nucleus to the site of cell division, (3) organization of the cytoplasmic site to undego division and (4) occurrence of cell division.

ACKNOWLEDGEMENTS

Our thanks are due to Prof. M. R. Suxena, Head, Department of Botany, Osmania University, for giving facilities and encouragement.

1. Clowes, F. A. L. and Juniper, B. E., *Plant Cells,* Blackwell Scientific Publications Ltd., Oxford and Edinburgh, 1968.
2. Johansen, D. A., *Plant Microtechnique,* McGraw-Hill Book Co., New York, 1940.
3. Kamia, N., *Ann. Rev. Plant Physiol.,* 1960, 11, 323.
4. Machlis, L. and Rawitscher Kunkel, E., "Mechanism of gametic approach in plants," *Fertilization,* Vol. I, Ed., Metz, C. B. and Monroy, A., Academic Press, London, 1967.
5. Maheshwari, P., *An Introduction to the Embryology of Angiosperms,* McGraw-Hill Book Co., New York, 1950.
6. Ramayya, N., "The development of trichomes in the compositae," *Recent Advances in Plant Anatomy,* Ed., Chowdhury, K. A., Hindustan Publishing Corp., Delhi-7, 1969.
7. — and Prabhakar, M., *Curr. Sci.,* 1973, 42, 376.
8. — and Rajgopal, T., *Jour. Osmania University (Sci.),* Golden Jubilee Vol., 1968, 4, 147.
9. Rebhum, L. I., "Saltatory particle movements and their regulation to the mitotic apparatus," *The Cell in Mitosis,* Ed., Levine, L., Academic Press, London, 1963.
10. Thaine, R,. *New Phytol.,* 1965, 64, 118.
11. Uphof, J. C. Th., "Plant Hairs", *Handb. Pflanzenanat.,* Band IV, Teil 5, Ed., Zimmermann, W. and Ozenda, P., Gebruder Borntraeger, Berlin, 1962.

INFLUENCE OF SCORPION VENOM ON ENZYME SYSTEMS OF SCORPION *HETEROMETRUS FULVIPES*

V. R. SELVARAJAN, Ch. RADHAKRISHNA MURTHY AND K. S. SWAMI*

Department of Zoology, S. V. University, Tirupati, 517502, India

ABSTRACT

Presence of cannibalistic behaviour amongst scorpions was observed. Addition of venom to the hepatopancreatic homogenate had no influence on the activity of succinate dehydrogenase while glutamate dehydrogenase was elevated. In the homogenate of cephalothoracic neuronal mass, the venom inhibited succinate dehydrogenase and increased glutamate dehydrogenase. These differential effects of venom are discussed.

INTRODUCTION

AN observation of cannibalistic behaviour in the scorpion, *Heterometrus fulvipes,* where one scorpion injects its venom and immobilizes the other, prompted us to study the effects of scorpion venom on the enzyme systems of scorpion tissues. Venom from different species of scorpions were shown to have six protein fractions[3–6]. Oommen and Kurup[4] have suggested that the toxicity exists in the fractions having cathodic mobility and the relative toxicity of the venom depends on the proportions of the cathodic protein fractions[7]. Earlier investigations in our laboratory have shown that the administration of scorpion venom into cockroach inhibits respiration and decreases body temperature, succinate and lactate dehydrogenase, and acetylcholinesterase activity levels in the muscle and ventral nerve cord[3]. However, no reports are available on the effects of scorpion venom on its own tissues. Two enzymes were chosen as representatives of oxidative and amino acid metabolisms.

To whom the reprint requests should be made.

MATERIALS AND METHODS

Scorpions were collected from local hilly terrain and were adapted to the laboratory conditions. They were kept in separate glass jars and were fed daily with cockroaches.

Venom was collected from freshly collected animals by applying electric shocks upto 15 V in the post-abdominal region with an Electronic Stimulator (Seemax, ST-5, Ambala). The venom was collected into a syringe and diluted with pH 7·4 (0·05 M) phosphate buffer. Protein level (2 mg/ml) was used as a check to obtain same dilution every time. Fresh venom was collected for each experiment.

Hepatopancreas and cephalothoracic nerve mass (referred as brain) were isolated from scorpions. Tissue homogenates were prepared in ice-cold 0·25 M sucrose solution in Potter-Elvehjem glass homogenizer. The homogenates were centrifuged

at 3000 rpm for 15 min. and the supernatant was used for assay.

Succinate dehydrogenase (succinate : acceptor oxidoreductase E.C. 1·3·991) and glutamate dehydrogenase (glutamate : NAD oxidoreductase E.C. 1·4·1·3) were assayed by modified dye reduction method[1]. 2·0 ml of the reaction mixture contained 50 μmoles of sodium succinate or 100 μmoles of sodium-L-glutamate, 100 μmoles of pH 7·4 phosphate buffer, 4 μmoles of INT [2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride] and 0·1 μmole of NAD (for glutamate dehydrogenase only). The reaction was started by adding 0·2 ml of 10% (W/V) hepatopancreatic homogenate or 0·5 ml of 1% brain homogenate. The incubation was carried out for 30 min. at 37° C, and the reaction was stopped with 5 ml of glacial acetic acid. The formazan formed was extracted overnight into 5 ml of toluene and the intensity of the colour was measured at 495 mμ with Bausch and Lomb Spectronic-20.

The experimental tubes received 0·1 ml of the venom while control tubes received same amount of distilled water. In preincubation studies, the homogenate was preincubated with venom and 100 μmoles of phosphate buffer (pH 7·4) for 10 min., and the aliquots were assayed for enzymic activity. Protein was determined by the method of Lowry *et al.*[2].

Results and Discussion

Succinate dehydrogenase of hepatopancreas was little affected by the venom while the brain enzyme was inhibited (Table I). The per cent inhibition was increased by preincubating the homogenate with venom and substrate could not protect the enzyme (Table I). Glutamate dehydrogenase activity was elevated by venom in both the tissues, but the

Table I

Levels of succinate dehydrogenase activity (expressed as micro moles of formazan/mg protein/hr) in normal and venom treated tissues of scorpion

	Hepatopancreas		Brain	
	Mean ± S.D.	Percentage Change	Mean ± S.D.	Percentage Change
Control	0·440 ± 0·004	Nil	0·320 ± 0·004	Nil
Homogenate *plus* venom	0·445† ± 0·007	Nil	0·157* ± 0·006	−50·9
Homogenate *plus* Succinate *plus* venom (preincubated)	0·440† ± 0·006	Nil	0·114* ± 0·003	−64·3
Homogenate *plus* venom *minus* Succinate (preincubated)	0·435† ± 0·005	Nil	0·094* ± 0·005	−70·6

S.D. = Standard Deviation, * = Statistically significant $P < 0·01$.
† = Statistically Non-significant. Number of samples tested, five in each case.

Table II

Levels of glutamate dehydrogenase activity (expressed as micro moles of formazan/mg protein/hr) in normal and venom treated tissues of scorpion

	Hepatopancreas		Brain	
	Mean ± S.D..	Percentage Change	Mean + S.D.	Percentage Change
Control	0·116 ± 0·015	−	0·042 ± 0·0004	−
Homogenate *plus* venom	0·205* ± 0·006	+ 76·7	0·073* ± 0·0007	+ 73·8
Homogenate *plus* venom *minus* glutamate (preincubated)	0·266* ± 0·005	+ 120·6	0·059* ± 0·0004	+ 40·4

S. D. = Standard Deviation, * = Statistically significant $P < 0·01$.
Number of the samples tested were five in each case.

per cent elevation was different in the two tissues during preincubation (Table II).

The observed differential effects of the venom in the two tissues may be due to (*a*) the tissue specific differences in the enzyme systems and/or (*b*) the possible presence of a detoxifying mechanism in hepatopancreas which nullifies the toxicity of the venom thereby the oxidative enzymes are little affected and the absence of such a mechanism in the neuronal tissue eventually makes the venom, a neurotoxin. Lack of substrate protection in succinate dehydrogenase suggests the possibility that the venom is acting at a site other than the active site.

A drop in succinate dehydrogenase may lead to a drop in the overall oxygen consumption of the neuronal tissue thus resulting in the anoxic state which may ultimately inhibit the total neuronal activity. The cessation of action potentials was observed by earlier investigators in the ventral nerve cord of cockroach[3]. The elevation of glutamate dehydrogenase activity in both the tissues suggests the stimulation of ammonia production, which by itself is lethal[8]. Due to the depletion of energy sources by decreased oxygen consumption, ammonia may not be metabolized further[8]. Further, the increased activity of glutamate dehydrogenase and decreased oxygen consumption may affect the ratio of NADH/NAD, which influences the energy charge of the cell[9]. Thus it appears the venom exerts differential effects on different tissues.

Financial aid from Department of Atomic Energy and USPL 480 is greatly appreciated by V. R. S. and Ch. R. K. M.

1. Nandakumar, N. V., Radhakrishnamurthy, Ch., Vijayakumari, D. and Swami K. S., *Ind. J. Expt. Biol.*, 1973 (In press).
2. Lowry. O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
3. Sasira Babu, K., Murali Krishna Dass, P. and Venkatachari, S. A. T., *Toxicon*, 1971, **9**, 119.
4. Oommen, P. K. and Kurup, P. A., *Ind. J. Exp. Biol.*, 1964, **2**, 78.
5. Fischer, F. G. and Bonn, H., 1957, *Z. Physiol. Chem.*, 1957, **306**, 269.
6. Weissmann, A., Shulov, A. and Shafria, E., *Experientia*, 1958, **14**, 175.
7. Nitzan, M. and Shulov, A., *Toxicon*, 1966, **4**, 17.
8. Harper, H. A., *Review of Physiological Chemistry*, Langley Medical Publications, Chicago, 1971.
9. Atkinson, D. E., *Enzymes* (Ed. Boyer. P. D.), Academic Press, New York, Vol. 1, 1970.