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## RESEARCH ARTICLES

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### HISTOLOGICAL ALTERATIONS IN THE REPRODUCTIVE, NERVOUS AND DIGESTIVE SYSTEMS OF *INDOPLANORBIS EXUSTUS* INTOXICATED WITH MOLLUSCICIDES

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#### ABSTRACT

The snails, *Indoplanorbis exustus* were exposed to niclosamide, a synthetic molluscicide and the crude water extract of *Brassia actinophylla* at the  $LC_{50}$  concentrations for 24 hours. The results showed similar histological alterations in the organs of reproductive, nervous and digestive systems of snails exposed to both molluscicides. In the ovotestes, there were reduction in number and degeneration of spermatozoa and oocytes. In the cerebral ganglia, there were a lysis of neuropile, significant increases in cell and nuclear diameters of neurosecretory cells, and enlargement of vacuoles in the cytoplasm. In the epithelium of digestive tract, there were reduction of cilia, increase in mucus secretion, dilatation of cells and fragmentation of muscle tissue.

#### INTRODUCTION

It has been observed that poisoning with molluscicides causes the snail either to retract into the shell and expell hemolymph or to become swollen and remain extended from the shell opening. The water balance of gastropod is thought to be under neurosecretory control. Trifenmorph has been shown to reduce neurosecretory activity in *Bulinus truncatus*, while the long-term exposure of the pulmonate, *Indoplanorbis exustus*, to barium chloride and copper sulfate also result in diminished neurosecretory activity<sup>1</sup>.

Molluscicidal action affecting water balance may take one of these forms. First, the snail may detect the presence of chemical and react by producing excess mucus or reducing the permeability of the external membrane. Second, the molluscicide may react directly with the external membrane<sup>2</sup>.

Zhou *et al.*<sup>3</sup> studied on the histological changes in the intestine, digestive gland and ovotestis of *Biomphalaria glabrata* treated with niclosamide and extract of *Eucalyptus camaldulensis*. They reported that in the intestine, the muscle fibers became relaxed and the gaps between the epithelial cells and the connective tissue appeared, resulting in the derangement of the ciliated cells. In addition, both mucous cells and ciliated cells appeared irregular in shape and some showed considerable shrinkage. In the digestive gland of *B. glabrata* treated with *E. camaldulensis*

and niclosamide, the digestive cells became irregular in shape. Some of the digestive cells were broken and the secretory material leaked into the lumen. In addition, the ovotestis of molluscicides treated snails showed reduction in numbers of spermatozoa and oocytes<sup>3</sup>.

The objective of the present study is to compare the histological alterations in the organs of the reproductive system (ovotestis), nervous system (cerebral ganglia) and digestive system (esophagus, intestine and digestive gland) of *I. exustus* intoxicated with niclosamide (Bayluscide) and the crude-water extract of *Brassaia actinophylla*. In addition, the macroscopic changes of *I. exustus* exposed to both synthetic and plant molluscicides will also be observed.

## MATERIALS AND METHODS

Laboratory -bred mature snails, *I. exustus* with the shell diameter of 1.5-1.8 cm were used in the experiment. Twenty snails each were exposed to synthetic molluscicide, niclosamide and the crude-water extract of *B. actinophylla* at the  $LC_{50}$  concentrations (0.009 mg/l and 23 mg/l, respectively). Control group consisting of another 20 snails was exposed to dechlorinated tap water. After 24 hours, ten live snails from each experimental group were removed from the shell and processed for histological study.

Organs in the reproductive (ovotestis), nervous (cerebral ganglia) and digestive systems (esophagus, intestine and digestive gland) were dissected out and fixed in Bouin's fluid for 5 h. The tissues were then washed several times with 70% alcohol, dehydrated in a graded series of ethanol, infiltrated with dioxane, and embedded in paraffin. Sections were cut on a rotary microtome at 5-6  $\mu$ m thickness and stained with hematoxylin and eosin. Special staining methods used were Periodic Acid Schiff (PAS) and chrome-hematoxylin-phloxine to identify mucopolysaccharides in the digestive system and neurosecretory cells in the cerebral ganglia, respectively<sup>4</sup>.

## RESULTS

The results show histological alterations of various organs in the reproductive, nervous and digestive systems of *I. exustus* intoxicated with niclosamide and the crude-water extract of *B. actinophylla*. These organs are ovotestis, cerebral ganglia, esophagus, intestine and digestive gland. In addition, macroscopic reactions of snails were also observed.

### 1. Macroscopic observation

The macroscopic visible reactions of snails intoxicated with lethal concentrations of niclosamide and crude-water extract of *B. actinophylla* were very similar. During the first hour, the snails still moved actively. After six hours, the tentacle tips of snails were bright red knobs. During this period, they secreted lucent mucus. The snails became inactive and immobile. After 12 hours, some snails died with bleeding. After 24 hours, some snails died during 48 hours of recovery period, whereas some snails could be recovered to normal. The gross anatomy of 24-hour niclosamide and *B. actinophylla* intoxicated snails was similar. There was a visible shrinkage of ovotestis, digestive gland, esophagus and intestine. However, the intestines of snails intoxicated with niclosamide were emptied while those of snails intoxicated with *B. actinophylla* were filled with green feces.

### 2. Ovotestis

The ovotestis of *I. exustus* is composed of a large number of acini (Fig. 1A). The wall of acini is composed of thin connective tissue of 2-3 cells thick. In each acinus, male and

female sex cells develop simultaneously.

**Control snails** In the ovotestis, the female germinal cells or ova are usually located at the periphery of the acini, and the male germinal cells are arranged in the center of acini (Fig. 1B).

The very early stage spermatogonia of male germinal cells that differentiate from the epithelium are frequently located near the atrium of an acinus while maturing stages generally line the wall of acinus from the atrium toward the apex (Fig. 1C). The maturing spermatozoa attaching to the bases of Sertoli cells are close to the area where the ova are developing (Fig. 1C). Spermatogonia contain chromatin rich nuclei which fill more than two-thirds of the cytoplasm which is rich in basophilic material. During the development of spermatogonia to spermatocytes, the cytoplasm gradually loses its basophilic material and becomes slightly acidophilic. Spermatocytes gradually develop into spermatids and spermatozoa whose cytoplasm gives rise to an acidophilic tail portion (Fig. 1C).

The female germinal cells are usually located at the apex of each acinus in small numbers and also differentiate from the epithelium. The mature ovum contains a round, slightly eccentric nucleus (Fig. 1D). The nuclei are round shaped and basophilic. They are situated on the margin of the cytoplasm. The cytoplasm of ovum which stains purple or bluish-purple in hematoxylin-eosin preparation, is coarsely granular (Fig. 1D). The nurse cells or follicle cells which are equivalent to the Sertoli cells of male germinal cells, adhere to the ovum to form an enveloping sac.

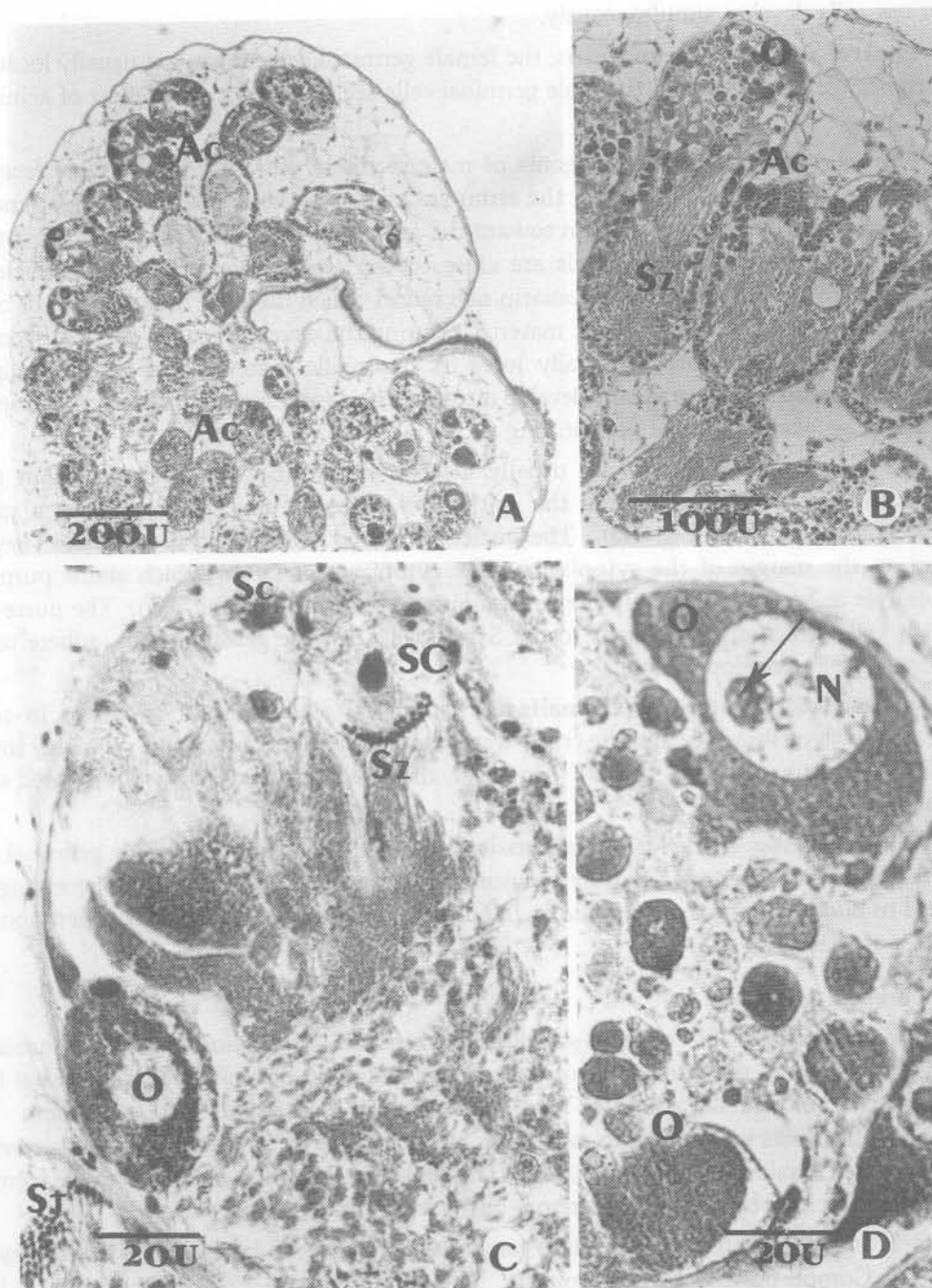
**Molluscicides intoxicated snails** Histological alterations of ovotestes in snails intoxicated with niclosamide and the crude-water extract of *B. actinophylla* are relatively similar after 24 hours of intoxication (Fig. 2). However, the effects of *B. actinophylla* appeared to be more severe.

In the ovotestes of molluscicides intoxicated snails, degeneration of male germinal cells and reduction of spermatozoa flagella are generally observed (Figs. 2B, 2C). Spermatogonia appeared to bud off from the epithelium. In addition, oocytes also showed degeneration and a decrease in size (Fig. 2C).

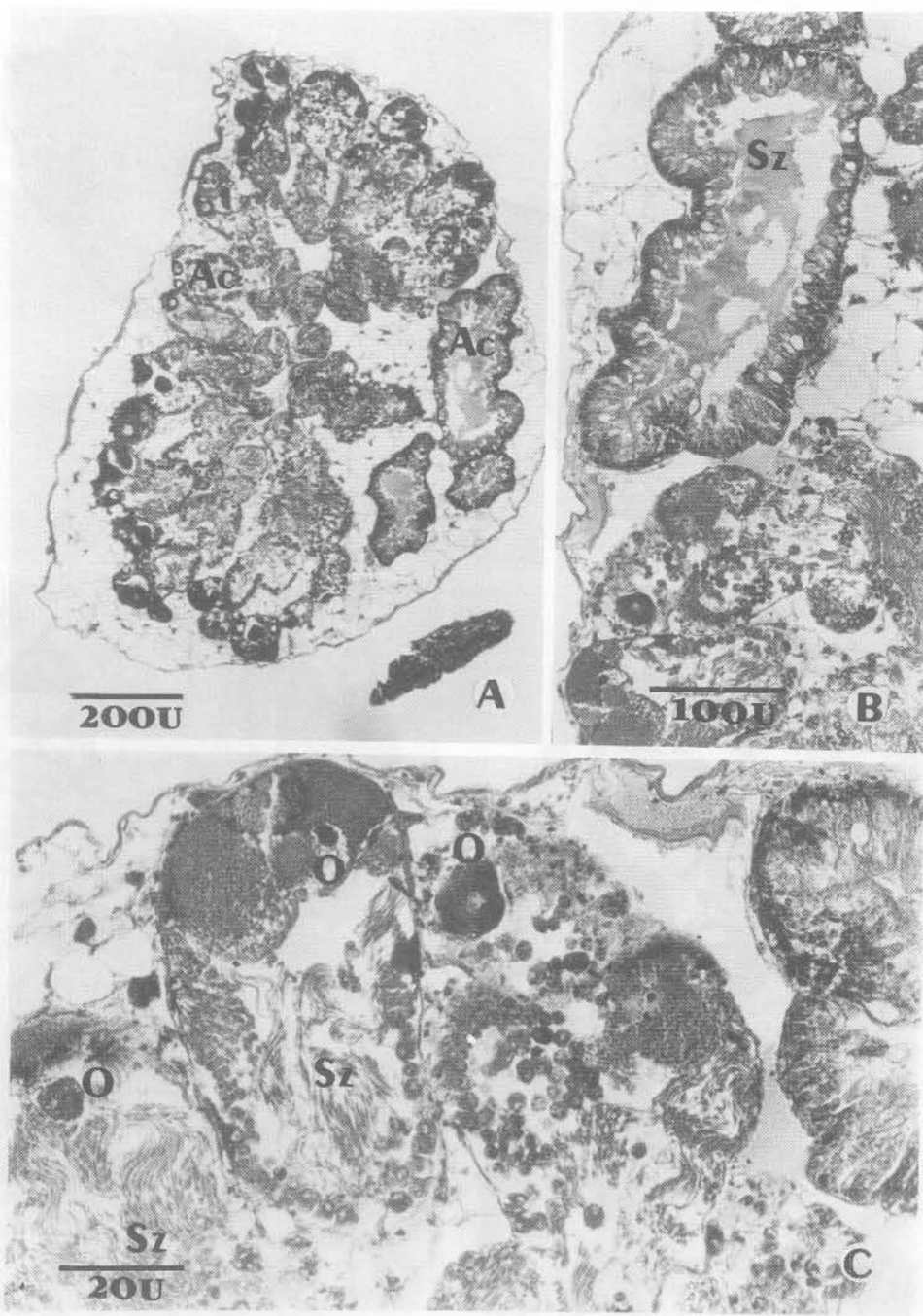
### 3. Cerebral ganglia

**Control snails** The cerebral ganglia are connected by the intercerebral commissure (Fig. 3A). Attached to the lateral side of the cerebral ganglia is a small lobe called lateral lobe. The epineurium of cerebral ganglia of *I. exustus* is rich in cellular and fibrous elements. The collagenous-like fibers run parallel to each other along the axis of the ganglion. Neurosecretory cells are located inside the ganglia (Fig. 3B). The center of the ganglion or neuropile contains nerve fibers sent out by the neurosecretory and ganglionic cells (Fig. 3A).

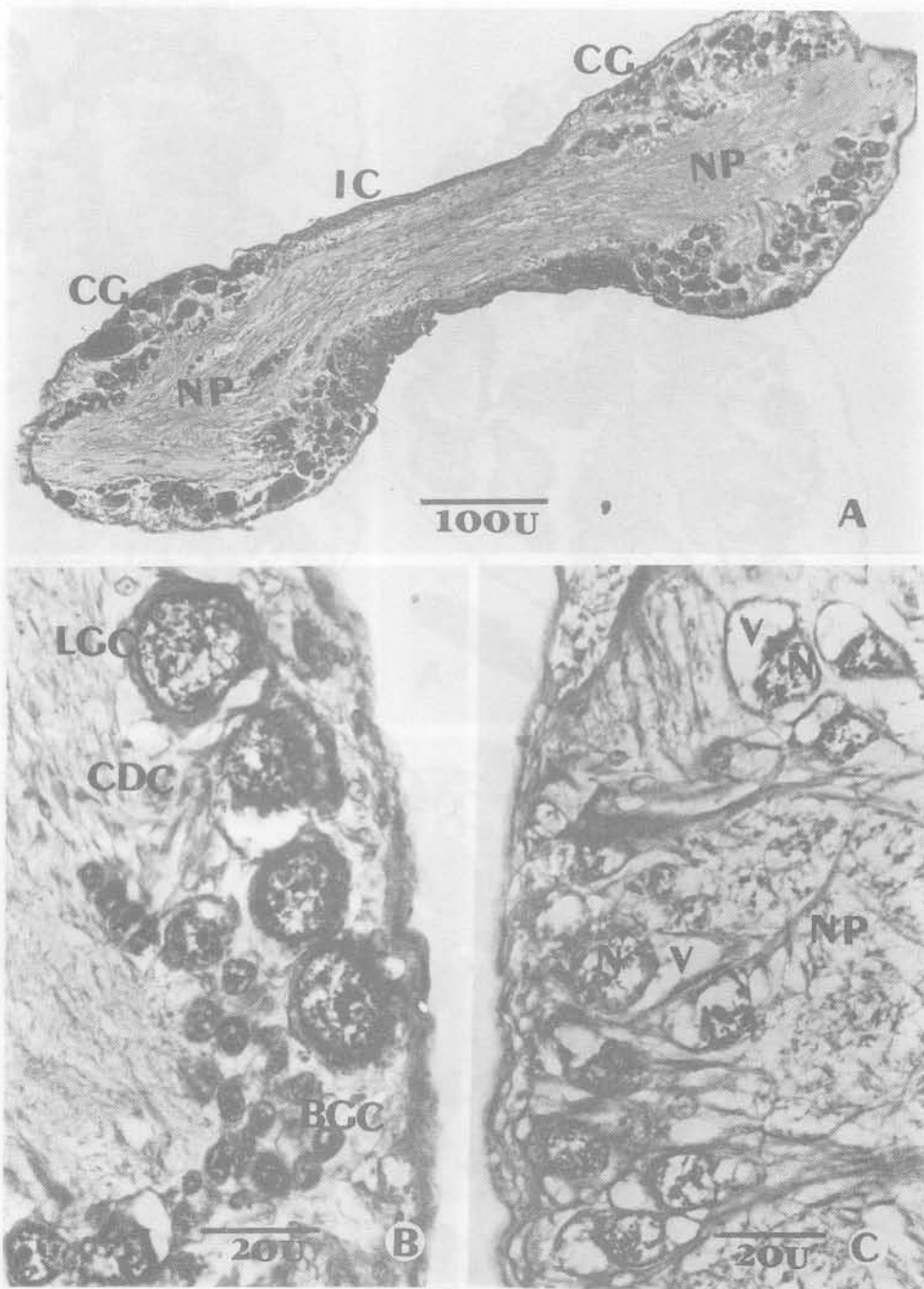
Neurosecretory cells in the cerebral ganglia of *I. exustus* can be divided into three types. The first is Gomori-positive light green cells. They are present in each ganglion in two large groups with the cell diameter of 22-45  $\mu$ m (Figs. 3B, Table 1). They are situated in the medio and latero-dorsal parts of the ganglia (Fig. 3B). The second is Gomori-positive bright green cells. This cell type occurs in the neuropile between the medio- and latero-dorsal cells (Fig. 3B). The diameter range of the bright green cells is 7-15  $\mu$ m (Fig. 3B, Table 1). The third type is caudo-dorsal cells. The diameter range of the caudo-dorsal cells is 4-10  $\mu$ m (Fig. 3B, Table 1). They lie caudo-dorsally in groups in the left and right ganglion, at the peripheral of cerebral commissure and transport their neurosecretory material to the periphery of the cerebral commissure.



**Fig. 1** Ovotestis of control snail, *I. exustus*. (A) Low magnification. The ovotestis is composed of a large number of acini (Ac). (B) The acini (Ac) contain male and female germ cells. O = oocyte, Sz = spermatozoa. (C) High magnification of acinus showing various stages of spermatogenesis. Spermatozoa (Sz) attaching to Sertoli cell (SC) are observed. Sc = spermatocyte, St = spermatid, O = oocyte. (D) High magnification of oocyte (O) with large nucleus (N) and nucleolus (arrow).



**Fig. 2** Ovotestis of molluscicides intoxicated snails. (A) Low magnification showing shrinkage of ovotestis and acini (Ac). (B) Medium magnification showing acinus with degenerated spermatozoa (Sz). (C) High magnification showing degeneration of oocytes (O) and spermatozoa (Sz).



**Fig. 3** Cerebral ganglia of control and molluscicides intoxicated snails. (A) Low magnification showing a pair of cerebral ganglia (CG) connected by intercerebral commissure (IC) in control snails. The center of ganglia contains nerve fibers or neuropile (NP). (B) Cerebral ganglion of control snails showing various types of neurosecretory cells : light green cell (LGC), bright green cell (BGC) and caudo-dorsal cell (CDC). (C) Cerebral ganglion of molluscicides intoxicated snails showing a lysis and disorientation of nerve fibers or neuropile (NP). Note the enlargement of vacuoles (V) in the cytoplasm. N = nucleus.

**Molluscicides intoxicated snails** Figure 3C shows histological alterations in the cerebral ganglia of *I. exustus* intoxicated with niclosamide and the crude-water extract of *B. actinophylla*. The present study emphasizes on the changes of neurosecretory cells after 24 hours of intoxication.

In general, there is a lysis and disorientation of nerve fibers or neuropile (Fig. 3C). Significant increases ( $P < 0.01$ ) are evident in cell and nuclear diameters of light green cells and bright green cells (Table 1). Cytological alteration observed in these neurosecretory cells is the enlargement of vacuoles in the cytoplasm (Fig. 3C).

**Table 1** Diameters of neurosecretory cells and their nuclei in the cerebral ganglia of *I. exustus*, control and molluscicides intoxicated snails, after 24 hours of exposure.

Snails	Cell diameter ( $\mu\text{m}$ )		Nuclear diameter ( $\mu\text{m}$ )	
	Light green cell	Bright green cell	Light green cell	Bright green cell
<b>Control snails</b>	24.35 $\pm$ 3.55	8.89 $\pm$ 1.05	15.32 $\pm$ 2.40	8.24 $\pm$ 1.01
<b>Niclosamide treated snails</b>	34.12 $\pm$ 2.93	10.61 $\pm$ 1.46	17.63 $\pm$ 2.95	9.50 $\pm$ 1.25
<b><i>B. actinophylla</i> treated snails</b>	31.47 $\pm$ 1.63	10.03 $\pm$ 0.88	17.30 $\pm$ 1.42	9.96 $\pm$ 0.84

#### 4. Esophagus

The esophagus is divided into proesophagus and postesophagus. The proesophagus gradually enlarges at the postesophagus with 1-2 bulbous (Fig. 4A).

**Control snails** The mucosal lining of the proesophagus of control snails consists largely of simple columnar cells which vary considerably in height, attaining their maximal length at the tips of the folds (Fig. 4B). They are uniformly provided with remarkably long cilia (Fig. 4B). Their nuclei are elliptical or oval which are rich in chromatin. They lie at the base of the cells and are highly acidophilic with conspicuous nucleoli (Fig. 4B). The cytoplasm is filled with acidophilic granules towards the apices of the cells and may contain several vacuoles (Fig. 4B). Mucous-secreting goblet cells are very scant (Fig. 4B). They contain oval or round nuclei situated at the basal part.

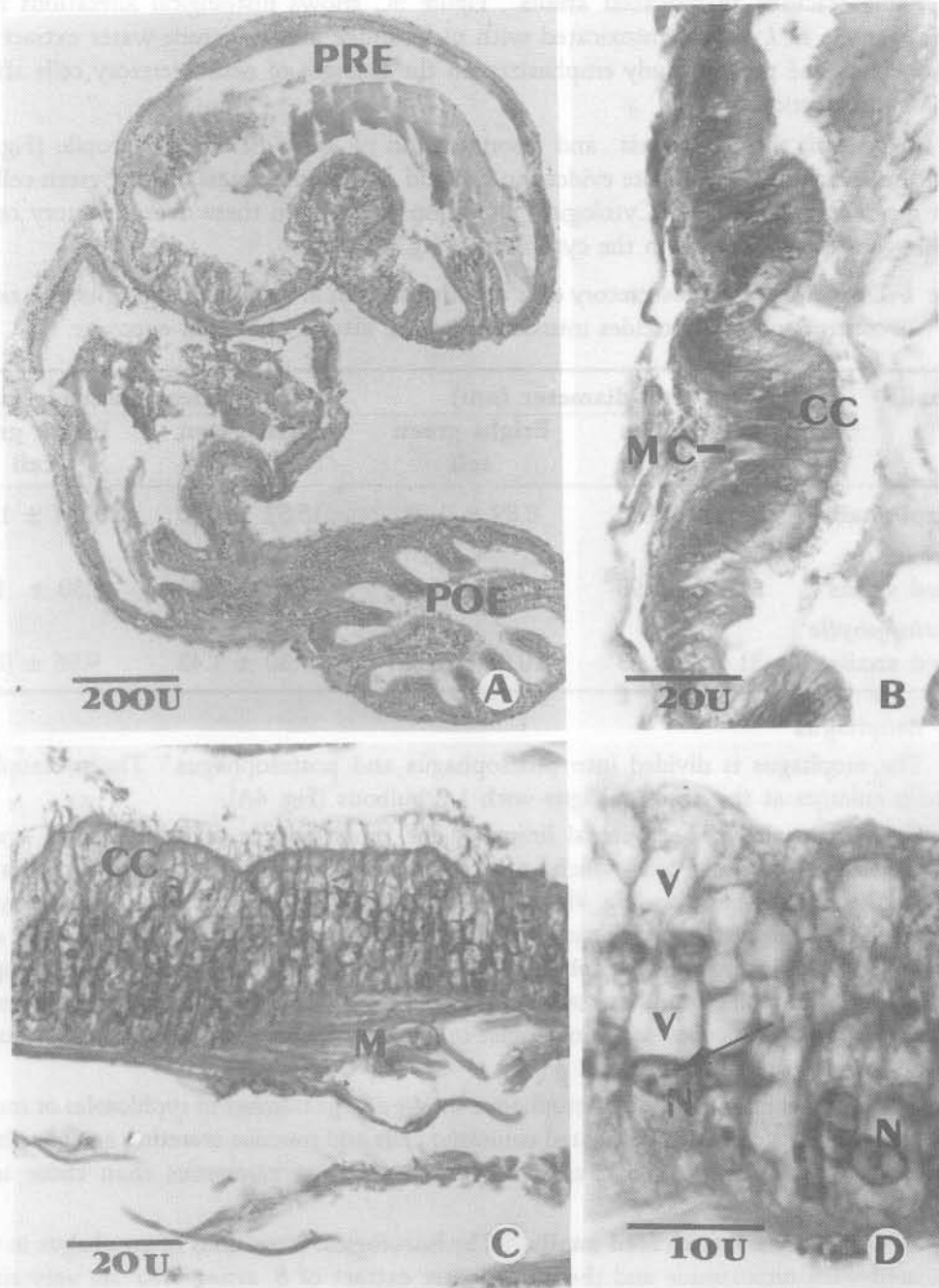
The mucosal lining of the postesophagus shows a large number of typhlosoles or mucosal folds (Fig. 5A). It is consisted of ciliated columnar cells and mucous-secreting goblet cells (Fig. 5B). The mucous-secreting goblet cells are relatively more numerous than those in the proesophagus.

**Molluscicides intoxicated snails** The histological alterations of esophagus in snails intoxicated with niclosamide and the crude-water extract of *B. actinophylla* are very similar. General reactions are more severe in the proesophagus (Figs. 4C,4D) than those in the postesophagus (Figs. 5C,5D). These reactions are reduction of cilia, increase in mucus secretion, dilatation of cells, enlargement of vacuoles and fragmentation of muscle tissue (Figs. 4C,4D,5C,5D). In addition, the nuclei have become dilated and lost most of their heterochromatin (Figs. 4D,5D).

#### 5. Intestine

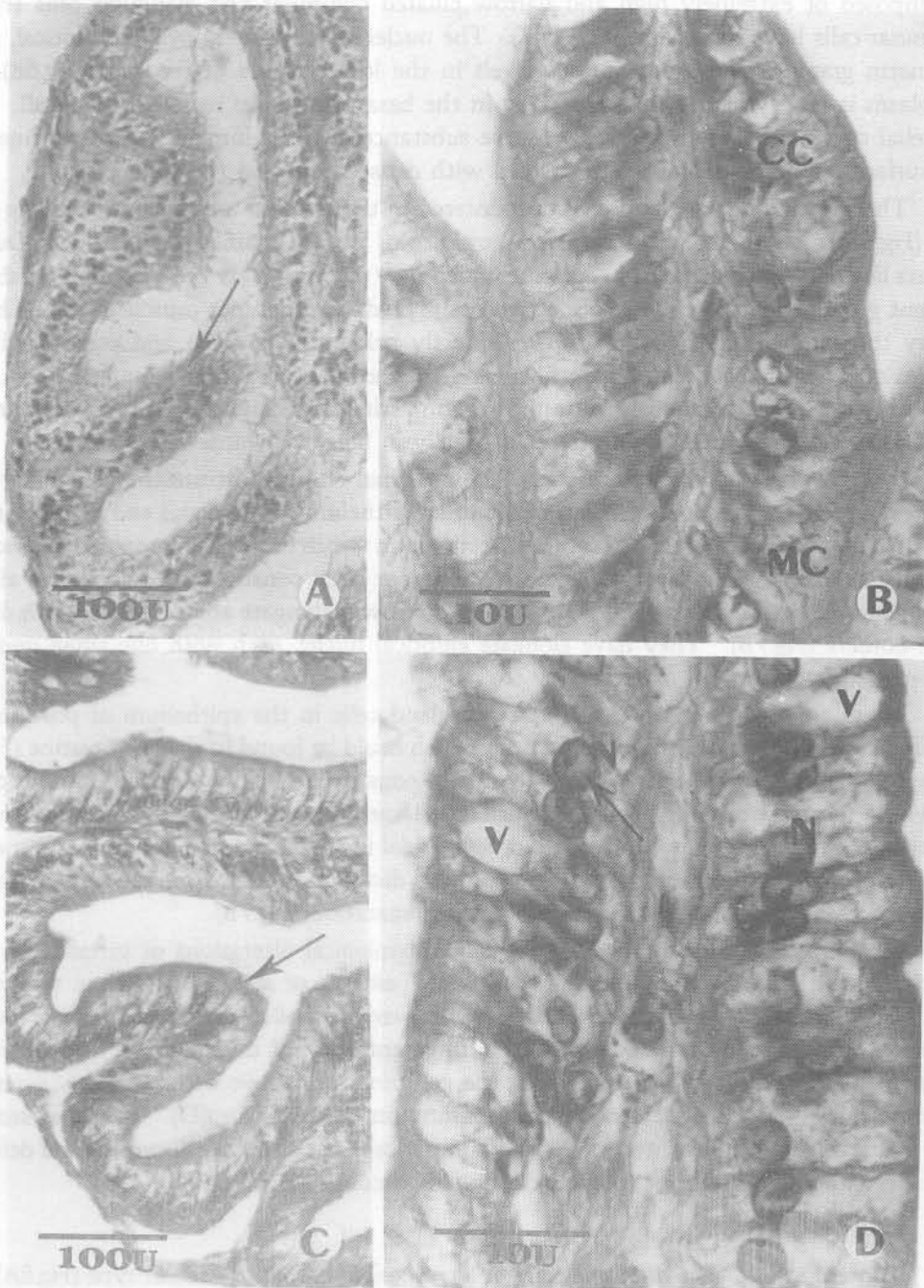
**Control snails** The internal lining of the prointestine in control snails displays an active secretory function. The epithelial cells form a pseudostratified epithelium which generally





**Fig. 4** Proesophagus of control (A,B) and molluscicides intoxicated (C,D) snails. (A) Low magnification showing proesophagus (PRE) and postesophagus (POE). (B) Medium magnification showing simple ciliated columnar epithelium which is composed of columnar cell (CC) and mucous-secreting goblet cell (MC). (C) Medium magnification showing epithelial lining of molluscicides intoxicated snails. Notice dilatation of columnar cells (CC) and fragmentation of muscle tissue (M). (D) High magnification of columnar cells in molluscicides intoxicated snails. Notice enlargement of vacuoles (V). The nuclei (N) have become dilated and lost most of their heterochromatin (arrow).





**Fig. 5** Postesophagus of control (A,B) and molluscicides intoxicated (C,D) snails. (A) Low magnification showing highly ciliated typhlosoles or mucosal folds (arrow) in control snails. (B) High magnification of columnar cells (CC) and mucous-secreting goblet cells (MC). (C) Low magnification of typhlosoles in molluscicides intoxicated snails showing loss of cilia (arrow). (D) High magnification of columnar cells. Notice enlargement of vacuoles (V), dilatation of nuclei (N) and reduction of heterochromatin (arrow).

is composed of extremely high and narrow ciliated columnar and glandular cells (Fig.6A). Columnar cells have broad apices (Fig.6B). The nuclei are elongately oval or elliptical, rich in chromatin granules and lie at various levels in the lower halves of the cells (Fig.6B). The cytoplasm is largely acidophilic, especially in the basal and apical regions of the cell. These epithelial cells discharge strong PAS-positive substance into the lumen of the intestine. The free surface of the epithelial cell is covered with dense short cilia (Fig.6B).

The most common gland cells encountered in the mucosa are mucous-secreting goblet cells (Fig.6B). These cells acquire a red-purple color in PAS stained preparation. Outer to mucosa lies a thick loose connective tissue which may contain a few brown to brownish-black pigment granules (Fig.6B). The connective tissue holds two distinct muscle layers. The first is very thin and difficult to discern, lies directly below the mucosa and consists of small bundles of inner longitudinal muscle fibers and the second, outer circular muscle fibers. The outer muscle layer continuously surrounds the intestinal tube and lies within the connective tissue coat. The prointestine decreases gradually in height towards the distal end.

Figure 7 shows the postintestine. The proximal end of postintestine is similar to the prointestine. It is composed of ciliated columnar epithelium. The distal end of postintestine is mostly composed of non-ciliated columnar epithelium which in turns is composed of absorptive and secretory cells. The absorptive cells or columnar cells, constitute the dominant element in the postintestinal mucosa (Fig.7A). They are narrowly elongate and columnar with distinct brush borders (Fig.7B). They have elongate elliptical nuclei, each with one small nucleolus, with moderate chromatin material (Fig.7B).

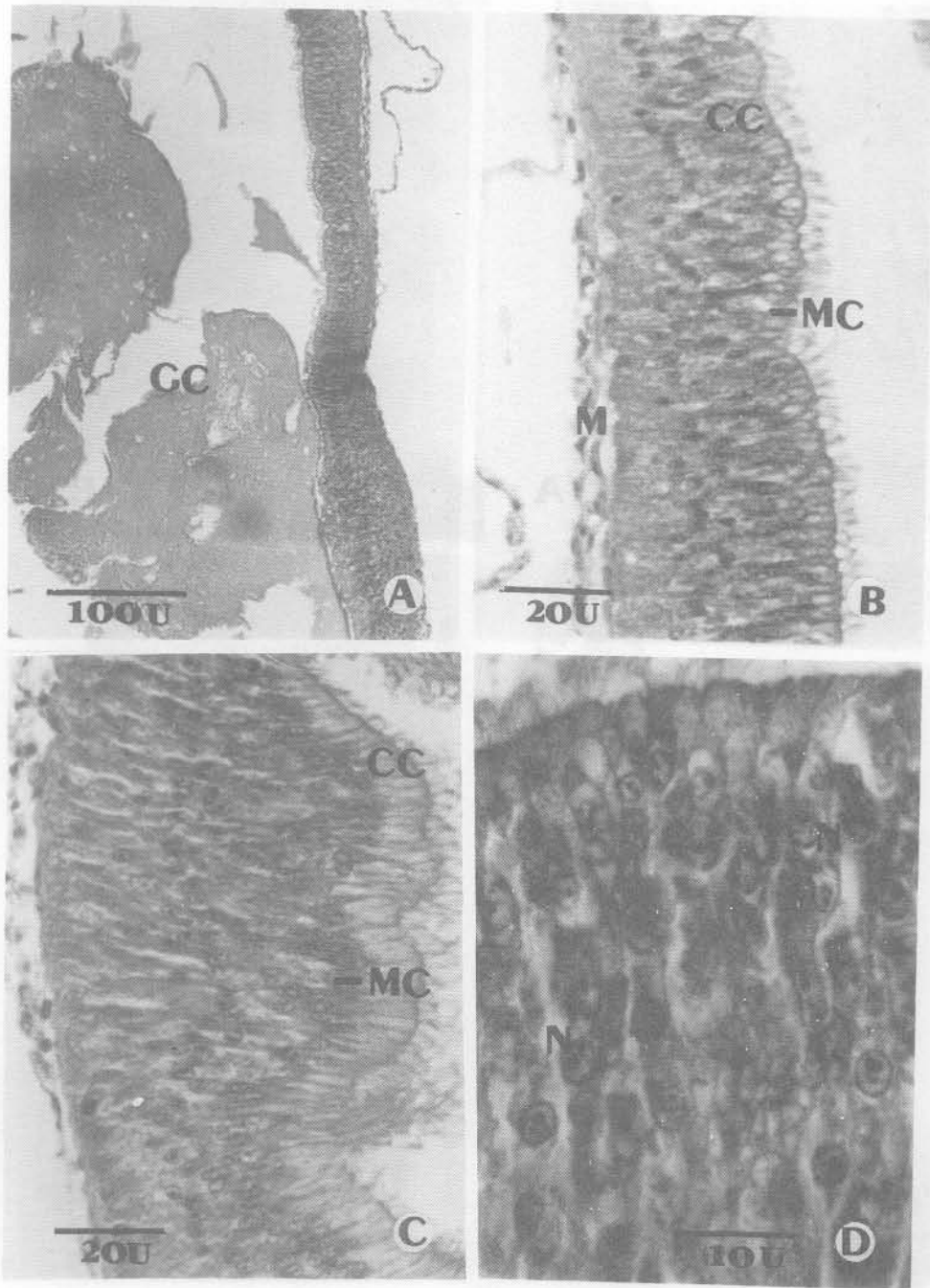
There are two main types of secretory gland cells in the epithelium of postintestine. The first type is mucous-secreting goblet cell which could be found in the prointestine (Fig.7B). The second type of gland cells is more abundant, consisting of elongated sacciform or clavate cell with a narrow neck (Fig.7B). The nucleus is large and may locate anywhere in the lower two-thirds of the cells. It is either ovoid or spheroidal in shape and rich in chromatin material. The cytoplasmic contents appear in the form of distinct, spherical refractive granules of a bright red color in hematoxylin - eosin stained preparation (Fig.7B).

**Molluscicides intoxicated snails** The histological alterations of intestine in snails intoxicated with niclosamide and the crude-water extract of *B. actinophylla* are very similar (Figs. 6C,6D,7C,7D). General reactions are more severe in snails exposed to *B. actinophylla*. In the prointestine, there are reduction of cilia, fragmentation of muscle tissue and an increase in mucus secretion (Fig.6C). In addition, the columnar cells have become highly vacuolated and their nuclei have dilated and lost their chromatin material (Fig.6D). The reactions seem to be less severe in the postintestine. The columnar cells have become distended and deformed in shape with the increase of mucous droplets in vacuoles (Figs.7C,7D).

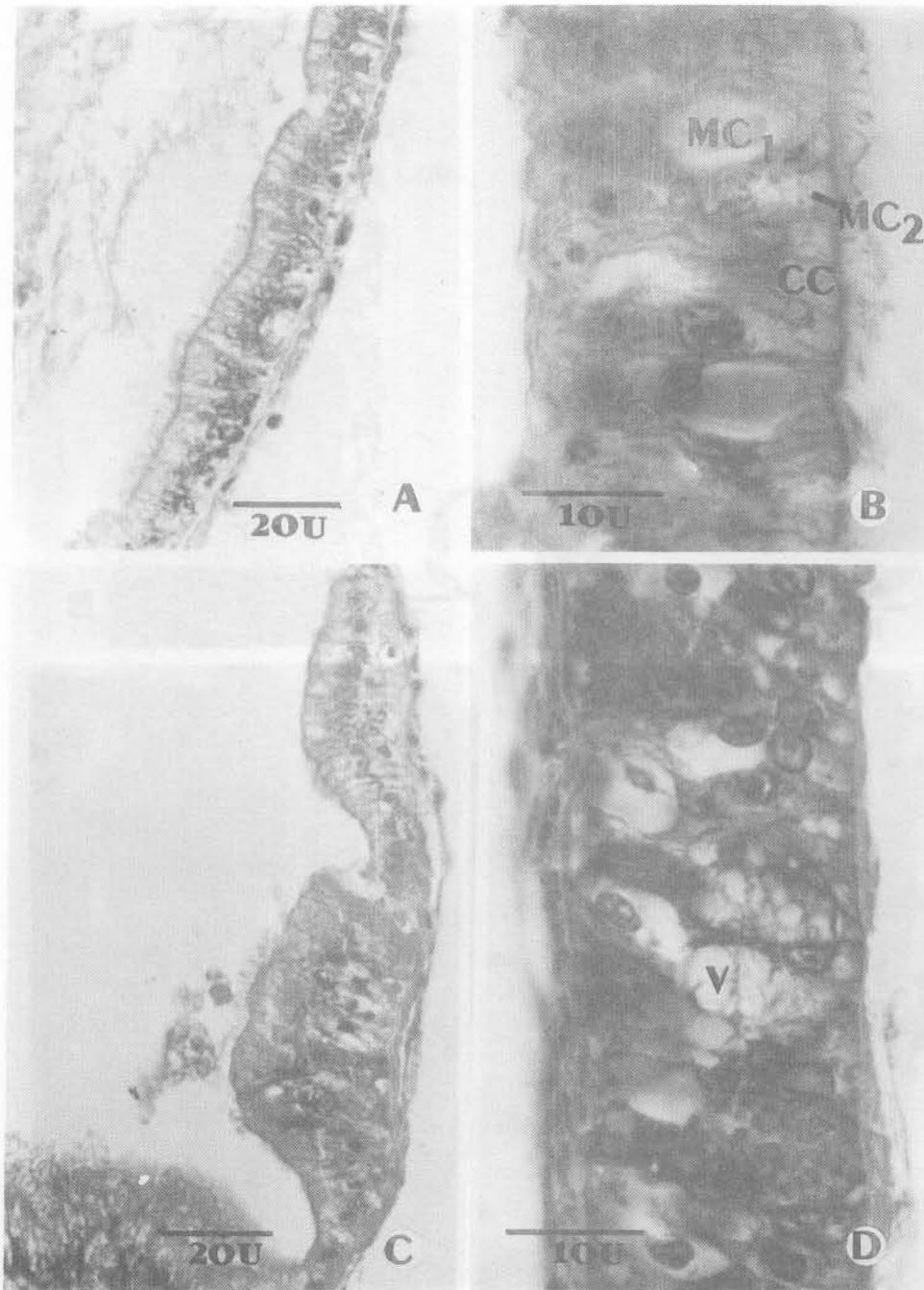
## 6. Digestive gland

**Control snails** The digestive gland is a compound branched tubular type (Fig.8A). The cells constituting the lining of the tubule are of four main types : digestive cell (acidophilic), thin cell (acidophilic), excretory cell (basophilic) and secretory cell (basophilic) (Fig.8B).

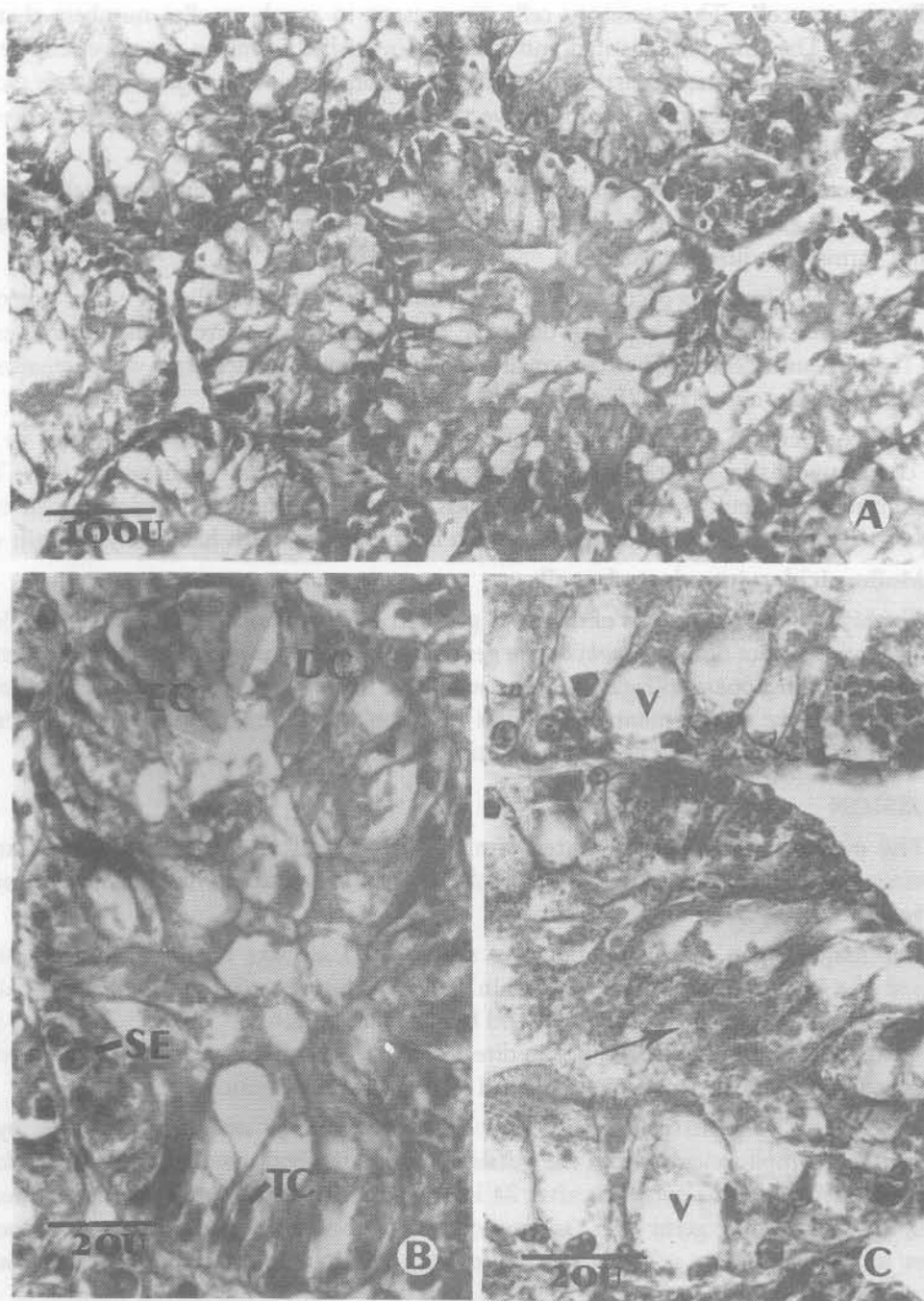
**Digestive cell** The digestive cells comprise the most numerous elements in the walls of the digestive gland tubules. They are long columnar and rest on a very thin basement membrane. They vary greatly in length within one and the same tubule. The nucleus is basal, usually oval, but may be elliptical, spheroidal, with one conspicuous nucleolus (Fig.8B). Inside the major part of the cell body, the cytoplasm shows various degrees of vacuolation and different kinds of granules (Fig.8B).



**Fig. 6** Prointestine of control (A,B) and molluscicides intoxicated (C,D) snails. (A) Low magnification showing pseudostratified ciliated columnar epithelium in control snails. GC = gut content. (B) Medium magnification of ciliated columnar (CC) and mucous-secreting goblet cells (MC). M = muscle tissue. (C) Medium magnification of epithelium in molluscicides intoxicated snails showing dilatation of columnar cells (CC) and mucous-secreting goblet cells (MC). (D) High magnification showing dilated nuclei (N) with reduction of heterochromatin.



**Fig. 7** Postesophagus of control (A,B) and molluscicides intoxicated (C,D) snails. (A) Medium magnification showing ciliated columnar epithelium in control snails. (B) High magnification showing columnar cells (CC) and mucous cells of two types (MC<sub>1</sub>, MC<sub>2</sub>). (C) Medium magnification of ciliated columnar epithelium in molluscicides intoxicated snails. (D) High magnification showing distended cells and increase of small mucous droplets in vacuoles (V).



**Fig. 8** Digestive gland of control (A,B) and molluscicides intoxicated (C) snails. (A) Low magnification of digestive gland which is a compound branched tubular type. (B) High magnification of the lining of tubule in control snails showing digestive cell (DC), excretory cell (EC), secretory cell (SE) and thin cell (TC). (C) High magnification of digestive gland cells showing enlargement of vacuoles (V) and a breakdown of cell apices (arrow).



**Excretory cell** The excretory cells are present in much smaller numbers than the digestive cells. They are quite distinct and easily distinguished from the digestive cells by their different morphological characteristic and staining reaction. Their shape is columnar or saccular and also markedly shorter than the digestive cells and therefore appear wedged in between groups of the digestive cells (Fig.8B).

The excretory cell has a characteristically large nucleus, with a distinct nucleolus. The cytoplasm of the excretory cell is usually crowded with a large number of excretory spherules of regular forms but different sizes.

**Thin cell** Thin cells occur only in low numbers, situated usually on either side of the secretory cells but also rarely between digestive cells. The cell is narrow, and extends to the tubule. The nucleus is central. There are small acidophilic granules which stain with hematoxylin-eosin present in the cytoplasm (Fig.8B).

**Secretory cell** Secretory cells usually occur singly in the corner of the tubules by lying on the connective tissue sheath. These cells are low and pyramidal in shape (Fig.8B). There is a large central nucleus with a single nucleolus. Except in the apical region, much of the volume of the cell is filled with spherules which are basophilic with hematoxylin-eosin stain.

#### **Molluscicides intoxicated snails**

The histological alterations of digestive gland in snails intoxicated with niclosamide and the crude-water extract of *B. actinophylla* are generally similar. The increase of mucus secretion in the lumen is quite apparent (Fig.8C). Some acini have undergone lysis. Cytological alterations in each cell type are enlargement of vacuoles in cytoplasm, a breakdown of cell apices and secretion of cell content into the lumen of acinus (Fig. 8C).

### **DISCUSSION**

The present study demonstrated similar histological alterations in the organs of reproductive, nervous and digestive systems of *I. exustus* intoxicated with niclosamide and *B. actinophylla*.

The major organ in the reproductive system of *I. exustus* is the ovotestis which is composed of a large number of acini. Within the acini, various stages of male germinal cells and Sertoli cells and female germinal cells and follicle cells are found. Since the ovotestis is the organ located inside the shell and is not in direct contact with molluscicides, alterations of the cells in the organ were not conspicuous until 24 hours after intoxication.

Alterations of germ cells inside the acini of ovotestis occurred probably by the transportation of molluscicides from the outside through the body wall of snails. Histological alterations observed in the ovotestis after 24 hours of intoxication were reduction in number of acini, degeneration of germ cells (spermatozoa and oocytes) and a decrease in chromatin granules in spermatogonia. Zhou *et al.*<sup>4</sup> also reported that in the ovotestis of *B. glabrata* intoxicated with niclosamide and *E. camaldulensis*, there was a reduction in number of acini, Sertoli cells, spermatozoa and oocytes. These cells also appeared degenerated<sup>4</sup>. However, in the ovotestis of *Lymnaea stagnalis*, degenerating cells may be influenced by other external conditions which may have an effect on oviposition and resorptive<sup>5</sup>. Moreover, Luis<sup>6</sup> has described degeneration of oocytes after the period of oviposition in *Arion ater rufus*. In the present study, intoxication was done in a short period. These factors might be less effective on decreasing in size of the ovotestis.

Histological changes of the neurosecretory cells in the cerebral ganglia induced by niclosamide and *B. actinophylla* are more conspicuous in the large neurosecretory cells, light



green cells and bright green cells. There were significant increases in cell and nuclear diameters and enlargement of vacuoles in the cytoplasm. Hanumante *et al.*<sup>7,8</sup> reported on the intoxication of *I. exustus* with barium chloride and copper sulfate for 24 hours. He also found the significant increase in nuclear diameters of light green cells and bright green cells ( $P < 0.05$ ).

There was a significant increase of cell dimension of neurosecretory cells of niclosamide and *B. actinophylla* intoxicated snails. This is shown by the enlargement of vacuoles. Hanumante *et al.*<sup>8</sup> also reported that there were a large number of vacuoles in neurosecretory cells of copper sulfate intoxicated snails but not in barium chloride intoxicated snails. It appears that niclosamide and *B. actinophylla* have some effects on the water balance of *I. exustus*. McCullough *et al.*<sup>1</sup> reported that water balance of gastropod was thought to be under neurosecretory control. Some kinds of molluscicides, barium chloride and copper sulfate, resulted in diminished neurosecretory activity. It may be that molluscicides cause stress on water balance or disturbance in metabolism or physiological function. Dorsett<sup>9</sup> reported that water balance was controlled by pleural ganglia. Hekstra and Lever<sup>10</sup> and Lever *et al.*<sup>11</sup> also suggested that neurosecretory material in the pleural ganglia of *L. stagnalis* involved in the hormonal control of water balance.

Molluscicides are used either as contact or as stomach poisons<sup>12</sup>. The pulmonate freshwater snails did not take up molluscicides via the gastrointestinal tract or the gills and a transport through the body wall is more probable<sup>13</sup>. Such a way of transportation through the body wall tends to induce muscle relaxation and a diminished ability to contract physically or tonically<sup>14-17</sup>. From macroscopic observation, *I. exustus* did not show any reaction from the first hour of intoxication by niclosamide and *B. actinophylla*. Therefore, it is believed that the snail does not only take up molluscicides by transport through the body wall but also through the digestive tract. The general histological alterations of epithelium of the digestive system are a rapid extrusion of mucus, reduction of cilia or microvilli and the increase fusion of vesicles and vacuoles. The secretion of mucus is considered to serve to dilute the toxin and may also have the capacity to detoxify molluscicides. Mucus passes through the digestive tract and is voided quickly due to the intensified mucous extrusion of the whole animal<sup>18</sup>. However, intensified exudation of mucus can also kill them. The including of mucus secretion would finally lead to a desiccation of the snail and loss of mucous cells would prevent production of the very mucus that protects the surface of the snails from desiccation<sup>19</sup>. Davies *et al.*<sup>20</sup> and Frain<sup>21</sup> believed that these activities of mucus production and extrusion were considered as a main mode of action of molluscicide, leading to a dehydration of snails.

The reduction of cilia or microvilli, the enlargement of vacuoles by the increase fusion of vesicles and vacuoles in the digestive cells of digestive gland or other ciliated columnar cells of digestive tract might result from the interaction of the lipophilic molluscicide with membrane. This interaction might induce changes in composition, fluidity and stability of membrane<sup>22</sup>. The intensified fusion between small and large vacuoles result in the increase in the number of large vacuoles after intoxication. Moore *et al.*<sup>23</sup> assumed that changes in membrane fluidity induced this altered rate of vesicle fusion.

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## บทคัดย่อ

จากการศึกษาด้วยกล้องจุลทรรศน์ถึงผลของสารเคมีฆ่าหอยนิโคซามาไนด์และสารสกัดจากใบหนวดปลาหมึก (*Brassia actinophylla*) ต่อการเปลี่ยนแปลงของเซลล์และเนื้อเยื่อในระบบทางเดินอาหาร ระบบสืบพันธุ์ และระบบประสาทในหอยคัน *Indoplanorbis exustus* พบว่าไม่มีความแตกต่างกันมากนัก แต่หอยที่ทดสอบกับสารสกัดจากใบหนวดปลาหมึกมีการเปลี่ยนแปลงของเซลล์และเนื้อเยื่อมากกว่าหอยที่ทดสอบกับนิโคซามาไนด์เล็กน้อย ในอวัยวะสืบพันธุ์มีการสลายตัวของสเปิร์มาโทไซด์และไข่ ส่วนในปมประสาทเซรีบรัลพบการเปลี่ยนแปลงของเซลล์ประสาทผลัดฮอร์โมนโดยการเพิ่มขนาดของเซลล์และนิวเคลียส การสลายตัวของนิวโรฟิล และการเพิ่มขนาดของแควคิวโลลในเซลล์ ส่วนการเปลี่ยนแปลงของเซลล์เยื่อทางเดินอาหารพบว่าการเพิ่มของจำนวนเซลล์ที่สร้างมิวคัส การเพิ่มขนาดของเซลล์ตามความสูง การเพิ่มขนาดของมิวคัสแควคิวโลล ซิเลียบนขอบเซลล์ด้านบนมีจำนวนลดลง เนื้อเยื่อกล้ามเนื้อฉีกขาด ส่วนการเปลี่ยนแปลงภายในเซลล์ของต่อมย่อยอาหารพบว่าการเพิ่มขนาดของเซลล์และแควคิวโลลและการสลายตัวของเซลล์บางส่วน