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Enhancement of Serum Somatomedin Activity and Cartilage Mitotic Activity in Snell Normal and Dwarf Mice Infected with *Spirometra erinacei* Plerocercoids

KUNINORI SHIWAKU[1], KAZUMITSU HIRAI, TAKAFUMI TSUBOI AND MOTOMI TORII

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Introduction

It is known that the plerocercoid of the genus *Spirometra* causes various biological effects in rodents. *S. mansonoides* plerocercoids accelerated the growth of mice, hamsters, thyroidectomized rats and hypophysectomized rats (Mueller, 1963, 1965, 1968), and increased somatomedin concentration in the serum (Garland *et al.,* 1971). Tsushima *et al.* (1974) reported that a substance in the incubation medium of *S. mansonoides* plerocercoids competed with human growth hormone for binding sites on rabbit liver membrane preparations. Meyer *et al.* (1965), Steelman *et al.* (1971) and Phares and Carroll (1982) reported that *S. mansonoides* plerocercoids stimulated a lipogenesis in hamsters, rats and hypophysectomized rats.

The plerocercoids of *S. erinacei* promoted the growth of mice, Snell dwarf mice and Chinese hamsters (Hirai *et al.,* 1978, 1983; Shiwaku and Hirai, 1982; Shiwaku *et al.,* 1983b). The rodents infected with *S. erinacei* plerocercoids showed no obesity and there was no growth-promoting effect on hypophysectomized rats infected with *S. erinacei* plerocercoids, indicating that the plerocercoids of *S. erinacei* and *S. mansonoides* differ in their effects. In our studies on the growth-promoting effects of *S. erinacei* plerocercoids in mice, we found that *S. erinacei* plerocercoid stimulated cell division in epiphyseal cartilage of mice, suggesting that this larva causes an increase in somatomedin levels in the serum (Shiwaku *et al.,* 1982). Subsequently, we reported evidence that some growth factors were present in the serum of mice infected with *S. erinacei* plerocercoids (Shiwaku *et al.,* 1986).

In present study, serum somtomedin activity and costal cartilage mitotic activity ([^{35}S] sulphate and [^{3}H] thymidine incorporation) were measured in both Snell normal and dwarf mice infected with *S. erinacei* plerocercoids in order to identify the properties of growth factors in the mouse serum.

Materials and Methods

Plerocercoids

Plerocercoids of *S. erinacei* were collected from two species of snake, *Elaphe quadrivirgata* and *Rhabdophis tigrinus,* captured in the southern part of Ehime Prefecture, Japan. These plerocercoids were stored in the subcutaneous tissue of golden hamsters.

Animals

Snell mice were purchased from the Jackson Memorial Laboratory, Bar Harbor, USA, in

Department of Parasitology, Ehime University School of Medicine, Shigenobu-cho, Ehime 791-02, Japan.
[1]*Present address: Department of Parasitology, Aichi Medical University, Nagakute-cho, Aichi 480-11, Japan.*
Offprint requests to: K. Hirai.
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1981. Dwarf mice and phenotypically normal mice were bred by mating these heterozygotes at the Institute for Comparative Medicine and Animal Experimentation, Ehime University School of Medicine. All animals were fed a diet of commercial food pellets (MF, Oriental Yeast Co., Japan). Water was available *ad libitum*. They were kept in plastic cages at a constant temperature of 24°C on a 12 hr day-night rhythm. Snell normal and dwarf mice were used between 5 and 8 weeks of age.

Experimental design

Snell normal and dwarf mice in the experimental groups were injected subcutaneously in the back with 10 scoleces in 0.4 ml of physiological saline solution containing 1000 U/ml penicillin-G and 0.5 mg/ml streptomycin. Animals in the control groups received a similar injection of saline containing antibiotics without larvae. Blood was collected by decapitation of normal mice and orbital puncture of dwarf mice at the 2nd and 6th weeks after infection. The serum obtained from each individual was pooled per group and stored at –20°C and the ribcage was dissected.

Serum somatomedin activity

Serum somatomedin activity and cartilage analysis were measured by a modification of the method of van Buul and Van den Brande (1978). F-12 medium (Nissui, Japan) was used in place of modified Krebs phosphosaline buffer described by van Buul and Van den Brande (1978) because of the ease of preparation and modifications (Shiwaku *et al.*, 1983a). The estimation was based on the uptake of [^{35}S] sulphate and [^{3}H] thymidine by the costal cartilage of homologous dwarf mouse. The tissue was obtained as follows: noninfected dwarf mice of 5–8 weeks old were killed, the ribcages were dissected and adherent muscle was removed. From each animal 12 fragments of costal cartilage were obtained and distributed three by three over a 24 well multidish (Nunc, Denmark) such that triplets of cartilage fragments from each animal were used only once for testing each serum. Preincubation in 0.5 ml of F-12 medium for 21 hr was followed by incubation in 0.5 ml of F-12 medium including of test substance for another 48 hr, the last 24 hr of which was in the presence of 1 μCi sodium [^{35}S] sulphate and 4 μCi [^{3}H]-methyl thymidine (Amersham, England) per ml. The assay was performed in triplicate. A blank value was obtained by incubation in 0.5 ml of F-12 medium excluding mice sera.

After incubation the cartilage fragments were boiled, washed for 2 hr, dried, weighed and dissolved in 0.5 ml formic acid at 80°C for 45 min. Ten ml scintillation cocktail (ACS II, Amersham) was added and the radioactivity was counted in an Aloka LSC903 Liquid Scintillation Spectrometer. Incorporation of radioactivity was expressed as d.p.m./mg dry weight of certilage.

Cartilage analysis

The ribcage was dissected and freed of adherent superficial muscle tissue. On either side the second through the sixth ribs were cut as closely as possible to the sternum and then each rib was again cut at the costochondral junction. The ribcage segments were incubated immediately after sacrifice for 21 hr in 0.5 ml of F-12 medium supplemented with 1 μCi sodium [^{35}S] sulphate and 4 μCi [^{3}H] methyl thymidine per ml and endogenous activity was measured.

In order to measure serum stimulated activity, preincubation for 21 hr was followed by a 48 hr incubation period in the presence of 16% human serum supplemented with the labeled compounds above mentioned for the last 24 hr. After incubation, the ribcage segments were processed as described above.

Statistical analysis

Analysis of variance was carried out by unpaired Student's *t*-test. Values of $P < 0.05$ were considered significantly.

Results

The measurement of incorporation of [^{35}S]-sulphate and [^{3}H] thymidine in costal cartilage was based on human serum concentrations, i.e. between a final concentrations of 1.58% and 40%, in order to select a linear portion of the dose-response curve (Figs. 1 and 2). This assay was sensitive to human serum concentrations as low as 16% as a function of the logarithm of the dose of serum. Incorporation with 16% human serum concentration was 2.82 of [^{3}H] thymidine and 3.63 of [^{35}S] sulphate against a blank, given as 1.00. Incorporation of both radioisotopes was highest in 2.56% mouse serum concentration, and incorporation of [^{3}H] thymidine decreased in 6.4%. Serum somatomedin activity in mice was therefore determined in a 2.56% serum concentration.

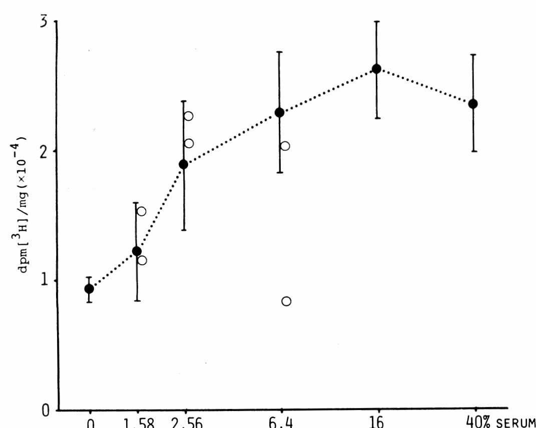

Fig. 1 Effect of normal human and mice sera on [^{3}H]thymidine incorporation in dwarf mouse costal cartilage.
(●) Normal human serum, mean of triplicate determinations ± SD.
(○) Normal mouse serum.

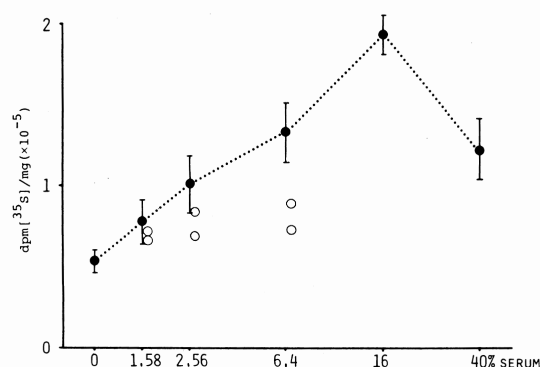

Fig. 2 Effect of normal human and mice sera on [^{3}H]sulphate incorporation in dwarf mouse costal cartilage.
(●) Normal human serum, mean of triplciate determinations ± SD.
(○) Normal mouse serum.

Serum somatomedin activity is summarized in Fig. 3. Serum somatomedin activity for [^{3}H] thymidine incorporation in normal mice was 21758 ± 2639 d.p.m./mg in 8-week-old and 16386 ± 1614 d.p.m./mg in 12-week-old mice. The activity in normal mice significantly decreased with age ($P < 0.01$). In dwarf mouse serum [^{3}H] thymidine incorporation was 12566 ± 620 d.p.m./mg in 8-week-old and 11375 ± 2031 d.p.m./mg in 12-week-old mice. Serum somatomedin activity in dwarf mice significantly decreased when compared with that in normals ($P < 0.01$). The [^{3}H] thymidine incorporation was 9221 ± 1118 d.p.m./mg in

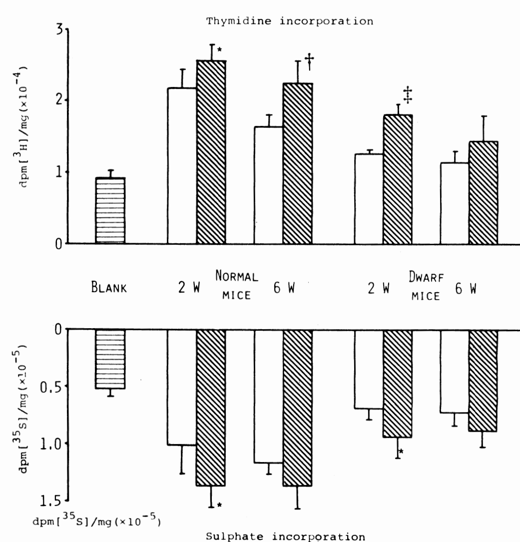

Fig. 3 Serum somatomedin activity of normal and dwarf mice infected with plerocercoids.
□ Control group, ▨ Infected group
Values are means ± SD. *$P < 0.05$, †$P < 0.01$ and ‡$P < 0.001$ compared with controls.

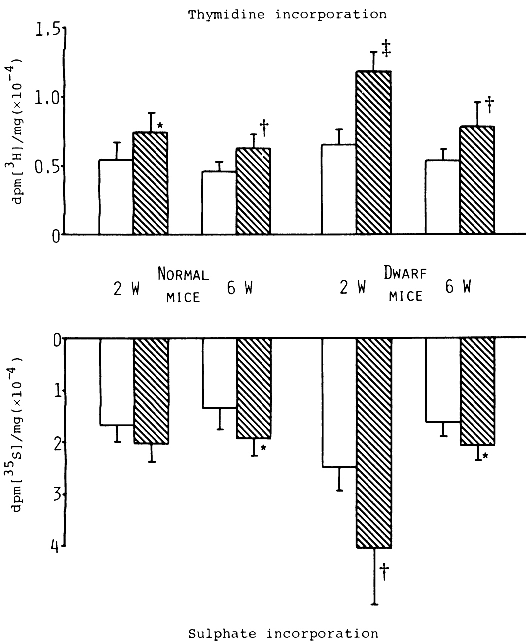

Fig. 4 Endogenous activity of costal cartilage from normal and dwarf mice infected with plerocercoids.

□ Control group, ▧ Infected group

Values are means ± SD. *$P < 0.05$, †$P < 0.01$ and ‡$P < 0.001$ compared with controls.

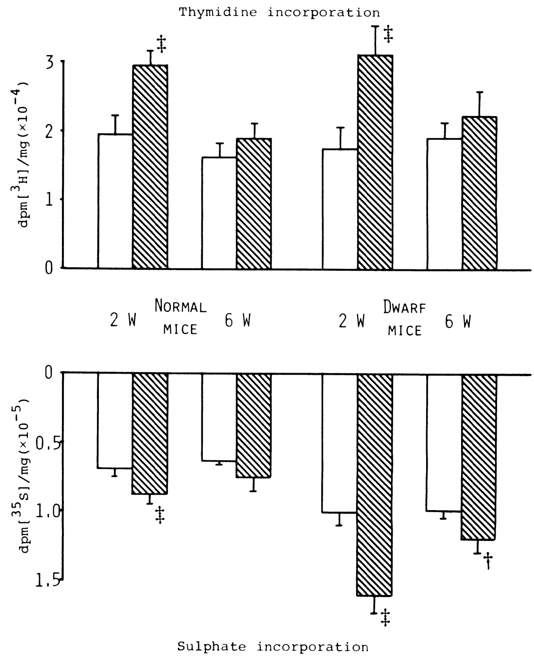

Fig. 5 Serum stimulated activity of costal cartilage from normal and dwarf mice infected with plerocercoids.

□ Control group, ▧ Infected group

Values are means ± SD. *$P < 0.05$, †$P < 0.01$ and ‡$P < 0.001$ compared with controls.

the blank. Serum somatomedin activity appeared to be 26.7% in dwarf mice compared to the normal littermates of 8-week age (Value in dwarf mice − Value in blank / Value in normal mice − Value in blank).

It was remarkable that in the 2nd week after infection, but not in the 6th week, serum somatomedin activity for [^{35}S] sulphate and [^{3}H] thymidine incorporation in the infected normal and dwarf mice was higher than in the control groups ($P < 0.05$). Dwarf mice infected with the plerocercoids showed a marked increase in serum somatomedin activity compared with infected normal mice.

Cartilage activity for sulphate and thymidine incorporation was demonstrated by endogenous and serum stimulated activities in normal and dwarf mice (Figs. 4 and 5). Infection with the plerocercoids induced significant increases in endogenous activity during the experimental period ($P < 0.05$), except for sulphate incorporation in normal mice in the 2nd week after infection. Infected normal and dwarf groups showed a significant increase in serum stimulated activity in the 2nd week after infection against the control groups ($P < 0.001$). The difference in both cartilage activities between the infected groups and the control groups was remarkable between in dwarf mice and normal mice, and also between at the 2nd week and the 6th week after infection.

Discussion

Injection with the serum from mice infected with *S. erinacei* plerocercoids caused increases in the weights of the liver and spleen of recipient mice, in the same manner as mice infected with the plerocercoids (Shiwaku *et al.*, 1986). Although this experiment showed that some growth factors were present in the serum of

mice infected with the plerocercoids, it was not clear what the properties of growth factors were. Since the effect of *S. erinacei* plerocercoids on the growth of Snell dwarf mice was similar to the effects of somatomedin or growth hormone (Shiwaku *et al.,* 1983b), serum somatomedin activity and cartilage mitotic activity were measured in Snell normal and dwarf mice infected with the plerocercoids in present study.

Somatomedin is a growth hormone-dependent peptide, circulating in serum, which directly causes skeletal growth. Its existence was proposed by studies of the action of growth hormone on hypophysectomized rat cartilage *in vivo* and *in vitro* (Salmon and Daughaday, 1957; Daughaday *et al.,* 1972). Somatomedin is now known to have many metabolic effects but only [^{35}S] sulphate or [^{3}H] thymidine incorporation into cartilage has been widely used for bioassay (Daughaday *et al.,* 1975).

Somatomedin stimulated thymidine and sulphate incorporation into the costal cartilage of Snell dwarf mouse (van Buul and Van den Brande, 1978). In order to obtain a homologous system, costal cartilage of Snell dwarf mice was used for measuring somatomedin activity of mouse serum according to van Buul and Van den Brande. Our assay system was sensitive to a serum concentration as low as 16% human serum and as low as 2.56% mouse serum. Serum somatomedin activity in dwarf mice showed a marked decrease, which appeared to be 26.7%, compared with that in normal littermates of 8-week-old. This degree of decrease in serum somtomedin activity of dwarf mice is similar to that rèported by van Buul and Van den Brande (1978). It seems that the decrease in serum somatomedin activity is due to a defective anterior pituitary gland (Bartke, 1965).

The plerocercoids enhanced serum somatomedin activity in normal and dwarf mice in the 2nd week after infection, but serum somatomedin activity in dwarf mice was not completely restored to a normal value. Serum somatomedin activity in infected normal and dwarf mice decreased with age and then became an insignificant value when compared to the controls in the 6th week after infection. The results show that the plerocercoids stimulated an increase in cartilage mitotic activity in the 2nd week after infection but did not stimulate such an activity in the 6th week. Probably the cause for the decrease in serum somtomedin activity and cartilage mitotic activity of infected normal and dwarf mice is due to an aging effect (van Buul and Van den Brande, 1978; Shiwaku and Hirai, 1982) and/or antibodies which prevent the induction of somatomedin (Garland *et al.,* 1971).

The results show that *S. erinacei* plerocercoids induced the mitosis of cartilage cells by an enhancement of serum somatomedin activity in their host. It is concluded that at least one of the growth factors in mouse serum infected with the plerocercoids is somatomedin. However, it is not clear at present whether somatomedin is secreted by the plerocercoids or not. Since Hirai *et al.* (1978) reported the existence of a lipolytic substance, similar in effect to a growth hormone, in the incubation medium of these larvae, it will be necessary to analyze a somatomedin-like and a growth hormone-like substance in the secretion of the plerocercoids in order to clarify the mechanism of growth-promoting effect of *S. erinacei* plerocercoids.

Summary

Our study presents evidence that some growth factors are present in the serum of mice infected with *S. erinacei* plerocercoids. Serum somatomedin activity and costal cartilage mitotic activity ([^{35}S] sulphate and [^{3}H] thymidine incorporation) were measured in Snell normal and dwarf mice infected with the plerocercoids in order to identify the properties of these growth factors.

Serum somatomedin activity appeared to be 26.7% in dwarf mice compared to the normal littermates of the same age. The plerocercoids significantly enhanced serum somtomedin ac-

tivity in the 2nd week after infection. Endogenous (21 hr incubation in medium with labeled compounds) and serum stimulated cartilage activity (21 hr preincubation in medium, 48 hr incubation with 16% human serum, supplemented with labeled compounds for the last 24 hr) was greater in Snell normal and dwarf mice infected with the plerocercoid than those in the control groups. It is concluded that *S. erinacei* plerocercoids induced the mitosis of cartilage cells by an enhancement of serum somatomedin activity.

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マンソン裂頭条虫擬充尾虫感染によるスネル正常および侏儒マウスにおける血清ソマトメジン活性と軟骨の分裂能の増加について

塩飽邦憲[1]　平井和光　坪井敬文　鳥居本美
(愛媛大学医学部寄生虫学教室)

マンソン裂頭条虫擬充尾虫感染ICR系マウスの血清を腹腔内投与したスネル正常および侏儒マウスでは，擬充尾虫感染マウスの場合と同様に成長の促進が観察された（Shiwaku *et al.*, 1986）．このマウス血清中に存在する成長促進因子を同定するために，マンソン裂頭条虫擬充尾虫感染スネル正常および侏儒マウス血清中のソマトメジン活性と軟骨の分裂能を，[^{35}S] sulphate と [^{3}H] thymidine のマウス肋軟骨への取り込みを指標にして測定した．

侏儒マウス肋軟骨を使用した生物学的検定法により，マウス血清中のソマトメジン活性を測定した．スネル侏儒マウス血清中のソマトメジン活性は，同週令のスネル正常マウスの活性の26.7%しか存在しなかった．マンソン裂頭条虫擬充尾虫は，スネル正常および侏儒マウス血清中のソマトメジン活性を，感染後2週では対照群に比較すて統計学的に有意に増加させていたが，感染後6週での増加は有意ではなかった．また，スネル侏儒マウスのソマトメジン活性は，マンソン裂頭条虫擬充尾虫感染によって正常マウスの水準には回復しなかった．

マウス軟骨の分裂能は，ヒト血清0%またはヒト血清16%存在下で，被験肋軟骨に [^{35}S]sulphate と [^{3}H]thymidine を添加して測定した．マンソン裂頭条虫擬充尾虫感染スネル正常および侏儒マウスの軟骨の分裂能は，測定系へのヒト血清添加の有無にかかわらず，感染後2週では増加していたが，6週での増加は2週ほど顕著ではなかった．

以上の結果より，マンソン裂頭条虫擬充尾虫は，スネル正常および侏儒マウスの血中ソマトメジン活性を増加させることによって，軟骨細胞の増殖を引き起こしていることが確認された．

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[1] 現所属：愛知医科大学寄生虫学教室，
愛知県愛知郡長久手町岩作