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In vitro and *In vivo* Effects of Praziquantel on Sparganum†

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=Abstract=An experimental study was performed to observe *in vitro* and *in vivo* effects of praziquantel on sparganum. For *in vitro* study, various concentrations of praziquantel solution (0.01, 0.1, 1, 10, 100 μg/ml) were prepared and spargana collected from the snake were incubated. For *in vivo* study, total 18 mice were fed each with 5-10 scolices of spargana, treated after 2-8 weeks with 100 mg/kg/day praziquantel for 1-5 days, and sacrificed 1 week later.

Following results were obtained:

1. After *in vitro* incubation with higher than 0.1 μg/ml praziquantel the spargana expanded laterally, lost their motility and finally became immobilized. Praziquantel revealed greater effects on the neck portion of spargana, which was severely disintegrated into fragments.

2. In *in vivo* study, the worm recovery rates from control and praziquantel-treated mice were not different each other, to be in the range of 60-80%, and all worms recovered were alive. This result represented a failure in the treatment of sparganosis of mice.

3. However, in mice treated with praziquantel, a histological observation sometimes revealed a significant degree of destructive changes in the tegument of worms as well as invasion of host cells such as eosinophils and histiocytes.

The results showed that praziquantel was highly effective in killing spargana *in vitro*, it did not show strong sparganocidal activity *in vivo* of mice by the drug regimen applied.

Key words: ***Sparganum, Praziquantel, Sparganocidal activity***

INTRODUCTION

Sparganum, a plerocercoid larva of dog or cat intestinal tapeworm (*Spirometra* sp.), is occasionally found from human subcutaneous tissues and/or visceral organs, being encapsulated in a granulomatous lesion. This tapeworm is distributed rather widely in the world but human sparganosis has been reported chiefly in southeast Asia and north America (Mineura *et* Mori 1980). In Korea, this disease is not uncommon and known to be contracted, in most cases, from consuming raw flesh and/or viscera of terrestrial snakes such as *Natrix tigrina lateralis* (Cho *et al.* 1975).

One of the important problems in clinical aspects of human sparganosis is its treatment, which usually depends on surgical removal of worms and involved tissues. No drug has been successful for the treatment of this larval tapeworm infection. Recently, however, praziquantel was introduced as a highly effective anthelmintic in treating a variety of trematode and cestode infections (Andrews *et al.* 1983; Lee *et* Chai 1985). This drug was especially appreciated for its therapeutic efficacy on dermal as well as cerebral cysticercosis, another kind of larval tapeworm infection of man (Rim *et al.* 1980; Park *et al.* 1982). But, there has been no report on the effects of praziquantel in killing sparganum.

For this reason, this study was performed to

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observe whether praziquantel has sparganocidal activity *in vitro* and/or *in vivo* and to know a possibility of medical treatment of human sparganosis.

MATERIALS AND METHODS

The spargana were obtained from subcutaneous tissues and/or viscera of the snake, *Natrix tigrina lateralis,* purchased from a local snake collector in Hoengseong-gun, Kangwon-do. Most active worms were used in two series of this experiment.

1. *In vitro* experiment

In each of small petri dishes containing various concentrations (0.01, 0.1, 1, 10 and 100 μg/ml) of praziquantel (Distocide®), which were diluted in Tyrode solution, 5 spargana were incubated at 37°C. After then, their activity as well as morphological changes, if any, were observed at various time intervals (5, 15, 30, 60 min. and 4 hrs. post-incubation) either by naked eye or under stereomicroscopy. Tyrode solution not containing praziquantel was used for control.

2. *In vivo* experiment

Each of a total of 18 mice (ICR strain), 35-42 g, was orally given with 5-10 scolices (or heads) of spargana. They were divided into 4 groups; 3 treatment groups by regimens of praziquantel (daily dose of 100 mg/kg for 1, 3 and 5 consecutive days) and an untreated control group.

Seven days after the last drug administration, the mice in all groups were sacrificed by cervical dislocation, put their skins off, and examined carefully the presence, location and activity of spargana, as well as histological morphology of worms and surrounding tissues. The activity of each worm was observed in small petri dishes containing phosphate buffered saline under stereomicroscopy. The histology of worms and tissues was observed with formalin-fixed specimens processed for routine hematoxylin and eosin staining.

RESULTS

1. *In vitro* experiment

Praziquantel appeared highly effective in killing spargana *in vitro,* with higher concentrations than 0.1 μg/ml (Table 1 & Figs. 1-3). Especially in 10 and 100 μg/ml, the spargana were rapidly immobilized, and complete lysis or disintegration of their neck portions ensued. In comparison, the worms in control group were consistently alive and revealed no morphological change until 4 hours of incubation.

In lowest praziquantel concentration, 0.01 μg/ml, the worms were active up to 5 minutes, but showed reduced activities after 15-30 minutes. They finally revealed only a weak movement of their posterior body later than 1 hour. In this concentration, however, no disintegration of worm body was observed. In higher concentrations such as 0.1 μg/ml or 1 μg/ml, the worm activity was more rapidly weakened, and the body expanded laterally to become dorsoventrally much flat and almost transparent after 5-15 minutes (Fig. 1). Later than 15-30 minutes, their head portion no more revealed movement, and lateral expansion or flattening of body was in its peak stage. The worms looked very soft in body texture and especially neck portions were completely disintegrated after 4 hours.

In 10 μg/ml, complete disintegration of the neck portion occurred as early as 1 hour after the incubation (Fig. 3). In 100 μg/ml, all of 5 worms incubated showed disintegrated necks within

Table 1. Chronologic observation of sparganum motility in various concentrations of praziquantel

Praziquantel concentration (μg/ml)	*Activity of sparganum by times after exposure				
	5min.	15 min.	30 min.	1 hr.	4 hr.
0(control)	+++	+++	+++	+++	+++
0.01	+++	++	+	+	±
0.1	++	+	+	±	−
1	++	+	+	±	−
10	+	+	±	−	−
100	+	+	+	−	−

*+++: very active, ++: moderately active, +: slightly active, ±: with minimal movement. −: no movement

Table 2. The result of sparganum recovery from experimental mice

Group (Days, treatment*)	No. mice used	No. mice examined**	Total No. spargana given	Total No. spargana recovered	(%)
control (0)	6	4	35	28	(80.0)
I(1)	4	1	5	3	(60.0)
II(3)	4	3	15	9	(60.0)
III(5)	4	3	15	12	(80.0)

*with daily dose of 100 mg/kg praziquantel

**A few number of mice in each group were dead due to unknown reason

15-30 minutes (Fig. 2). In this concentration almost whole worm body was completely disintegrated in 1 hour.

From these results, it was considered that the minimum effective concentration of praziquantel to kill spargana *in vitro* was 0.1 μg/ml (Table 1).

2. *In vivo* experiment

From 4 control mice, 28 (80.0%) of 35 spargana introduced were successfully recovered from 3 locations of subcutaneous tissues (Table 2 & 3), *i.e.,* nearby the head, neck (Fig. 4) and trunk. All recovered worms showed active movements. In histopathological sections, spargana and surrounding granulomas were shown (Fig. 5). The granuloma was chiefly consisted with inflammatory cells such as plasma cells, eosinophils and histiocytes. However, no special change was recognized in the morphology of worms (Fig. 6).

The recovery rate of spargana from praziquantel-treated mice was not different from that of control group and in the range of 60-80% (Table 2). There seemed no dose (drug)-dependence in worm recovery rates. More than half of worms were found from subcutaneous tissues nearby the neck portion, followed by the trunk, head and abdominal wall (Table 3). All recovered spargana from control and treated groups revealed active movements and signs of life, which represented a failure of praziquantel in killing spargana *in vivo* of mice.

An interesting observation in the treated mice, however, was that histopathological sections of worm granulomas revealed significant features of tegumental deteriorations in spargana (Figs. 7-9). Even in 1-day treatment (100 mg/kg) group, tegumental layers of a sparganum were seen to be closely attached to the wall of the surrounding granuloma (Fig. 7). Another noteworthy finding was that, in a mouse treated for 3 days (total 300 mg/kg), a part of the tegumental layer of a sparganum

Table 3. Subcutaneous locations of spargana in mice

Group*	No. spargana found from			
	Head	Neck	Trunk	Abdominal wall
control	2	14	12	0
I	0	3	0	0
II	0	4	4	1
III	2	6	4	0
Total	4	27	20	1

*Same group as in Table 2.

was being absorbed into the host tissue (Fig. 8). Invasion of host cells such as eosinophils and/or histiocytes into the tegumental layers of spargana was not an uncommon finding (Fig. 9).

These results showed that, though praziquantel failed to kill spargana *in vivo* of mice, the worms were damaged at their teguments, probably due to the drug action, which allowed host cells to invade into the tegumental layers.

DISCUSSION

In clinical aspects of human sparganosis, it has long been a problem to make its diagnosis and treatment easier, which have usually depended upon surgical intervention. As for the diagnosis, however, the serodiagnostic tool of enzyme-linked immunosorbent assay (ELISA) was recently applied and proved to be highly useful and feasible (Kim *et al.* 1984). However, the problem of its treatment by chemotherapy, has not been solved. In 1933 Cornet tried to treat sparganosis by injecting 2-4 ml procain in 40% ethanol into the worm granuloma (Seo 1978), however, it seemed not a feasible method. More recently, Torres *et al.* (1981) used mebendazole and praziquantel for the treatment of proliferative sparganosis, a fulminating disease caused by branching spargana (*Sparganum*

prolifierum), but they reported an unsatisfactory result. Chai *et al.* (1986) treated a sparganosis case (not proliferative) with praziquantel, but the result was unsatisfactory either.

In the present study, however, praziquantel appeared highly effective in killing spargana, especially in *in vitro* condition. As low as 0.1 μg/ml solution was satisfactory not only for immobilization of worms but also for complete disintegration and lysis of their body. This result was quite comparable with the report on the plerocercoid of *Diphyllobothrium latum,* a morphologically closely related species to sparganum (*Spirometra* sp.), which was killed in greatly higher praziquantel concentration of 600 μg/ml (Bylund *et al.* 1977). If it was true, sparganum should be much more sensitive to praziquantel than the plerocercoid of *D. latum.* As reported in other larval and/or adult tapeworms such as *Hymenolepis nana* (Becker *et al.* 1980), *H. diminuta, Taenia taeniaeformis,* and *Echinococcus granulosus* (Becker *et al.* 1981), the most sensitive part of sparganum affected by praziquantel was its neck portion.

Nevertheless such a rapid and excellent sparganocidal activity *in vitro,* praziquantel failed to kill spargana *in vivo* of experimentally infected mice, although were recongnized some tegumental damages on the worms. It is difficult to give a plausible explanation on that. Speculations may be in several ways, one of them is the short follow-up period of only 1 week in this study. But most of all, the dose (or regimen) of praziquantel given to mice might have been insufficient to kill spargana completely. It is a general concept that the therapeutic dose of praziquantel should be much higher to treat tissue parasites than gut-infecting ones (Andrews *et al.* 1983; Lee and Chai 1985). For example, as much as 500-750 mg/kg in total dose was required for muscular or cerebral cysticercosis in pigs, while only 10-15 mg/kg single dose was required for intestinal trematode or cestode infections of animals or man (Andrews *et al.* 1983; Lee and Chai 1985). But in this study, though maximum 500 mg/kg (100 mg/kg for 5 days) was given to 4 mice, the result was not good. In human sparganosis, two courses (at 1 week interval) of treatment each with 225 mg/kg dose were tried in a man but the result was also poor (Chai *et al.* 1986). Therefore, it should be verified whether higher dose could bring about a successful result in the treatment of sparganosis.

In other aspects, it should be ruled out that the distribution or concentration of praziquantel in subcutaneous tissues may be insufficient after an oral administration. At present, however, there is no direct evidence concerning poor distribution of praziquantel in subcutaneous tissues of mice. Andrew (1976) titrated the amount of praziquantel in muscles of mice and reported that the concentration was higher than 100 μg/g (tissue) during 15-60 minutes after the administration of 250 mg/kg *per os.* It is of interest how much difference could exist in the drug concentrations between muscles and subcutaneous tissues of mice. Steiner *et al.* (1976) reported that, in the rat host, praziquantel concentrations in muscles, fat tissues, and skins were approximately the same. Therefore, it is roughly suggested that the drug concentration in subcutaneous tissues of mice should have been at least higher than 10 μg/g in this study, much higher than the minimum effective concentration *in vitro,* within 1 hour after the administration of 100 mg/kg *per os.* An indirect evidence for this suggestion seems that dermal cysticercosis responded well to the praziquantel treatment (Rim *et al.* 1980), in spite that the location of cysticercus is also in the subcutaneous tissues and it always has a cyst wall of host origin (Chi and Chi 1978) which may rather interfere with the penetration of the drug. Sparganum may not have such a distinct wall or a capsule (Chi *et al.* 1980; Choi 1984). Nevertheless, praziquantel did not show killing effects on spargana infected in mice.

Consequently, it seems that the failure should be explained in a more speculative way. A rough speculation is that there may exist certain chemical barriers between spargana and surrounding tissues. Tissue concentrations of divalent cations such as calcium seem to be an important factor, since the anthelmintic action of praziquantel is known to be calcium-dependent (Andrews *et al.* 1983). Depletion of such cations might occur in the subcutaneous tissues nearby the worms because of their probable requirement of calcium and/or other chemicals to form calcium corpuscles within their body. The growth (of host) promoting factor (Mueller 1963; Shiwaku and Hirai 1982), or others produced by the spargana might interfere with the activity of praziquantel. Further studies are necessary to elucidate this point.

Still a promising observation in this study, was alterations in the tegumental integrity of spargana, probably due to the action of praziquantel. Into the damaged teguments host cells such as histiocytes

were invading. This feature is just like that reported in *Paragonimus westermani*, the lung fluke, in the lung of dogs treated with praziquantel (Chiu *et al.* 1982). However, according to them, *P. westermani* worms were dead and already in the process of necrosis 7 days after the treatment, while not in the present study with spargana.

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= 국문초록 =

스파르가눔에 대한 프라지콴텔의 살충효과 : 시험관내 및 실험감염 마우스 체내에서의 관찰

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프라지콴텔(praziquantel)이 스파르가눔(sparganum)에 대하여 殺蟲作用을 나타내는지를 관찰함으로써 스파르가눔症(sparganosis)의 藥物治療 가능성을 알아보았다. 殺蟲作用은 시험관內(*in vitro*) 및 실험감염 마우스 體內(*in vivo*)의 2가지 조건하에 관찰하였고 꽃뱀(유혈목이 : *Natrix tigrina lateralis*)에서 획득한 스파르가눔을 실험에 사용하였다. 프라지콴텔의 시험관내 농도는 0, 0.01, 0.1, 1, 10 및 100 μg/ml로 하였고 마우스는 蟲體 頭部(scolex or head)를 경구감염시킨 다음 2~8週後 1일 100 mg/kg 프라지콴텔을 1일, 3일 또는 5일간 투여하고 1週後 蟲體回收 狀況 및 蟲體形態 등을 관찰하였다.

그 結果는 다음과 같다.

1. 시험관내에서 스파르가눔은 프라지콴텔의 作用으로 蟲體가 옆으로 팽대하면서 얇아졌고, 運動性을 상실하였으며, 결국 頸部(neck)를 중심으로한 蟲體 파괴 또는 分解에 이르는 심한 손상을 받아 사멸하였다. 이러한 완전한 殺蟲效果는 프라지콴텔 0.1 μg/ml 이상의 농도에서 뚜렷하였다.

2. 그러나 마우스 체내에서는 약제가 완전한 殺蟲作用을 나타내지 못하여 투약하지 않은 대조군과 같은 蟲體回收率(60~80%)을 보였고, 回收된 蟲體도 활발히 살아있었다. 다만 약제를 투여한 마우스의 病巢 조직표본에서 다소의 蟲體 表皮層(tegumental layer) 손상과 손상된 부위로 침입하고 있는 宿主의 好酸球 또는 組織球(histiocyte) 등이 관찰되었다.

이상의 결과는 프라지콴텔이 시험관내에서 강한 스파르가눔 殺蟲作用을 가지는 반면 마우스 체내에서는 1일 100mg/kg 용량의 1~5일간 투여로 즉각적인 殺蟲作用을 보이지 않았음을 의미하였다.

EXPLANATIONS FOR FIGURES

Fig. 1. Spargana incubated in 0.1 μg/ml praziquantel solution for 15 minutes. Lateral expansion and flattening of worm body (arrows) and loss of their tegumental texture are seen. (Scale: mm)

Fig. 2. *Ibid,* incubated in 100 μg/ml concentration for 30 minutes. A sparganum is already seen to be disintegrated at its neck portion (arrows). (Scale: mm)

Fig. 3. A sparganum incubated in 10 μg/ml for 1 hour. Disintegration and lysis of its neck is conspicuous (arrow). (Scale: mm)

Fig. 4. A sparganum found from a subcutaneous tissue near the neck of a mouse (arrow). Control group. (Scale: mm)

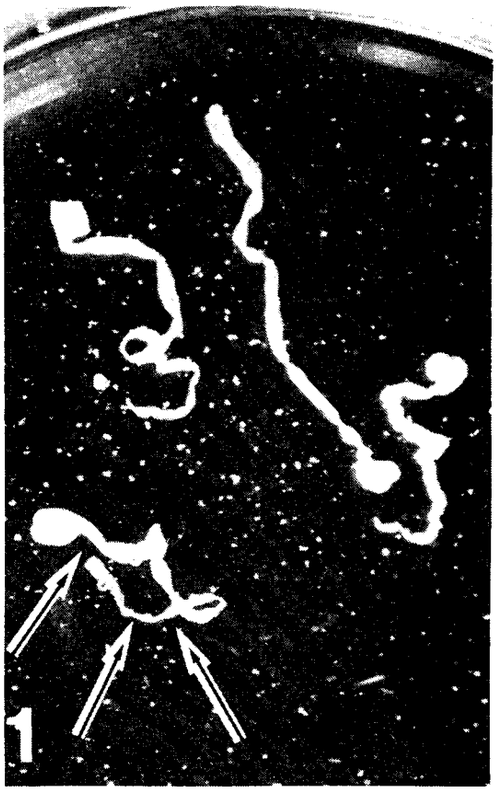
Fig. 5. Section of a subcutaneous granuloma of a control mouse. A sectioned sparganum and surrounding host tissue are seen, and the tegument of the worm is remained intact. Histiocytes and eosinophils are abundant in the granuloma wall. x40

Fig. 6. *Ibid,* higher magnification of the lower portion of Fig. 5. The worm tegument is free from host cells (arrows). X100

Fig. 7. Section of a granuloma of a praziquantel-treated mouse. Tegumental layers of the worm are in close contact with the granuloma wall (arrows). 1-day treatment group. x40

Fig. 8. Section of another granuloma in treated group. A degenerating part of a sparganum is seen to be under absorption into the host granuloma tissue (arrows). 3-day treatment group. x200

Fig. 9. *Ibid,* other portion. The junction between host and parasite is hardly discriminated, due to the invasion by host cells (arrows) into the tegumental layer of a sparganum. x400



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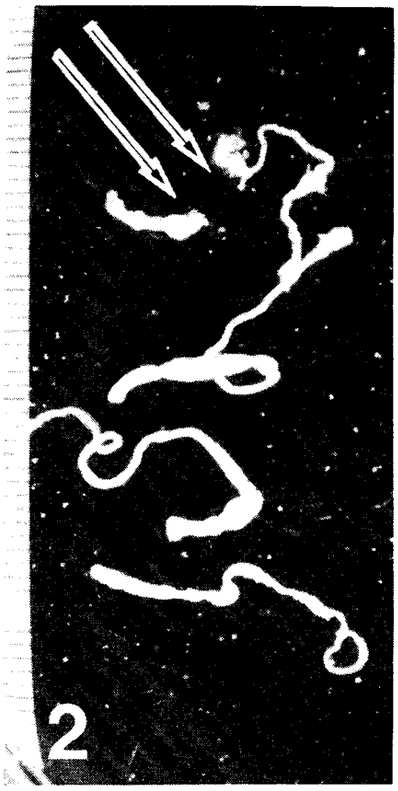

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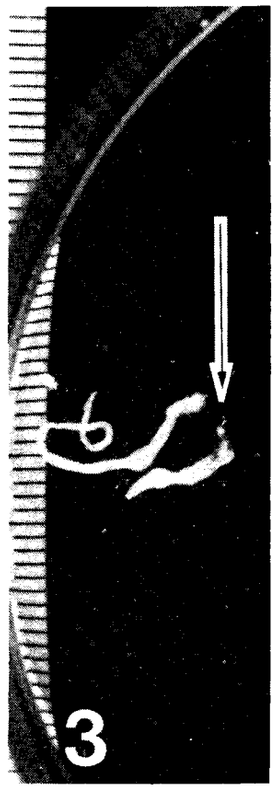

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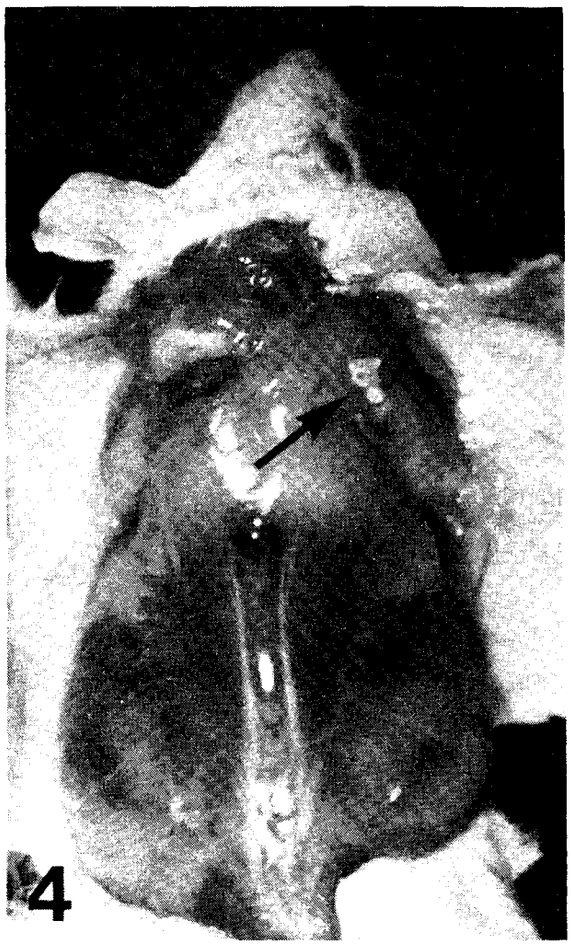

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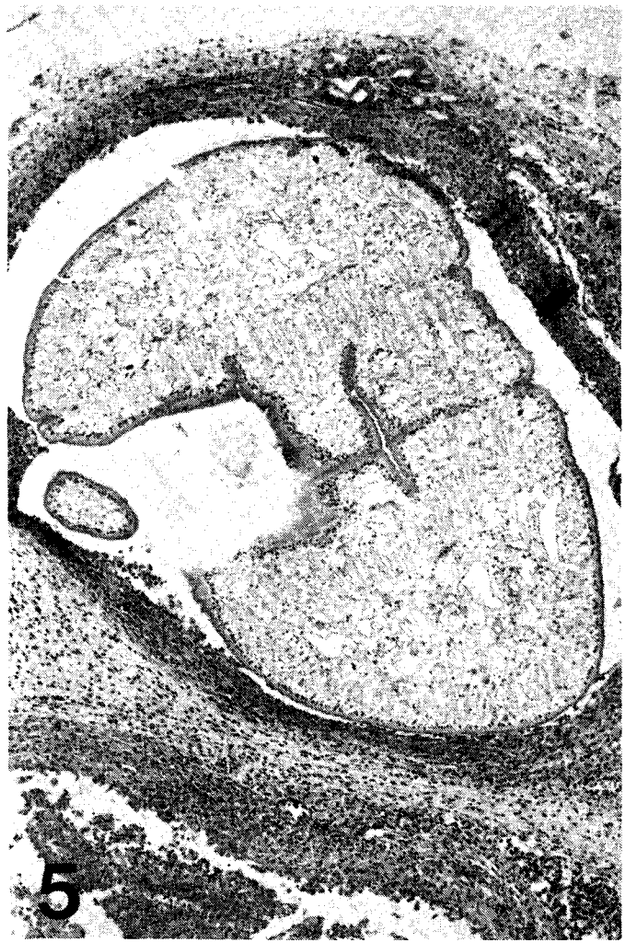

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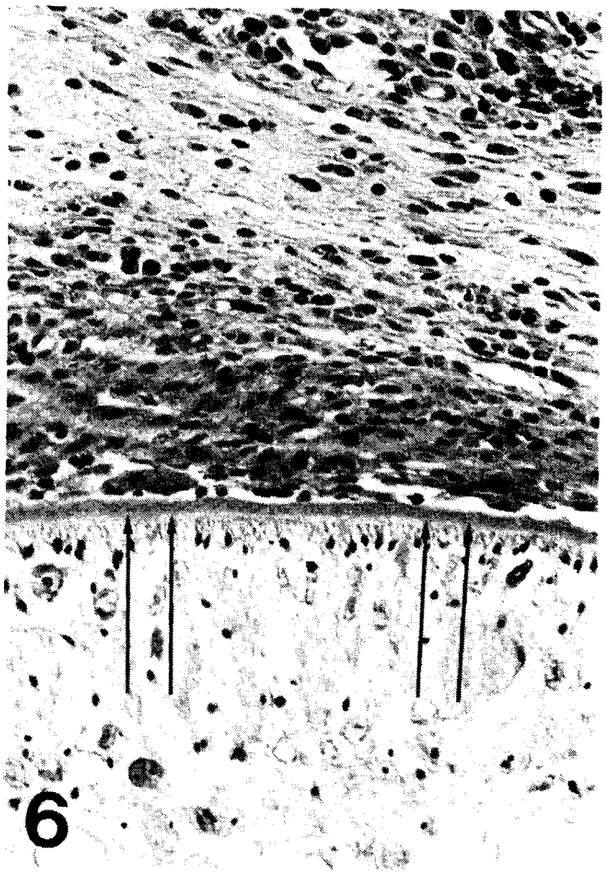

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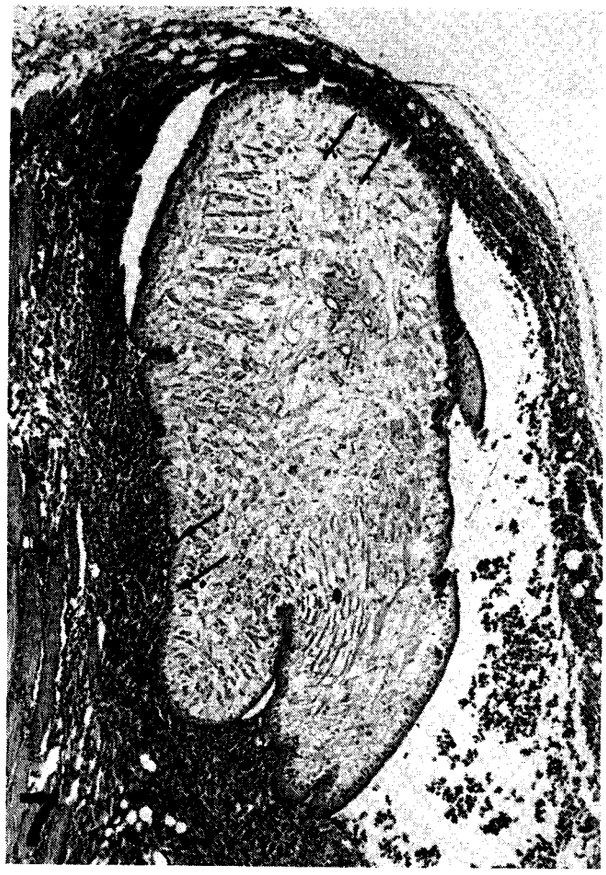

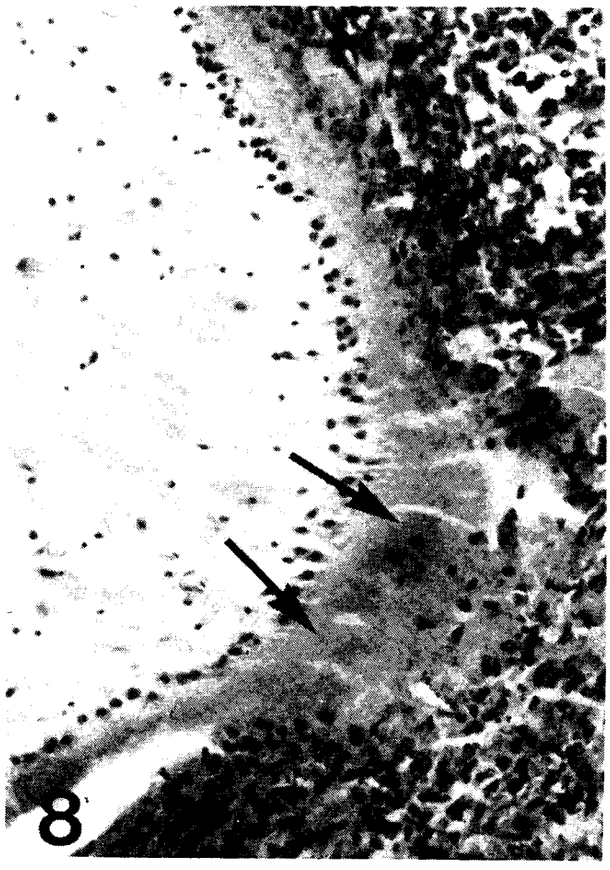

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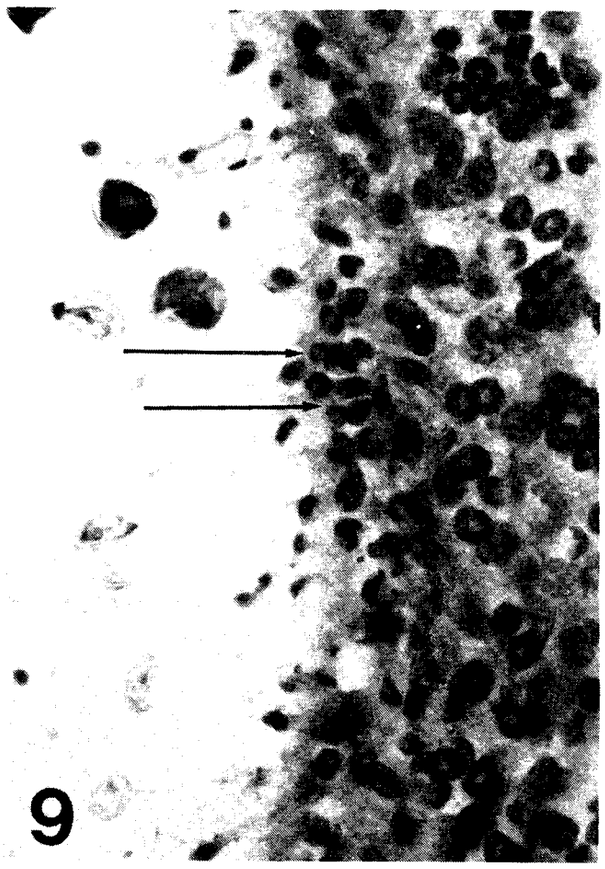

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