THE ENZYMES OF THE EGYPTIAN SCORPION AND ALLIED SNAKE VENOMS AS TOOLS IN PHYSIOLOGICAL RESEARCH.

Thesis Submitted for
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BY

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(1) Effects of Echis carinatus venom on tissue and blood histamine and their relations to local tissue reactions and eosinophil changes. Toxicon (1968) Vol.6, p.51.

(2) Studies of phospholipase A & B activities of Egyptian snake venoms and a scorpion toxin. Toxicon (1969) Vol. 6, p. 293.

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PART I

INTRODUCTION

GENERAL INTRODUCTION

The scope of venom research has always been a most interesting field throughout the world. Early studies on venoms of snakes and scorpion were primarily directed towards the explanation of the mode of action of such venoms and attempting to find out a proper line of treatment of snake-bites and scorpion stings. The last two decades have witnessed great advances in the knowledge of the physiological effects and biochemical properties of venoms. Prominent among these was the study of several enzyme activities and their relation to some biological consequences following snake bites, notably toxicity, hemolysis, promotion or retardation of blood clotting and local tissue changes.

Snake venoms are considered to be some of the most important sources of many enzymes. The enzyme phospholipase A is known to be present in almost all venoms, by splitting the lipids in the membrane of red blood cells, it produces hemolysis, one of the serious effects of bites by such poisonous snakes. Release of histamine, adrenaline and acetylcholine and the formation of lysolecithin and prostaglandins are other effects attributed to venom phospholipase A.

Venom proteinases contribute to the production of shock following snake bites, by damaging the capillary walls with

loss of blood from the vascular bed, together with hemocoagulation. By its spreading action hyaluronidase enzyme facilitates the absorption of the venom from the site of envenomation and speeds up the process of poisoning. To the enzyme cholinesterase was ascribed the neuromuscular blocking action of the venom. Phosphatases, present in most venoms render the prey helpless by depriving it fromits immediate source of energy, ATP; added to this is the marked vasodilator effect by the products of their reaction, therefore participating in the production of shock. Still there are however, other enzymes in snake venoms which are of physiological, biochemical and pharmacological interest.

Scorpion venom also contains various enzymes, but they are fewer and less active than those in snake venoms. Progress in research on venom enzymes was achieved by the introduction of various fractionation techniques, by which many enzymes could be obtained in an almost pure state. This opened new vistas for the use of snake venoms in the field of biology, medical sciences and therapeutics.

V E N O M S

The venoms employed in this work were obtained from the Serpentorium of the Physiology Department, Faculty of Medicine, Ain-Shams University.

The venoms employed were those of Naja haje, Naja nigricollis, Walterinnesia aegyptia, Cerastes vipera, Cerastes cerastes, Echis carinatus, and Echis coloratus. The snakes were allowed to inject venom directly through a nylon diaphragm into an ice-cold container. The venom was immediately dried in a desiccator in vaccum at 5°C.

The Egyptian scorpion, Buthus quinquestriatus, venom was obtained by stimulating the glands with an induction shock, using a method after Strassberg and Russell (1962):

The scorpion was held with its cephalic-caudal axis in a vertical position by means of a pair of forceps placed lightly across the prosoma. Stimulating forceps were placed about the distal intersegmental membrane of the metasoma, and the metasoma was unfurled so that the vesicle and the aculens were directed into a collecting ice cold beaker. By the use of an electric stimulator set at 300 V and connected to the forceps, the intersegmental membrane was stimulated at a frequency of five shocks per second over a period

of 30-160 seconds. This stimulation produced 1 to 20 drops of venom from each scorpion. The venom obtained was immediately dried in a desiccator over anhydrous calcium chloride granules, in vaccuo at 5°C.

For assay the venom was dissolved in physiological saline immediately before use in the required concentration.

In some experiments fresh liquid venom was used immediately after collection.

Preparation of Chemically Purified Scorpion Toxin After Mohamed (1944):

A number of dried telsons were mixed with a small quantity of quartz sand wetted with one or two ccs. of tenth normal hydrochloric acid solution and ground thoroughly in a mortar for about half an hour. The finely ground mass was extracted with a decinormal hydrochloric acid using 30 ccs at a a time. Five extractions were sufficient to remove all the toxin. The insoluble residue was removed by decantation. The combined decanted extracts were neutralized with stirring with normal caustic soda. The toxin was precipitated as picrate by the addition of saturated picric acid solution and allowing the mixture to stand for 24 hours.

The clear liquid was separated by decantation and the

residue was extracted with 80 % acetone. Several extractions were necessary to remove all the soluble picrates. The picrate was further converted into the hydrochloride by precipitation of the combined extracts by adding excess of pure acetone and few drops of concentrated hydrochloric acid. The precipitate was centrifuged and filtered, washed first with more dry acetone and then with dry ether and finally dried in vaccum.

AIM OF THE PRESENT WORK

This work was initiated to make a general survey of the enzymatic activities of the various Egyptian snake venoms as well as scorpion toxin, and to elucidate some of their properties and their role in the production of the manifestations of envenomation. To gain more information about the role played by these enzymes in relation to the biological effects of venoms, separation of the different venom components was tried, and the enzymatic activities of the various fractions studied, as well as the lethal effects of each fraction. We have deliberately used the isolated venom enzymes as tools to unveil some physiological effects of venom poisoning.

Furthermore, the role of venom enzymes in the production of local tissue damage and histamine release by tissues has been investigated. We also tried the effects of some enzyme inhibitors on venom enzymes as well as on the lethality and local effects of snake venoms.

P A R T I I

ENZYMATIC STUDIES OF EGYPTIAN SNAKE VENOMS AND SCORPION TOXIN.

INTRODUCTION

THE presence of enzymes in snake venoms was not known until 1881, when de Lacerda reported proteolytic enzyme activity in Bothrops jararaca venom. In the subsequent years other enzymes, notably phospholipase A, were identified in the venoms of poisoncus snakes. The work done, in nearly fifty years, on venom enzymcs was reviewed by Houssay (1930) in a comprehensive article. Contribution of enzymes to the venom toxicity was first suggested by Iyengar et al (1938) and Kellaway (1939). Since then, rapid advances in our knowledge in this field has been achieved and the biological effects of most of the enzymes are being recognized.

Phospholipase A & B enzymes:

As early as 1898, Stephens and Myers observed the ability of snake venoms to lyse red blood cells. The presence of a small amount of plasma was found to be necessary for this effect (Flexner and Noguchi,1902); Kyes (1902) succeeded in demonstrating the ability of lecithin to replace plasma in this respect. Lüdecke (1905) pointed to the enzymatic degradation product of lecithin as being the agent responsible for the hemolytic action of snake venoms. Delezenne and Ledebt (1911) established conclusively that the remaining part of the lecithin molecule is , itself, the lytic substance ,

usually referred to as lysolecithin. The venom factor responsible for the conversion of lecithin into lysolecithin has thus been shown to be an enzyme, now called phospholipase A. This enzyme, also attacks cephalin resulting in the formation of lysocephalin which is also lytic for erythrocytes (Levene and Rolf, 1923).

Earlier studies seemed to show that the ester bond in the alpha position was the primary site of attack by the enzyme (Hanahan et al, 1954; Long & Penny, 1957), but later it has been found that lysolecithin is formed by the removal of the fatty acid bound in the beta position (Hanahan et al, 1960; Marinetti et al, 1960, de Haas and Van Deenen, 1961).

Phospholipase A has been detected in all snake venoms tested so far (Zeller, 1951; Neumann, 1953; Slotta, 1955; Jaques, 1955; Balozet, 1957), in bee venom (Neumann and Habermann, 1954), and in scorpion venom (Balozet, 1957). Nevertheless, Master et al (1963) reported the absence of phospholipase A in the venom of Indian scorpions. Ibrahim (1967) also denied the presence of phospholipase A in the venom of the Sudanese scorpion Leiurus quinquestriatus.

The enzyme phospholipase B catalyzes the splitting of a fatty acid radicle from lysophosphatids with the formation of glycerophosphoryl choline. Dorey and Pearson (1964) reported