

# Comparative Studies on the Structural Changes in Some Kind of Snails During the Estivation and Trematoda Infection

## I. Structural Changes of Normal *Biomphalaria glabrata* During the Estivation

Soon-Hyung Lee, M.D.\*

Department of Parasitology and Institute of Endemic Diseases,  
College of Medicine, Seoul National University

### INTRODUCTION

Schistosomiasis is possibly the most important of the helminthic diseases because of its worldwide distribution and the extensive pathologic changes produced by the parasites. It is believed about 118 million persons are affected by this condition and 354 million persons are exposed to this infection (Wright, 1968). Human schistosomiasis designates a group of diseases produced by 3 species of bloodflukes belonging to the family Schistosomatidae, i.e., *Schistosoma japonicum*, *S. mansoni* and *S. haematobium*.

The infection is acquired by the contact of fresh water with free-swimming cercariae which are liberated from the snail intermediate hosts. Thus the distribution of suitable snail host is of considerable significance in the epidemiology and control of schistosomiasis.

Of 3 species of schistosomes in man, *S. mansoni* is the most common in the Western Hemisphere. Several species of planorbid snails in the Western Hemisphere are capable of transmitting *S. mansoni*. Among them, *Biom-*

*phalaria glabrata* is probably the most important species as a snail intermediate host of *S. mansoni*.

It is well known that an aquatic snail, *B. glabrata*, had a tendency to climb spontaneously out of water and estivate. This spontaneous estivation provides insurance against drought or chemical molluscicides. Thus an estivating *B. glabrata* has an excellent chance of survival, because at the end of the dry period the new population is started by only a few surviving snails. This phenomenon is very important to maintain the infection in certain endemic areas.

On the estivation of *B. glabrata*, many studies of previous authors (Barbosa, 1956; Barbosa & Barbosa, 1958; Barbosa & Olivier, 1958; Cridland, 1967; Olivier, 1956a & b, & 1960; Olivier & Barbosa, 1955, 1956 & 1958; Olivier et al., 1954) declared that interruption of growth and development by an extended period of reduced metabolic activity occurred in the estivating snails and their survival rates were also decreased. Studies on the physiology of the estivating snails demonstrated that decrease in glycogen content (Magalhaes & Almeida, 1956) and oxygen consumption (Magalhaes, 1953) occurred in estivating *B. glabrata* snails.

In general, according to their conclusions,

\* Present Address: Department of Parasitology, College of Medicine, Chung-Ang University.

\*\* This study was supported by Research grant 73-300-6 from the China Medical Board of New York, Inc.

<Received for Publication on June 15, 1976>

the estivating snails lost their weight and died more rapidly than the snails in water. Some evidences demonstrated that *S. mansoni* stopped its development when its host snails went into estivation. The host snails were found to lose infection or to carry immature infection after variable period of estivation (Barbosa & Barbosa, 1958; Barbosa & Coelho, 1953 & 1955; Barbosa & Olivier, 1958; Olivier et al., 1954). Richards (1967 & 1970) claimed that the estivation appeared to be genetically determined and involved physiologic and morphologic adaptations prior to spontaneous climbing out of water.

Therefore, all the structural changes and metabolic alterations occurred in the snails during the process of estivation may be reflected upon impairment of cellular function and changes of structure in host snail tissues. But little is known about the structural changes in the snail, *B. glabrata*, particularly in the estivating period. The objectives of this experiment are to elucidate the changes and differences occurred in some organs of *B. glabrata* snails during the process of artificial estivation. Growth rate, survival rate and histopathological changes are observed in comparison with their normal structures of the control snails.

## MATERIALS AND METHODS

### Origin and Classification of Snails

Puerto Rican strain of *B. glabrata* susceptible to *S. mansoni* were used. A total of 72 snails, shell diameter in the range of 4.0 mm ~10.0 mm and wet weight in the range of 13 mg~180 mg, were divided into following 2 groups, and each group included 36 snails.

Group I: Normal control snails which were kept in water throughout the experimental period.

Group II: Estivating snails kept out of water for 12 weeks. After the estivation, they were put into water for 4 weeks.

### Care of Snails

Snails were reared in 17×13 cm plastic boxes with covers, in dechlorinated water and fed romaine lettuce and dried leaves for the normal control, Group I. Each box contained 12 snails, and a half of water in the box was exchanged once a week (Fig. 1). For the estivating snails, Group II, moist soil was contained in the boxes instead of water. After placing the snails on the surface of moist soil (Fig. 2), these boxes were covered and placed shady shelves in the laboratory under the controlled temperature (24.5°~26.0°C) and humidity (50%~57%), and let them dry gradually. The relative humidity of the boxes which contained the estivating snails varied from 98% in the beginning to 62% at the end of estivation.

### Growth Rate

Both groups of snails were measured their shell diameter with a ruler and were also measured their wet weight with a torsion balance after wiping off excess water from the surface with filter paper. The mathematic means of each group were calculated. For the calculation of per cent increase or decrease of diameter and wet weight, following formula was applied.

% increase (or decrease)

$$= \frac{\text{measurement of } n^{\text{th}} \text{ week}}{\text{initial measurement}} \times 100$$

### Survival Rate

Survival of snail was determined by observing the heartbeat of snail under a dissecting

microscope; for the estivating groups, revival by putting the snail back into water and observing for reactivation was additionally checked. And the rate was calculated by a formula shown below;

% survival

$$= \frac{\text{No. of snails alive}}{\text{initial No.} - \text{No. of snails sampled}} \times 100.$$

For the estimation of growth rate and survival rate, the snails were measured every week throughout the experiment.

### Histopathology

Sampling of snails from both groups was done in 3rd week, 8th week, 12th week and 16th week after the estivation. Conventional techniques of Bouin fixation, paraffine embed-



Fig. 1. Snail rearing box for the control group (Group I).

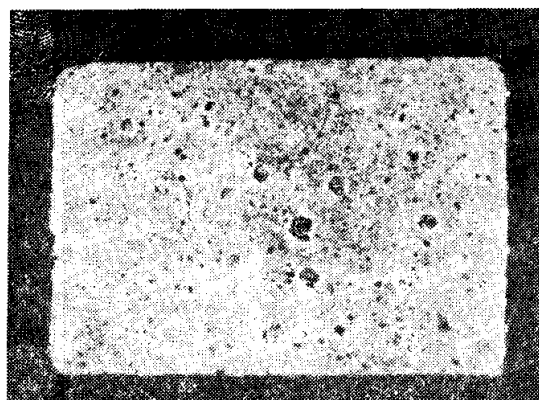


Fig. 2. Box for the estivating snails (Group II).

ding, and hematoxylin-eosin staining were used for the histopathological portion of this study, according to the manual of Luna, 1960.

## EXPERIMENTAL RESULTS

### Growth Rate

The growth rates of control snails kept in the water (Group I) and estivating snails kept out of water (Group II) were presented in Table 1.

The mean diameter of control snails was slightly smaller than that of estivating group at the beginning of experiment. But according to the progress of experiment, the mean diameter of control snails became larger throughout the experimental period, while the mean diameter of estivating snails remained nearly the same size. Figure 3 showed that the mean diameter of control snails grew rapidly to 44% of initial size within first 5 weeks and then the increasing rate turned rather gradual. The negligible increase of the mean diameter in estivating snails was observed for whole 12 weeks of estivation, but when the snails were put back into water, they grew very rapidly and got about 47% increase of their initial diameter during the period of 4 weeks.

Table 1. also showed that the mean wet weight of control snails kept in water increased gradually, whereas that of estivating snails lost their weight during the estivation period. Per cent increase of wet weight in both groups was illustrated in Fig. 4.

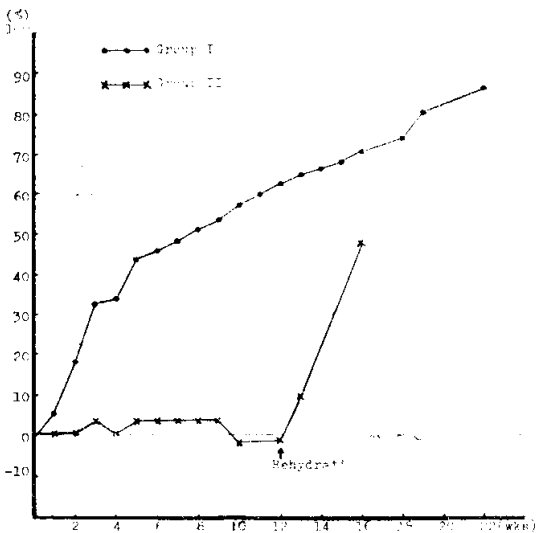
The control snails gained more than 3 folds of their initial weight at the end of experiment, while the estivating snails lost about 40% of their initial weight during 12 weeks of estivation period. The loss of wet weight in estivating group occurred within the first 2 weeks of estivation and after then, that level

**Table 1.** The mean diameter, mean wet weight and mean percent survival of snails both in Group I (control) and Group II (estivating snails)

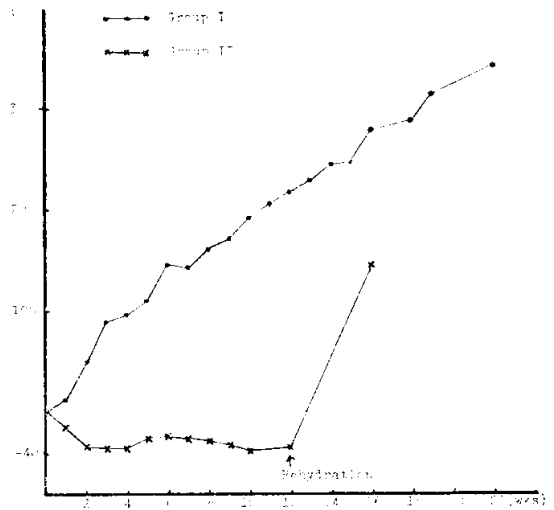
Exp. weeks	Group I				Group II			
	SD	WW	No. SA	SR	SD	WW	No. SA	SR
0	6.02	41.22	36	100	6.60	43.61	36	100
1	6.39	46.06	35	97.2	6.64	36.03	36	100
2	7.12	61.75	33	91.7	6.64	29.36	36	100
3	7.99	78.44	27	79.4	6.87	28.46	28	82.4
4	8.04	80.74	27	79.4	6.61	28.27	15	44.1
5	8.65	86.74	27	79.4	6.85	33.60	10	29.4
6	8.79	101.42	26	76.4	6.85	33.60	10	29.4
7	8.92	99.96	26	76.4	6.85	33.00	10	29.4
8	9.10	108.09	23	71.9	6.85	31.70	10	29.4
9	9.24	111.61	23	71.9	6.68	30.25	8	23.5
10	9.47	119.91	23	71.9	6.50	27.00	6	17.7
11	9.60	126.44	23	71.9	—	—	—	—
12	9.78	131.00	23	71.9	6.52	28.40	5	14.7
13	9.92	135.83	23	71.9	7.25	50.00	2	6.3
14	10.01	142.09	22	68.8	—	—	—	—
15	10.09	142.32	22	68.8	—	—	—	—
16	10.29	156.59	22	68.8	9.75	105.00	2	6.3
17	—	—	—	—	—	—	—	—
18	10.45	159.77	22	68.8	—	—	—	—
19	10.84	170.25	21	64.5	—	—	—	—
20	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—
22	11.21	182.06	17	54.8	—	—	—	—

SD: The mean shell diameter in mm  
No. SA: Number of snail alive

WW: The mean wet weight in mg  
SR: The mean percent survival



**Fig. 3.** The percent increase in diameter of control snails (Group I) compared to that of estivating snails (Group II).



**Fig. 4.** The percent increase or decrease of wet weight in both snail groups; estivating snails (Group I) and their control snails (Group II).

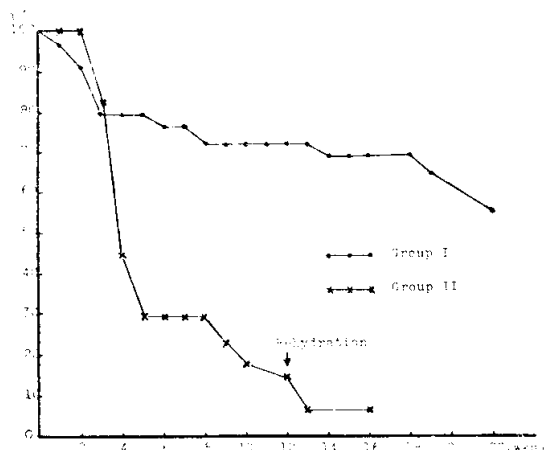


Fig. 5. The mean percent survival of snails in both control snails (Group I) and estivating snails (Group II).

was maintained till they were put into water again.

### Survival Rate

The survival rates of Group I and II were shown in Table 1 and Fig. 5. At the beginning of experiment, about 20% of the control snails kept in water died within 3 weeks, but the mortality slowed down gradually and they showed 55% of survival rate at the end of experiment. In the estivating snails, the number of dead snail increased after 2 weeks of estivation and the survival rate sharply dropped to 30% level when the snails were kept out of water for 5 weeks. At the end of experiment they showed the level of 6.3%.

### Histopathological Changes

For the control snails (Group I), histopathological observation showed no abnormalities in the hepatopancreas, ovotestes, seminal vesicle, kidney and rectal ridge throughout the experiment just the same as the description of Pan (1958).

In the hepatopancreatic region, a section of normal compound tubular gland structure could be seen (Fig. 6). From the magnification of

Fig. 6, the normal hepatic lobules which were consisted of mostly digestive cells and lime cells filled with secreting materials in their glandular lumens (Fig. 7). The digestive cells were tall and were in 3rd stage of secretory function, and lime cells were also distinct just as the description of Pan (1958).

The moderately simplified glandular structure and loss of parenchyme could be observed the hepatic region of estivating snails kept out of water for 3 weeks (Fig. 8). Among the hepatic lobules of this snail, there were still functioning lobules (Fig. 9) but some of them had degenerating hepatic epithelium in parts.

As the progress of estivation, the loss of parenchyme, degenerative changes of epithelium and simplification of glandular structure were getting worse. In the case of the estivated snail for 12 weeks, the loss of hepatic parenchyme and degeneration of hepatic epithelium were extreme (Fig. 10). When the estivating snails were returned to water again, the hepatic lobules began to regenerate actively and composed nearly normal appearance similar to their control snails. Figure 11 showed that the hepatic lobules almost refilled the entire hepatic region and some what hyperactive hepatic lobules monitored in high power magnification (Fig. 12).

The hepatic duct, which normally has simple columnar epithelial lining and which is raised in number of foldlike elevations, lost their characteristics during the period of estivation. The scanty fold-like structure and decrease of cell weight were noted in the estivating snails. After return of those snails into water, they restored their typical columnar epithelium of hepatic duct (Fig. 13).

The architecture of ovotestis also a compound tubular structure. The ovotestes of a control snail kept in water showed numerous

acinar structure (Fig. 14), and the acinus contained ovum, male germinal cells, growing spermatids and mature spermatozoa (Fig. 15).

During the estivation, the reduction of parenchyme was the rule in the snails kept out of water. The ovotestes of a estivating snail kept out of water for 3 weeks maintained the acinar structure but there were scanty reproductive cells inside the acinus. As the progress of estivation, the ovotestes of estivating snails lost more parenchymal tissue, and so the number of acini remained small in Group II snails kept out of water for 12 weeks (Fig. 16).

But when the estivating snails were put back into water again after the estivation, the acinar structure of ovotestes restored and refilled with plenty of reproductive cells (Fig. 17). And sometimes, those acini seemed to be more active than those of snails kept in water.

The normal seminal vesicle of control snails kept in water usually contained numerous mature spermatozoa in its lumen (Fig. 18). During the progress of estivation, the seminal vesicle of estivating snails showed scantiness of spermatozoa in its lumen space (Fig. 19 & 20). Scantiness of spermatozoa in the seminal vesicle seemed to be proportional with the duration of exposure to dessication.

The seminal vesicles of estivating snails were refilled with massive spermatozoa after rehydration, and were compatible with the restoration of ovotestes in that period (Fig. 21).

According to Pan (1958), the wall of the saccular portion of kidney formed many prominent folds and the folds consisted of 2 epithelial sheets with a blood space between them. In control snails, such kinds of structures were clearly seen in the saccular portion of kidney (Fig. 22 & 23). Among the estivating snails, the epithelial sheets of saccular portion changed into abnormal architecture. In the

earlier stage of estivation, the blood space of between 2 epithelial sheets became narrower and epithelial cells began to degenerate. At the later stage of estivation, structure of folds and epithelial sheets were destroyed and showed irregular arrangements (Fig. 24). After return to water, the saccular portion became compact and the folds started to regenerate showing regular arrangement of the epithelial sheets (Fig. 25).

The epithelial cells of tubular portion of the kidney were low columnar to cuboidal and epithelial sheet of this portion showed a wavy appearance in control snails kept in water (Fig. 26). This portion showed the least pathological change during the process of estivation (Fig. 27). Almost the same histological characteristics were maintained throughout the experiment in both groups of snails.

The loose vascular connective tissue of rectal ridge contained considerable amount of pigment cells and crystalline concretions in normal control snails (Fig. 28). It seemed to be contained more pigment cells and crystalline concretions with larger size during the estivation (Fig. 29), and even after return to water again in the estivating snails.

## DISCUSSION

In present experiment, the growth rates between control group kept in water (Group I) and the estivating snails (Group II) showed distinctive differences. The mean diameter of control snail group increased continuously throughout the period of experiment, while that of the estivating group showed negligible increase during the snails were kept out of water. But when the estivating snails were put into water again. They grew rapidly. The fluctuation of the mean diameter of the estiva-

ting snails were not regarded real change but merely the product of statistical manipulation.

On the weight loss of *B. glabrata* kept out of water, Olivier and Barbosa(1958) reported that weight losses among the snails at the lower relative humidities were greater. In present experiment, the loss of mean wet weight was observed in the estivating snails kept out of water, while their controls got weight gain continuously during the whole period of experiment. When the estivating snails were put back into water again, then they started to get their wet weight rapidly. The fluctuation of mean wet weight of estivating snails during they were kept out of water was also interpreted as an effect of statistical manipulation with insufficient number of the remained snails.

From the previous reports of Olivier(1956a) and Olivier and Barbosa(1958), it was found that weight loss and survival of the snails were dependent on the relative humidity. In one experiment, *B. glabrata* kept out of water at 92% of relative humidity lost 14% of their original weight in 140 days while those at 82% of relative humidity lost 22%, and those at 65% of relative humidity lost 22%, and those at 65% of relative humidity lost 26% (Olivier, 1956a). On the other hand, snails in the same experiment kept at 56% of relative humidity lost 18% of their original weight in only 40 days. In this experiment, the estivating snails lost their weight to the level of about 40% at 62% of relative humidity at the end of 12 weeks of estivation. Loss of weight in the estivating snails of this experiment seemed greater than that of previous reports, but this difference was considered as a result originated from the difference of snail strains. Olivier (1956a & b) and Barbosa(1956) declared that snails from different localities showed striking

differences in their ability to survive out of water.

During the estivating period the snails retract into their shells and some of the snails secrete fine membranes across the shell opening which might protect them from water loss. Richards (1963 & 1967) suggested that this apertural lamellae present in some *B. glabrata* were frequently associated with the estivation and were influenced by genetic factors. So loss of wet weight and survival rate of estivating snail were not constant even in the same strain. In present experiment, the apertural lamellae of the estivating snails were not investigated.

The survival rate of control snails (Group I) was much higher than that of the estivating snails (Group II) through out the experimental period, except the first 3 weeks. It could be considered that the estivating snails died more off rather in later period of estivation when relative humidity reached lower level.

Generally speaking, the results obtained in present experiment revealed somewhat similar tendencies in comparison with the results of previous authors (Barbosa, 1956; Barbosa & Dobbin, 1952; Cridland, 1967; Olivier, 1956a & b; Olivier & Barbosa, 1955 & 1958) though there were some discrepancies in parts.

The normal histology of *B. glabrata*(Group I) kept in water coincided with the studies of previous authors (Pan, 1958, 1963 & 1965; File, 1971). On the histopathological changes of the estivating snails, very little is known. So the histopathological changes of certain organs such as hepatopancreas, ovotestes, seminal vesicle, kidney and rectal ridge could be only presumed from the previous studies of the metabolic alterations in estivating snails. Recently Beaver and Malek(1973) observed the morphological changes of estivating *B. glab-*

*rata*, and confirmed that the simplified branching of the hepatopancreas through the experiment of 1 month estivation. But they found that there were still remained intact ovotestes with the content of reproductive cells and the seminal vesicles with full of sperms in the estivating snails. This differences would be elucidated by further experiment with longer period of estivation.

From the results of present experiment, it can be easily imagined that the reduction of parenchymal tissue of various important organs would cause metabolic impairment, retardation of growth, lowered fecundity, and finally death of snails. But declaration of direct causes of such phenomena and confirmation of detailed contributing factors will be obtained by further extensive studies of this line. Beaver and Malek(1973) has wondered whether this histopathological changes were merely caused by dehydration and/or starvation during the estivation.

It can be thought, at present, that further experiment will elucidate the ultimate mechanism of estivation and histopathological changes of ultrastructure in conjunction with the altered metabolism in those estivating snails.

#### SUMMARY AND CONCLUSIONS

This study was undertaken to elucidate the changes and differences in *Biomphalaria glabrata*, the most important snail host of *Schistosoma mansoni* in Western Hemisphere, during the process of estivation. The growth rate (increase in shell diameter and wet weight), survival rate and histopathology were observed during the 22 weeks of experimental period in comparison with the control snails.

Two groups of snails, each group consisted of 36 snails, were used for the present experi-

ment. One group of snails was undergone artificial estivation, and other group was applied as normal control reared in the water.

The results obtained were as follows:

1. The growth rate of estivating snails (Group II) was consistently lower than that of control snails (Group I) kept in the water.
2. The growth rate rose rapidly when the estivating snails were put into water again after 12 weeks of estivation.
3. Survival rate of the estivating snails was lower than that of their controls.
4. During the estivation, the reduction of parenchyme and degenerative pictures were prominently observed in the hepatopancreas, ovotestes, seminal vesicle and saccular portion of kidney. And little change was observed in the rectal ridge and tubular portion of kidney in the estivating snails.
5. After return of the estivating snails into water, they almostly restored their normal structures within 4 weeks.

#### ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Dr. Paul C. Beaver (Tulane University) whose interest, guidance and constructive criticism were of great assistance in this study. He is also indebted to Dr. Byong-Seol Seo (Department of Parasitology, College of Medicine, Seoul National University) and Dr. Emile A. Malek (Tulane University) for all the help, kindness and able guidances throughout the experiment. Sincere thanks are extended to Dr. Maurice D. Little (Tulane University) for lending him the photographic equipments.

The author would like to express his sincere thanks to China Medical Board of New York, Inc., enabled him to perform this study.



»國文抄錄«

夏眠이 만손住血吸虫 中間宿主 *Biomphalaria glabrata* 에 미치는 影響

서울大學校 醫科大學 寄生蟲學教室 및  
風土病研究所

李 純 炯

만손住血吸虫(*Schistosoma mansoni*)의 貝類 中間  
宿主인 *Biomphalaria glabrata*가 夏眠(estivation)  
을 할때 일어나는 變化를 알기 위하여, 對照群인 第 I  
群 및 夏眠群인 第 II 群 各 36마리씩의 *B. glabrata*를  
16~22週間 飼育하면서 그 成長率, 生存率 및 病理組織  
學的 變化를 比較 觀察하였다.

實驗期間中 對照群은 물속에서, 미리 處理한 단풍잎  
과 배추로 飼育하였으며, 夏眠群은 젖은 흙 表面에 放  
置한채 아무 飼料도 주지 않고 12週間 人工的으로 夏眠  
시켰다가 다시 물속에서 4週間 對照群에서와 같은 方法  
으로 飼育하였다.

1. 第 I 群(對照群)은 實驗期間中 貝殼直徑 및 무게  
가 계속 增加하였으나 第 II 群(夏眠群)에서는 夏眠中 貝  
殼은 커지지 않았으며 무게는 오히려 減少하였다.

2. 夏眠群을 12週間的 夏眠後 다시 물속에서 4週間  
飼育하였을 때 貝殼 및 무게는 急速히 增加하였다.

3. 生存率은 實驗 第 3 週까지는 兩群에서 비슷하였  
으나, 第 4 週後부터는 夏眠群이 對照群에 比하여 현저  
히 低下하였다.

4. 夏眠群에서의 組織學的 變化로는 消化腺, 生殖腺  
및 腎臟囊狀部 實質組織의 萎縮減少 및 細胞變性이 현  
저하였으며 貯精囊의 內容(精子)이 減少하는 것을 볼  
수 있었다. 腎臟管狀部 및 rectal ridge에서는 僅少한  
變化가 있을 뿐이었다.

5. 夏眠群을 夏眠이 끝난後 다시 4週間 물속에서 飼  
育했을때 이 모든 變化가 正常組織으로 還元하는 것을  
觀察할 수 있었다.

以上の 結果로 미루어 만손住血吸虫 流行地域에 있어  
서 가뭄등 不適當한 環境條件으로 인하여 夏眠을 하게  
될 때 *B. glabrata*는 그 成長發育이 停止되고 生存率  
이 低下하나 다시 雨期를 맞이하면 極少數의 殘存貝들  
이 急速히 成長繁殖하므로써 만손住血吸虫症의 流行을  
維持할 수 있다고 생각된다.

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### EXPLANATION OF THE PLATES

- Fig. 6. The hepatopancreatic region of a control snail kept in water (Group I). A: hepatopancreas, B: ovotestes.  $\times 35$ .
- Fig. 7. Hepatic lobules of control snail with the epithelium at 3rd stage.  $\times 100$ .
- Fig. 8. Hepatic region of estivating snail (Group II), kept out of water for 3 weeks. Note the simplified hepatic lobules.  $\times 35$ .
- Fig. 9. Hepatic lobules of estivating snail, kept out of water for 3 weeks. Some functioning lobules are still remained.  $\times 100$ .
- Fig. 10. Hepatic lobules of estivating snails kept out of water for 12 weeks. Note the extreme flattening and thinning of hepatic epithelium.  $\times 100$ .
- Fig. 11. Hepatic region of estivating snails kept in water for 4 weeks, after 12 weeks of estivation. Note the regeneration of hepatic lobules.  $\times 35$ .
- Fig. 12. Magnification of Fig. 11. Note the regenerated hepatic epithelium and functioning cells (3rd stage).  $\times 100$ .
- Fig. 13. Hepatic duct epithelium of estivated snails kept in water for 4 weeks, after 12 weeks of estivation. Note the regenerated epithelium.  $\times 450$ .
- Fig. 14. Ovotestes of control snails (Group I) kept in water.  $\times 35$ .
- Fig. 15. A acinus from the ovotestes of control snails kept in water. It contained ovum (A), male germinal cells (B) and mature spermatozoa (C).  $\times 450$ .
- Fig. 16. Ovotestes of estivating snail kept out of water for 12 weeks (Group II). Note the scantiness of acini and abnormal appearance.  $\times 100$ .
- Fig. 17. Ovotestes of estivated snail kept in water for 4 weeks, after 12 weeks of estivation. Note the active regeneration of acini filled with reproductive cells.  $\times 100$ .
- Fig. 18. Normal seminal vesicle from the control snail kept in water.  $\times 450$ .
- Fig. 19. Seminal vesicle of estivating snail kept out of water for 3 weeks showing scantiness of sperms in its lumen.  $\times 100$ .
- Fig. 20. Seminal vesicle of estivating snail kept out of water for 8 weeks. Note the scantiness of sperms in its lumen.  $\times 450$ .
- Fig. 21. Seminal vesicle of estivated snail kept in water for 4 weeks, after 12 weeks of estivation. Its lumen was filled with numerous sperms.  $\times 100$ .
- Fig. 22. Saccular portion of control snail kept in water showing normal architecture.  $\times 100$ .
- Fig. 23. Normal epithelial sheet of saccular portion of kidney in control snail.  $\times 450$ . A: epithelial sheet, B: blood space and C: lumen.
- Fig. 24. Saccular portion of kidney in estivating snail kept out of water for 8 weeks showing destruction of normal architecture.  $\times 100$ .
- Fig. 25. Saccular portion of estivated snail kept in water for 4 weeks, after 12 weeks of estivation. Note the rearrangement of epithelial sheets.  $\times 100$ .
- Fig. 26. Kidney epithelium of tubular portion of kidney in control snail kept in water.  $\times 450$ .
- Fig. 27. Epithelium of tubular portion of kidney in estivating snail kept out of water for 8 weeks.  $\times 450$ .
- Fig. 28. The rectal ridge of control snail showing a little pigment cells and crystalline concretions.  $\times 100$ .
- Fig. 29. The rectal ridge of estivating snails kept out of water for 8 weeks. Note the massive accumulation of crystalline concretions.  $\times 100$ .

