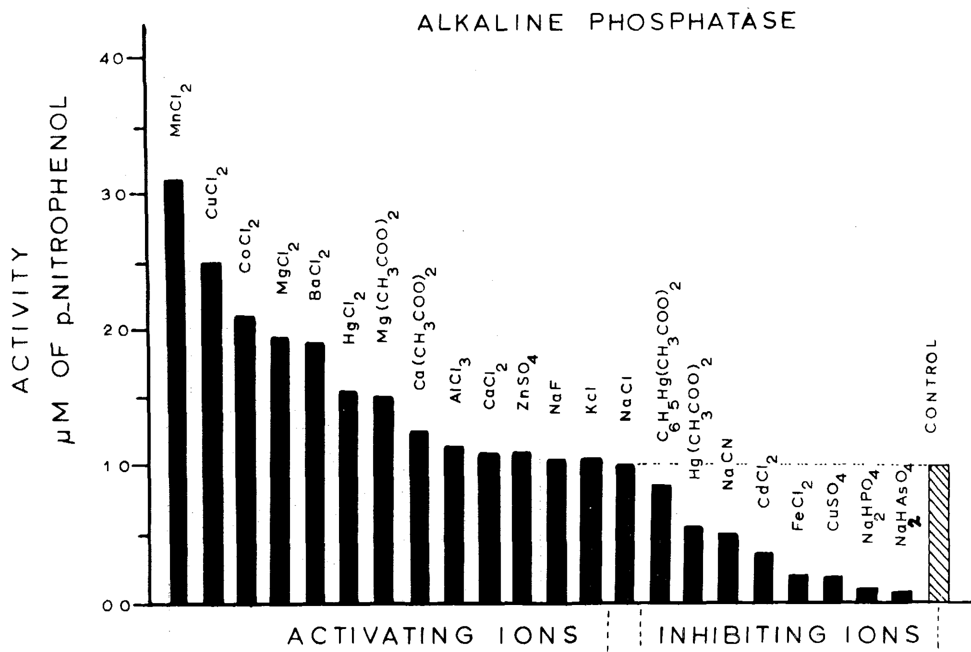

FIGURE 8. Influence of activating and inhibiting ions on the rate of alkaline-phosphatase activity.

DISCUSSION

The optimum pH for alkaline phosphatase was found to be 7.4. This pH value is comparable with 7.3 reported by Ashrafi (1960) for the alkaline phosphatase in the whole homogenate of the stable fly, *Stomoxys calcitrans* (L), same procedure.

The enzyme activity was linear with the increase of the homogenate concentration. Fitzgerald (1949) showed a similar relationship with respect to alkaline phosphatase in the egg of the grasshopper, *Melanoplus differentialis*, (Thomas). Although the tissue and the substrate are different, both of these are hemimetabolous insects and belong to the same order.

A zero-order reaction (fig. 3) was found at 0.0006 M concentration of *p*-nitrophenylphosphate disodium, while Ashrafi (1960) reported the same at 0.0012 M of the same substrate with the stable fly. The Km value for this enzyme was found to be 1.4 x 10^{-4} M, whereas Ashrafi (1960) reported the Km value to be 4.0 x 10^{-4} M for stable fly phosphatase. Lambremont and Schrader (1964) reported the Km value to be 9.39 x 10^{-4} M for pyrophosphatase in the boll weevil. The Km values reported by Wojtczak (1956) and by Hodgson and Kumar (1964)

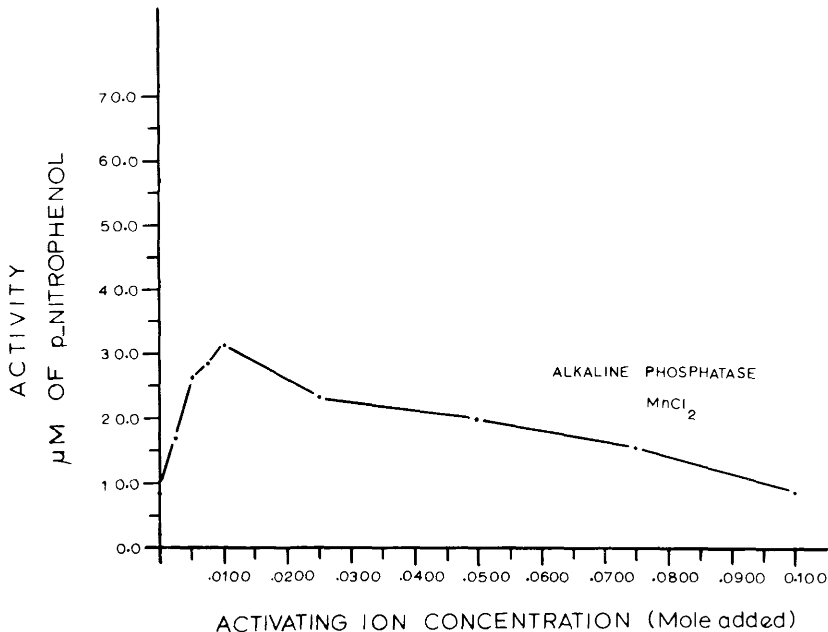

FIGURE 9. Optimum manganese-ion concentration required for alkaline phosphatase activity.

differ appreciably from the present value. This may be due either to the difference of the substrate or to the source of enzyme (i.e. insect species used). The low Km value supports the view of Gilbert and Huddleston (1965) that *p*-nitrophenylphosphate disodium is required in lesser amounts than other substrates. Moreover, this value also indicates that the concentration of the substrate (0.0014 M) used during the experiments was adequate for maximum hydrolysis.

A zero-order kinetics was maintained for 25 minutes. This is in agreement with the observations of Ashrafi (1960), who used the same procedure. Moreover, the incubation period (30 minutes) used for testing inhibitors and activators was near the point of maximum hydrolysis and within the period of 40 minutes, after which the rate of hydrolysis decreased considerably. The difference in the optimum incubation period, as compared with the reports of Rockstein (1956) and of Lambremont and Schrader (1964), may be due to differences in species and/or substrates.

Maximum hydrolysis of *p*-nitrophenylphosphate disodium occurred at 40°C and a sharp decrease above that temperature supports the findings of Sizer (1943). In the present problem, the Q_{10} values (mentioned in the results) coincide with those reported by Rockstein and Herron (1951) and by Ashrafi (1960).

Analysis of the results shown in Figure 8 indicates that $MnCl_2$, $CuCl_2$, $CoCl_2$, $MgCl_2$, $BaCl_2$, $HgCl_2$, $Mg(CH_3COO)_2$, and $Ca(CH_3COO)_2$ activated the enzyme. $MnCl_2$ activated the enzyme threefold, whereas, $CuCl_2$, $CoCl_2$, $MgCl_2$, and $BaCl_2$ activated the system to approximately twice its basic activity. Ashrafi (1960) reported activation by Fe^{++}, Co^{++}, Cd^{++}, Mn^{++}, and Mg^{++}. In the present case, $FeCl_2$ and $CdCl_2$ were found to be inhibitors. This indicates that the enzyme, alkaline phosphatase, found in the desert locust is of a slightly different nature than the phosphatase reported by Ashrafi (1960) in the stable fly. Further analysis indicates that $CuCl_2$ activated the enzyme, whereas, $CuSO_4$ inhibited it. It is suggested that inhibition by $CuSO_4$ is due to $SO_4^=$ rather than to Cu^{++}. Inhibition

by Cu^{++}, as reported by Lambremont and Schrader (1964), is due either to the difference in insect species or to the concentration of the salt.

As another example of both activation and inhibition by the same cation, $HgCl_2$ activated the enzyme and $Hg(CH_3COO)_2$ inhibited it. This indicates that the inhibition by $Hg(CH_3COO)_2$ may be due to CH_3COO^-, rather than to Hg^{++}. This view is supported by the fact that more activation was produced by $MgCl_2$ than by $Mg(CH_3COO)_2$. Mg^{++} is present in both compounds and so the factor decreasing activation is the CH_3COO^- which has replaced Cl^-. Hodgson and Kumar (1964) reported Hg^{++} as an inhibitor, which may be due either to the species difference or to the higher concentration used, because, beyond a certain optimum concentration, activating ions may start inhibiting (Lambremont, 1959). It was also observed that NaCl had no effect, while NaCN, Na_2HPO_4, and $NaHAsO_4$ inhibited the activity. This suggests that inhibition by the latter three compounds is due to the anions, rather than to the Na^+ cation. Cyanide has been reported as an inhibitor by Fishman, Green, and Ingilis (1962), and by Lambremont and Schrader (1964) for the alkaline phosphatase of other animals. Although calcium and fluoride have been reported as inhibitors by Lambremont and Schrader (1964) and zinc by Fishman *et al.* (1962), they produced no effect in the present study.

CONCLUSIONS

On the basis of these results, it may be concluded that the alkaline phosphatase of the intestine of the desert locust may be closely related to those derived from the stable fly, the blow fly, and the boll weevil. Despite the small variations observed with respect to optimum pH, Km value, incubation period, temperature, and behaviour with salt solutions, this enzyme may be grouped among the alkaline phosphatases (3.1.3.1) according to Dixon and Webb (1964).

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