



THE HAEMOLYSINS OF CHIRONEX FLECKERI AND

CHIOPSALMUS QUADRIGATUS

THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Submitted by

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S U M M A R Y



The modern concepts concerning the structure and metabolism of the red cell membrane have been reviewed, and the function of the membrane in relation to the transport of water, anions and cations have been discussed.

In this survey only those agents which produce haemolysis in vitro have been included. Such agents act directly on the red cell membrane and cause an increase in permeability and an osmotic imbalance between the cell and the surrounding medium, leading to rupture and release of haemoglobin.

Substances producing haemolysis in vitro are of diverse origins and compositions. They have been described under the categories of physical, chemical, biological and immunological agents. Occasionally these agents have physical and chemical properties which explain their mode of action; such as enzymes or chemical agents reacting with membrane constituents; or they have surface properties which allow them to penetrate and disorganize membrane structure. A number of model systems have been described which might be used to investigate properties of haemolysins and their mode of action.

The haemolysin of the box jellyfish Chironex fleckeri was initially examined using aqueous extracts of the tentacle. The haemolysin is a protein which was unstable in dilute solutions at room temperature. However, the tentacles and their extracts could be stored at temperatures of -20°C . or below for long periods without loss of activity.

Exclusion chromatography was used to separate tentacle extracts into two fractions, one which was

haemolytic (molecular weight, 70,000) and the other with lethal activity (molecular weight, 150,000). When administered parenterally the haemolytic fraction was also lethal producing cardio-respiratory failure. The skin necrotizing activity of the whole tentacle extract appeared to be confined to the haemolytic fraction.

Although the tentacle extracts contained a potent haemolysin, no clinical evidence of intravascular haemolysis has been described in human envenomation. This was considered to be due to the presence of inhibitory substances in the plasma and the instability of the lysin at normal body temperature.

The properties of the haemolysins of C. fleckeri and Chiropsalmus quadrigatus were compared. Although they were both proteins with a molecular weight of 70,000 approximately, they could be distinguished from each other by the relatively greater stability of C. fleckeri lysin at temperatures above 5 deg. There was evidence also that the mode of action of the two lysins was different. Antisera prepared against both extracts showed that the antihemolysins were species specific and no common antigenic properties were detected.

The mode of action of the haemolysin of C. fleckeri was examined. It was not an enzyme and it did not appear to have any unusual surface properties to explain its cytolytic action. The haemolysin showed no interactions with monolayers of individual components of the red cell membrane using surface pressure and surface potential measurements. However, there appeared to be specific complexing with a component in a mixed monolayer derived from red cell lipids. This might explain the mode of action of the haemolysin, but this observation would need further verification in monolayers prepared from individual membrane lipids.

DECLARATION

The studies described in the experimental section of this thesis have been reported in several papers (see below). All the research has been carried out under my direction, and I have been responsible for the preparation of the manuscripts and the work reported in those articles which bear my name alone or in which I appear as the principal author. In those papers where I appear as the minor author with H. D. Crone, he has been responsible for the biochemical aspects of the work and I have been responsible for the toxicological investigations. All the instrumentation and techniques developed for the investigations on monolayers have been my responsibility.

I declare that the thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and that to the best of my knowledge and belief the thesis contains no material previously published or written by any other person, except when due reference is made in the text of the thesis.

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