IMMUNOLOGICAL PROPERTIES OF GONADS OF *CHICOREUS RAMOSUS*

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INTRODUCTION

The role of antibodies in the immune system of a living organism assumes importance since these are recognized as foreign substances that are responsible for the initiation of the immune responses. These antibodies are known to agglutinate erythrocytes and presumed to be extracted only from plants (Stillmark, 1906). However, the exploration of marine bioactive agents for medicinal use has led to the search for antibody-like substances from marine organisms especially invertebrates since 1902 (Shimizu and Kamiya, 1983). Many marine invertebrate groups were investigated for antibodies or antibody-like substances and as a result, substances providing immunological protection, were identified from marine molluscs (Johnson, 1964; Prokop *et al.*, 1965) and fish ova (Prokop *et al.*, 1968). Further investigations revealed the extracts of the marine mollusc to be important sources of RSP's (receptor specific protein) which were considered as a primitive fixed specificity protection system (Gold and Balding, 1975).

The haemolymph of *Megathura crenulata* (Rowley & Rowley, 1968) and the blood of *Crassostrea virginica* has been reported to possess a powerful non specific agglutinin and the latter was presumed to play a significant role in the phagocytosis of substances injected into the organism (Tripp, 1966). Whole body extracts of clam *Saxidomus giganteus* were reported to possess the A antigen (Johnson, 1964). Extracts and eggs of the mollusc *Aeolidia papillosa* has been found to possess anti B agglutinin. Antibody-like substances (Agglutinins) were also reported from marine gastropods like *Trochus niloticus, Turbo speciosus, Haliotis asinina, Aplysia* sp (McKay, 1969) and *Buccinum undulatum* (Uhlenbruck *et al.*, 1970). Despite search for antibody-like substances, attempts to produce antibodies from the salivery gland extracts of the blue ringed octopus *Hapalochlaena maculosa* did not afford any significant protection against it (Sutherland *et al.*, 1970).

Hitherto, marine molluscs have formed an important source for antibodies. Hence, an attempt was made to study the immunological properties of the gonad extract of the muricid gastropod *Chicoreus ramosus*, by raising antibodies in rabbits. Presence of the immunologically related protein was verified by CIEP.

MATERIALS AND METHODS

Collection of samples

Samples of gastropods were collected from Mandapam, Gulf of Mannar region, southeast coast of India by diving at a depth of 10-30 m. The collected animals were kept in sea water for some days before use. The shells were broken opened with a hammer and the gonad part excised from the animal. Gonads excised from about 50 samples were minced and preserved in ethanol.

Preparation of extract

The gonads, minced in ethanol were homogenized in a Waring blendor in an equal volume of ethanol and distilled water. The homogenates were filtered. The filtrate was re-extracted with ethanol after overnight percolation and filtered. The filtered solution was centrifuged and the supernatant solution was evaporated to dryness at reduced pressure at 40±3°C. The crude extract, mixed with Tween 80 and diluted with sterile saline, was taken as the test solution for injection in rabbits.

Immunization of the rabbits

White rabbits, weighing 2-4 kg were injected with the homogeneous mixture of the gonad isolated

from *C. ramosus*. The marginal vein of the rabbit ear, located at the outer edge of the dorsal side of the ear, was considered for injection. The area to be injected was shaved off with a sharp razor blade and cleaned with 70% alcohol. For the 1st injection 1 ml of the gonad extract suspended in Tween 80 and saline was injected intravenously near the tip of the marginal vein so that scar tissue would not prevent injected material from entering the circulation. The next booster doses were given 5 times at 4 days intervals. Sterile syringes of 1 ml capacity was used for each intravenous injection.

Bleeding

After 1 week of the last injection blood was collected from the marginal vein. The area to be punctured was shaved off and washed with alcohol. The vein was punctured with a sterile syringe and the blood (5 ml) was collected in a sterile screw cap tube. Serum was isolated and collected by centrifugation. The isolated serum was stored at -20°C for further use.

Immuno-electrophoretic analysis

Immuno diffusion was carried out on agarose gel as described by Carpenter (1965). Melted agarose was poured on a microscopic slide covering it with 2.5 ml of agarose in barbital buffer, pH 8.2 (500 mlN 10^{-1}Sodium barbital, 150 mlN 10^{-1}HCl, 350 ml distilled water). When the agar was hardened, wells (holes) of 4-5 mm diameter and a trough of 1mm were cut off using well cutters. The cut agar was removed using a Pasteur pipette. The wells were filled with antigen, using a 0.5 ml syringe and 27 gauge needle. This slide was placed between the buffer reservoirs such that the ends of the slide were connected to the buffer solutions by a 2.5 cm filter paper strip.

Immuno electrophoresis was carried out in barbital buffer for 45 minutes with 5 mA current per slide. The slide was removed and the trough was filled with antiserum. The slide was placed in a level position above the moistened filter paper in a Petri dish and allowed to develop precipitation for 24 hrs. The agar was dried and stained to preserve the precipitin lines.

RESULTS AND DISCUSSION

When a bacterial, fungal or foreign animal cell is introduced as an immunogen, it is broken up in the host animal and many of its surface and internal components become immunogenic (Eisen, 1990). Immuno diffusion analysis of the slide filled with antigen and antisera suspended in barbital buffer exhibited a visible result of a serological reaction between the soluble protein and antiserum. After 24 hrs, a precipitin line was obtained between the soluble protein and the antisera confirming the homogeneity of the antigen. Therefore, this indicates that the antibodies in the antiserum (A_s) against the normal antigen has diffused to produce lines of precipitation. Antigens used for these tests were derived from microorganisms, plants and animals. The amount of the antibody in the antiserum was estimated by substracting the amount of antigen in the mixture with the largest precipitate from the total amount of precipitate since this precipitate contains all, or nearly all the antigen and antibody in the mixture (Carpenter, 1965). The amount of antibodies had relatively high titre value which means a higher production of antibodies. This agrees well with the fact that increasing amounts of antigen yielded greater antibody production but not in proportion to the increments of antigen (Topley, 1930).

Protective antibodies detected and titrated were challenged passively against the immunized rabbits with multiple lethal doses of pathogenic agents, and revealed considerable protection against the challenge dose compared to normal animals. For this trials, rabbits were used as test animals since they were considered to be less sensitive or resistant to repeated injections than guinea pigs (Hamre *et al.*, 1943; Desomer *et al.*, 1955). Marine organisms considered to be rich sources of macromolecular compounds have been examined mainly for haemagglutinin activity and many agglutinins have been identified from the marine molluscs. The reason for this presence of agglutinins has been presumed due

to the genetic capability of the organism (Rogers, 1977).

Most of the agglutinins so far identified were from extracts prepared from ova and eggs of marine organisms (Rogers, 1977). Extract prepared from the gonad of *C. ramosus* revealed interesting results with increasing amount of antibodies, which further attributes to a new form of immunotherapy to assist in the control of immunodeficiency or perhaps even to cure diseases.

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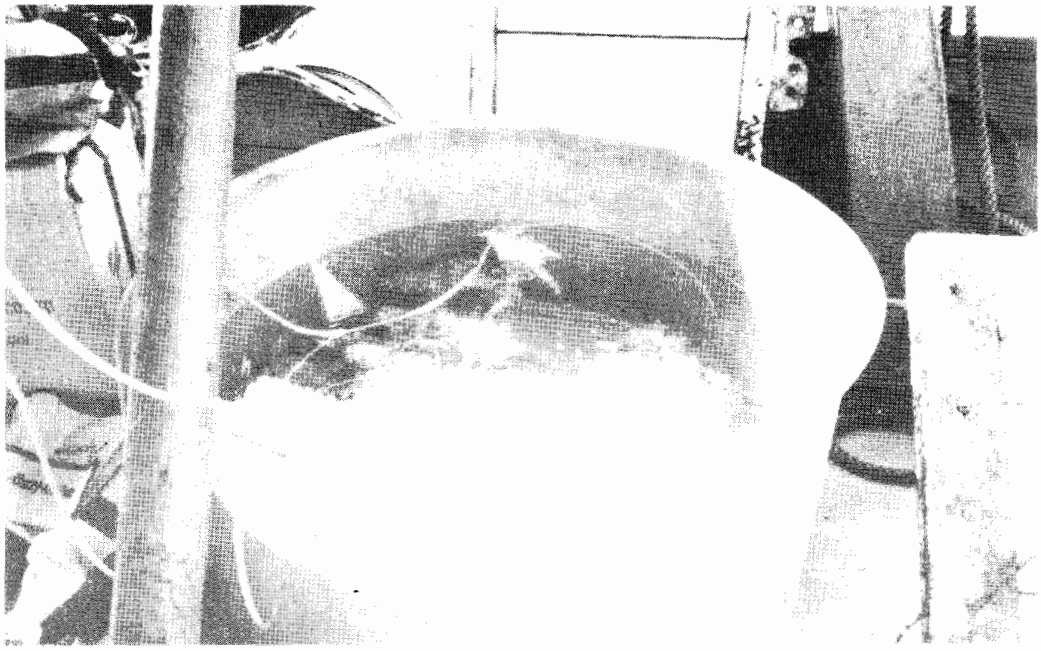
Field environmental in *C. ramosus* fishing area, Thailand



Field collecting at Ko Talibong, Thailand