Growth Hormone-Like Activity in Hypophysectomized Rats Implanted with *Spirometra Mansonoides* Spargana (34453)

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Mueller (1) and Mueller and Reed (2) have recently described the stimulation of growth in hypophysectomized and hypothyroid rats induced by the subcutaneous implantation of *Spirometra mansonoides* spargana (SMS). Their observations strongly suggested a growth hormone-like activity. The studies reported herein confirm and extend their observations in hypophysectomized rats. A comparison of SMS and growth hormone-stimulated growth revealed many common attributes.

Materials and Methods. Hypophysectomized male rats (Charles River), approximately 90–105 g in weight, were implanted subcutaneously with 12–15 scoleces of *Spirometra mansonoides* spargana (SMS) about a week after operation. Another group of animals from the same shipment were used as controls. Animals were weighed individually at 2, 4, 7, 9, and 10 days. They were sacrificed on the eleventh day, and bled from the abdominal aorta using heparinized syringes. Various tissues were weighed and the tibias were processed according to the method of Greenspan *et al.* (3).

The plasma samples (SMSP) from two identical experiments were pooled and frozen until used. In a subsequent experiment hypophysectomized rats were given an intraperitoneal injection (1 ml) of either saline, control plasma, SMSP, or 100 μg/day of a highly purified bovine growth hormone (1 USP U/mg). On the eleventh day the animals were sacrificed; and various tissues were removed and weighed. One tibia was used to measure the epiphyseal width, while the other was decalcified, embedded, sectioned, and treated with Picro-ponceau stain. The femur was also removed, dissected free of tissue, and placed in an oven at 110° for 18 hr and dry weight was determined.

To ascertain the effect of varying the dose, SMSP was administered to hypophysectomized male rats in amounts from 0.1 to 1.0 ml/day for 10 days. The tibias were removed, and the width of the epiphyseal cartilage was measured (3). A log dose response was obtained.

Uptake of $^{35}SO_4^{2-}$ by costal cartilage. Hypophysectomized rats were given either saline, growth hormone, or plasma from SMS-infected animals subcutaneously for 3 days. Immediately after the last injection, 25 μCi of ^{35}S-labeled sulfate was administered intraperitoneally. Eighteen hr later costal cartilage was removed and handled according to the general method described by Salmon and Daughaday (4). The cartilages were then oven dried overnight at 60°, weighed, and solubilized in 1.2 ml of formic acid. The solutions were brought to a constant volume with distilled water, and a 0.2-ml aliquot was placed on a 1 × 3 in. strip of Whatman No. 1 paper and air dried overnight. The paper strips were then placed in counting vials containing 20 ml of toluene–Liquifluor and counted in a Packard Tri-carb liquid scintillation spectrometer (314E).

Results. Spargana-implanted animals. Figure 1 compares the growth curve of animals implanted with spargana with that of untreated animals. The growth rate is comparable to that reported by Mueller (1). The treated animals gained approximately 30% of their starting body weight during the treatment period.

Table I summarizes the tissue responses from spargana-implanted animals. Of particular interest is the effect on the thymus, kid-

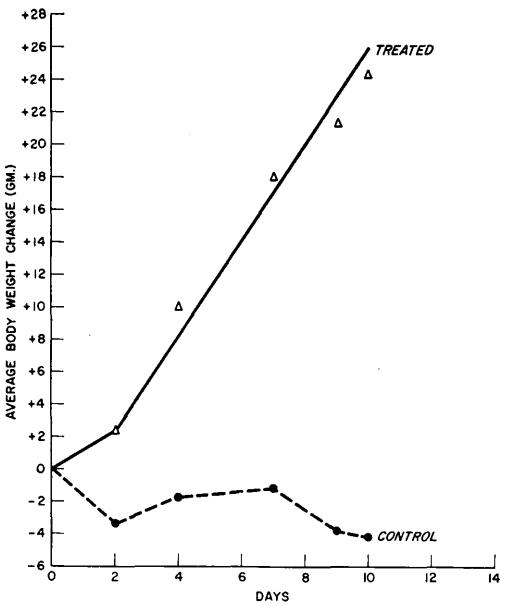

FIG. 1. Comparison of the growth curves of hypophysectomized rats implanted with *Spirometra mansonoides* spargana and untreated controls.

TABLE I. Response of Hypophysectomized Rats to Subcutaneous Implants of *Spirometra mansonoides* Spargana.

Index	Response	
	Control	Treated
Body wt (initial) (g)[a]	95.7	92.8
(sacrifice)	91.6	117.1
change	−4.1	+24.3
Kidney wt (mg)	637	778
(/100 g of body wt)	698	663
Thymus wt (mg)	247	371
(/100 g of body wt)	267	316
Liver wt (g)	3.31	4.35
(/100 g of body wt)	3.60	3.70
Tibia dry wt (mg)	129	134
epiphyseal width (μ)	143 ± 4	303 ± 22
Adrenal wt (mg)[b]	9.8	12.6
(/100 g of body wt)	10.4	11.0
Testes wt (mg)[b]	244	374
(/100 g of body wt)	263	329
Seminal vesicle wt (mg)[b]	11	14
(/100 g of body wt)	12	12
Ventral prostate wt (mg)	9.2	11.2
(/100 g of body wt)	9.8	10.0

[a] Control, 11 animals; treated, 16 animals.

[b] Data from five animals in each group.

ney, and liver weights. These responses when combined with the doubling of the width of the epiphyseal cartilage, suggested a growth hormone-like action. The adrenal, seminal vesicle and ventral prostate weights were not significantly changed. The slight stimulation of the testes weight may be a nonspecific growth effect, since histological examination of the testes did not show significant morphologic differences. The thyroid gland of treated animals were microscopically normal when compared with untreated hypophysectomized controls.

Plasma from SMS animals. Mueller (personal communication) stated that plasma from hypophysectomized rats implanted with SMS will also elicit a body weight response when administered to other hypophysectomized rats. This has also been confirmed. Table II gives the data obtained as a result of daily injections of SMSP into hypophysectomized rats for 10 days. Growth hormone (100 μg/day) was employed for comparative purposes, and plasma from untreated hypophysectomized rats was used as a control. Figure 2 depicts body weight changes in hy-

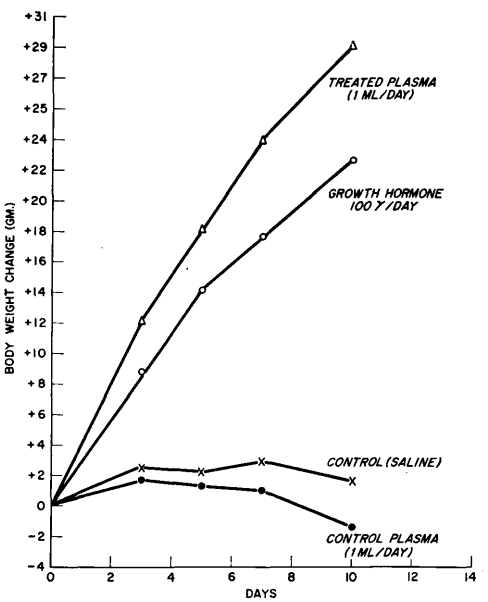

FIG. 2. Comparison of the growth curves of hypophysectomized rats with plasma from *Spirometra mansonoides* spargana implanted rats and those given 100 μg of growth hormone.

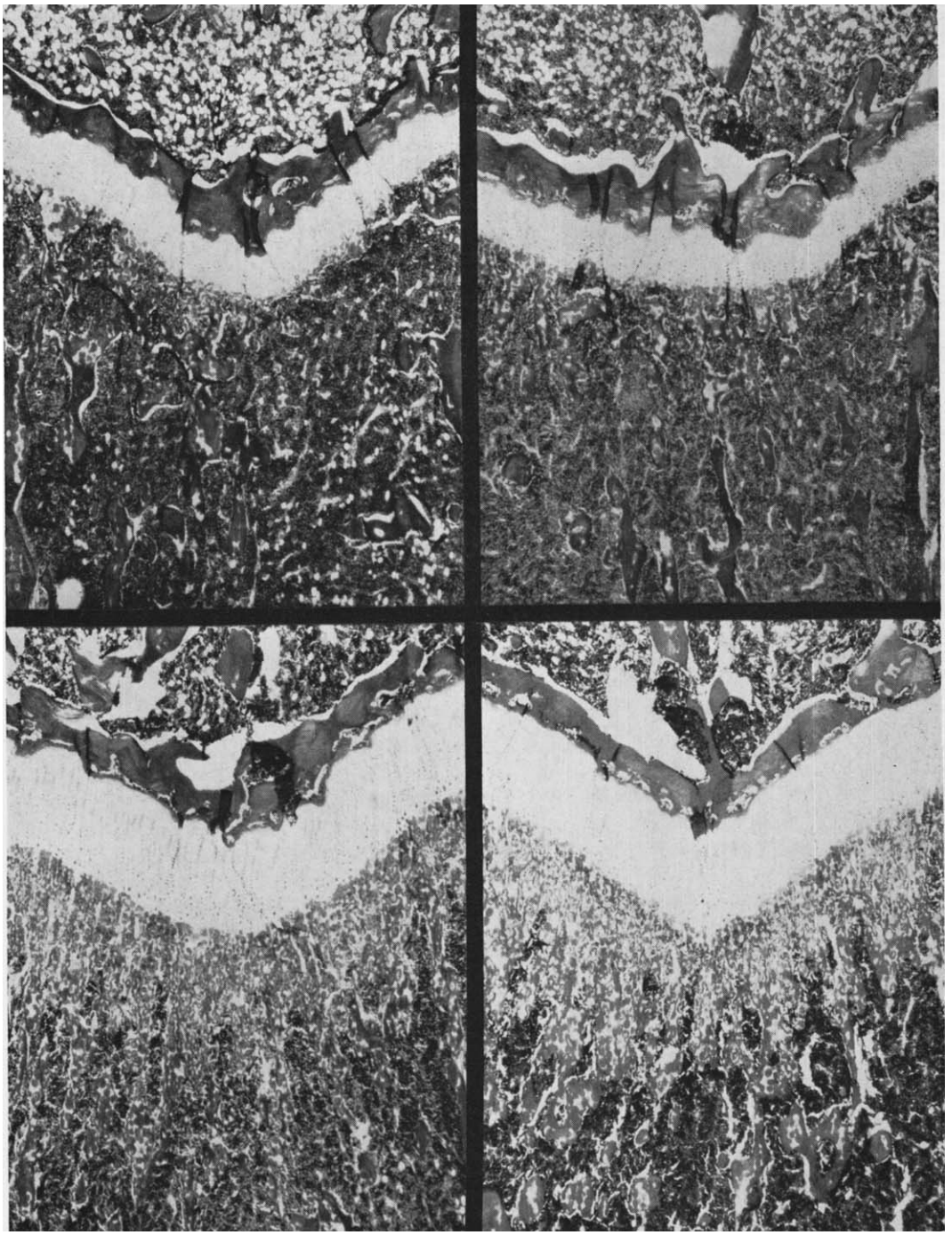

FIG. 3. Comparison of tibial cartilage response of hypophysectomized rats treated for 10 days with growth hormone (100 μg/day) and plasma (1 ml/day) of *Spirometra mansonoides* sparagna-implanted rats. (upper left), untreated control; (upper right), plasma from unimplanted rats; (lower left), 100 μg of growth hormone; (lower right), 1 ml of plasma from spargana-implanted rats.

TABLE II. Response of Hypophysectomized Rats to Growth Hormone and Plasma from SMS-Treated Rats.[a]

Group	No. of animals	B.W. change (g)	Kidney (mg)	Thymus (mg)	Liver (g)	Tibia width (μ)	Femur dry wt (mg)	Fat pad (mg)
Control, saline	6	+1.7	704	233	3.73	137 ± 3.9	186	507
GH, 100 μg/day	6	+22.7	783	378	4.37	280 ±10.6	208	523
Test plasma, 1 ml/day	5	+29.2	813	412	4.80	275 ± 8.6	207	793
Control plasma, 1 ml/day	3	—1.3	668	228	3.31	134 ± 6.3	177	460

[a] Values represent average of the group.

pophysectomized rats treated with either saline, control plasma, treated plasma (SMSP), or growth hormone. The growth response for 1 ml/day of SMSP was approximately equivalent to the response obtained with 100 μg of bovine growth hormone and equivalent to that seen in the spargana-implanted animals (Fig. 1).

It was noted that, with the exception of the epididymal fat pad weights, the responses of the animals treated with SMSP were comparable to those of growth hormone; whereas, those receiving hypophysectomized control plasma were similar to saline-injected animals.

Tibias from all four groups were examined histologically. Figure 3 shows representative photomicrographs of one animal from each group. It is apparent that the response of the SMSP-treated animals is indistinguishable from that seen with growth hormone.

Administration of SMSP to hypophysectomized rats using doses of 0.1–1.0 ml/day resulted in an incremental change in tibial cartilage width which was a function of the logarithm of the dose (Fig. 4). The slope of the response was similar to that obtained with purified growth hormone.

$^{35}SO_4^{2-}$ uptake in costal cartilage. Table III summarizes the data obtained when

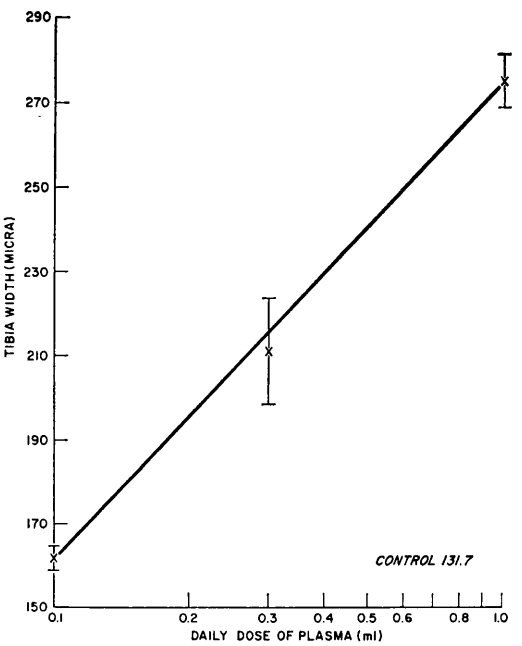

FIG. 4. Dose–response curve of tibia response of animals treated for 10 days with varying doses of plasma from *Spirometra mansonoides* spargana implanted rats; ranges indicate SE of mean.

TABLE III. The Effect of Bovine Growth Hormone and Plasma from Spargana-Implanted Rats upon the Uptake of ^{35}S Sulfate by Costal Cartilage in Hypophysectomized Rats.

Group	Dose	Animals/group	Uptake of $S^{35}O_4^{2-}$ (av cpm/mg of cartilage ± SE)
Control	Saline	6	83.4 ± 2.4
Bovine growth hormone	50 μg	6	308.9 ± 4.9
Spargana plasma	1 ml	6	174.1 ± 2.7

SMSP-treated hypophysectomized rats were given $^{35}SO_4^{2-}$. The increase in sulfate uptake is similar to that reported by Daughaday and Kipnis (5) and others for growth hormone.

Discussion. Mueller (1) postulated that the observed growth effect may be comparable to that of growth hormone. The data from our laboratory fully confirms this view. The question must be raised as to the mechanism of action. Growth hormone secretion *per se* by SMS seems unlikely but cannot be excluded. Another possible explanation is that a substance is released which causes sulfation factor to be produced. Daughaday and Kipnis (5) showed that growth hormone has this action. Alternatively, the growth factor could be sulfation factor like.

The rate and degree of growth seen with the plasma from SMS-treated animals is striking. The growth hormone equivalents are approximately 100 μg/ml. This quantity of activity in 1 ml of SMSP is similar to that which is present in a single normal rat pituitary and far greater than that found in normal rat plasma.

Mueller (1) also reported that the growth effect of the SMS disappears after approximately 4 weeks. This was also confirmed in our laboratory. Furthermore, it was demonstrated that such animals still respond to the administration of growth hormone. This suggests that the disappearance of the growth effect is not an exhaustion of the body's ability to respond due to overstimulation. The skeletal system of the rat responded to SMS and SMSP. There was an increase in tibial width as well as increased bone weight suggesting calcium deposition. However, no calcium data are available to date.

The mechanism of action of the SMS and SMSP growth responses is being investigated. These fundamental studies should provide basic information regarding the regulation of growth processes in the body.

Summary. The subcutaneous implantation of *Spirometra mansonoides* spargana into hypophysectomized rats produced a striking increase in body weight and tibial cartilage width comparable to that noted with growth hormone. Other tissue responses confirmed this similarity. Injection of plasma from implanted rats also produced a growth hormone-like response including a stimulation of the uptake of $^{35}SO_4^{2-}$ by costal cartilage.

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