ISSN : 2321-9602

Indo-American Journal of Agricultural and Veterinary Sciences




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Indo-American Journal of Agricultural and Veterinary Sciences
ISSN 2321–9602 www.iajavs.com

Histological Investigations of the Changing Activity of Snails Throughout Their Lives

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Abstract

The larger terrestrial mollusc species are cultivated specifically for human consumption. They are now often eaten across the world. Histology has several applications in animal and human health care. This technique's central value lies in its ability to detect and characterize the existence of formations and inclusions. It's purpose is to help figure out the make-up and development level of this procedure. Histology is the study of tissue samples under a microscope. The processed material is next examined and described by the expert in the form of histology preparations. Consequently, we suggested examining snails using histology. The overall health of snail populations may be determined using this approach. The research used specimens of the snail species Helix aspersa maxima, Helix aspersa muller, and Helix pomatia. A total of 10 snails from each species were chosen at four distinct times throughout their active life cycle (immediately after hibernation, 5 days after waking up, 14 days after waking up, and July). We started with summertime histology investigations of snails since that is when they are most active. Therefore, the experiment's snails were collected in July. If the snail consumes and lives normally, its digestive gland will have tubules lined with hemolymphatic sinuses and hemocytes, divided by intertubular connective tissue. Therefore, we focused on the structure of the digestive system in our investigation. Each tubule has a ring of circular muscular tissue protecting it. The epithelium that lines the digestive gland tubules has three distinct cell types: digestive cells, calcium cells, and excretory cells. The digestive gland's tubular epithelium is composed almost entirely of digestive cells. Cells in the digestive tract contain circular or oval nuclei at their bases. During this time, the snail's digestive gland ceases all digestive activity and its cells undergo a metamorphosis into excretory structures. There is no outward discharge of metabolic byproducts. When they build up, they completely block all passageways throughout a snail's body, including the lumen of glandular tubules, excretory ducts, the stomach, and the intes- tines. Snails' metabolisms speed up after a meal. It increases the production of digestive enzymes, the efficiency of the excretory system, and the quantity of waste products. It results in the thorough flushing out of the digestive system of any waste products that may have built up while the body was at rest. The digestive gland has been fully restored to its original configuration.

Keywords: terrestrial mollusks, periods of the year, laboratory tests, cell structure, digestive tract.

1. Introduction

Terrestrial mollusks are an ecological group of creatures that makes up about 35,000 modern species. They belong to the Gastropoda class and are an important part of many ecosystems as they process much of the dead organic matter,distributing some substances and forming the diet basis for many animals (Köse et al., 2015; Peña et al., 2017; Cilia & Fratini, 2018; Becker et al., 2021).

Large species of terrestrial mollusks are used for human consumption and are bred for this purpose. They have be- come a part of national cuisine in many countries. Terrestri- al mollusks are the most successful model objects for many studies due to limited mobility and settlement (both active and passive) (Hamlet et al., 2012; Lobo-da-Cunha, 2019; Noothuan et al., 2021).

Improving the quality of meat products produced in un- stable raw materials conditions requires solving several practical tasks in the field of veterinary medicine. There is a search for modern, scientifically based, objective, and ac- cessible laboratory methods for determining the freshness ofsnails. Another issue, namely, the study of some processes concerning snails, is partially studied (Hamed et al., 2007; Noothuan et al., 2021).Thus, we proposed a histological method of studying snails. This method can provide the data of snails' state as a whole.

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ISSN 2321–9602 www.iajavs.com

Histology is a branch of biology that studies the struc- ture, life activity, and development of living organism tissues, i.e., the structure at the tissue level. With the help of histology, it is possible to study the development and differ- entiation of cells and tissues, adaptation at the cellular and tissue levels, the problems of regeneration of tissues and organs, etc. (Mohammadein et al., 2013; Ali & Said, 2019).

Histological examination is a very accurate method of laboratory diagnostics, which studies the changes in tissues at the cellular level under a microscope. This method is one of the most effective and modern methods of diagnostics (Habib, 2018). It is complex and involves different technol- ogies of material processing and different methods of microscopy. Histology is used in many fields of humane and veterinary medicine (Parvate & Thayi, 2017). Its main es- sence is that this method can diagnose the presence of for- mations and inclusions. It is used to determine this process's structure and stage of development. Samples of different tissues can be used as material for histological examination. The specialist analyzes and describes the histological prepa- rations obtained after processing the material (Pirger et al., 2004; Amal & Abdel-Rahman, 2020).

DSTU 7353:2013 “Meat. Method of histological deter- mination of freshness and degree of ripening” was devel- oped by the staff of the Institute of Food Resources of the National Academy of Agrarian Sciences (IFR NAAS) to increase the objectivity of the examination. It describes the essence of the method; measuring equipment, test equip- ment, ancillary equipment, materials, and reagents; rules for reagents preparation, selection and preparation of a test sample for the research; research process; the processing of results; registration of research results, safety and personnel requirements. However, due to this DSTU, it is impossible to study and determine all processes occurring in the bodyof snails (DSTU 7353: 2013).

With the help of this research, it is possible to determine the stage of the digestive gland, its structure, functioning, and the strategy for using snails in the future (Hamed et al., 2007).

Nowadays, a detailed ultrastructural study of snails and the study of changes in organs at different periods remain relevant.

We decided to study the structure of digestive cells in snails at different periods after waking. It helps to differenti-ate and analyze them according to specially developed and scientifically sound morphological criteria.

2. Materials and methods

Snails of the species *Helix aspersa maxima*, *Helix asper-sa muller*, and *Helix pomatia* were the material for the study. Ten snails of each species were selected for the ex- periment at different periods: immediately after hibernation, five days after wakening, 14 days after wakening, and at the time of their active life – in July.

Before a histological examination, pieces of organs were carefully removed and fixed in a 10 % formalin solution. They were washed with water from the fixative. Dehydra- tion was performed in alcohols of different strengths: 70 °C, 80 °C, 90 °C, 96 °C and in absolute alcohol for 12 hours.

Then the material was kept in a solution of alcohol with chloroform (1:1) for 30 minutes, in chloroform for 1 hour, and in chloroform with paraffin (1:1) for 1 hour in a thermo-stat at 37 °C.

Paraffin was poured into a thermostat at 57 °C for 15 minutes each.Histological sections were made on a rotary microtome MPS-2 3–4 μm thick and stained with hematoxylin-eosin. For this purpose, sections were deparaffinized in xylene for 20 minutes, dehydrated with 96 °C alcohol, washed with distilled water for 1 minute, and stained with hematoxylinfor 10 minutes. Then they were washed with tap water,differentiated in 1 % sulfuric acid solution, washed with water, stained with 1 % eosin solution, washed with distilledwater, and dehydrated with 96 °C alcohol. In the last stage, they were put in a 25 % polystyrene solution and covered with a cover glass.

Preparations stained with hematoxylin-eosin were sub- jected to review microscopy to assess the histoarchitectonicsof the digestive gland under the microscope Axioskop 40 (Zeiss).

It should also be noted that the histological picture in an- alyzing experimental preparations from different species of snails was the same. Thus, the type of snail did not affect thestudy.

3. Results and discussion

First, we conducted histological studies of snails during their active life, i.e., in the summer months. Therefore, for this purpose snails for the experiment were taken in July.

The digestive gland of mollusks is involved in the extra- cellular and intracellular digestion of food material, absorp- tion of nutrients, storage of lipids, glycogen, and minerals, and plays an essential role in detoxification.

It was determined that if the snail eats and has an every- day life, its digestive gland generally consists of digestive tubules separated by intertubular connective tissue contain- ing hemolymphatic sinuses and hemocytes. Thus, in ourresearch, we studied the structure of the digestive gland.

A circular muscle layer surrounds each tubule. There are three different cell types in the epithelium lining the tubules of the digestive glands, which differentiate into digestive cells, calcium cells, and

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ISSN 2321–9602 www.iajavs.com

excretory cells.

Digestive cells are the most numerous cellular compo- nent of the tubular epithelium of the digestive gland. Basallylocated nuclei of digestive cells have a round or oval shape (Fig. 1).

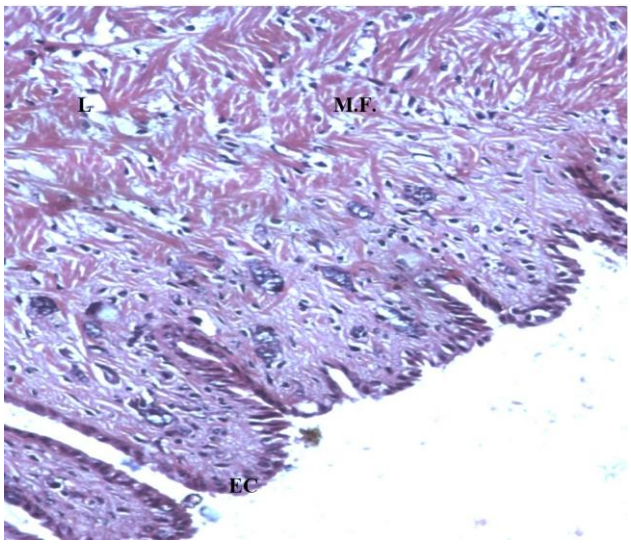
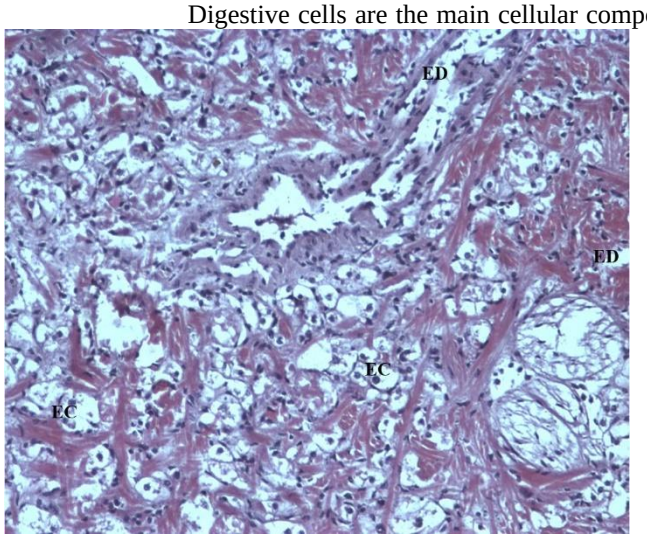


Fig. 1. Histological section of *Helix pomatia* snail in July.Magnification 10×/0.25. L – lumen, M.F. – muscle fibers,
E.C. – epithelial cover

Figure 1 shows that muscle fibers surround the digestive tubules. Each tubule has a lumen and well-defined epithelial cover.

Digestive cells are the main cellular component of the digestive tubules of the gland, and they are involved in criti- cal morphofunctional changes following the digestive activi-ty of the snail. They are round or oval and have one nucleo- lus. They contain two types of granules, shown in Fig. 2.



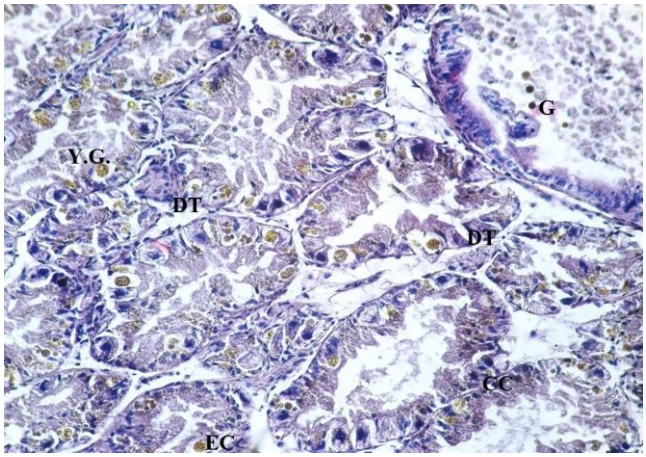


Fig. 2. Digestive gland of *Helix aspersa muller* snail in summer. Magnification 10×/0.25. D.T. – digestive tubules,CC – calcium cells, G.G. – green granules, Y.G. – yellow granules, E.C. – excretory cells

Green granules are mainly in the vacuoles and are lim- ited to the distal edge of the cells, and yellow granules - are in the basal area.

Calcium cells are less involved in the digestive process compared to other cells. They are located either separatelyor in pairs in the corners of the tubules and have a pyramidalshape.

Excretory cells have a spherical shape. They are charac- terized by their presence in the vacuole and occupy almost their entire volume.

Thin cells are rarely observed in microscopic specimens.

They are distributed randomly among other cell types.

During the rest period, the digestive gland of snails is characterized by a complete loss of digestive function and the transformation of digestive cells into excretory ones. Metabolic products are not excreted into the environment.

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ISSN 2321–9602 www.iajavs.com

When they accumulate, they fill all cavities in the body of snails, namely the lumens of glandular tubules, excretory ducts, stomach, and intestines (Fig. 3).

There is a gradual restoration of digestive cell function and digestive processes in snails that wake up after anabio- sis. Under the influence of digestive enzymes, the excretory mass accumulated during anabiosis is broken down, lique- fied, and excreted into the environment. There are also dif- ferent types of hemocytes identified in the vacuoles. They can be of different shapes: round, oval, or pear-shaped, with a large nucleus, and occupy almost the entire volume of the vacuole (Fig. 4).

Food intake in snails increases the level of metabolism. It stimulates the differentiation of digestive cells, the func- tional activity of excretory cells, and the volume of excreto- ry products. It leads to the complete liberation of the diges- tive tract per- forms such functions as food uptake, extracellular and intra- cellular digestion, secretion, excretion, and

from accumulated excretion products during the rest period. The structure of the digestive gland is wholly restored (Fig. 5).

Fig. 3 Histological section of the *Helix aspersa maxima*

snail immediately after wakening. Magnification 10×/0.25.

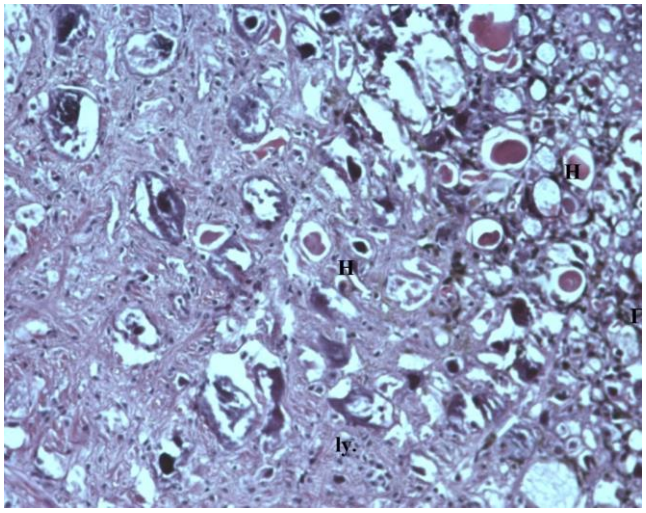
E.C. – excretory cells, E.D. – excretory duct.

Fourteen days after anabiosis, cells of the digestive tu- bules completely recovered. They have a well-defined shape. Digestive cells are packed with green and yellow granules. Hemocytes are partially observed.

The hepatopancreas (digestive gland) of mollusks is a multifunctional organ. According to some authors, it

osmoregulation, and it plays an essential role in detoxification. The digestive glands of mollusks are known

as target organs for toxicants. The changes in cytoarchitectonics of the digestive gland in snails are used as a



biomarker of induced toxicity. Thus, anystructural damage to the digestive gland affects animals different.

Fig. 4

Histological section of the *Helix pomatia* snail five days after wakening. Magnification 10×/0.25. H – hemato-cytes, D.C. – digestive cells.

It was found that the digestive gland of snails is repre- sented by three types of cells: digestive, excretory, and cal- cium. Their ratio is different and depends on the stage of the snail's life.

There is a standard 19496-2013 called “Meat and meat products. The method of histological examination”. It is used only in Armenia, Kazakhstan, Moldova, and other

countries. It is used for meat of all slaughtered animals and poultry, meat products, and canned meat. However, only due to the histological method is it possible to determine the freshness and degree of meat maturation and the structure and composition of meat products.

Therefore, the authors proposed this method to deter- mine the state of the digestive gland in snails to obtain data on their further use. In this case, the histological method is based on the determination of microstructural parameters of the digestive gland, identification of the components of the analyzed samples following their microstructural features, and the compliance of these components to histological specimens. The study of the digestive gland characterizesthe state of the snail as a whole.

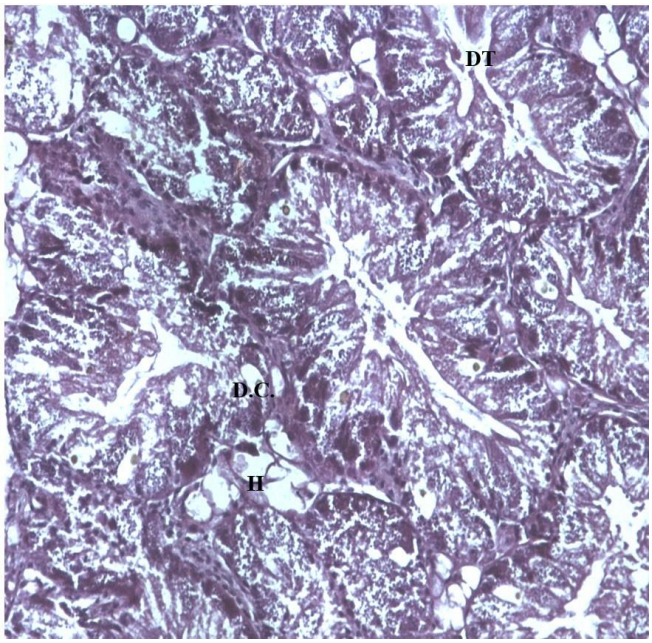


Fig. 5.

Histological section of the *Helix muller* snail 14 days after wakening. Magnification 10×/0.25. D.T. – diges-tive tubules, D.C. – digestive cells, H – hematocytes.

The disadvantage of this method is that it is pretty ex- pensive. It requires special equipment (materials, reagents, equipment, etc.) and a well-qualified specialist with appro- priate education.

According to some authors, bioindication of freshwater pollution can be carried out based on the histopathological examination of the organs and tissues of mollusks, namely ampullaria. Therefore, the prospect of further research will be the study of *Helix aspersa maxima* snails, *Helix aspersa muller* snails, and *Helix pomatia* snails as bioindicators of the environment.

4. Conclusions

Histological studies confirm the possibility of their use to study microstructural changes in the organs of snails at different stages of their life. Therefore, it is essential to conduct research to identify morphological changes in the organs during hibernation and at different periods after snails' waking and compare the results. The digestive gland of mollusks is one of the organs that can be used as a markerin various types of research. Histological studies of snails at different periods of life can be studied on one species, as thetype of snail does not affect the result. Studying the digestive gland of snails at the histological level makes it possibleto determine its state and the possibility of using snails for food. We found that it is possible to use snail meat for food only 14 days after the anabiotic state. This research method can be used as one of the methods of application in some pathological assumptions about snails. Data obtained by the histological method can be used to study snails' digestive glands for control and their use in manufacturing various dishes.

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