# EFFECTS OF THE PROTEINS INVOLVED IN REGENERATION ON THE ANTIOXIDANT ENZYME ACTIVITY OF PHAGOCYTES IN THE HOLOTHURIAN *EUPENTACTA FRAUDATRIX*

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**Cells of the immune system play an important role in the recovery processes in tissues, and they are considered as regulators of regeneration (Yushkov, 2017). In vertebrates, macrophages have ability to undergo activation to pro-inflammatory M1 (classically activated) or anti-inflammatory M2 (alternatively activated) phenotypes (Italiani, Boraschi, 2014).**

**Holothurian *Eupentacta fraudatrix* (Fig. 1) is a prospective species for mariculture as a resource for pharmaceutics. The two types of phagocytes (P1 and P2) of the holothurian are known to have different functional activities and markers similar to the M1 and M2 macrophages respectively (Dolmatova, Ulanova, 2019) .**

**Objective: Studies on the influence of some proteins expressed during regeneration on the activity of antioxidant system enzymes of *E. fraudatrix* phagocytes.**

## Materials and methods

***E. fraudatrix* specimens with a body length of 4 to 6 cm were collected in winter in Vostok Bay (Peter the Great Bay, Sea of Japan). The extract of holothurian tissues (EH) was obtained according to Dolmatova et al. (2014).**
**The main stages of the experiment are shown in the scheme below (Fig. 2).**

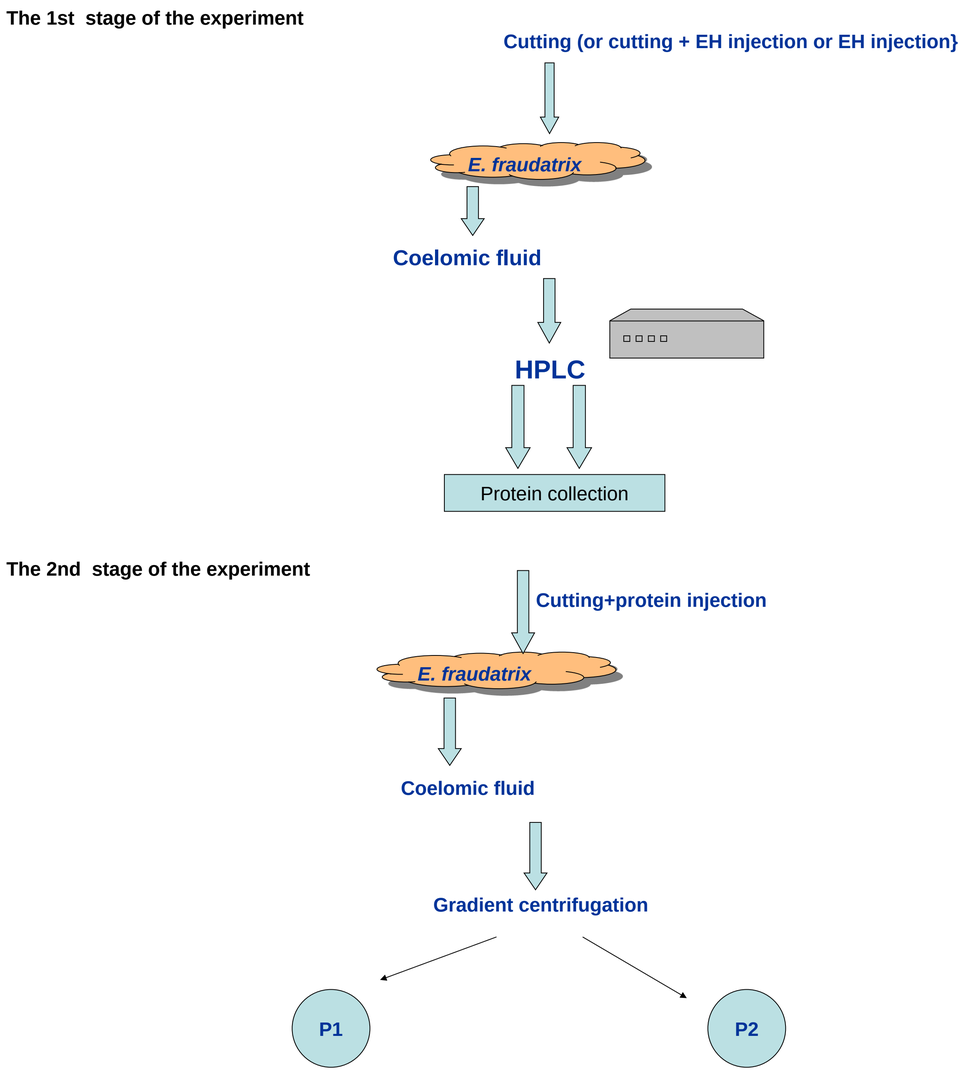


**Fig. 2. Scheme of the experiments.**

**The concentrations of P1 and P2 phagocytes were determined in Goryaev chamber. Activities of catalase, glutathione reductase (GR), and glutathione-S-transferase (GT) were determined in the two types of phagocytes with spectrophotometric methods as described earlier by Dolmatova et al. (2004). The difference between the groups was considered significant at P<0.05.**

## Results and discussion

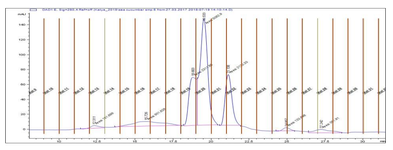

**Fig.3. An example of a chromatogram of coelomic fluid.**

**At stage 1, chromatogram (an example is shown in Fig. 3) analysis revealed that when EH was added, the most significant changes in the concentrations of proteins were noted for the two unidentified proteins. The level of the first (Protein 1) increased in the wounded animals compared to control, and the second protein (Protein 2), by contrast, disappeared in unwounded specimens.**

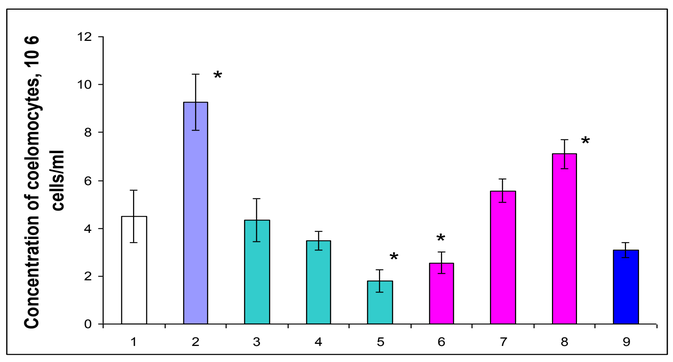


**Fig. 4. Concentrations of coelomocytes after injection of proteins (Protein 1 and Protein 2) isolated. 1- PBSN, 2-wound, 3- wound+Pr1 low, 4 wound+Pr1mid, 5 wound+Pr1 high, 6 wound+Pr2 low, 7 wound +Pr2 mid, 8 wound+Pr2 high, 9- wound +bovine serum albumine (BSA).**
*** P<0.05 compared to the control.**

**The proteins 1 and 2 had opposite concentration-dependent effects on the concentration of cells in the coelomic fluid (Fig. 4).**

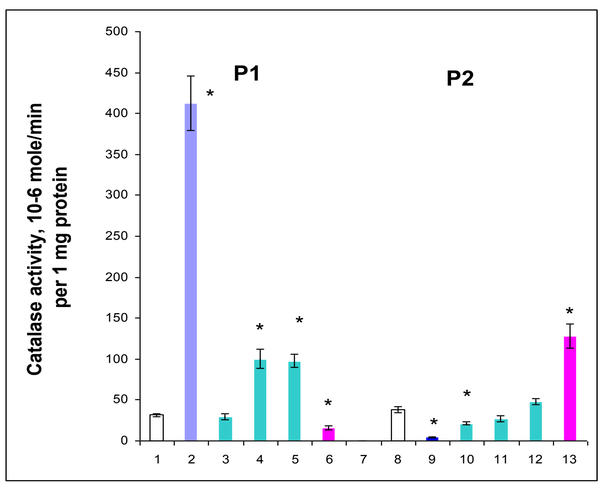


**Fig. 5. Catalase activity in the two types of phagocytes (P1 and P2) treated with Protein 1 (Pr1, low, middle and high concentration) and Protein 2 (Pr2).**
**1,8-control (PBSN); 2,9-injury +PBSN; 3,10-injury+ Pr1 (low); 4,11-injury+Pr1(mid); 5,12-injury +Pr1(high); 6,13-injury+Pr2.**
*** P<0.05 compared to the control.**

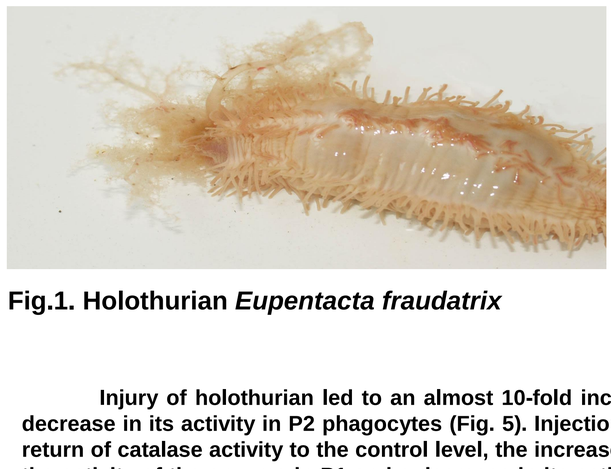

**Fig.1. Holothurian *Eupentacta fraudatrix***

**Injury of holothurian led to an almost 10-fold increase in the activity of catalase in P1 and, conversely, to a decrease in its activity in P2 phagocytes (Fig. 5). Injection of Protein 1 at the lowest concentration studied led to the return of catalase activity to the control level, the increase in concentration caused a significantly smaller increase in the activity of the enzyme in P1 and a decrease in its activity in P2 cells. At the same time, the activity of the enzyme in P1 remained higher than in P2 all the time. Protein 2 at a medium concentration stimulated activity in P2 and decreased activity in P1 phagocytes.**

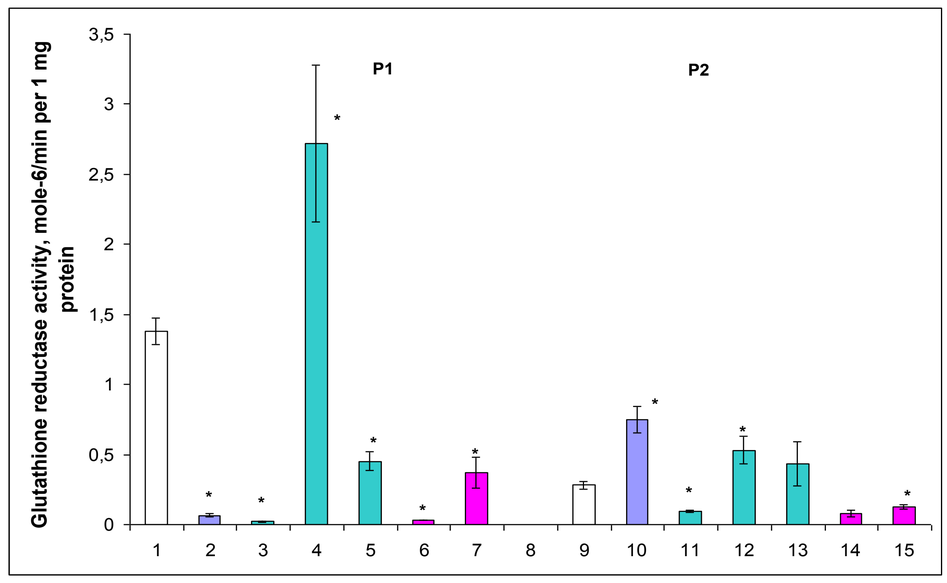


**Fig. 6. Glutathione reductase activity in the two types of phagocytes (P1 and P2) treated with Protein 1(low, middle and high concentrations) and Protein 2 (middle and high concentrations).**
**1,9-control (PBSN); 2,10-injury +PBSN; 3,11-injure+ Pr1 (low); 4,12-injury+Pr1(mid); 5,13-injury +Pr1(high); 6,14-injury+Pr2 (mid); 7,15-injury+Pr2 (high).**
**P<0.05 compared to the control.**

**GR (Fig. 6) activity of P1 and P2 cells significantly decreased and increased respectively in wounded animals compared to controls. Protein 1 suppressed the enzyme activity compared with control and wounded animals in both types of phagocytes at the lowest of concentrations and significantly stimulated the activity in P1, but not in P2, at the average concentration. A further increase in concentration was accompanied by a decrease in enzyme activity in P1 below its level at wounding, but with no change in P2 phagocytes. Protein 2 at the highest concentration significantly suppressed the enzyme activity compared to that at wounding in both cell types, and at the average concentration also suppressed it, but the enzyme activity in P2 cells was higher than in P1.**

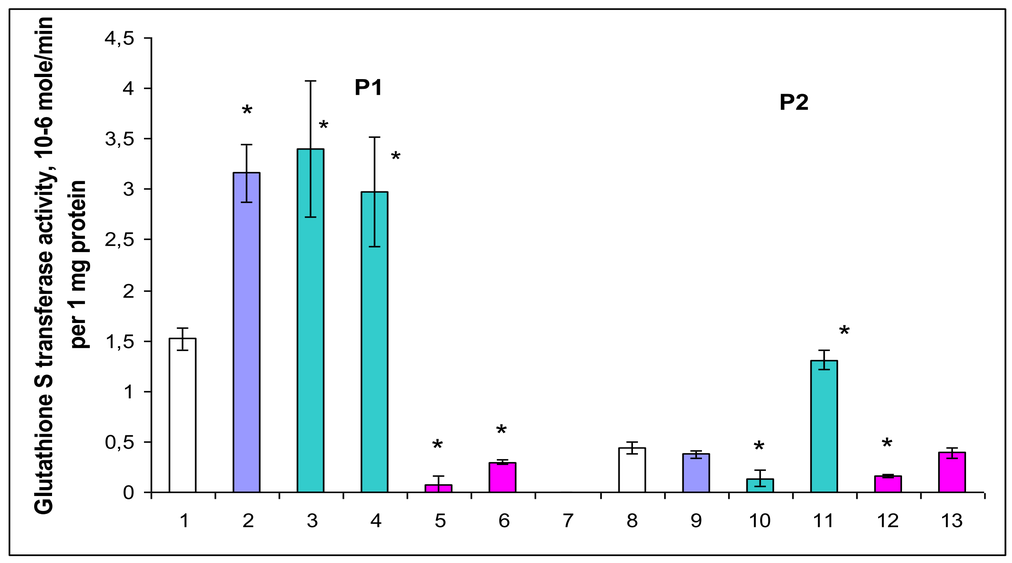


**Fig. 7. Glutathione S-transferase activity in the two types of phagocytes (P1 and P2) treated with Protein 1(middle and high concentrations) and Protein 2 (middle and high concentrations).**
**1,8-control (PBSN); 2,9-injury +PBSN; 3,10-injury+ Pr1 (mid); 4,11-injury +Pr1(high); 5,12-injury+Pr2 (mid); 6,13- injury+Pr2 (high).**
*** P<0.05 compared to the control.**

**GT activity (Fig. 7) significantly increased at wounding compared with control in P1 but not in P2 phagocytes. The low concentration of Protein 1 significantly decreased enzyme activity in both types of phagocytes, and with increasing its concentration there was a return of enzyme activity to the level at wounding (medium concentration) and even an increase in enzyme activity in P2. However, a significantly higher activity in P1 compared with that in P2 was maintained. Protein 2 significantly reduced enzyme activity compared with wounding at both concentrations studied in an inverse concentration relationship, with activity in P2 being higher than in P1 (while an inverse ratio of enzyme activity in the two phagocyte types was observed in the control).**

## Conclusions

1. **Tissue damage induces increase in the number of coelomocytes, variations in protein concentrations in coelomic fluid and the opposite changes in activities of catalase, glutathione reductase and glutathione S transferase in P1 and P2 phagocytes.**
2. **Injection of EH into wounded specimens induced the increase in concentration of two proteins compared to wounded and uninjured individuals.**
3. **Proteins 1 and 2 influenced coelomocyte concentration and activities of antioxidant enzymes in phagocytes in different concentration dependences.**
4. **Protein 2, considered as a protein mediator of the wound-healing effect of the extract, produces a shift in the functional activity of phagocytes toward its predominant activation in P2 phagocytes.**

## Literature

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