

## The changes of histopathology and serum anti-sparganum IgG in experimental sparganosis of mice

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**Abstract:** The present study is intended to observe the chronologic changes of experimental sparganosis by histopathological observation and detection of circulating anti-sparganum IgG antibody using ELISA. Each of 25 mice was infected with five spargana, and they were examined after 1, 2, 4, 10 weeks or 6 months from infection. The followings are summarized results.

1. The plerocercoids were detected in the subcutaneous tissue of the trunk, neck or axilla, but a few often extended into the skeletal muscle. The recovery rates were 72% at the first week, 80% at the second week, 95% at the fourth week, 92% at the tenth week and 100% at the sixth month. The larvae grew slowly in both length and weight until 6 months.

2. Histopathologically, most of the larvae were observed alive in the soft tissue or skeletal muscle. Numerous eosinophils, neutrophils, lymphocytes and plasma cells were infiltrated focally around the worms by the second week, but they surrounded the worms to form a layer of inflammatory reaction after 4 weeks of infection. Also histiocytes and fibroblasts began to appear around the inflammatory cells at 4 weeks. After 10 weeks, the worms encircled by a thin fibrous layer were found. After 6 months, the worms were surrounded by either fibrous tissue or active inflammatory cells. The inflammation looked more severe in the tracks left by the worms, rather than around the worms.

3. The level of anti-sparganum IgG antibody in the serum showed an increase by the fourth week, and a rapid and continuous increase was observed thereafter by the tenth week after infection. The high level of the IgG antibody was maintained up to 6 months forming a plateau curve.

The present results suggest that the tissue reaction and antibody production in subcutaneous sparganosis become distinctive by the fourth week after infection.

**Key words:** mouse, sparganum, recovery, growth, histopathology, serum IgG, ELISA

### INTRODUCTION

Sparganum is the plerocercoid larva of *Spirometra* sp., which, in the larval stage, infects

humans. In America, larvae of *S. mansonioides* are known to infect humans, and *S. erinacei* is an oriental species, although there is still a debate whether *S. erinacei* and *S. mansoni* are synonymous or not (Mueller, 1974; Lee *et al.*, 1984; We, 1989). Its definitive host is carnivorous mammals, including dogs and cats, and

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the first intermediate host is the copepod, but the second host is a rather wide spectrum of vertebrates that include amphibians, reptiles, avians and mammals. Humans usually are a second intermediate host, though there are some cases of adult *Spirometra* infection (Suzuki *et al.*, 1982; Lee *et al.*, 1984).

Presently, diagnosis of sparganosis is made by biopsy or serology, and radiologic approaches are greatly helpful in cases of neurosparganosis (Ishii, 1973; Kim *et al.*, 1984; Chang *et al.*, 1987; Anegawa *et al.*, 1989). Today, the number of known cases of sparganosis in Korea is increasing mainly thanks to more accurate diagnosis. Its pathologic impact in each case depends upon the location of parasitism. Histopathologically, the lesion is composed of a worm in a tunnel surrounded by inflammatory cells, and histiocytes and granulation tissue. Such findings by serology or histology were found in chronic sparganosis. However, the chronologic changes of histopathological and serologic findings of experimental sparganosis are not known yet. The present study was undertaken to follow the changing pattern of serum IgG antibody level and histopathological course of experimental sparganosis in a model of mice.

## MATERIALS AND METHODS

The spargana were collected from the subcutaneous tissue of the snake, *Rhabdophis tigrina tigrina*. Actively moving worms were selected and stored in physiological saline, and their scoleces were used for infection. Each of the ICR mice of 20 g weight was infected with five heads of spargana through a gavage needle. The mice were bred in the laboratory, and 5 mice were sacrificed after 1, 2, 4, 10 weeks or 6 months. Their blood was sampled for serology, and their carcasses were grossly examined for the spargana. Some of the lesions were processed *in situ* for histological observation, and others were recovered for parasitological examination. The sera were examined for the level of anti-sparganum IgG antibody by

micro-ELISA following the methods of Kim *et al.* (1984). The coating antigen was the saline extract of whole spargana total protein of which was 0.45 mg/ml.

## RESULTS

### 1. The distribution and recovery of spargana in mice

Almost all of the worms were found in the subcutaneous tissue (Fig. 1), but a few of them extended into the adjacent muscle. The recovery rate was 72% at 1 week and 100% at 6 months after infection. Most of them were found in the subcutaneous tissue of the neck, axilla and back of trunk (Table 1). They became longer and heavier from 7.3 mg at 1 week to 47.1 mg at 6 months (Table 2).

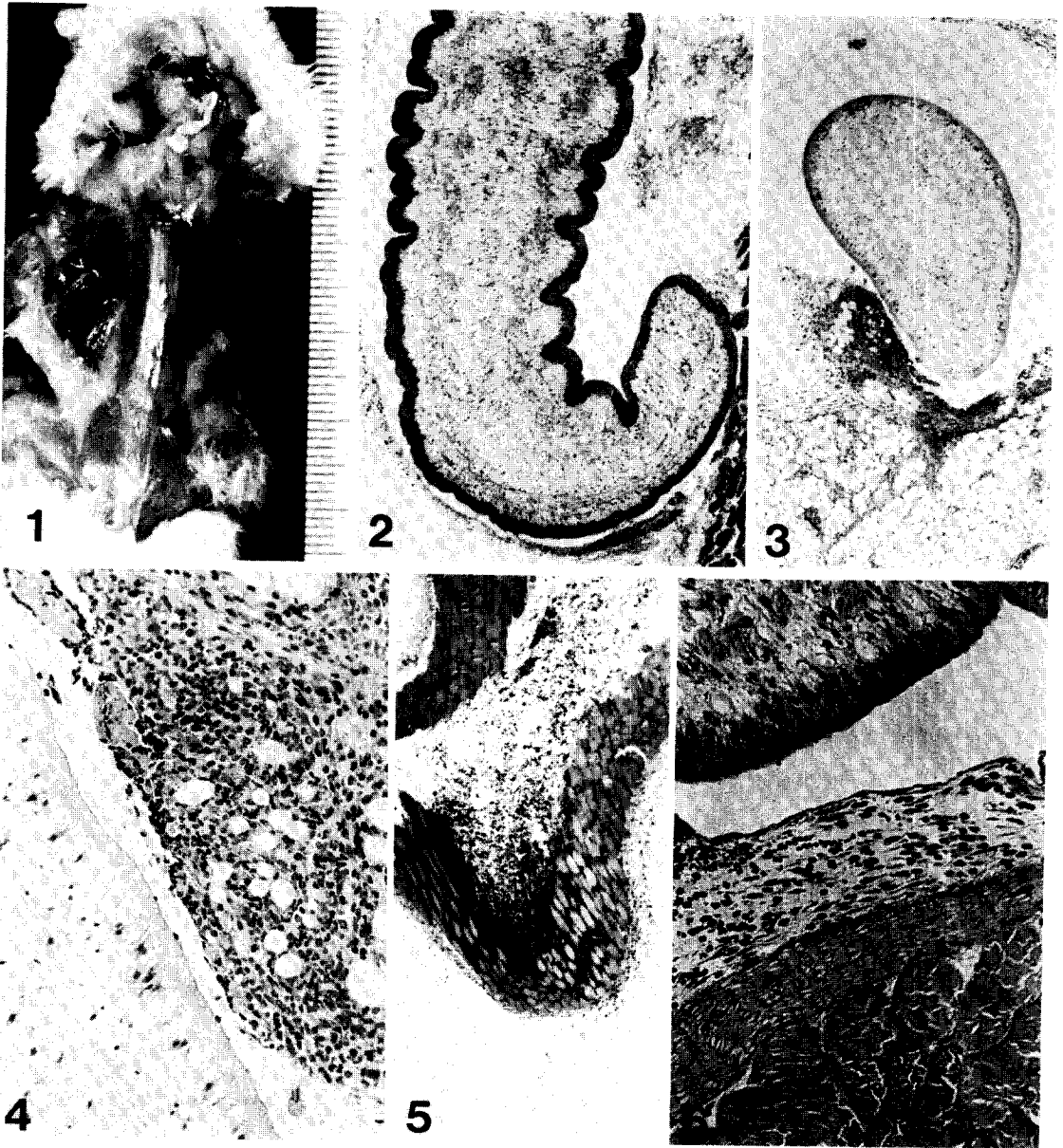
### 2. Histopathological findings

**A) 1 week after infection:** The worms were in the subcutaneous soft tissue or in the membrane of the skeletal muscle. There were inflammatory cells and necrotic debris focally (Fig. 2).

**B) 2 weeks after infection:** The worms were in the subcutaneous soft tissue or adipose tissue with focal infiltration of the inflammatory cells (Fig. 3). The cells were mainly neutrophils or eosinophils, and a few histiocytes (Fig. 4). A tunnel-like space without a worm, which was regarded as a remnant made by a moving sparganum, was heavily infiltrated by numerous inflammatory cells (Fig. 5).

**C) 4 weeks after infection:** The worm became longer and folded to be found as several sections in a lesion. Inflammatory cells surrounded the worm completely and extended to the adjacent muscles. In the outer layer, fibroblasts proliferated to form a layer (Fig. 6). The vacant tunnels were filled with fibrinous exudate and numerous inflammatory cells.

**D) 10 weeks after infection:** The worms grew in length and width. All of their sections included calcareous corpuscles. Neutrophils,



**Fig. 1.** A sparganum in the subcutaneous tissue of a mouse(arrow head), 2 weeks after infection.

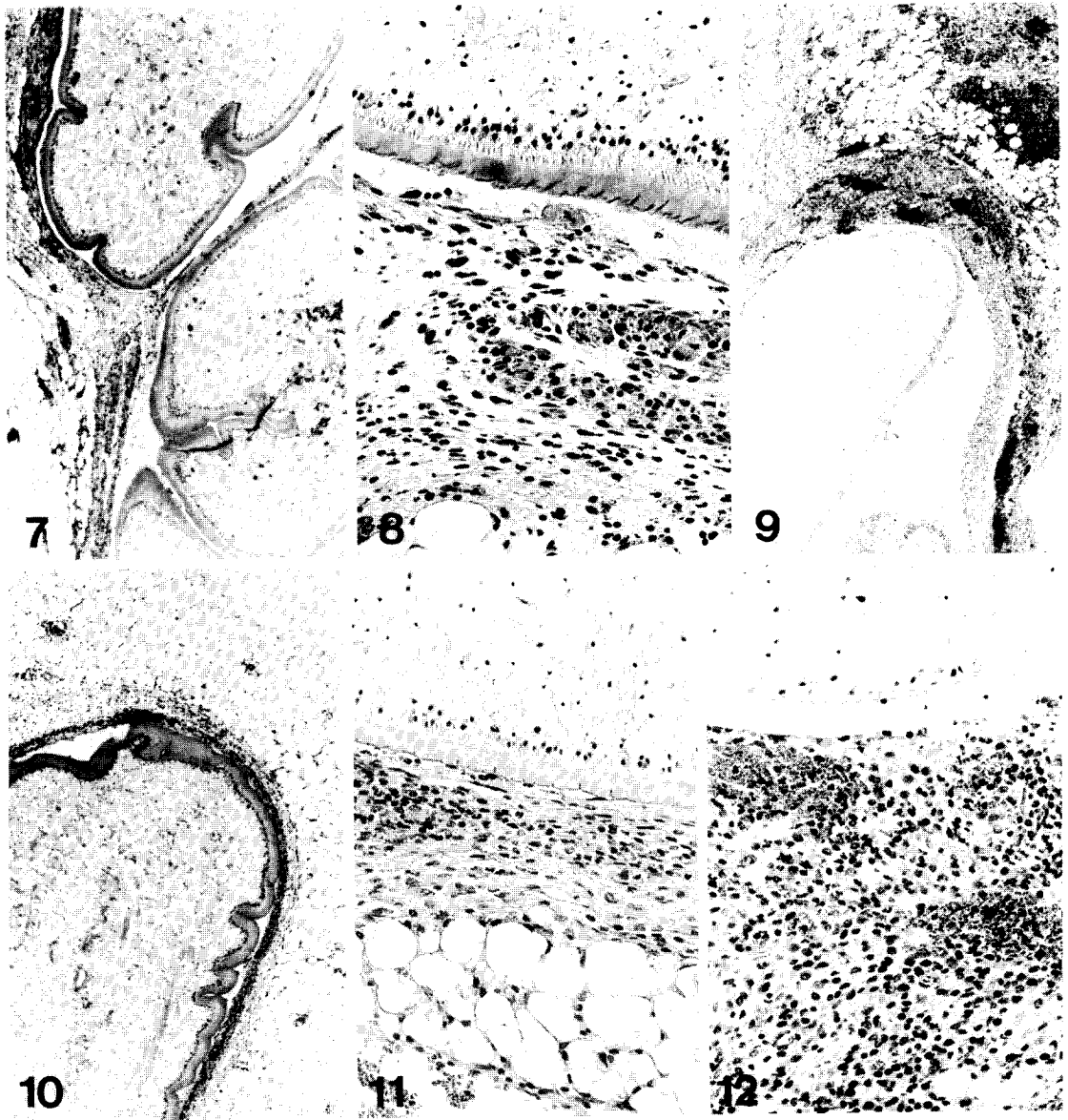
**Fig. 2.** A sectioned sparganum in the muscle fascia, 1 week after infection,  $\times 40$ .

**Fig. 3.** A worm in a tunnel in the adipose tissue with focal infiltration of inflammatory cells, 2 weeks after infection,  $\times 40$ .

**Fig. 4.** High power view of the focal inflammation,  $\times 200$ .

**Fig. 5.** Massive infiltration of inflammatory cells in an empty tunnel in the muscle, 2 weeks after infection,  $\times 40$ .

**Fig. 6.** The layer of fibroblasts around the worm, 4 weeks after infection,  $\times 200$ .



**Fig. 7.** Three sections of a sparganum in the subcutaneous tissue surrounded by inflammatory cells and fibrous tissue, 10 weeks after infection,  $\times 40$ .  
**Fig. 8.** The layer of foreign body giant cells and fibroblasts in the surrounding tissue,  $\times 200$ .  
**Fig. 9.** A sparganum in the muscle with severe inflammation, 6 months after infection,  $\times 40$ .  
**Fig. 10.** A sparganum in the subcutaneous adipose tissue surrounded by a thin layer of inflammatory cells and fibrous tissue, 6 months after infection,  $\times 40$ .  
**Fig. 11.** High power view of the surrounding tissue, 6 months after infection,  $\times 200$ .  
**Fig. 12.** A focus of cell infiltration, RBCs, eosinophils, lymphocytes and histiocytes, 6 months after infection,  $\times 200$ .

**Table 1.** Numbers and sites of spargana in experimental mice infected with five heads each

Duration of infection	No. of exp. mice	No. of spargana from the subcutaneous tissue of								
		head	neck	back	chest	abdomen	axilla	limbs	total	(%)
1 week	5	1	6	2	1	0	7	1	18	(72)
2 weeks	4	1	12	1	0	1	1	0	16	(80)
4 weeks	4	0	3	5	3	0	8	0	19	(95)
10 weeks	5	2	5	12	1	1	3	0	23	(92)
6 months	6	3	11	3	2	2	9	0	30	(100)
<b>Total (%)</b>	<b>24</b>	<b>7 (6.6)</b>	<b>37 (34.9)</b>	<b>23 (21.7)</b>	<b>7 (6.6)</b>	<b>3 (2.8)</b>	<b>28 (26.4)</b>	<b>1 (0.9)</b>	<b>106 (100)</b>	<b>(88.3)</b>

**Table 2.** The weights of spargana recovered from mice during the periods of infection

Duration of infection	No. of worms measured	Weight(mg)/sparganum		
		range	mean	S.D.*
1 week	11	2.8~23.6	7.3	5.5
2 weeks	10	7.8~23.7	13.8	5.3
4 weeks	8	12.0~50.3	26.7	11.3
10 weeks	10	23.8~49.2	40.1	7.5
6 months	21	21.6~85.8	47.1	17.2

\* S.D.; standard deviation

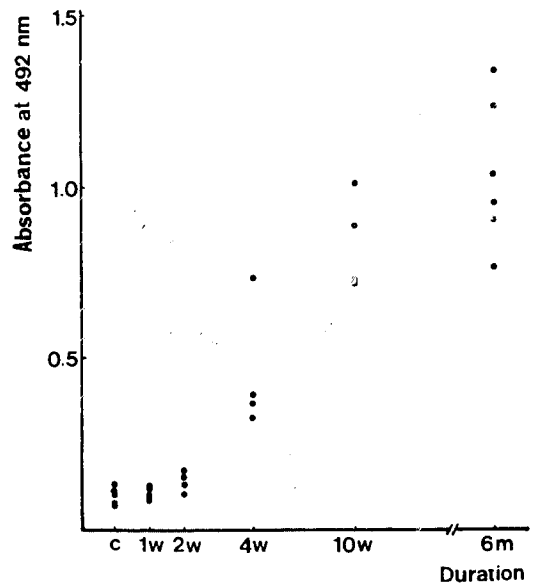
eosinophils and lymphocytes encircled the worms to form an abscess, and then histiocytes, giant cells and fibroblasts formed the next layer(Fig. 7). The outermost layer was composed of granulation tissue and fibrous tissue(Fig. 8).

**E) 6 months after infection:** The foci of abscess-like infiltration of the cells were still around the worm, and some cells adhered to the worm surface. Also, destruction of the tissue, inflammation and exudation were observed which were the findings of active host reaction in acute sparganosis (Fig. 9). However, most of the worms were surrounded by the fibrous tissue to form a layer of worm capsule or focal infiltration of chronic inflammatory cells (Figs. 10, 11 & 12). The cut surfaces of the worm were intact, as found in all of the other groups.

### 3. Anti-sparganum IgG antibody in sera by micro-ELISA

The absorbances at 492 nm were  $0.101 \pm 0.018$  in the control mice,  $0.108 \pm 0.017$  in the mice

of the one-week infection group, but increased to  $0.456 \pm 0.103$  after four weeks and  $1.060 \pm 0.201$  after six months (Table 3 & Fig. 13).



**Fig. 13.** Anti-sparganum IgG antibody levels in serum by the duration of infection.

**Table 3.** Anti-sparganum IgG antibody levels in sera of mice during the periods of infection

Duration of infection	No. of mice	Optical density	
		Range	Mean ± S.D.
control	5	0.079—0.124	0.101 ± 0.018
1 week	5	0.089—0.131	0.108 ± 0.017
2 weeks	4	0.106—0.170	0.141 ± 0.024
4 weeks	4	0.327—0.735	0.456 ± 0.103
10 weeks	5	0.721—1.324	0.955 ± 0.232
6 months	6	0.767—1.347	1.060 ± 0.201

## DISCUSSION

Human sparganosis is usually found as a subcutaneous lump with inflammation at the trunk (Swartzwelder *et al.*, 1964; Cho *et al.*, 1975; Chi *et al.*, 1980). In rare occasions, it involves the vital organs or central nervous system (Min *et al.*, 1976; Chi *et al.*, 1980; Kittiponghansa *et al.*, 1988). Neurosparganosis is clinically important because of various neurological complications. Preoperative diagnosis of neurosparganosis remained impossible until a couple of years ago when specific antibody test and neuroradiological imaging techniques were applied (Fan *et al.*, 1986; Chang *et al.*, 1987; Anegawa *et al.*, 1989).

The primary habitat of sparganum in the present experimental mouse model is also the subcutaneous tissue. All of the 106 recovered worms were in the subcutaneous tissue, and the tails of a few worms extended into the skeletal muscle. The peritoneal cavity, thoracic cavity and brain were examined, but no sparganum was found. Choi(1984) observed the distribution pattern of spargana in mice for two weeks after infection. They penetrated the stomach or intestine within one hour after oral ingestion. Most of them were in the peritoneal cavity, but a few arrived at the subcutaneous tissue after one day. All of them were in the subcutaneous tissue after one week. They were found at the sites of the head, neck and trunk. The present results showed 72~100% recovery rates by each duration of infection, and most of them were at the neck (34.9%), axilla (26.4%) or back of the trunk (21.7%). The present findings and Choi's(1984) results suggest that the larvae invade the stomach wall just after ingestion, and then they migrate into the subcutaneous tissue of the neck, axilla or trunk. The low recovery rates in the early phase of infection in the present study may be due to incomplete detection of the small worm during dissection.

The larvae grew continuously after infection

until six months of this observation period. The larvae weighed 7.3 mg in the first week and became heavier following a sigmoid curve. Such a rapid and remarkable growth of spargana is resulted from growth of the posterior parts to the neck. The growth means rapid cell divisions and active metabolism, even though the worm is in larval stage.

At the first 2 weeks of infection the inflammation was rather mild, as the inflammatory cells were infiltrated focally around the worm. However, much more inflammatory cells and exudates were found in empty tunnels through which the worms had migrated rather than around the worms. The tunnels appear to just undergo the process of active healing after the worms pass through. Therefore, the empty tunnels are older lesions than those containing the worms, and such a time lag may partly make the difference in the intensity of inflammation. Another speculation is that the difference in the degree of inflammation may suggest the ability of the worm to produce any anti-inflammatory substance. However, such an effect if any seems not so potent because numerous inflammatory cells are around the worms. Contrary to this, secretion of the chemotactic factor for eosinophils and neutrophils by sparganum was detected and purified already (Horii, 1989). Since the sparganum is well-known for its ability of secretion of the growth hormone-like substance (Mueller, 1974; Salem and Phares, 1986) or its anti-thyroid function (Hirai *et al.*, 1987), the activity of secreting such various biological substances deserves to study peculiarly to sparganum.

The inflammation was very severe after 4 weeks sometimes extending into the nearby muscles. The active inflammatory layer was squeezed by histiocytes and fibroblasts. In infections after 10 weeks or later, the inflammatory layer was surrounded by the granulation tissue and fibrous layer. Histopathologically the lesion becomes chronic from four weeks after infection. However, the active inflammation process containing infiltration of numerous eosinophils was

also combined at 6 months after infection, which was regarded as a newly-made focus by the migration of the worms. In the lesions of 6 months infection, the tangled mass of a worm and surrounding inflammation was observed as encircled by a thin layer of fibrous tissue.

As for the histopathological characteristics of human sparganosis, Chi *et al.* (1980) described that the lesion was localized around the worm and was differentiated from that of septic abscess in three points. First, most of the centers of the lesions were empty or partly filled with exudate or blood. Second was the irregular inside margin probably due to the worm motility. Third, the surrounding tissue was composed of inflammatory cells, and of histiocytes and fibroblasts outside. In the present study of mouse sparganosis, the granulation layer and fibrous capsule were observed in addition to that of Chi *et al.* (1980). However, the worm capsule is not thick enough to localize the worm inside. The texture and thickness of the capsule is quite different from that of paragonimiasis or hydatid cyst. Such a similar pattern of layers around a worm was also recorded in rabbit anisakiasis (Hong and Lee, 1987).

Anti-sparganum IgG antibody in serum increased distinctively after four weeks from infection. After that, the absorbance by micro-ELISA was at its highest level in the tenth week, and the high level was maintained for six months of the present observation. The chronologically changing pattern of the serum IgG level was quite similar with that of neurosparganosis in cats (Wang, 1988). The elevated level of the serum IgG may last as far as the live worm gives antigenic stimulation. The elevated level of the serum IgG antibody in cat paragonimiasis was noticed at 40~50 days after infection and was at its peak after 140~180 days (Choi *et al.*, 1986). In rabbit anisakiasis, it began to increase at 20 days and reached at the highest at 30~60 days and then decreased (Hong and Lee, 1987). The difference of the chronologic patterns of specific serum antibody level to those parasites may originate

from the differences of duration needed for their migration into the habitat and of the viability after infection. The present result of ELISA for the serum anti-sparganum IgG antibody reveals that serodiagnosis of sparganosis is valuable after 4 weeks from infection. It also takes 4 weeks to induce chronic inflammatory reaction in the experimental sparganosis.

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= 국문초록 =

## 마우스 피하 스파르가눔증에 있어서 감염 경과에 따른 조직병리학적 병변 및 혈청 항체가 의 변화 양상

서울대학교 의과대학 기생충학교실 및 풍토병연구소, 피부과학교실\* 및 법의학교실\*\*  
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스파르가눔증(sparganosis)은 *Spirometra*의 plerocercoid 유충이 인체에 감염되었을 때 유발되는 질병으로 국내에서 드물지 않게 관찰되는 조직내 기생 유충증이다.

이 연구에서는 마우스에 실험적으로 피하 스파르가눔증을 만들고 경시적으로 병변의 조직학적 소견의 변화와 효소면역법에 의한 혈청 항체가를 관찰하여 이 질병의 병소 형성 과정을 관찰하였다. 배에서 얻은 총체를 5마리씩 마우스에 경구 감염시키고, 1, 2, 4 및 10주와 6개월 후에 도살하여, 총체의 분포와 수를 육안으로 확인하고 피하 감염부위를 조직표본으로 만들어 현미경으로 관찰하였다. 이와 동시에 효소면역법을 이용하여 혈청 내 항스파르가눔 IgG 항체가를 측정하여 아래의 결과를 얻었다.

1. 총체 회수율은 감염 후 1주와 2주에 각각 80% 이었으나 조금씩 증가하여 6개월에는 100%이고, 전체 평균 88.3%가 회수되었다. 총체의 대부분은 목과 몸통의 피하조직에서 검출되었고 피하조직에서 골격근에까지 침범한 것도 일부 관찰되었다. 감염 기간이 경과할수록 총체가 성장하여 그 무게가 처음에 평균 7.3 mg에서 6개월 후에는 47.1 mg으로 증가하였다. 감염기간과 총체의 분포와는 차이가 없었다.

2. 감염 후 1주와 2주에 총체 주위에 염증세포가 국한되어 소수 관찰되었으나, 총체가 지나간 부위로 추측되는 곳에 많은 세포가 관찰되기도 하였다. 감염 4주 이후에는 총체가 있는 조직 내의 공간 주위로 파괴된 조직세포와 중성구, 호산구, 림프구등의 염증세포가 많이 침윤되어 있고, 일부에서 삼출액도 관찰되었다. 감염 후 4주와 10주에는 총체와 이에 인접한 염증세포층의 주위를 조직구(histocytes)와 섬유아세포가 층을 이루어 둘러싸고 있었다. 감염 후 6개월에 관찰한 바, 대부분의 총체 주위에서 염증세포가 줄고 섬유소에 의한 총낭(worm capsule)이 얇게 형성되어 있었다. 그러나 이 때에도 총체 주위에 농양을 만들거나 심한 염증소견을 보인 부분도 있었다. 모든 감염군에서 총체는 형태학적으로 온전하고 칼슘 과립을 갖고 있었다.

3. 혈청 내 IgG 항체가는 평균 흡광도가 감염 후 1주에 0.108, 2주에 0.141이었으나 4주에 0.456, 10주에 0.955, 6개월에 1.060으로 증가하였다.

이상의 결과를 종합하면 스파르가눔 감염 후 4주가 경과하면 염증반응이 강하게 나타나고, 조직구와 섬유세포가 관찰되기 시작하며, 혈청 내 IgG 항체가도 유의하게 증가함을 알 수 있었다. 총체가 이동하지 않을 경우에 약 10주가 경과하면 총체 주위에 섬유소가 증식하여 총낭을 형성하고 이 기간 이후에는 항체가의 증가도 완만하였다.