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# HISTOPATHOLOGICAL CHANGES IN THE TISSUES OF THE FRESH WATER SNAIL GYRAULUS LAEVIS (ALDER) PRODUCED BY PARAMPHISTOMUM LARVAL STAGES

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**Paramphistomum gelişme dönemlerinin tatlısu sümüklüsü Gyraulus laevis (Alder) dokularında meydana getirdiği histopatolojik değişiklikler.**

**Özet:** *Tatlısu sümüklüsü* Gyraulus laevis *(Alder) ve Paramphistomum serkerleri ile ilgili seri araştırmaların ikincisi olan bu çalışma, Paramphistomum gelişme dönemlerinin sümüklüdeki lokalizasyonlarını ve özellikle sümüklü dokularında neden oldukları histopatolojik değişiklikleri saptamağa yöneliktir.*

*Binoküler disseksiyon mikroskobu kullanılarak yapılan kontrollerde saydam olan kabuk yapılarından ötürü sümüklü içersindeki sporokist, redi, serker gibi gelişme dönemlerini görerek enfekte* G. laevis'*lerin ayrımı mümkün olabilmektedir.*

*Histopatolojik yoklamalar için ayrılan örnekler sert kabuk kısımları uzaklaştırıldıktan sonra Bouin sıvısında tespit, alkol serilerinden geçirilerek dehidre edilmiş ve ksilolde saydamlaştırıldıktan sonra parafin bloklara alınmıştır. Bu bloklardan* 8–12 *μm kalınlığında kesitler yapılmış ve hematoksilen–eozin ile boyanmıştır.*

*Kesitlerin incelenmesinde; Paramphistomumların sporokist, redi ve serker gibi gelişme dönemlerine başlıca sindirim bezi ve ovotestis'lerde rastlanmıştır. Bu lokalizasyonlara bağlı olarak sindirim bezi tüplerinin sayısında belirgin bir azalma olduğu, kaybolan doku kısımlarının yerinde parazit gelişme dönemlerinin bulunduğu gözlenmiştir. Ovotestis'i çevreleyen kan sinüslerinde de Paramphistomum gelişme dönemlerinin yerleştiği, foliküller içerisinde sporokist ve redilere rastlandığı, dolayısıyla cinsiyet hücrelerinin de zarar gördüğü saptanmıştır. Bunlara ilaveten,*

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*ayak ve böbrekte daha az olarak Paramphistomum gelişme dönemlerine rastlanmıştır. Paramphistomum gelişme dönemleriyle enfekte sümüklülerde, sindirim bezi ve ovotestislerin ençok zarar gören iki organ olduğu saptanmıştır.*

**Summary:** *The present paper is the second in the series dealt with the fresh water snail* Gyraulus laevis *(Alder) and Paramphistomum larval stages. This work was directed towards an investigation of the occurrence of the Paramphistomum larval stages within the snail* G. laevis *and more particularly their histopathological effects on the snail tissues.*

Gyraulus laevis *parasitized with Paramphistomum larval stages could be positively identified by detecting the different developing stages of the parasite such as sporocysts, rediae and cercariae through the transparent shell using binocular dissecting microscope.*

*For histopathological examinations, representative snails were fixed using Bouin's fluid, dehydrated, cleared and paraffin embedded. Sections were cut at* 8–12 *microns in thickness and stained with haematoxylin and eosin.*

*The results indicated that the majority of the Paramphistomum larval stages, sporocysts, rediae and cercariae, parasitized* G. laevis *were located mainly in the region of the digestive gland and ovotestis. These two organs were severely damaged by the presence of Paramphistomum larval stages. There was a considerable reduction in the number of the digestive gland tubules. Their tissues were replaced by the parasite. On the other hand Paramphistomum larval stages settled in the blood sinuses surrounding the ovotestis. Sporocysts and rediae were occurred inside the tubules and invaded the acini damaging the sex cells. In addition foot, and kidney were affected by Paramphistomum larval stages.*

## Introduction

The vital activities of an animal whose body is invaded by other organism become very greatly disturbed. One can expect to find significant changes induced by the foreign organism, such alternation from the normal can be expected as mechanical, histopathological, physiological, biochemical, even sometimes morphological changes (2,14, 19,20,24,26).

Mechanical changes are recognized as the local structure disturbance; morphological changes such as abnormal proliferation of the tissues and cyst formation; physiological and biochemical effects are expressed in the limitation or modification of the normal processes of the host, the histopathological changes described as the damages which the foreign organisms produce in their hosts tissues (5,17,18,25).

The mollusca is a particularly large and remarkable diverse phylum in the animal kingdom. Many species of gastropod molluscs suffer digenetic trematode larval infections. The histopathological effects of the parasite on the snail host have been studied by many authors (1,9,10,11,15,16,22).

One is impressed by the degree of damage which the larval trematodes produce in the snail hosts, and at the same time one is equally impressed by the tolerance of the snail to these infections.

The sporocysts and rediae of trematodes affect the genitalia, albumen gland, the prostate, the uterus, the sperm duct, and indirectly affect the reproductive capacity of the parasitized snail (15,16).

The damages that larval flukes cause are produce when the larval stages migrate through the various tissues or by the consumption of the larvae of absorbed nutritive material. The amount of these damages depend on whether the infection is light, moderate or heavy and by accumulation of large amount of toxic waste products excreted by the parasite. Digenetic larval trematodes are differ in the extent of the harm they cause to the host. Some cause little damage on the host, others are very destructive and the pathological changes in the host tissues are very pronounced. It can be stated in general that the trematodes which posses a redial stages in the life cycle are more injurious than others to their snail hosts (2,6,25,26).

Cheng and Snyder (7) reported the destructive effect on the digestive gland of *Heliosoma trivolvis* infected with *Glypthelmins pennsylvaniensis*. Bourns (3) indicated that larval trematodes parasitized *Lymnaea stagnalis* harm their host to the extent of inhibiting its growth and shortening its life span. Schmid et al. (23) on infected *Planorbis planorbis* with *Paramphistomum cervi* larval stages showed an increased in mortality and decrease in fertility. Histopathological studies were made on the details of sporocysts, rediae and cercariae of *Paramphistomum cervi* in the snail host *Planorbis planorbis* and its reac-

tions on host tissues. Damages of the host caused by the parasite were found mainly in the ovotestis whereby its function was severely affected (8).

Recently Kamel et al. (13) on the occurrence of xiphidiocercariae and their histopathological effects on the tissues of the fresh water snail *Lanistes carinatus*, showed that in heavily parasitized snails the gonad were completely destroyed and replaced by the parasite, the digestive gland was highlv reduced in addition the ctenidia and kidney were also affected by xiphidiocercariae.

The present study is the second of a series that deals with the fresh water snail *Gyraulus laevis* (Alder) and the Paramphistomum larval stages.

The present investigation was carried out to observe the location and the histopathological effects of Paramphistomum larval stages on the tissues of the snail host *Gyraulus laevis*.

## Materials and Methods

*Gyraulus laevis* snails were collected from Eskişehir Çifteler State Farm, west of Ankara, Turkey. The snails were transported to the laboratory and they were kept in plastic aquaria containing dechlorinated tap water. Snails were fed on fresh lettuce leaves.

Parasitized specimens with Paramphistomum larval stages could be positively identified and separated from unparasitized ones by detecting the different developing stages of the parasite such as sporocysts, rediae and cercariae through the transparent shell of the snail *G. laevis* using binocular dissecting microscope, or by keeping individual snail in a container half filled with dechlorinated water for one hour exposed to light to allow mature cercariae to emerge (13,21).

For histopathological examinations, representative specimens were fixed using Bouin's fluid, which proved to be satisfactory for this work. The material was then dehydrated, cleared and paraffin embedded. Sections were cut at 10–12 microns in thickness and stained with haematoxylin and eosin.

## Results

In the fresh water snail *Gyraulus laevis* the digestive gland is in the form of a spirally coiled cone, which extends down to the posterior

end of the visceral mass, and occupies the greater part of the visceral hump which fills the shell spire above the body whole. It is light greyish or rather blackish in colour. The stomach lies embedded in the digestive gland, while the ovotestis extend over the right side and can be differentiated from the digestive gland by its colour and structure. (Fig. 1).

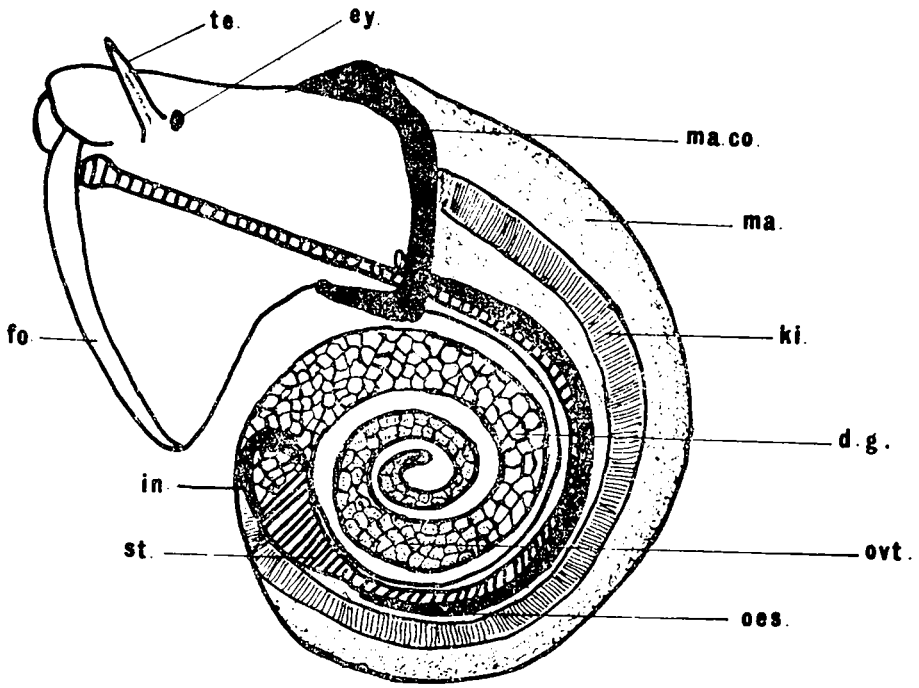


Fig. 1. Left side of *Gyraulus laevis* (Alder) removed from the shell
(Kabuksuz *Gyraulus laevis* (Alder)'in sol yanı)

d.g.-digestive gland (sindirim bezi)
ey.-eye (göz)
fo.-foot (ayak)
in.-intestine (barsak)
ki.-kidney (böbrek)
ma.-mantle (manto)
ma.co.-mantle collar (manto yakası)
oes.-oesophagus (özefagus)
ovt.-ovotestis (ovotestis)
st.-stomach (mide)
te.-tentacle (tentakül)

Normally the digestive gland consists of collecting tubules. Each tubule consists of two types of cells, the digestive cells (absorptive) and the excretory cells. The digestive cells are numerous and contains basely placed nuclei with the cytoplasm firly evenly distributed throughout the cells. These cells have been defined by various authors as prefixes liver-ferment, glandular and absorptive. On the other hand, the excretory cells are less abundant and occur between the digestive cells. The nuclei of the secretory cells are relatively large, the cells contain densely granular cytoplasm. The function of the excretory cell is to extract metabolic excretory products from the visceral haemocael and to pass them into the digestive gland tubule lumen, from where they are passed into the alimentary canal (Figs.2 and 3).

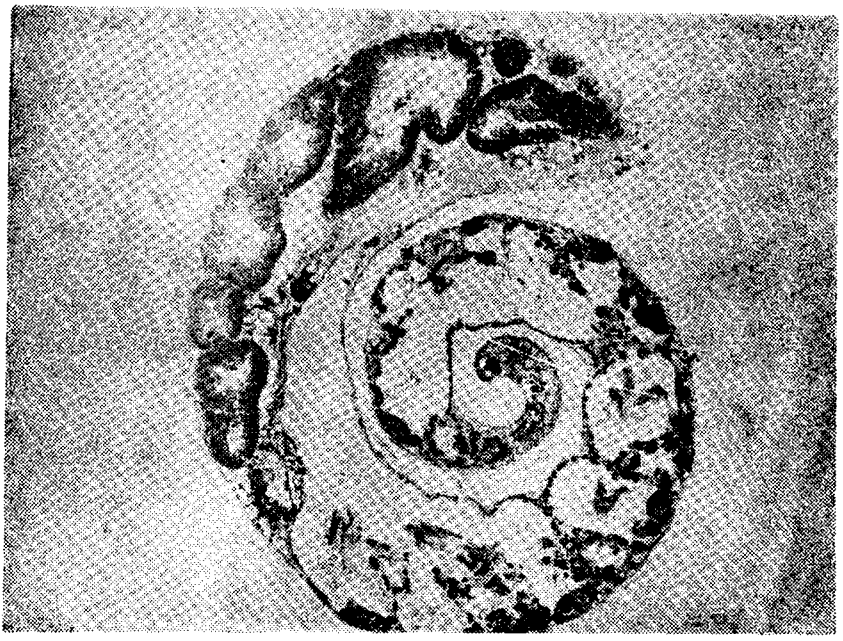

Fig. 2. Digestive gland and ovotestis of unparasitized *G.laevis* (x 40).
(Parazitsiz *G. laevis*'in sindirim bezi ve ovotestis'i)

In parasitized *G. laevis* with Paramphistomum larval stages the growth and development of Paramphistomum larval stages in the inter-lobular spaces of the digestive gland leads to displacement of the lobes and loss of their branched structure with subsequent degeneration

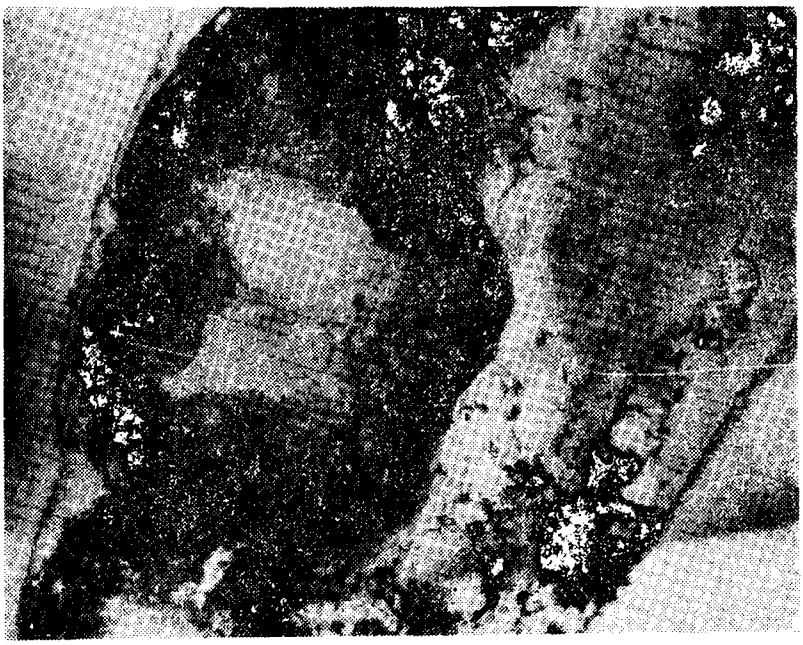

Fig. 3. A digestive gland tubule of unparasitized *G.laevis* (x 200).
(Parazitsiz *G.laevis*'in sindirim bezi tüpü)

of the epithelium lining the tubules. There was a considerable reduction in the number of the digestive gland tubules. The nuclei of the digestive cells migrate away from the cell-base and there was a decrease in the contained granular food store. The distal walls of these cells often break down and some of these cells content were released into the lumen of the tubules (Figs.4,5 A and B).

The pulmonates snails *Gyraulus laevis* are hermaphrodite. The ovotestis, which is the reproductive organ in the hermaphroditic snails produces both eggs and sperms in compartment called acini, is the other organ in the visceral mass situated at the end of the spiral animal (Fig.1). The ovotestis or hermaphroditic gland consists of several follicles or acini at the end of the spiral of the snail. Each acinus is enveloped in a sheath of squamous epithelium and a thin connective tissue lies between the acini and the covering epithelium of the mantle. In each acinus of the ovotestis, a number of immature oocysts, a few mature ova, and bundles of sperms can be detected (Figs.1,2 and 6).

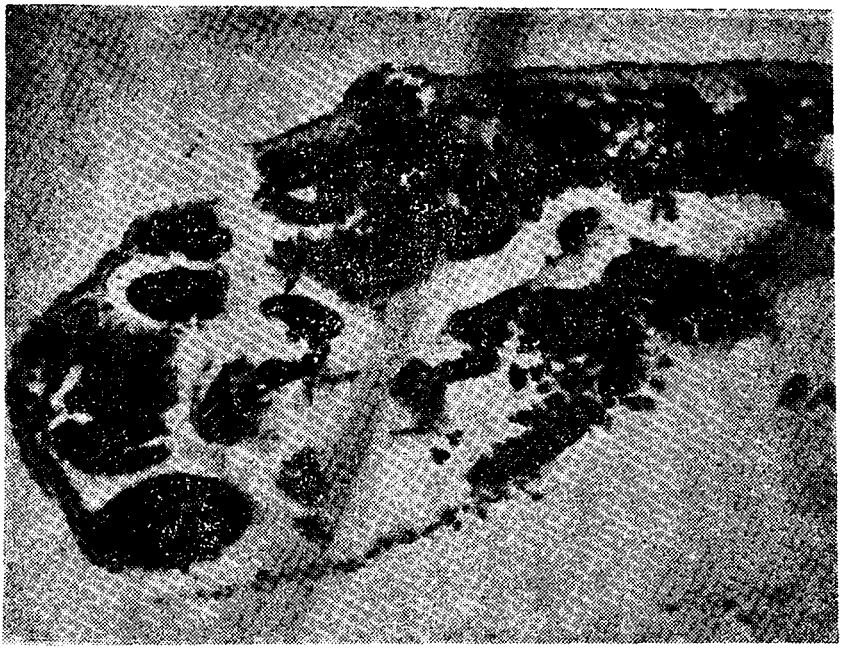

Fig. 4. Moderate parasitized digestive gland of *G.laevis* (x 100).
(*G.laevis*'in orta derecede parazitli sindirim bezi)

It was observed during the present investigation that the majority of Paramphistomum larval stages, sporocysts, rediae and cercariae, parasitized *G. laevis* were located in the region of the digestive gland and ovotestis. The pathological changes of the ovotestis in parasitized specimens can be summarized in the following steps: Paramphistomum larva stages settled in the blood sinuses surrounding the tubules of the ovotestis. In some cases the rediae occurred inside the persisting tubules and invaded the acini damaging the sex cells. The parasite inhibited the normal gametogenesis through the disruption of normal vascularization, crowding and may be toxicity (Fig.7). The shape of the eggs within the acini were not uniform and the lumen of the tubules becomes filled with degenerated protoplasm and nuclei. The tissue of the ovotestis in parasitized *G. laevis* with Paramphistomum larval stages is most severely damage when rediae are present. The ovotestis of most *G. laevis* specimens examined during the present study were greatly destroyed and replaced by the larval stages. It is of interest to point out that the accessory genital organs in heavily parasitized snail became reduced and in extreme cases became invisible.

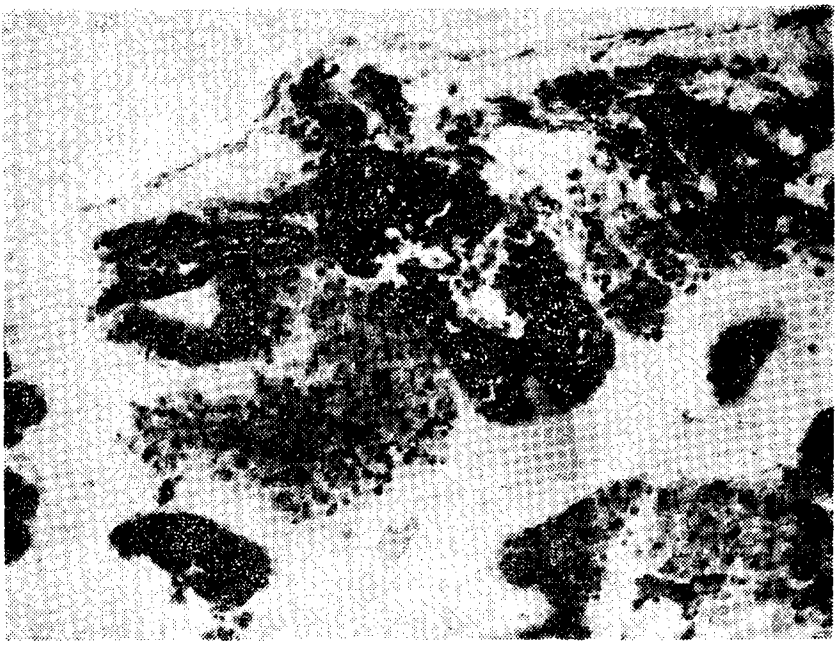

Fig. 5. A) Replacement of the digestive gland tubules by the parasite (x 200).
(Sindirim bezi tüplerinin yerini alan parazitler).

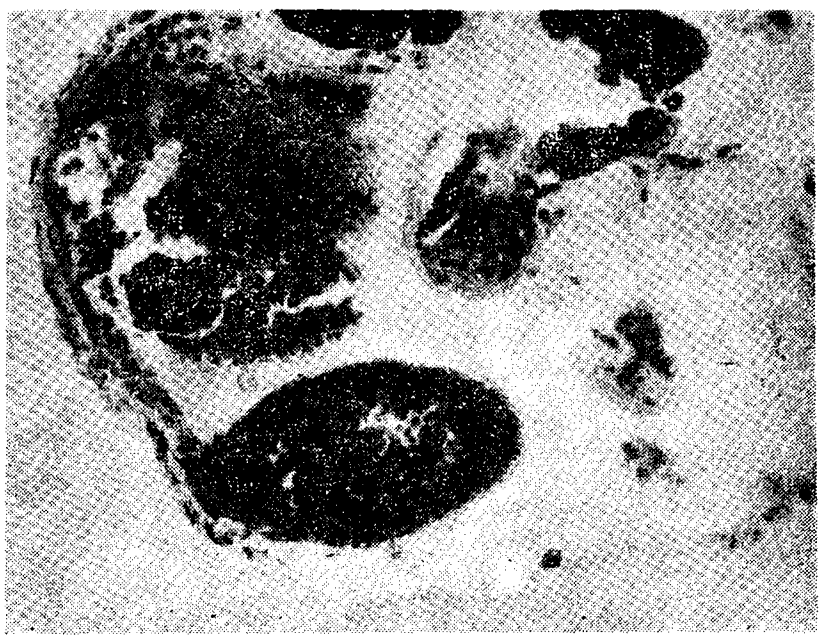

Fig. 5. B) Disappearance of most of the digestive gland tubules (x 200).
(Sindirim bezi tüplerinin çoğunun kayboluşu)

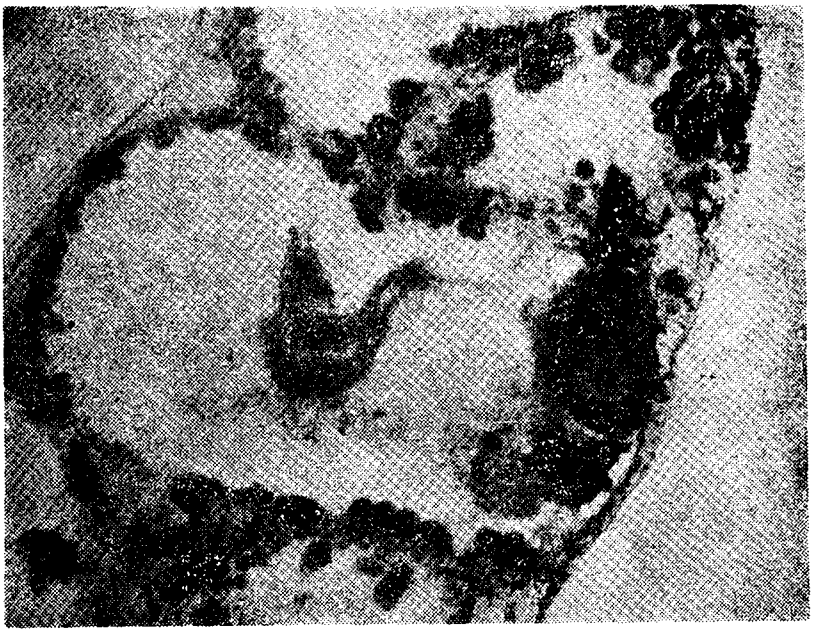

Fig. 6. Ova and sperms of unparasitized ovotestis (x 200).
(Parazitsiz ovotestis'te yumurta ve spermler)

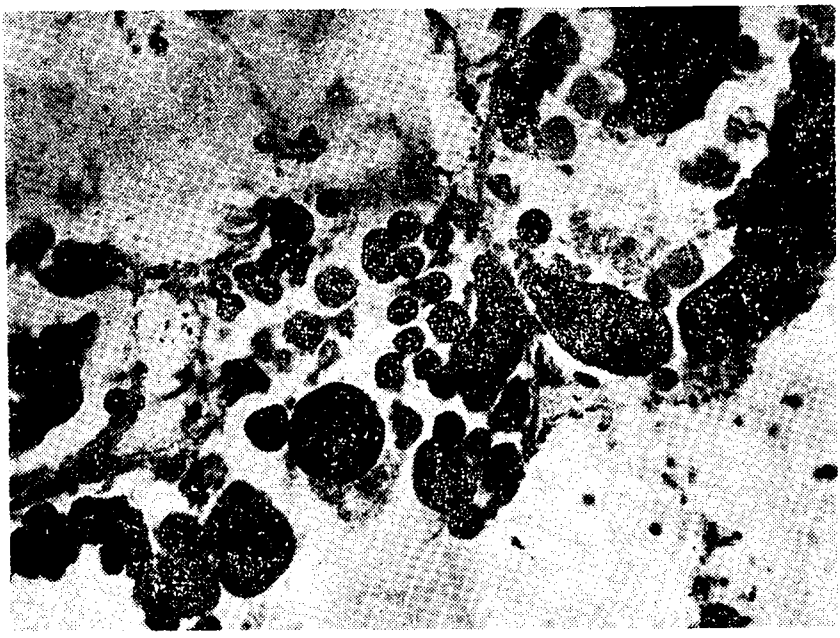

Fig. 7. Sporocysts and rediae invading the acini of the ovotestis (x 200).
(Ovotestis foliküllerinde sporokist ve rediler)

The headfoot of the snail *Gyraulus laevis* is surrounded by an epithelial layer of columnar cells. The goblet cells are found beneath the columnar cells, but open between them. The core of the foot is made of a large number of thin muscle fibers running singly in various direction throughout a dense vascular connective tissue (Figs.1 and 8).

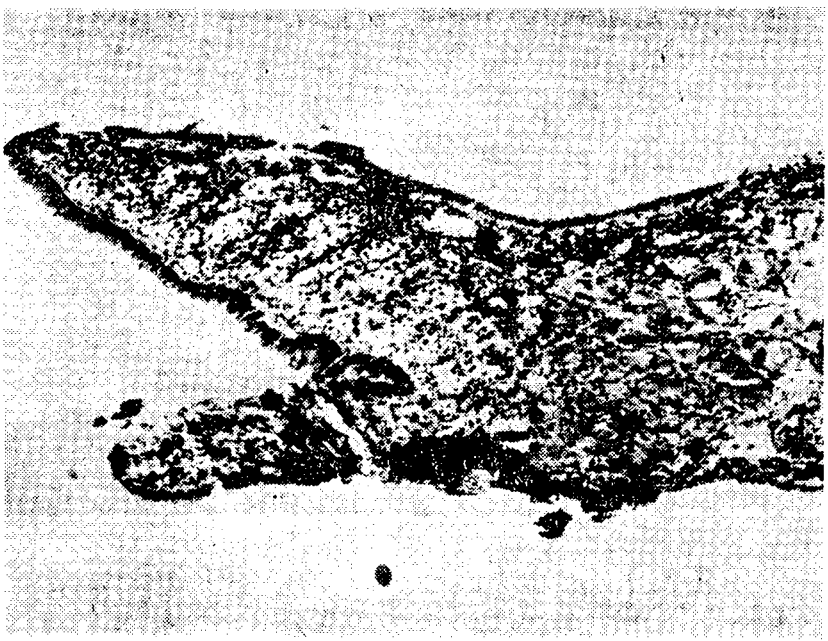

Fig. 8. Headfoot of unparasitized *G.laevis* (x 100)
(Parazitsiz *G.laevis*'in başayağı)

In parasitized *G. laevis* with Paramphistomum larval stages, it is clear (Fig.9) that the parasite located in the region of the headfoot. The sporocyst in the headfoot causes swelling and deformations which are particularly easy to detect. In compact tissues the sporocysts may bring about localized degenerative changes due to the pressure upon the muscular tissues of the foot (Fig.9).

The healthy kidney of the fresh water snail *Gyraulus laevis* is adhering to the inner surface of the mantle and extending distally to the pericardium. It is elongated and is as long as the respiratory cavity. The kidney terminates in a short, thick, tubular ureter, which curves on itself and opens into the respiratory cavity near the mantle collar.

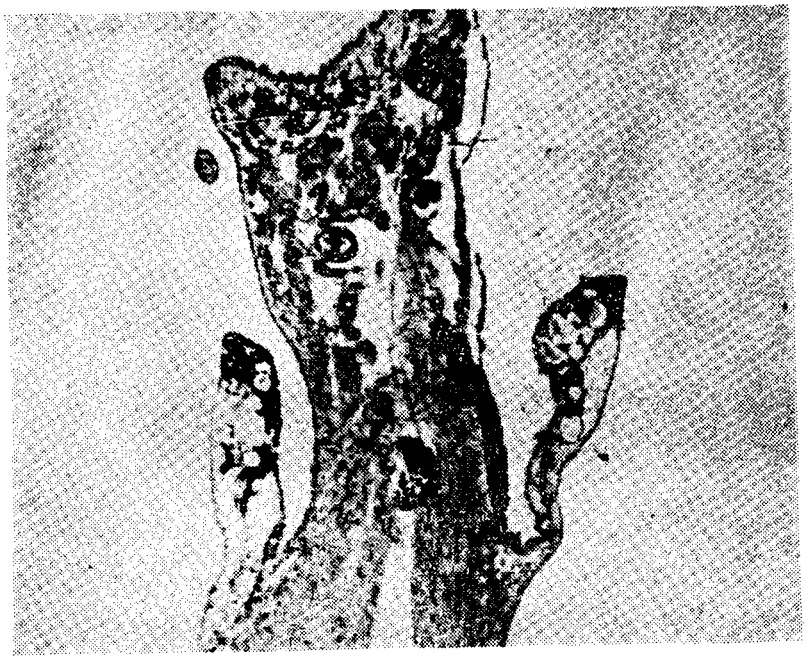

Fig. 9. Position of the parasite in the headfoot (x 40).
(Başayağında parazitlerin lokalizasyonu)

The epithelial part of the kidney consists of columnar to cuboidal cells resting on a basement membrane and is supported by a layer of connective tissue and muscle fibers. Vacuoles are found in the epithelial kidney cells (Figs.1 and 10).

Within the kidney of parasitized *G. laevis* with Paramphistomum larval stages, little disturbance was found during the present investigation. In some cases of parasitized snails, the sporocysts and rediae were carried in the haemolymph to the kidney and lodged near its tissue causes mechanical disturbance by pressing upon the tissue of this organ (Fig.11).

During the present investigation it is very important to note that sporocysts, rediae and cercariae of Paramphistomum parasitized *G. laevis* were also detected in the respiratory cavity and around the digestive tract particularly oesophagus and sometimes in the blood sinuses in the region of the genital tracts. It seems likely that the rediae are transferred by the haemolymph to every organ in the visceral mass of the snail *G. laevis* (Figs.12 and 13).

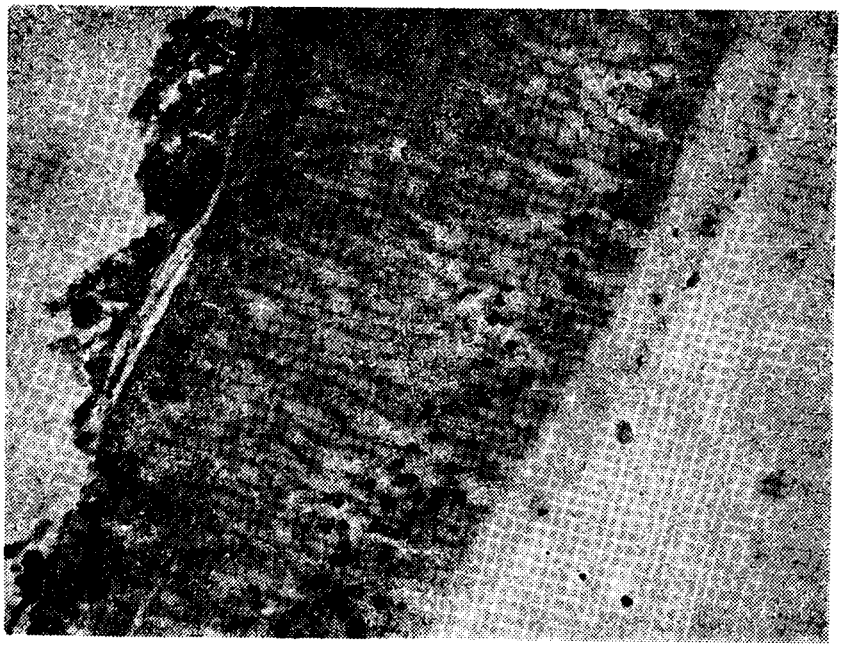

Fig. 10. Unparasitized kidney of *G.laevis* (x 200).
*(G laevis*'in parazitsiz böbreği)

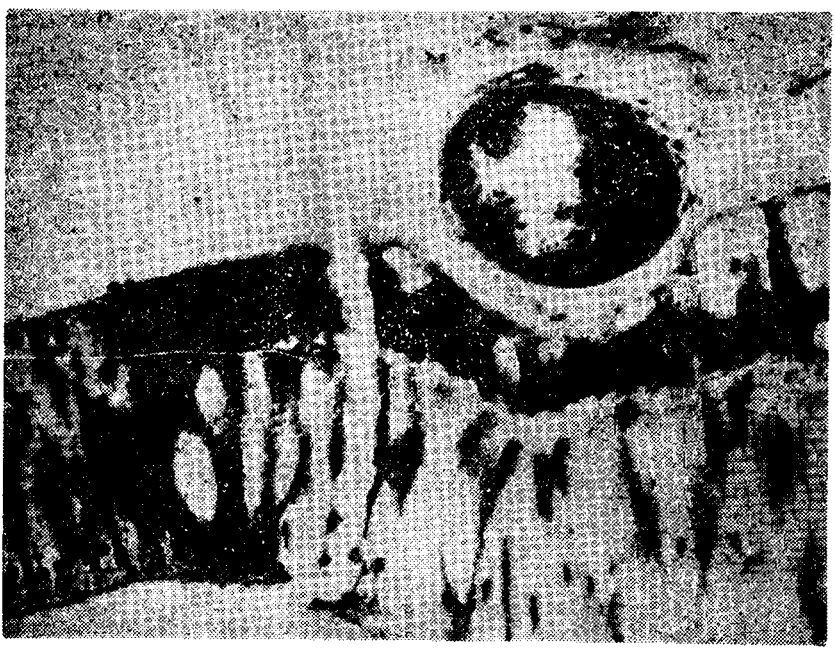

Fig. 11. The location of the parasite in kidney (x 200).
(Böbrekte parazit lokalizasyonu)

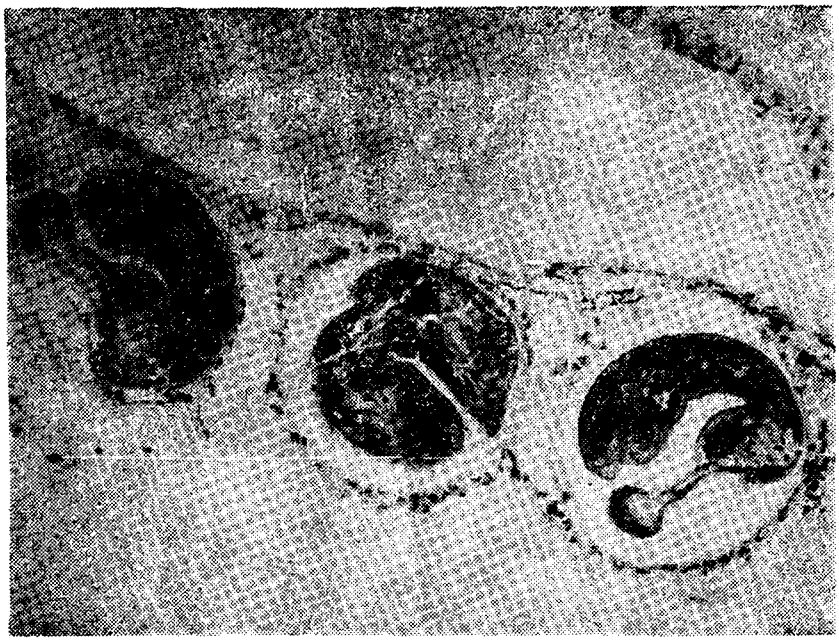

Fig. 12. The location of rediae in the mantle cavity (x 100).
(Manto boşluğunda redilerin lokalizasyonu)

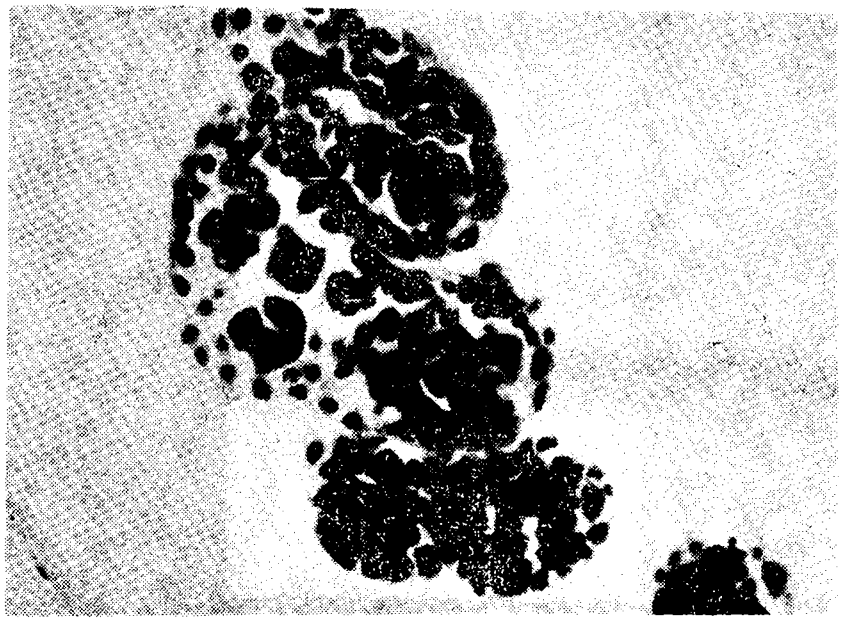

Fig. 13. Rediae of Paramphistomum (x 200).
(Paramphistomum redileri)

As a result of infection in *G. laevis* it was observed that there is a heavy production and agglomeration of excretory granules, necrosis of cells of the digestive gland tubules and proliferation on intertubules connective tissues.

## Discussion and Conclusion

The effects of digenetic larval trematodes on their molluscan hosts moderately well documented (25). Some larval trematodes are known to attack the hosts tissues directly. Others develop in certain organs and still others merely exert an indirect influence on the host tissues. Increasingly more investigators are studying the effects of larval trematodes on their molluscan hosts. This topic has been reviewed by some authors (2,5,7,25). The range of effects varies from none to considerable, the latter being the role.

The effects of larval trematodes on their molluscan hosts can be categorized in four groups. The effect on host's digestive gland, the effect on the host's gonad and reproductive structure, the effect on the general physiology and biochemistry state of the host, and cellular responses on the part of the host.

During the course of the present investigation the infection of *G. laevis* with Paramphistomum larval stages, sporocysts, rediae and cercariae, has profoundly damaging effects on the tissues of the visceral mass of *G. laevis*. The tissues of the digestive gland and ovotestis in *G. laevis* were most severely damage by the presence of sporocysts, rediae or cercariae of Paramphistomum. The histopathology of infected gastropod digestive gland has been the subject of great number of authors and reviewed by Wright (25) and Becker (2). The destructive damages described in the present study are in full agreement with the histopathological changes described by previous authors (10,11,12,22).

It was observed during the present study that there are some differences in the effects produced by sporocysts and rediae, because, while in the former all of the uptake of nutrients takes place through the tegument, in rediae this method is supplemented by active feeding with the pharynx and consequently more mechanical damage is done. The present investigation indicated that the rediae of Paramphistomum attack and consume the host's ovotestis very quickly after the infection was established. Infection with Paramphistomum, which possesses redial stages, caused most direct damage especially as these tended

to be evenly distributed throughout the digestive gland and ovotestis of *G. laevis*. It seems likely that the rediae feed on both ovotestis and digestive gland tissues. Rees (20) mentioned that when female *Littorina littorea* are infected with cercariae of *Himasthla seconda*, rediae seem to be full of eggs and yolk sucked from the ovarian tubules by their powerful muscular pharynx.

Wright (26) mentioned that the miracidia which enter the more compact muscular tissues of the foot are less successful in establishing an infection than those which get into the open spaces of the headfoot regions and the mantle. It was also observed by Burgu (4) that *Paramphistomum cervi* miracidia penetreted the intermediate snail host *Planorbis planorbis* only through the mantle cavity and not from the headfoot or tentacles.

It is of interest to note that the present investigation indicated that the Paramphistomum miracidia penetrate the muscular tissues of the headfoot of the snail *G. laevis* and successfully established the infection. The results presented in this study may be due to the fact the snails *G. laevis* are small in size and the tissues of the headfoot regions are soft and less compact.

The present study indicated that the parasite tend to become concentrated in the lower part of the visceral mass of *G. laevis* where they form a "blocking layer" this layer isolates the upper part of the visceral mass and deprives most of the digestive gland and ovotestis of their normal haemolymph supplies with consequent degeneration due to starvation and accumulation of excretory products.

It seems likely that during the development of the Paramphistomum larval stages within the snail *G. laevis*, the parasite pass from one stage to another and migrate into all parts of the snail tissues causing great damage to most of the host tissues.

Finally, intermolluscan larval trematodes do deplete their host carbohydrate, lipid and amino acid (2,25) which causes great harm to the host. In fact, infected molluscs generally do not survive as long as uninfected ones.

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