Z. Ernährungswiss. **20**, 44–54 (1981)
© 1981 Dr. Dietrich Steinkopff Verlag, Darmstadt
ISSN 0044-264 X

The Egyptian Organization for Biological Products and Vaccines, Agouza (Cairo)

Acid and alkaline phosphomonoesterases in Egyptian snake venoms

F. Hassan, M. F. S. El-Hawary, and *A. El-Ghazawy*

With 4 figures and 3 tables

(Received September 16, 1980)

The action of snake venom phosphatases has been suggested by Zeller (23) to be related to shock symptoms. Investigations on the activity, property, and characteristics of phosphomonoesterases in various snake venoms have attracted the attention of many workers, Kay (9), Lundsteen and Vermehren (14), Shinowara et al. (18).

Studies on different snake venoms by a number of workers – Yang and Chang (22), Laskowski et al. (12), Kaye (10), Bjork (3) and Jimenez-Porras (8) – showed certain variations that proved to be of remarkable value when considering the variable effects of different snake venoms.

No similar studies on any of the Egyptian snakes could be traced in the available literature.

The present study deals with investigations on acid and alkaline phosphatases in four of the most common species in Egypt, namely: two viperids, *Cerastes cerastes* and *Cerastes vipera,* and two elapids, *Naja haje* and *Naja nigricollis.*

Materials and methods

Venoms used were milked in Agouza snake farm (Cairo) and venoms collected were either used fresh or frozen at – 20 °C, then desiccated under vacuum as described by Hassan and El-Hawary (6). Venom solution of suitable concentration was adopted following pilot experiments, these were: 5 % for the two vipers and 1 % for the two elapids, prepared in physiological saline.

King-Armstrong method as described by Wootton (21) was used for the determination of the activities of both acid and alkaline phosphatases.

Optimum pH values were investigated in the range of 3–7 for acid phosphatase or 7–12 for alkaline phosphatase. Optimum substrate concentrations were studied in the range of 0.001–0.200 M under the obtained pH optima. The effect of activators and inhibitors was studied by the addition of 0.1 ml of either reagent to the venom buffer mixture in a final concentration of 0.01 M. Effect of heat was studied by heating the venom solutions for 10 minutes at different temperatures between 10 and 60 °C, then estimating the residual activity.

Results and discussion

Table 1 shows the levels of acid and alkaline phosphatase activities in the investigated snake venoms in comparison to human serum. The mag-

Table 1. Mean values for acid and alkaline phosphatase activities in snake venoms as compared to those of human serum.

Sample	Acid phosphatase activity $\times 10^{-3}$		Alkaline phosphatase activity $\times 10^{-3}$	
	range	mean	range	mean
Human serum	0.38– 0.52	0.46±0.04	1.28– 2.22	1.75±0.29
C. cerastes venom	0.69– 0.92	0.80±0.05	1.45– 2.37	2.00±0.24
C. vipera venom	0.54– 0.83	0.70±0.06	0.95– 2.03	1.50±0.27
N. haje venom	2.95– 4.22	3.60±0.29	23.5 –42.2	30.00±4.50
N. nig. venom	12.6 –21.5	16.00±2.32	6.00–13.80	10.00±1.38

Mean of 10 estimations on different samples.
King-Armstrong units/1 mg protein.

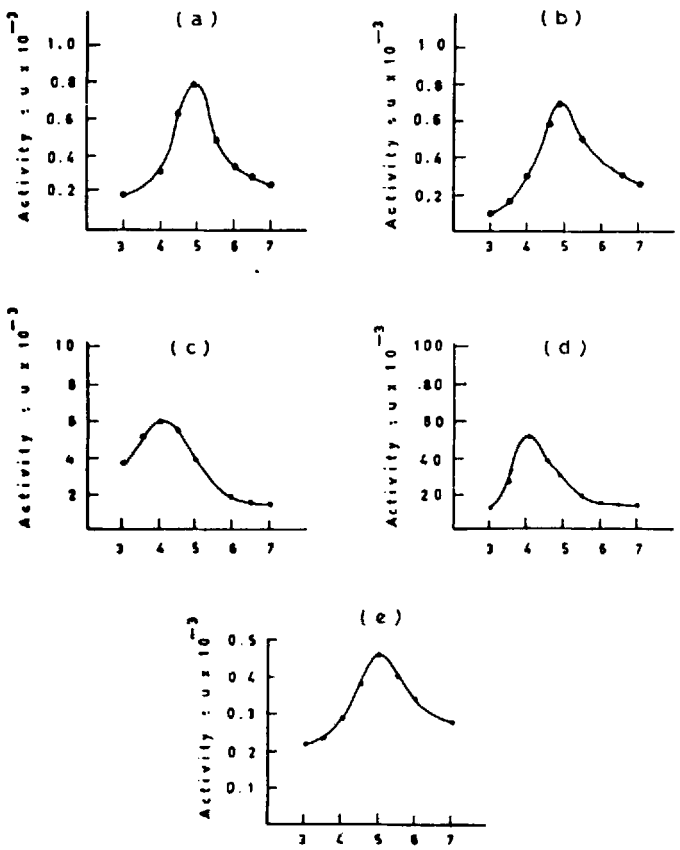

Fig. 1a–e. pH – acid phosphatase activity curves of the investigated snake venoms compared to that of human serum (a) C. cerastes; (b) C. vipera; (c) N. haje; (d) N. nigricollis and (e) human serum.

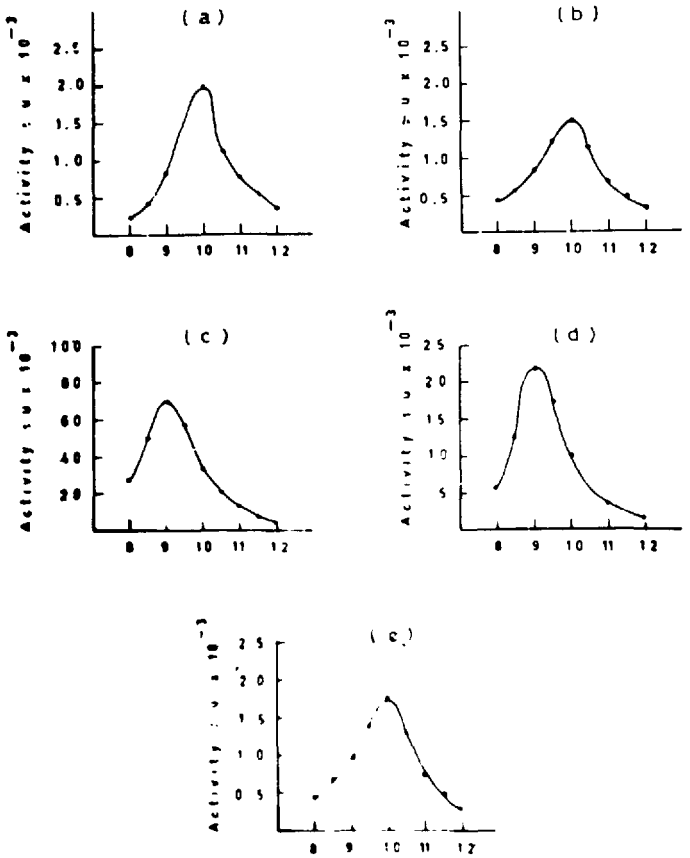

Fig. 2a–e. pH – alkaline phosphatase activity curves of the investigated snake venoms and human serum (a) C. cerastes; (b) C. vipera; (c) N. haje; (d) N. nigricollis and (e) human serum.

nitude of the activity was in the order of Elapidae > Viperidae; of interest, the activity of both enzymes was more or less the same in the two viperids; while their strength varied remarkably between the members of elapids. N. nigricollis only showed acid phosphatase activity exceeding its alkaline

Table 2. Optimum substrate concentrations and their corresponding Km values.

Sample	Acid phosphatase		Alkaline phosphatase	
	Optimum substrate conc. (M)	Km	Optimum substrate conc. (M)	Km
C. cerastes	0.100	0.028	0.100	0.025
C. vipera	0.100	0.025	0.100	0.026
N. haje	0.125	0.037	0.150	0.029
N. nigricollis	0.150	0.042	0.125	0.038
H. serum	0.010	0.004	0.010	0.005

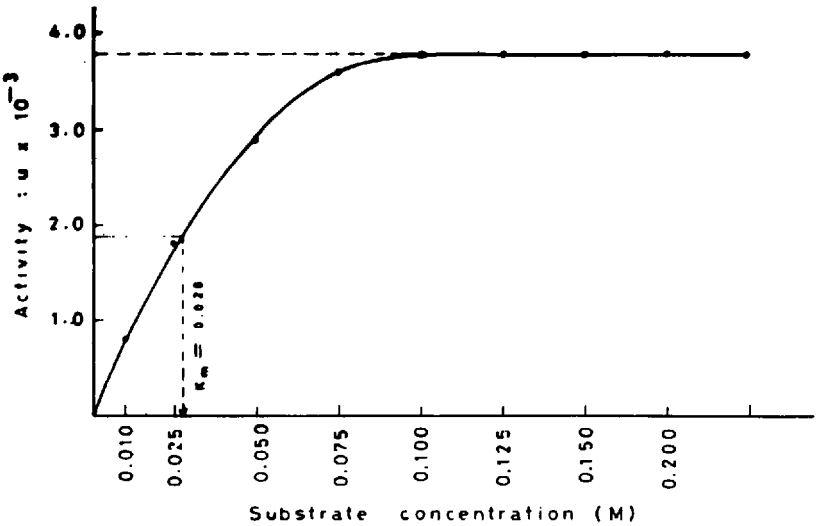

Fig. 3a. Substrate concentration – acid phosphatase activity curve for venom of C. cerastes.

one. This is parallel to similar results reported by Tu and Chua (20). When the activity of both enzymes in the snake venoms was compared to those of human serum, the former seemed to be a very rich source, especially elapids supporting similar findings by Richards et al. (15).

Figures 1 and 2 show that the optimum pH for acid and alkaline phosphatases are 4.9 and 10.0 in the 2 vipers, while 4.0 and 9.0 for the 2

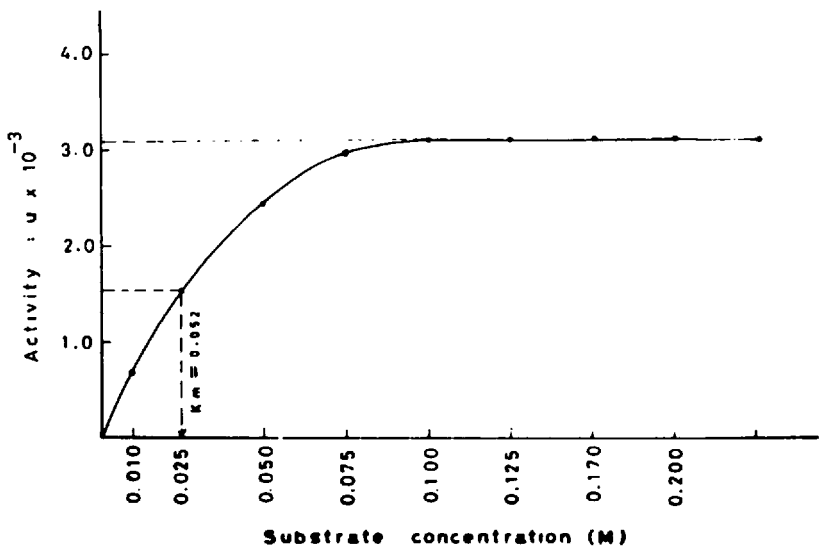

Fig. 3b. Substrate concentration – acid phosphatase activity curve for venom of C. vipera.

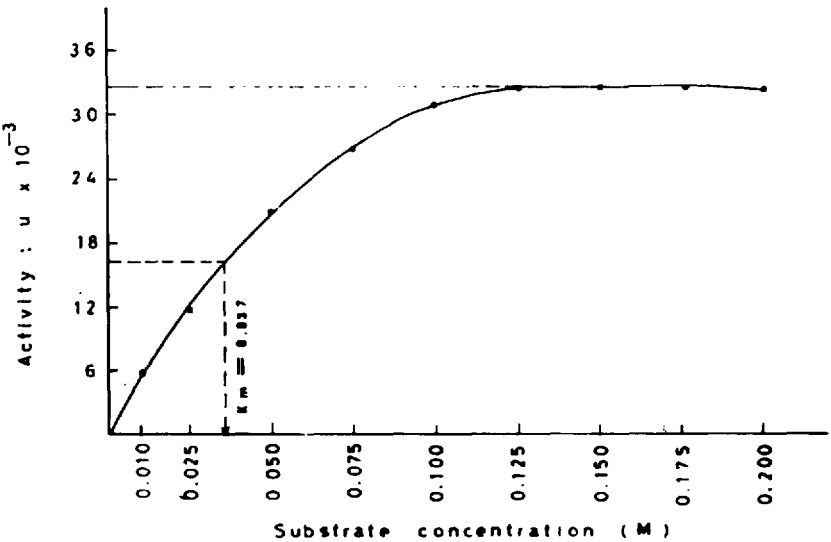

Fig. 3c. Substrate concentration – acid phosphatase activity curve for venom of N. haje.

elapids respectively. These optima are close to those reported by *Tu* and Chua (20), working on 3 crotalids and 3 elapids, and Setogouchi et al. (17), working on sea snakes. Comparable results for human serum enzymes are 4.9 and 10.0, respectively (Wootton 21).

Optimum substrate concentrations and their corresponding Km values (Michael-Menten constants) for both activities in tested venoms are shown

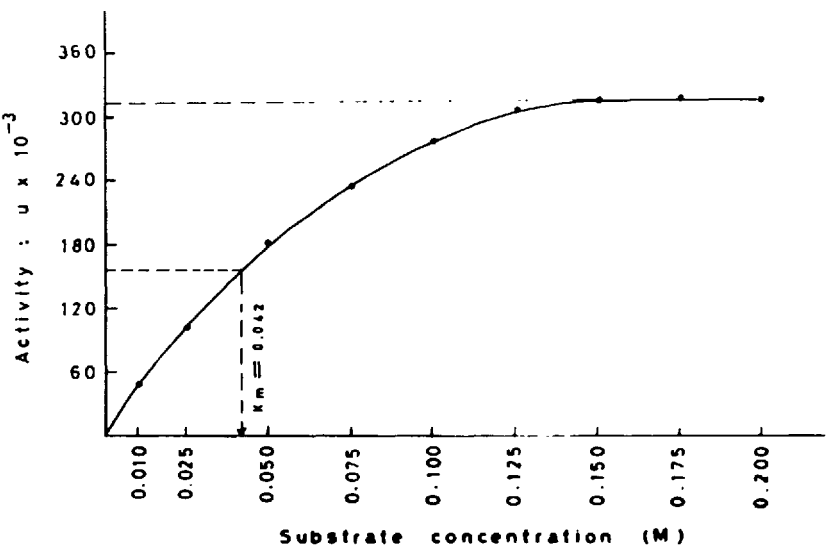

Fig. 3d. Substrate concentration – acid phosphatase activity curve for venom of N. nigricollis.

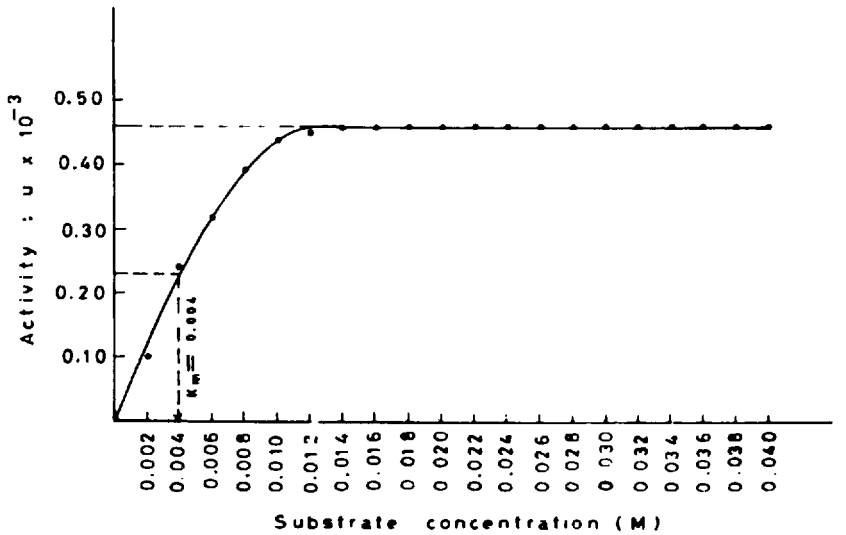

Fig. 3e. Substrate concentration – acid phosphatase activity curve of human serum enzyme.

in table 2. Both values exceed those for corresponding enzymes. The high venom-enzyme content and activity may reflect their essential role, at least partially in precipitating the toxic effects of the venoms.

Linear relationship could be demonstrated between enzyme activity and temperature in the range of 37–40 °C; beyond this range deterioration in the enzyme activity occurred. Brown (4) noted that the esterases of Crotalus atrox are inactivated between 50 and 75 °C when heated for 1 hour Setogouchi et al. (17) showed that the phosphatases of L. semifasciata are heat-labile when heated for 10 minutes at 57 °C.

Table 3 shows the effect of the activators and inhibitors on the activity of both enzymes in the tested venoms as compared to those of human serum. Mg^{++} activated both enzymes of either venoms and human serum. Similar findings were reported by Roche (16), Jenner and Kay (7) and Davis (5) when working with mammalian erythrocytes. On the other hand, Kutscher and Wolbergs (11) could not prove the activation of Mg^{++} on acid phosphatase when working with the prostatic enzyme or human erthrocytes. However, Abul-Fadl and King reported a slight inhibitory effect of Mg^{++} on the prostatic acid phosphatase. Laskowski et al. (13) found that nonspecific alkaline phosphatase of *Bothrops atrox* venom is activated by Mg^{++}. The activatory effect of this ion might explain its addition to the reaction mixture in the experiments of Setogouchi et al. (17) in the venom of *L. semifasciata.*

Zn^{++} was found to be with no effect on the activity of all venoms' acid phosphatase, and the viperids' alkaline phosphatases, but proved to be inhibitory for elapids' alkaline phosphatases.

Arvy (2) isolated a purified alkaline phosphatase enzyme from islet tissue of the pancreas of *vipera aspis* and *vipera berus* and demonstrated the presence of zinc in the islet cells. Suzuki (19), working on thirteen

Table 3. Acid and alkaline phosphomonoesterase activities in snake venoms as compared to those of human serum under effect of Mg^{++}, F^{--}, and EDTA.

Experiment	C. cerastes		C. vipera		N. haje		N. nigricollis		N. serum	
	Ac. ph.	Alk. ph.	Ac. ph.	Alk. ph.	Ac. ph.	Alk. ph.	Ac. ph.	Alk. ph.	Ac. ph.	Alk. ph.
Mg^{++}	137 %	140 %	142 %	145 %	153 %	148 %	147 %	152 %	145 %	150 %
Zn^{++}	100 %	100 %	100 %	100 %	100 %	21 %	100 %	15 %	112 %	100 %
F^-	zero	100 %	zero	100 %	zero	100 %	zero	100 %	zero	100 %
EDTA	100 %	100 %	100 %	100 %	37 %	100 %	28 %	100 %	100 %	100 %

Enzyme-activity estimated in crude material without any interference is considered 100%.

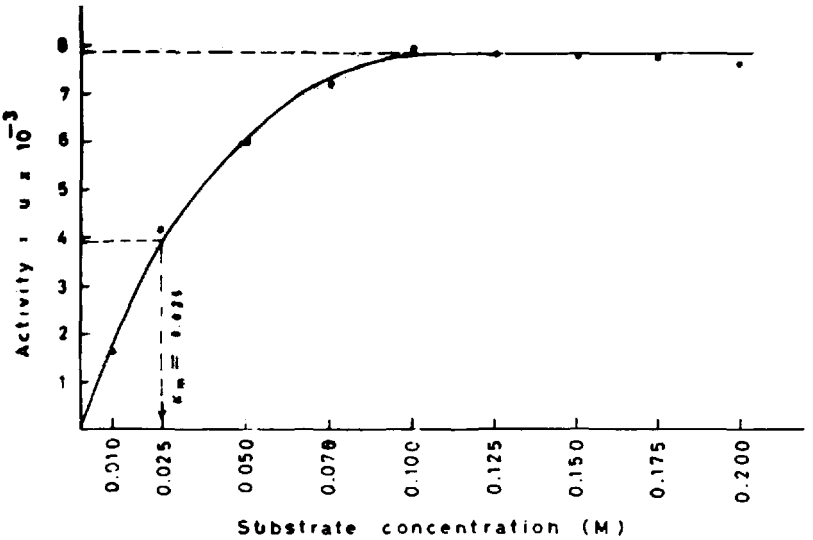

Fig. 4a. Substrate concentration – alkaline phosphatase activity curve for venom of C. cerastes.

venoms of Japanese and Formosan snakes, demonstrated the presence of considerable amounts of zinc and small amounts of magnesium in these venoms by emission spectrophotometry; and reported markedly inhibitory effect of Zn on alkaline phosphatase activity of all venoms studied. Tu and Chua (20), working on five elapids and three crotalids, reached similar conclusions.

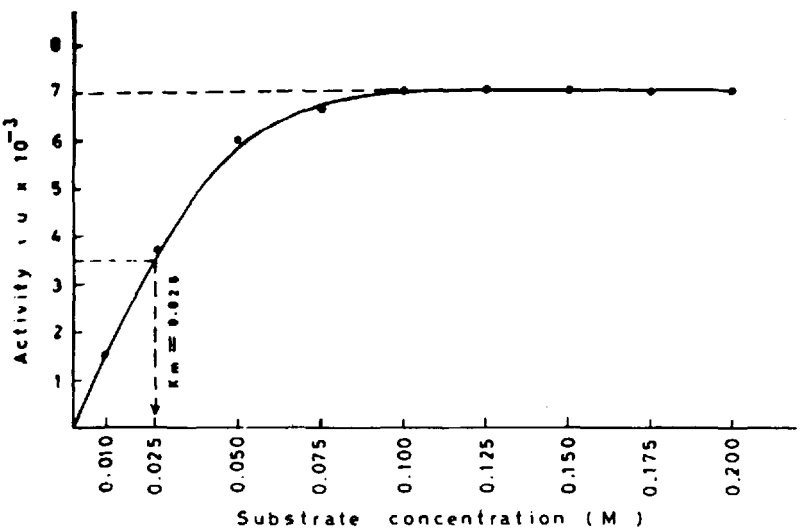

Fig. 4b. Substrate concentration – alkaline phosphatase activity curves for venom of C. vipera.

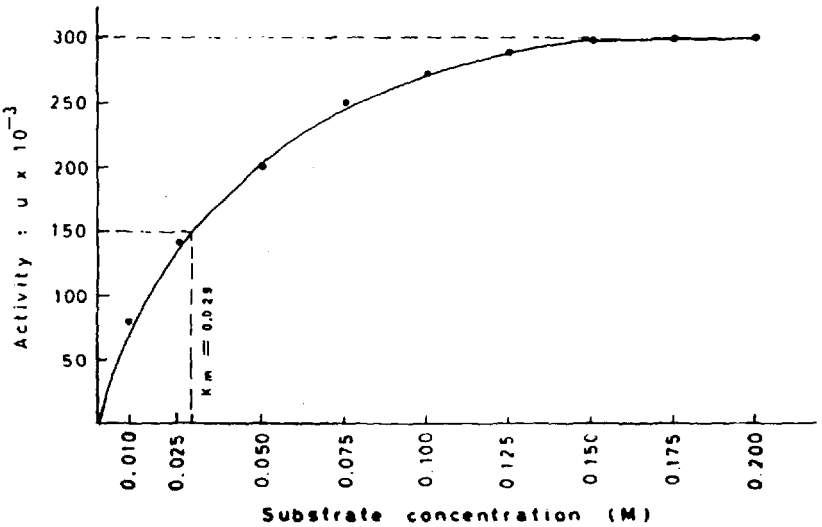

Fig. 4c. Substrate concentration – alkaline phosphatase activity curve for venom of N. haje.

Fluoride ions showed no effect on viperid alkaline phosphatases, but exhibited slight activatory effect on elapid alkaline phosphatase; on the contrary, snake-venom acid phosphatases were completely inhibited by fluoride ions. Similar observation was reported by Tu and Chua (20) when working on alkaline phosphatase of *N. haje samarensis* and *N. haje.*

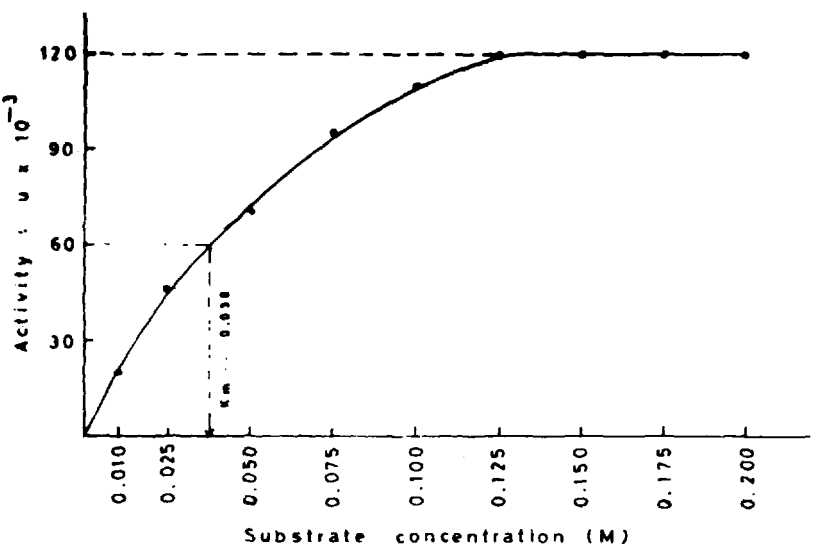

Fig. 4d. Substrate concentration – alkaline phosphatase activity curve for venom of N. nigricollis.

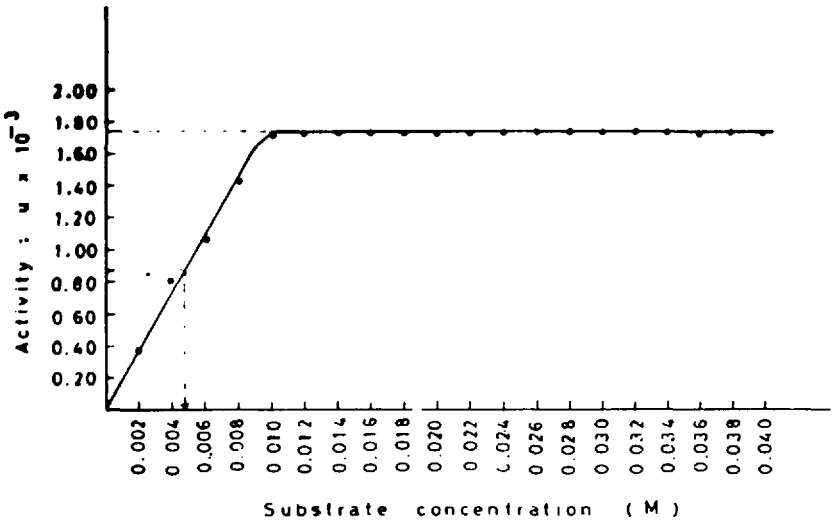

Fig. 4e. Substrate concentration – alkaline phosphatase activity curve for human serum enzyme.

The effect of EDTA on the activity of different venom acid and alkaline phosphatases was extremely variable. Only acid phosphatase of *N. haje* and *N. nigricollis* were inhibited while alkaline and acid phosphatase of the viperid enzymes were not influenced.

In conclusion, it is apparent that venom acid and alkaline phosphatases are substrate specific, but differences were observed in their pH optima, substrate concentration, and behaviour towards activators and inhibitors. This may suggest the existence of different isoenzyme patterns, which needs further investigations. In addition, such variations seem to support the idea of biochemical classification rather than the morphological one.

Summary

Non-specific acid and alkaline phosphomonoesterases could be demonstrated in two viperids (*Cerastes cerastes* and *Cerastes vipera*) and two elapids (*Naja haje* and *Naja nigricollis*). The latter could be a natural source for the production of these enzymes.

The activities of both enzymes in elapids were greater than in viperids. *N. nigricollis* was the only to show acid phosphatase activity exceeding its alkaline one.

The optimum pH values recorded for acid phosphatase was 4.0 and 4.9 and for alkaline phosphatase 9.0 and 10.0 in viperids and elapids, respectively.

Optimum substrate concentration for both enzymes in viperids was 0.01 M, while for acid phosphatase in *N. haje* and *N. nigricollis* it was 0.125 and 0.150 M; and for their alkaline phosphatases the values were 0.150 and 0.125 M, respectively.

Mg^{++} behaved as an activator for both enzymes in all venoms investigated, while Zn^{++} showed either no or slight activating effect. Fluoride ions as well as EDTA showed certain inhibitory action.

Both enzymes in the crude venoms were heat-labile.

Key words: elapids, viperids, phosphomonoesterases

References

1. Abul-Fadl, M. A. M., E. J. King: Biochem. J., **45,** 51 (1949).
2. Arvy, L.: Comp. Soc. Biol., **153,** 8 (1959).
3. Bjork, W.: Biochim. Biophys. Acta, **49,** 195 (1961).
4. Brown, J. H.: Toxicon, **4,** 99 (1966).
5. Davis, D. R.: Biochim. J., **28,** 529 (1934).
6. Hassan, F., M. F. El-Hawary: Egypt. J. Phys. Sci., **1/1,** 19 (1974).
7. Jenner, H. D., H. D. Kay: Brit. J. Exp. Path. **13,** 22 (1932).
8. Jimenez-Porras, J. M.: Toxicon, **2,** 155 (1964a).
9. Kay, H. D.: Biochem. J., **22,** 1446 (1928).
10. Kaye, M. A. G.: Biochim. Biophys. Acta **38,** 34 (1960).
11. Kutscher, W., H. Wolbergs: Z. Physiol. Chem., **236,** 237 (1935).
12. Laskowski, M., G. Hagerty, U. R. Laurila: Nature **180,** 1181 (1957).
13. Laskowski, E., W. Bjork, SR. M. Laskowski: Biol. Chem. J., **238,** 2477 (1963).
14. Lundsteen, E., E. Vermehren: C. R. Trav. Lab. Carlsberg, Serie chim. **21,** 147 (1936).
15. Richards, G. M., G. Du Vaire, M. Sr. Laskowski: Biochemistry **4,** 501 (1965).
16. Roche, J.: Bull. Soc. Chim. Biol. **13,** 841 (1931).
17. Setoguchi, Y., S. Morisawa, F. Obo: Acta Medica Univ. Kagoshima **10,** 53 (1968).
18. Shinowara, G. Y., L. M. Jones, H. L. Reinhart: Biol. Chem. **142,** 921 (1942).
19. Suzuki, T.: Butantan Symp. Interance **33,** 519.
20. Tu, A. T., A. Chua: Comp. Biochem. Physiol. **17,** 297 (1966).
21. Wootton, I. D. P.: Microanalysis in Medical Biochemistry, 4th ed. (London 1964).
22. Yang, C. C., L. T. Chang: J. Formos. med. Ass., **53,** 609 (1954).
23. Zeller, E. A.: The Enzymes (Summer, J. B., and Myrback, K., eds.) vol. 1, Part 11, p. 987 (N.Y. 1951).

Authors' address:

Prof. Dr. *M. F. S. El-Hawary,* Head of Biochem. Dept., National Research Centre, Dokki, Cairo (Egypt)