Preliminary Studies on the Sporozoan Parasites in Oysters on the Southern Coast of Korea

by

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南海岸굴의 寄生性 胞子虫 豫備調査

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굴 養殖이 성해짐에 따라 각종 疾病이 流行되리라 염려되어 굴에 기생되는 *Minchinia* sp.의 기생충 조사를 했다.

1970년에는 金海 송정리 송지식 굴과 巨濟島 法洞里 垂下式 굴을 調査했고 1971, 1972년에는 巨濟島 어구 추봉, 가배 어장의 굴만을 調査했다. 總480 個体의 굴을 組織學的으로 各장기 마다 觀察했으나 *Minchinia* sp.는 檢出되지 않았다.

송정리 송지식굴과 巨濟島 垂下式굴을 比較할 때 組織上 若干의 變化는 있었다.

INTRODUCTION

The commercial oyster common to Korean waters is *Crassostrea gigas*. Today, this valuable natural food resource is produced in the Republic of Korea by several methods. Particularly, the hanging culture method has become more prevalent since it began to be used in Korea in 1963. The oytser production is now one of the most important national fisheries programs with the full support of government, industry, and cooperatives.

In accordance with the national policy to develop and expand the oyster production, studies on the oyster diseases have been conducted in the oyster growing areas in Kyung-Sang-Nam Province during the period from June 1970 to October 1972. Special attention has been paid to the haplosporidan parasites of oysters by means of histological examinations. Fortunately none was found the case of oyster mortality caused by the sporozoan parasites.

The incidences of *Minchinia nelsoni* and *M. costalis* in oysters were reported by a good number of investigators (Andrews, Wood and Hoese, 1962; Wood and Andrews, 1962; Haskin, Canzonier and Myhre, 1965; Haskin, Stauber and Mackin, 1966; Andrews, 1966, 1967; Haskin, Couch, Farley and Rosenfield, 1966; Farley, 1967)

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MATERIALS AND METHODS

The oyster samples were collected at two stations in Songjung-ri, Gimhae and Bupdong-ri, Geoje-do in 1970 (Fig. 1). Beginning in June, 1971, the samples were collected from three stations in Geoje-do, mainly Chubong, Gabae and Eogu (Fig. 2).

Samples from each locality were placed on ice immediately after collections and delivered to the microbiology laboratory of Pusan Fisheries College. Oysters were fixed in Bouin's and Davidson's fixatives, sectioned and stained with Harris hematoxylin-eosin, Ziehl's fuchsin and Malloy's triple stain (Farley, 1965; Couch and Rosenfield, 1968). Single sections of 6μ from the area of gills, stomach and gonad of each oyster were examined for *Minchinia* sp.. Major organs and structures present in each slide were gills, gonad, stomach, digestine diverticulum, intestine suprabranchial chambers, demibranch and mantle. These were specifically checked along with connective tissue epithelium and blood vessels. All the slides were observed in bright field illumination. Each slide was initially scanned at 35X magnification to ascertain the presence, location and position of the various structures. Selected portions of each slide were checked at 150X, primarily to determine gonad condition, incidence and distribution of leucocytes, and to scan for damaged or abnormal areas in extensive tissues such as gastrointestinal columnar epithelium and gills. Individual organs were then checked at 600X with emphasis on changes in tissues and cells and the presence of bacterial, protozoan or fungal parasites. Selected areas were sometimes observed at 1500 X, primarily for nuclear aberrations or divisions, phagocytic activity of leucocytes, or the speciation and reproductive activity of parasites.

RESULTS AND DISCUSSION

The highest surface water temperature at Gimhae and Geoje-do areas was 28° to

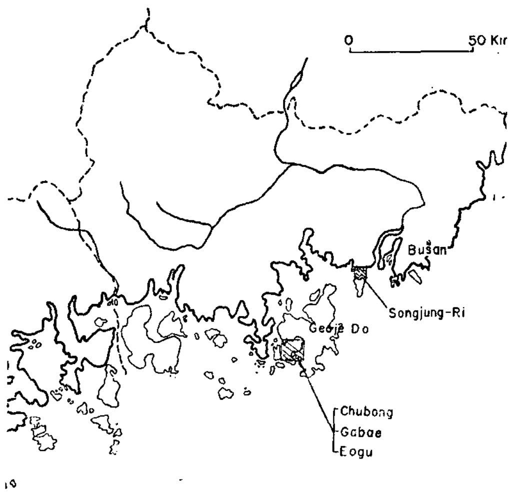

Fig. 1. Location of study areas.

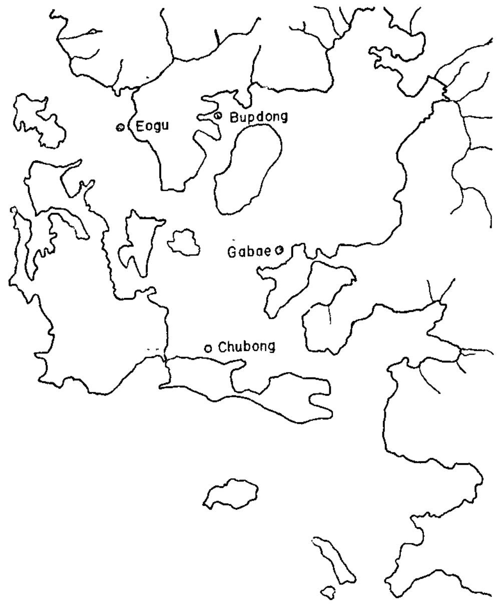

Fig. 2. Location of study areas in Geoje Bay.

29°C in August, 1970. However, the lowest temperature was different between the stations, that is 5°C at Gimhae and 8°C at Geoje-do in February, 1970.

The results of the present survey on the incidences of *Minchnia* sp. in living oysters collected from Gimhae and Geoje-do were shown in Table 1. Of the 480 samples examined no *Minchinia* sp. infections were found in living oysters.

Table 1. Records of *Minchinia* sd. Live Oysters in Kyung Sang Nam Province(1970 to 1972)

Area	Location	Date sampled	No. of oyster examined	Cases of *Minchnia* infections
Gimhae	Songjung-ri	16 June 1970	30	0
		25 July 〃	30	0
		25 Aug. 〃	30	0
		15 Sep. 〃	30	0
		5 Oct. 〃	30	0
Geoje-do	Bupdong-ri	13 June 1970	30	0
		17 July 〃	30	0
		2 Oct. 〃	30	0
		25 Nov. 〃	30	0
		23 Dec. 〃	30	0
		Subtotal	300	0
Geoje-do	Chubong	9 June 1971	10	0
		10 June 〃	10	0
		1 July 〃	10	0
		10 July 〃	10	0
	Gabae	9 June 1971	10	0
		16 June 〃	10	0
		1 July 〃	10	0
		10 July 〃	10	0
	Eogu	9 June 1971	10	0
		16 June 〃	10	0
		1 July 〃	10	0
		10 July 〃	10	0
		Subtotal	120	0
Geoje-do	Chubong	6 Oct. 1972	20	0
	Gabae	6 Oct. 〃	20	0
	Eogu	6 Oct. 〃	20	0
		Subtotal	60	0
		Total	480	0

Although the present survey shows no incidences of *Minchinia* sp. in living oysters, histological examinations of the various organs and tissues of *Crassostrea gigas* are summarized as follows:

Gill Lamella

Each lamella of a gill consists of a great number of tubular filaments arranged at right angles to the axis of the gill. Fig. 3 shows the arrangement of filaments in alternating grooves and ridges. The number of filaments on a single fold of an adult *C. gigas* is not constant, and three types of filaments are distinguishable by their position, shape, and dimension. The larger, or principal filaments are located at the bottom of the groove between the plica. In cross section they have a triangular shape with tow bulky chitinous rods forming two sides of this triangle. The rods are fused at the apex but are separated at the base, which contains a narrow blood vessel. The two transitional filament, one on each side of the principal one, are smaller and differ in shape from the ordinary filaments(Fig. 4) which form the rest of the plica. Sometimes the differnce is insignificant(Fig. 5). In general the ordinary filaments seen on cross section are elongated and club-shaped units.

Throughout their length the filaments of each plica are joined at the bases by regularly spaced interfilamentar junctions, which consist of narrow bands of vascular connective tissue.

Fig. 6 shows swollen gill lamellae and distension of the hemolymph sinuses of the gill of oysters collected from the Gimhae station. This is often so severe that the lamellae and even entire demibranchs become grossly distended and the water tubules are collapsed.

Gastric Shield

The stomach wall in front of the opening to the mid-gut and style sac is covered by a thin tough, irregulary shaped membrane made of translucent and slightly striated material (Fig. 7). The structure rests on a prominent epithelial ridge, of narrow columnar cells with oval nuclei, rich in chromatin. The cells are devoid of cilia. The shield is made of two portions of different size, joined together by a narrow middle piece. The thicker portions of the shield lies over the peak of the ridge and is underlined by the tallest cells in the area. On both sides of the peak the epithelium flattens and at the edges changes into the typical ciliated lining of the stomach. The surface of the shield is roughened by the remnants of food particles embedded in it.

In some instances the cilia began to slough. At about this time the gut becomes greatly distended, there is a marked leucocytic infiltration just beneath the basement membrane and the underlying Leydig cells undergo karyolysis as shown by Fig. 8.

Digestive Diverticula

The digestion and absorption of food in the oyster are primarily intracellular, process which takes place in the digestive diverticula. The digestive diverticula consist of irregular brownish-green lobules, which together with the oesophagus and stomach constitute the ovoid visceral mass. These lobules, found together by a interlobular connective tissue stroma, are exposed on removal of the mantle. They consist of ducts and tubules which fill all the interspaces about the digestive tract proper. A transverse section of typical tubule of the digestive diverticula is shown by Fig. 9.

Changes of uncertain organ were seen in the di estive diverticula and other digestive organs of oysters from the Gimhae station. These changes generally appeared as a thinning of the walls in the smaller digestive diverticula resulting from shrinkage and loss of cells in the epithelial lining (Fig. 10.).

Gonad

Toward the end of the second breeding season the primary gonad is transformed into a definite ovary or testis (Fig. 11). Fig. 12 shows the sex cells in the follicle wall of the gonad of the oyster. Eggs in the mature ovary of oysters are pear-shaped and compressed. Many of them are attached to the follicle wall by long, slender peduncles (Fig. 13). Fig. 14 shows the gradual increase in the number of male sex cells from the wall of the follicle toward the center.

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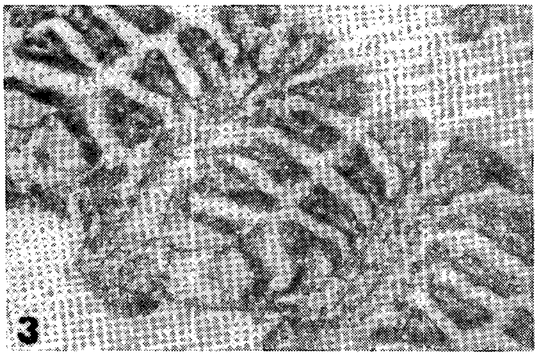
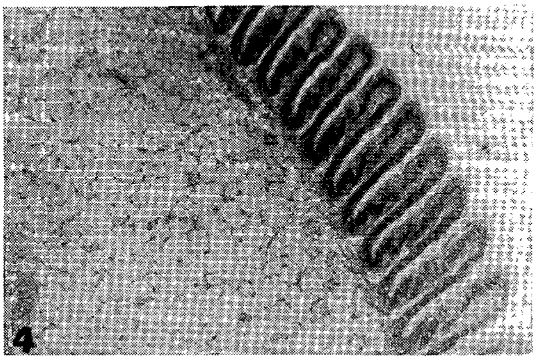
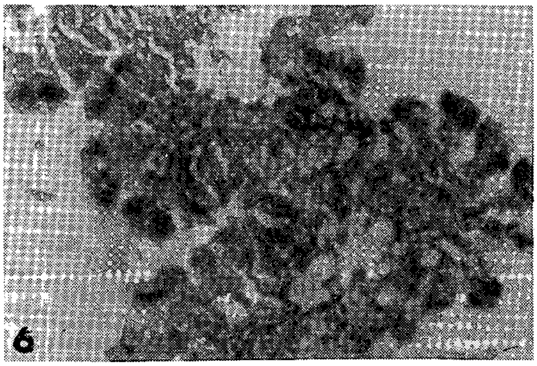
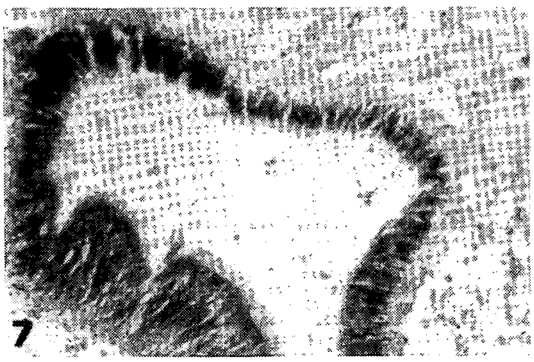








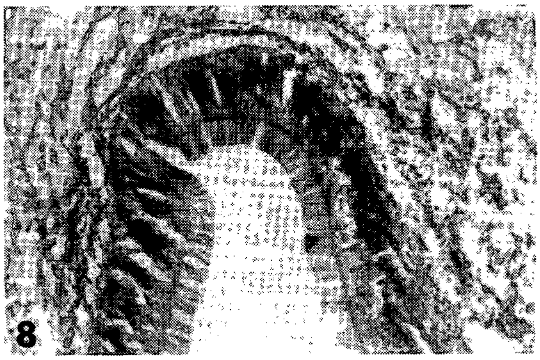
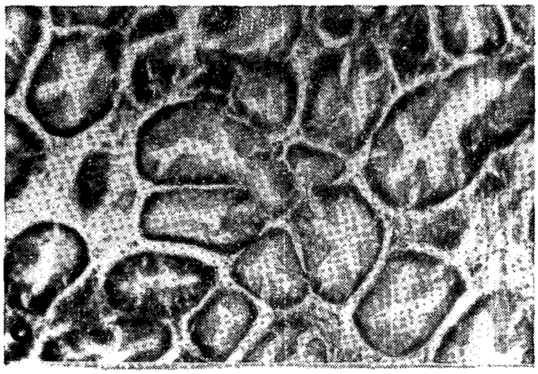
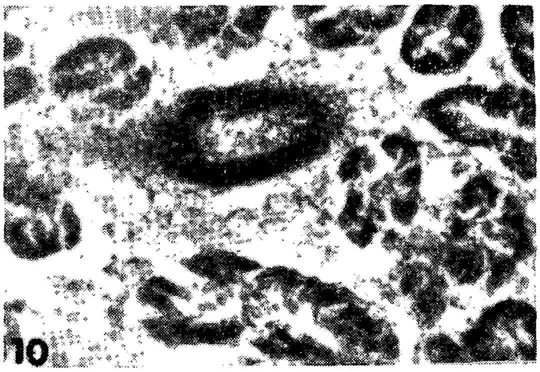
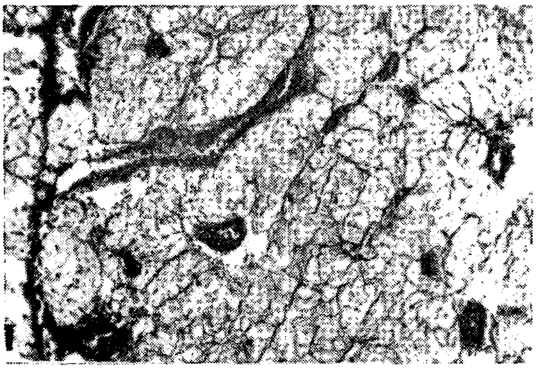


Figure 3. Low magnification of a normal gill. 150X.
Figure 4. Cross section of the interfilamentar junction. 150X.
Figure 5. Enlarged cross section of the interfilamentar junction (labial palp). 200X.
Figure 6. Low magnification of a gill sinuses swollen so excessively that the water tubes are reduced to this channels. 150X.
Figure 7. Cross section of the mid-gut. 150X.
Figure 8. Cross section of the wall of the stomach. 150X.
Note disorganization of epithelium and leucocytic infiltration beneath basement membrane.







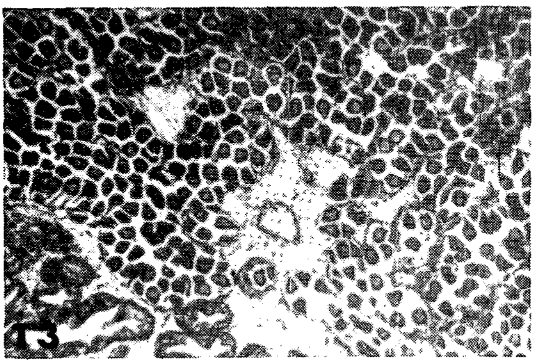
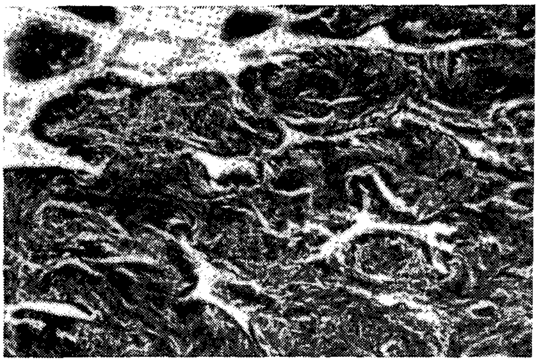




Figure 9. Normal digestive gland diverticula for comparison. 150X.

Figure 10. Digestive gland diverticula. 150X. Note fragmentation and sloughing of epithelium and karyolysis of nuclei in Leydig cells.

Figure 11. Section of a gonad of at an early stage of differentiation. 150X.

Figure 12. Section of a gonad. 150X. Note the larger, clear cells are ovogonia, the smaller ones are undifferentiated cells of germinal epithelium.

Figure 13. Photomicrograph of eggs in follicles of the ovary at the begining of the spawning season. 150X. Ovocytes and small indifferentiated cells line the wall; mature eggs are either free or connected to the wall with long peduncles.

Figure 14. Photomicrograph of a cross section of the follicle of a fully matured sperms. 150X.