

## Asterina Gibbosa: Immune Reactions after Injections of Alkaline Phosphatase

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### ABSTRACT

The sea star *Asterina gibbosa* was injected with the enzyme Alkaline phosphatase twice (1 injection per week). 5 days after the last injection, an immunocytochemical test was performed with the axial organ; it was finally observed in T.E.M. It revealed a positive reaction : an anti-alkaline phosphatase antibody was created.

**Keywords:** sea star; alkaline phosphatase antibody ; immunocytochemical test.

### INTRODUCTION

IN 1973, Leclerc (Ref.1) described, for the first time, a positive immunocytochemical reaction which was obtained with the sea star *Asterina gibbosa*, after injection of HRP (Horse-radish peroxydase).

Another enzyme used as antigen was then tested: the alkaline phosphatase. In this paper we report this last experiment

### MATERIALS AND METHODS

Five *Asterina gibbosa* (Asterids, Echinodermata) were injected twice with 100µl of a solution (250µg/ml) each of Alkaline phosphatase (Sigma), on the day 1, then on the day 8. They were sacrificed on the day 13. 5 Controls were injected with HRP (Horse-radish peroxydase) (Ref .1). 5 Controls were not injected. All were kept in aquarium in running sea water.

As it was said, on day 13, all were sacrificed; axial organs were removed and treated after fixation by immunocytochemical test. But this time incubation was made in an antigenic solution of Alkaline phosphatase at 250 µg/ml. The following step was for all axial organs the same: incubation in the solution (pH=9) according the method of Hugon and Borgers (Ref.2):

Buffer Tris maleate 0, 2 M : 2ml

Beta. Glycerophosphate Na 1, 25 % : 2 ml

Distilled water: 4, 7 ml

PbNO 3: 1, 3 ml

After washing, dehydration (from alcohol 70 to alcohol 100°) Inclusion was performed (Ref.3)

Cut were done. Observations were made in T.E.M with a Hitachi.

### RESULTS

Specific labelling appears in animals treated with Alkaline phosphatase in the perinuclear space and ergastoplasmic cisternae (C E) (Fig. 62-63)

No specific labelling occurs in controls.

We note in controls and in treated animals, a labelling at the level of peroxysomes (P). (Fig. 60-61)

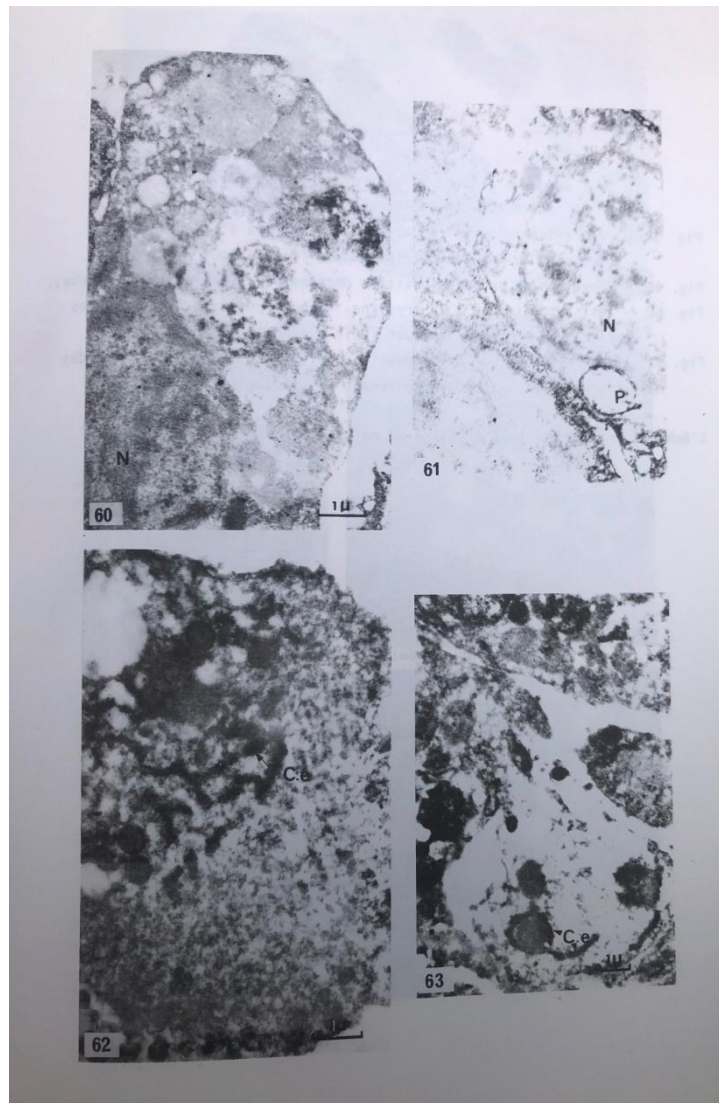
### CONCLUSION DISCUSSION

Specific labelling is shown in alkaline phosphatase treated animals and suggests an ANTIBODY reaction. It recalls the obtained one with Horse-radish peroxydase (HRP) (Ref.1).

No crossed reactions occur in the sea star immune system between HRP and Alkaline phosphatase enzymes.

We know now that this system includes sea star T and B lymphocytes (Ref.4) and an Invertebrate Primitive Antibody (IPA) (Ref.5), to be correlated to « anti-alkaline phosphatase antibody.

From day to day, new discoveries appear in this Invertebrate immune system as shown by the sea star humoral response to alkaline phosphatase.



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