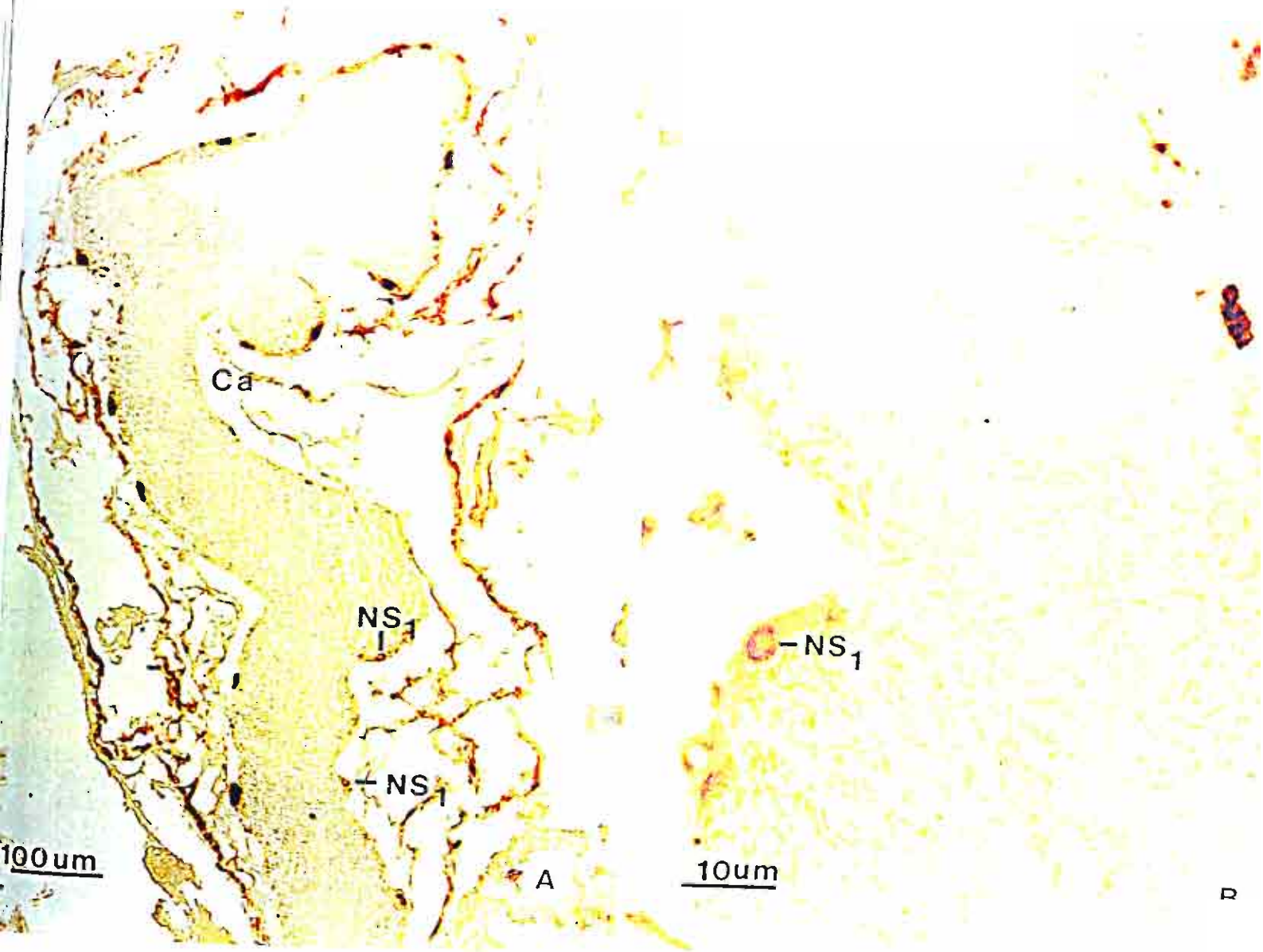
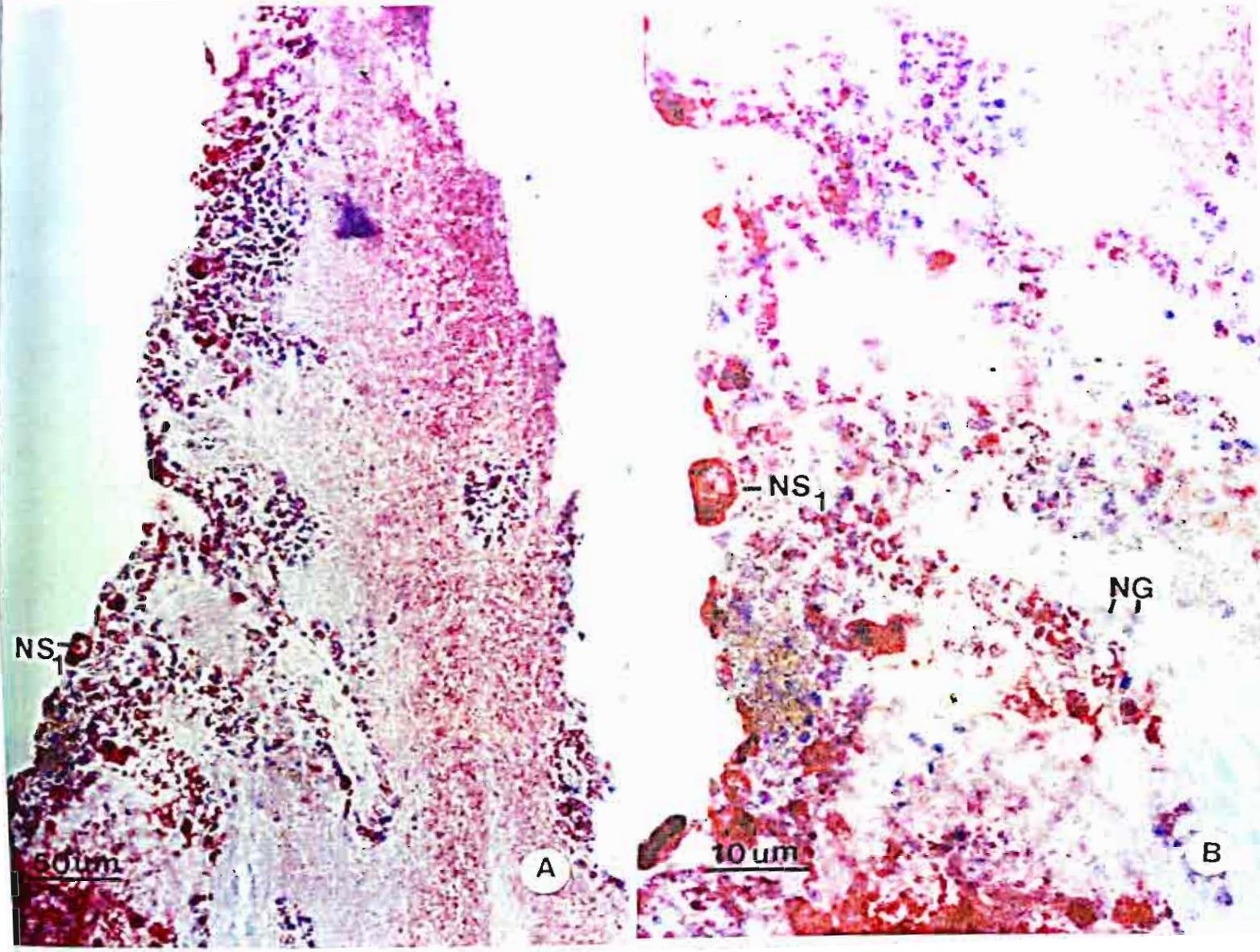
รูปที่ 2 แสดงปมประสาทซีรีบรัลย้อมด้วย anti- αELHโดยวิธี immunoperoxidase

A-B. Sections ที่ย้อมด้วย anti- αELH ปรากฏว่า NS. จะติดสีน้ำตาลเข้ม


Ca
NS1
NS1
NS1
100um
A
10um
B

รูปที่ 3 แสดงปมประสาทซีรีบรัลย้อมด้วย anti- αELHโดยวิธี alkaline phosphatase

A-B. Sections ที่ย้อมด้วย anti- αELH ปรากฏว่า NS_1 จะติดสีน้ำตาลแดงเข้ม


NS1
60um
A
NS1
NG
10um
B

ภาคผนวก

Localization of egg-laying hormone in the cerebral ganglia of a tropical abalone, *Haliotis asinina* Linnaeus

Amporn Thongkukiakul

Department of Biology, Faculty of Science, Burapha University, Chonburi 20131, Thailand

Abstract Localization of abalone egg-laying hormone (ELH) was performed by immunoperoxidase and alkaline phosphatase techniques using polyclonal antibody to recombinant abalone egg-laying hormone (αELH) of *H.rubra* as prob. Anti-αELH exhibit strong binding which implies the presence of αELH to NS_1. The antibody was used to show that the18.5 kDA neuropolypeptide accumulated in the secretory granules.

KEYWORDS: *Haliotis asinina*, immunocytochimistry, egg-laying hormone

INTRODUCTION

Neurosecretory cells controlling egg-laying have been identified and well characterized in both pulmonate: opisthobranch *Aplysia californica*[1] and the *Lymnaea stagnalis*.[2] In *A. californica* the bag cells, located on connective tissue near the abdominal ganglion, are the source of the egg-laying hormone (ELH). In *L.stagnalis* the caudodorsal cells (CDCs), located in cluster in the cerebral ganglia, synthesized the ovulation hormone, caudodorsal cell hormone (CDCH)[3]. Hanh[4] also reported that the neurosecretory cells in the cerebral ganglia of *H. discus hannai* have role in reproduction. In present study we use antibodies raised against peptides from *H. rubra* to identify the neurosecretory cells that involved in reproduction of *H.asinina*.

MATERIALS AND METHODS

Animals

The adult abalone *H.asinina*, with a shell length of 4-5 cm, were obtained from the Institute of Marine Science. They were anesthetized with 5% MgCl, after which their shell were removed. The cerebral ganglia were dissected out.

Immunocytochemistry

For immunostaining, cerebral ganglia were frozen in TissueTek in the cryostat at -25° C and sections at 6 μm. Sections were dried, rinsed three times in TBS, and incubated 1 hr at room temperature in primary antibody consisting of mouse polyclonal antibody against recombinant *H.rubra* egg-laying hormone[5].They were then rinsed three times in TBS, incubated in biotinylated rabbit anti-mouse IgG, with a dilution 1:100 for 30 minutes. The sections were then washed in TBS, covered with the streptavidin-peroxidase conjugated (Zymed) in the same buffer, with dilution 1:100 for 30 minutes. Finally, the sections were immersed in the substrate solution containing 5 ml of 0.03% (W/V) 3,3' diaminobenzidine (DAB) plus 17 μl H_2O_2, for 15 minutes or AEC chromogen. Finally, the

sections were washed several times with distilled water, and some were counter-stained with hematoxylin being mounted in buffer glycerol, and observed in an Olympus Vanox microscope.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot

For all separations and blotting, using the Mini-V 8 * 10 Vertical Electrophoresis System . Proteins were visualized by Coomassie staining. For MW determination, SDS-PAGE Molecular Weight Standards, Broad Range were use (Bio-Rad). The separated polypeptides were electrophoretically transferred to 0.45 μm pore nitrocellulose paper during 60 min at 90 C to method of Towbin[6]. Immunodetection was preformed using mouse anti-*H.rubra* and alkaline phosphatase labeled anti-mouse immunoglobulin (Zymed) and detected with 5-Bromo-4-Chloro-3-Indolyl phosphate / Nitroblue tetrazodium salt substrate (Zymed).

Results

By using immunoperoxidase and alkaline phosphatase methods, the presence of brownish staining suggested that there are strong and specific bindings of anti-αELH in the neurosecretory cells type 1. The brownish stain is distinct on the neurosecretory granules (Fig.1A,1B;2A,2B), in contrast to the control sections which are completely unstained. However, there appear to be a lower concentration of these positive in the connective tissue around the ganglia.

By using immunobloting, the antibody was used to show that the18.5 kDA neuropolypeptide accumulated in the secretory granules (Fig.3A).

Discussion

The expression of the egg-laying peptide gene in the bag cells of abdominal ganglion, atrial ganglion and pleural ganglion of *A. californica*[7] and in caudodorsal cell in the cerebral ganglia of *L.stagnalis*[8] has been detected by *in situ* hybridization, using radiolebeled cDNA probes to ELH mRNA. In contrast to egg-laying hormone of pulmonate and opithobranch little known about the origin of the egg laying hormone in abalone. Histological studies in *H. discus hannai* show that the number of neurosecretory cells, especially type 1 and 7, in pleuro-pedal ganglia were correlated with the induction of spawning[4]. From the evidence gathered in the present study I, therefore, would like to suggest that the neurosecretory cells of the ganglia can synthesize ELH.

References

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Fig 1. Light micrographs of the cerebral ganglia, stained with anti-αELH by immunoperoxidase method. (A,B) anti–αELH exhibit staining in the NS_1.

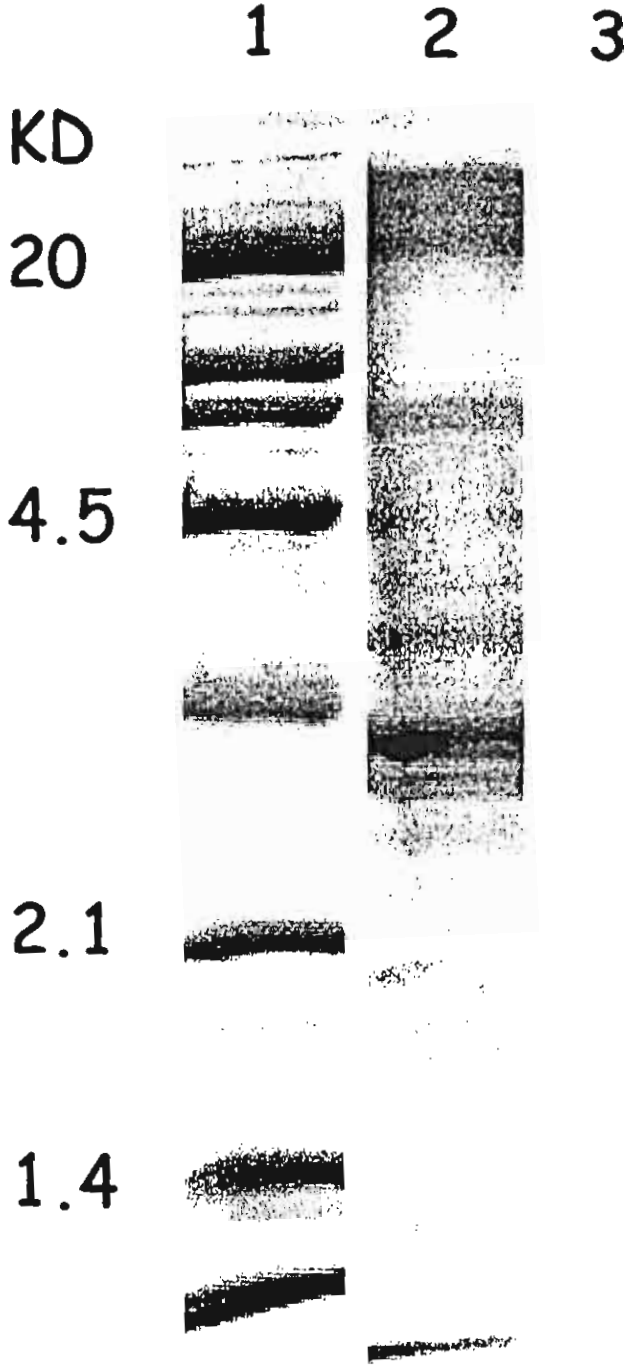

1
2
3
KD
20
4.5
2.1
1.4

Fig 2. Light micrographs of the cerebral ganglia, stained with anti-αELH by alkaline phosphatase method and counter stained with hematoxylin. (A,B) anti--αELH exhibit staining in the NS_1.

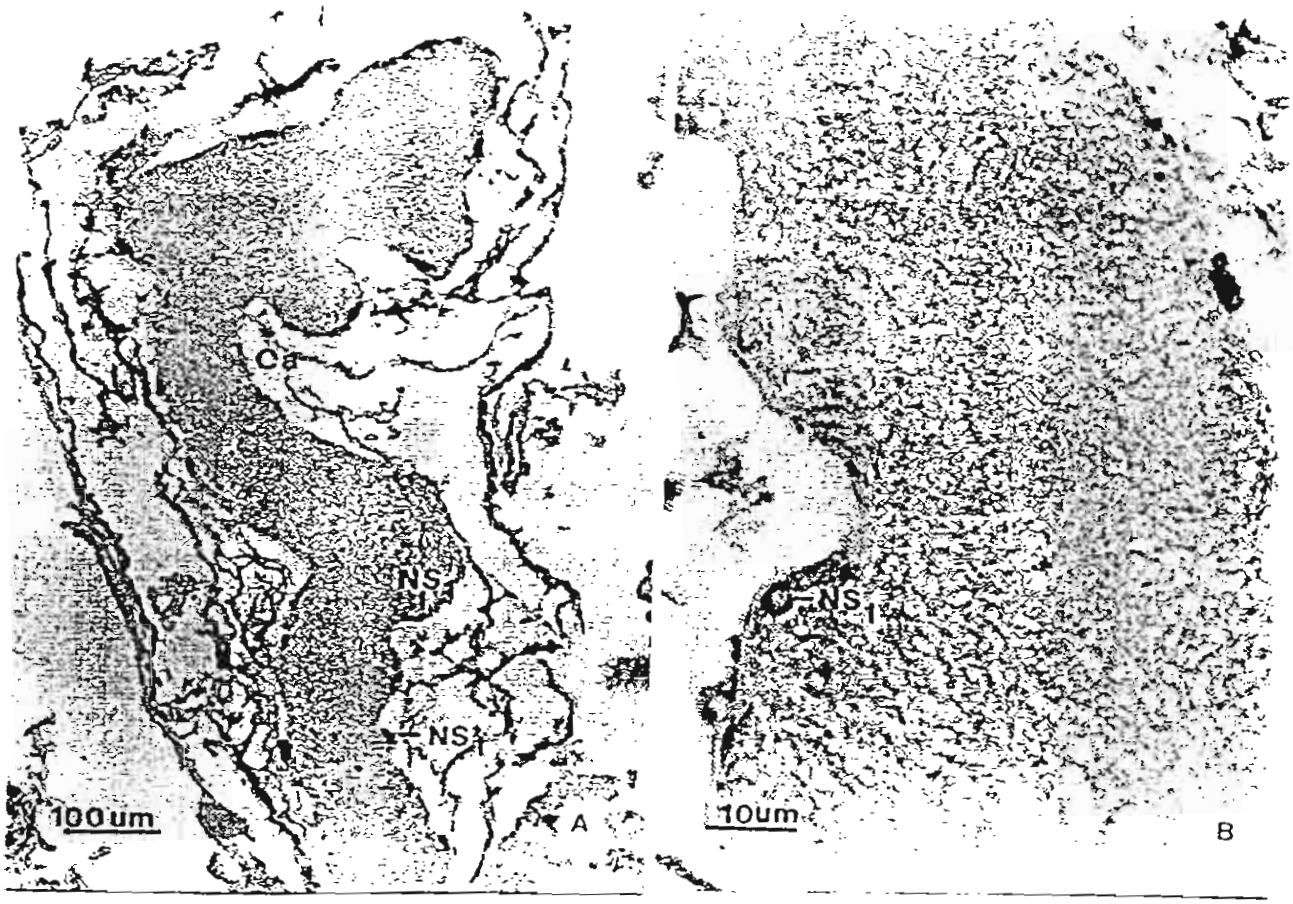
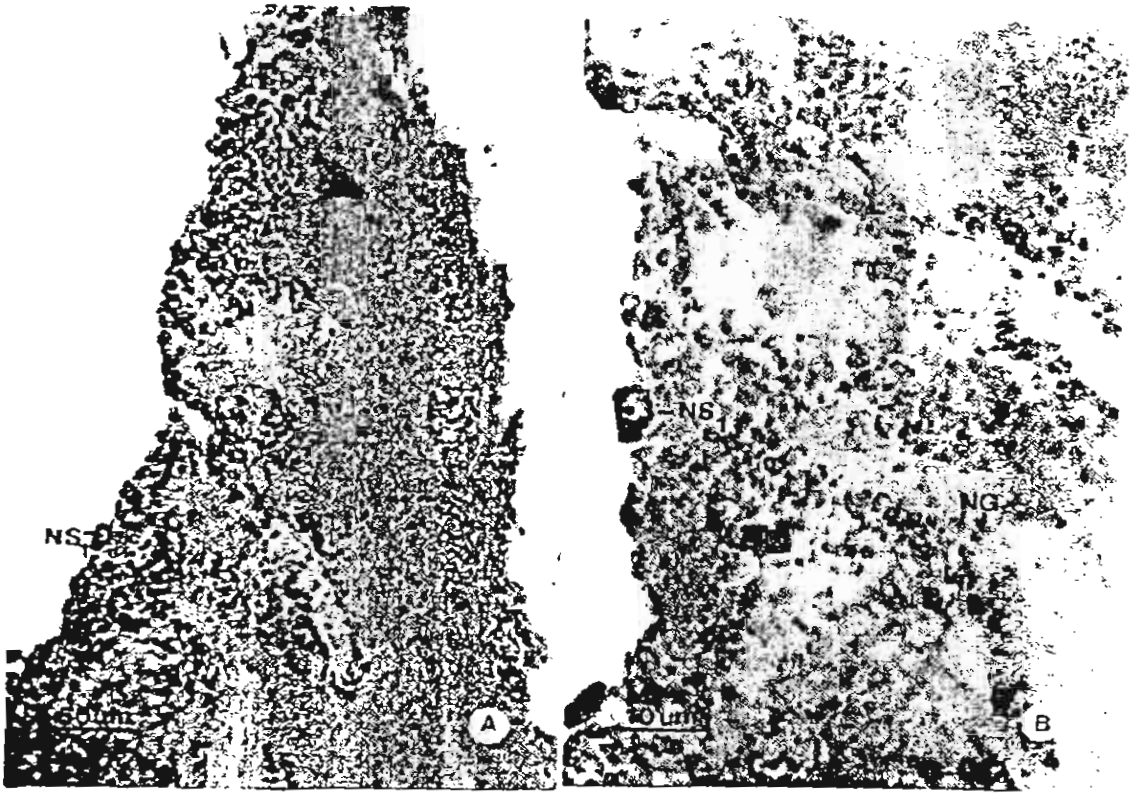

Ca
NS
NS
100um
A
NS
10um
B

Fig 3. *H.asinina* neuropeptides were separated by SDS-PAGE, and stained with Coomassie Blue (Lane 2), or blotted onto nitrocellulose and incubated with anti-*H.rubra* (Lane 3)


NS
A
NS
NG
B