

## Responses in Hypophysectomized Rats Implanted with Plerocercoids of the Tapeworm, *Spirometra mansonioides* (37269)

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Growth stimulation in hypophysectomized (hypox) rats implanted with plerocercoids (spargana) of *Spirometra mansonioides* was first observed by Mueller (1). This initial observation has been amply verified and a number of alterations in the metabolism of the host animal have been noted (2-6). The serum collected from hypox rats with implanted plerocercoids also contains the active growth factor and some of the responses produced in the hypox rat by the administration of active serum, plerocercoids and bovine growth hormone (BGH) are described in the present report.

**Methods.** Male hypox and intact control rats of the Sprague Dawley strain weighing approximately 80 g were obtained from the Hormone Assay Laboratories in Chicago. All animals were housed in a constant temperature room with controlled lighting (12 hr photoperiod) and were fed a purified sucrose-casein diet (6). Plerocercoids of the tapeworm *S. mansonioides* were obtained from the fresh carcasses of mice that were maintained for this purpose. Scolexes clipped from the plerocercoids were prepared for sc injection into host animals by washing them in sterile physiological saline and suspending them in fresh saline containing 10,000 units of penicillin G and 12 mg of streptomycin/ml.

The concentrations of various serum constituents in host animals were determined on fresh blood obtained by cardiac puncture. The specimens were analyzed immediately, using the standardized techniques available in the clinical laboratories of the University

Hospital.<sup>2</sup> Serum taken from hypox rats implanted with scolexes was pooled and stored at  $-5^{\circ}$  for later use as a source of the plerocercoid growth factor (PGF) and is designated as WHRS (wormy hypox rat serum), as described by Garland, Ruegamer and Daughaday (4).

Liver glycogen levels were determined by the procedure of Pfeleiderer (7) and glucose concentrations were measured by the method of Bergmeyer and Bernt (8). Serum total cholesterol concentrations were measured by the method of Sperry and Webb (9) and total liver lipid concentrations were measured gravimetrically as total solids appearing in a Bloor extract of individual samples of fresh liver.

**Results. Expt 1.** The first experiment was undertaken to determine whether plerocercoids produce any major changes in several serum and liver constituents.

Four animals were placed in each of the experimental groups cited in Table I and the scolexes were injected sc into the host animals on Day 0 of the experiment. At the end of 3 wk, the only significant difference observed between control and plerocercoid-implanted hypox rats was a dramatic increase in the liver glycogen concentration of implanted animals. No significant differences were noted between intact controls and intact implanted animals and plerocercoids did not restore the serum BUN or liver glycogen concentrations of hypox rats to those levels seen in intact controls.

<sup>1</sup> This study was supported in part by Grant AM 13579 from the NIH, U.S. Public Health Service.

<sup>2</sup> The authors are deeply indebted to Dr. John Henry, Professor and Director of Clinical Pathology at the SUNY, Upstate Medical Center, Syracuse, New York for these determinations.

TABLE I. Serum and Liver Responses in Rats Implanted with Plerocercoids (mean  $\pm$  SE for 4 rats/group).<sup>a</sup>

Group	Serum (per 100 ml)						Liver		
	Glucose (mg)	BUN (mg)	Alk. P-ase (KA units)	Ca (mEq)	Chol (mg)	TG (mg)	Glycogen (mg/g)	Chol (mg/100 g)	Total lipids (g/100 g)
Hypox controls	164 $\pm$ 20	48 $\pm$ 3	13.7 $\pm$ 1.6	0.54 $\pm$ 0.05	125 $\pm$ 12	45 $\pm$ 4	2.6 $\pm$ 0.1	358 $\pm$ 87	9.4 $\pm$ 1.3
Hypox + 15 scolexes	130 $\pm$ 12	37 $\pm$ 4	11.6 $\pm$ 1.5	0.50 $\pm$ 0.05	125 $\pm$ 10	50 $\pm$ 6	21.6 $\pm$ 0.9	258 $\pm$ 54	8.1 $\pm$ 1.2
Intact controls	166 $\pm$ 15	26 $\pm$ 2	12.3 $\pm$ 1.2	0.58 $\pm$ 0.05	95 $\pm$ 13	125 $\pm$ 10	41.7 $\pm$ 3.2	272 $\pm$ 67	7.9 $\pm$ 1.1
Intact + 15 scolexes	151 $\pm$ 11	21 $\pm$ 2	13.0 $\pm$ 1.7	0.57 $\pm$ 0.04	120 $\pm$ 12	140 $\pm$ 22	33.1 $\pm$ 3.0	260 $\pm$ 38	8.7 $\pm$ 1.2

<sup>a</sup>The abbreviations are defined as follows: BUN = blood urea nitrogen, Alk. P-ase = alkaline phosphatase in KA = King Armstrong units, Ca = calcium, Chol = cholesterol, and TG = triglycerides.

When the experimental animals were killed by decapitation, a number of organs or tissues were quickly removed and weighed. Tissue weights were calculated as percentage of body weight and are summarized in Table II. The liver, spleen, thymus and testes of plerocercoid-implanted animals enlarged at the same rate as the whole body mass since these tissue weights expressed as percentage of body weight remained the same as hypox controls. The kidneys and hearts of plerocercoid-implanted rats actually decreased in relative proportion to the body mass whereas the epididymal fat increased significantly. All of the tissues and organs studied were much smaller in the hypox control and im-

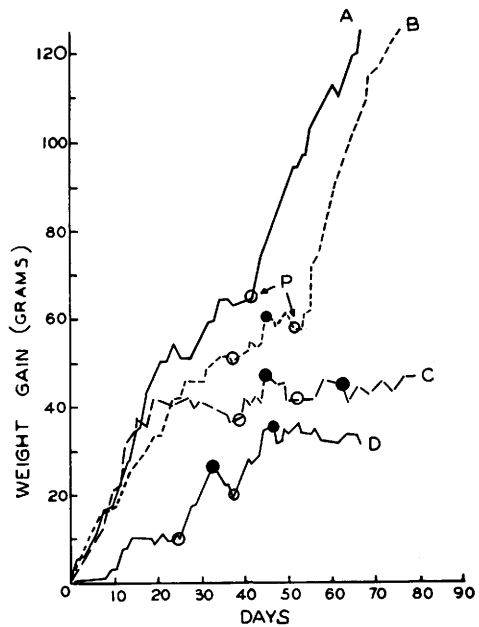


FIG. 1. Representative growth curves for hypox animals receiving WHRS, BGH or plerocercoids. (A) Received 1 ml WHRS/day for the first 10 days and was injected with 15 plerocercoids (P) on Day 42. (B) received 1 ml WHRS/day for the first 10 days, followed by 1 mg BGH/day from Days 38 (O) to 45 (●) and finally injected with 15 plerocercoids (P) on Day 52. (C) received 1 ml WHRS/day for the first 10 days, followed by 1 mg BGH/day from Days 38 (O) to 45 (●) and again from Days 52 (O) to 62 (●). (D) received 2.5  $\mu$ g L-thyroxine/100 g body weight/day for the first 10 days, followed by 1 mg BGH/day from Days 24 (O) to 32 (●) and again from days 37 (O) to 47 (●).

TABLE II. Tissue Responses in Rats Implanted with Plerocercoids (mean  $\pm$  SE for 4 rats/group).<sup>a</sup>

	Hypox controls	Hypox + 15 scolexes	Intact controls
Initial body wt (g)	80 $\pm$ 10	82 $\pm$ 10	75 $\pm$ 6
Final body wt (g)	81 $\pm$ 11	142 $\pm$ 22	168 $\pm$ 12
Tissue (mg/100 g body wt)			
Liver	3600 $\pm$ 92	3600 $\pm$ 71	5700 $\pm$ 320
Kidneys	670 $\pm$ 18	540 $\pm$ 31	940 $\pm$ 62
Spleen	170 $\pm$ 15	190 $\pm$ 14	330 $\pm$ 20
Heart	320 $\pm$ 4	280 $\pm$ 26	440 $\pm$ 31
Epididymal fat pad	600 $\pm$ 25	780 $\pm$ 68	910 $\pm$ 85
Thymus	280 $\pm$ 13	270 $\pm$ 39	390 $\pm$ 40
Testes	225 $\pm$ 13	230 $\pm$ 17	1160 $\pm$ 76

<sup>a</sup> The kidney and heart weights of hypox rats with scolexes are significantly smaller than those of hypox controls ( $p < .005$ ) and the epididymal fat pad weight is greater in scolex-implanted animals than in hypox controls ( $p < .005$ ).

planted animals than in the intact controls.

*Expt 2.* Since the hypox rat is devoid of growth hormone, it is important to compare the growth response produced by growth hormone with that produced by plerocercoids or WHRS. A number of combined treatments were tried, and the results of these studies are summarized in Figs. 1 and 2. Daily injections of 1 ml of pooled WHRS into hypox rats produced good growth responses (see rats A-C), whereas daily sc injections of 1 mg BGH<sup>3</sup> produced relatively small growth responses (see rats B, C, G). On the other hand, the sc injection of 15 scolexes into animals A and B (Fig. 1) resulted in a dramatic stimulation of growth. It can be further observed that rat G failed to show a significant growth response to BGH but a good response was obtained with injections of WHRS following the BGH treatment. Daily sc injections of 2.5  $\mu$ g L-T<sub>4</sub>/100 g body weight (rat D) failed to produce a significant growth response although BGH administration following T<sub>4</sub> administration resulted in appreciable growth.

*Expt 3.* Several attempts have been made to develop a dose response relationship in hypox rats between the dose level of PGF

<sup>3</sup> The authors thank the Endocrinology Study Section, NIAMD for a supply of bovine growth hormone. The growth hormone was dissolved in slightly alkaline saline for sc injection.

and the growth response. Because of the growth responses obtained in Expt 2 when as little as 0.1 ml WHRS was injected daily (rat F) and when a single injection of 2 ml was given (rat G), it was decided to inject from 0.1 to 1 ml WHRS/day for 4 consecutive days. Serum volumes of less than 1 ml

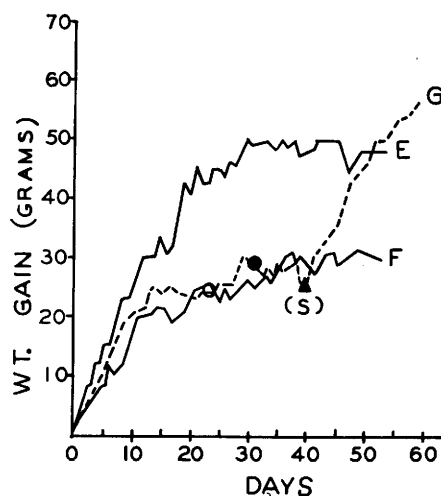


FIG. 2. Representative growth curves for hypox animals receiving different doses of WHRS. (E) Received 0.5 ml WHRS/day for the first 10 days. (F) received 0.1 ml WHRS/day for the first 10 days. (G) received a single injection of 2 ml WHRS on Day 0, followed by 1 mg BGH per day from Days 22 (○) to 31 (●) and finally 1 ml of WHRS/day from Days 40 to 50.

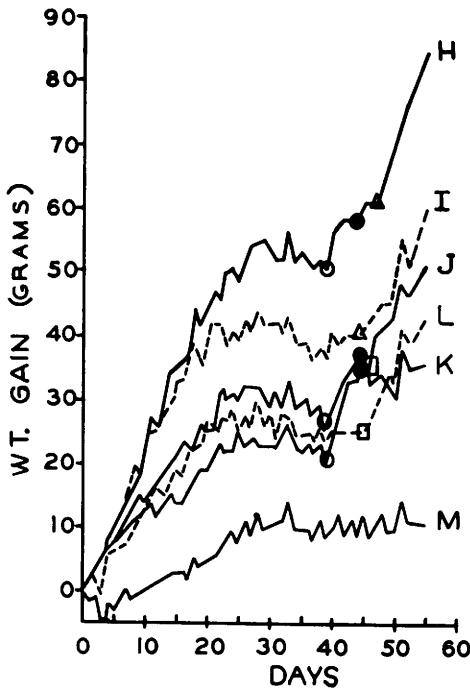


FIG. 3. Representative growth responses in hypox rats injected with various amounts of WHRS. (H) Received 1 ml WHRS/day for the first 10 days followed by 1 mg BGH/day from Days 40 (○) to 45 (●), and was injected with 15 plerocercoids on Day 48 (△). (I) received 1 ml WHRS/day for the first 4 days and was injected with 15 plerocercoids on Day 45 (△). (J) received 0.5 ml WHRS/day for the first 4 days, followed by 1 mg BGH/day from Days 38 (○) to 45 (●), and followed by injections of 1 ml WHRS/day from Days 46 to 55. (K) received 0.2 ml WHRS/day for the first 4 days, followed by 1 mg BGH/day from Days 38 (○) to 45 (●). (L) received 0.1 ml WHRS/day for the first 4 days, followed by injections of 1 ml WHRS/day from Days 45 to 55. (M) served as the control and received a single injection of 1 ml physiological saline on the first day.

were diluted with saline solution to make a final injection volume of 1 ml. From the growth response curves summarized in Fig. 3, it appears that animals receiving 1 ml WHRS per day for 10 days (rat H) do not grow faster than animals receiving 4 injections of 1 ml WHRS (rat I). As in Expt 2, BGH administration produced an inferior growth response (rats H, J, K) but the animals remained very responsive to plerocercoids (rats H and I) and to additional injections of 1

ml WHRS/day (rats J and L).

*Discussion.* In an effort to obtain some insight as to how PGF may work in bringing about a growth response in hypox rats, the concentrations of several substances in serum and liver were measured in control and plerocercoid-implanted hypox animals (Table I). The only difference observed was in liver glycogen concentration, which was much higher in plerocercoid-implanted hypox rats than in hypox controls.

Relatively small differences were seen between the weights of several organs or tissues of plerocercoid-implanted hypox rats and hypox controls when the tissue weights were compared on a percentage of body weight basis (Table II). However, the relative organ weights of both hypox control and implanted animals remained much smaller than those of intact controls and it may be concluded that PGF produces a generalized growth response in hypox rats but it does not possess trophic or hormonal activity.

From the data presented in Figs. 1-3, it would appear that PGF is a more potent growth stimulator than bovine growth hormone (BGH). In contrast to growth hormone, PGF apparently has a long half-life because the growth response continued for 10-20 days following the last injection of WHRS. After a "rest period" of 20 or more days following the last WHRS injection, the animals were responsive to both plerocercoid implantation and to a new series of WHRS injections (Figs. 1-3). It has been postulated (1, 3-5, 11-13) that plerocercoids induce an immune response in the host animal and that antibody production neutralizes PGF activity. However, no one has reported on what happens to growth in hypox animals injected daily with PGF for a period of 6 wk or more. Consequently, it is not known whether animals injected with PGF will plateau in growth at about 5 or 6 wk as do plerocercoid-implanted animals. In any event, hypox animals injected for 10 days with PGF as WHRS are still responsive to either fresh serum injections or implantation with plerocercoids following a "rest period" of 20 days or more.

On the basis of the data presented in Fig. 3 and other unpublished data, it appears that growth response in the hypox rat does not

offer a practical method for assaying crude materials for PGF activity. The potential problems involved in establishing a PGF dose-growth response relationship are: (a) the extremely small dose-dependent differences, if any, between growth rates or final body weight gains; (b) the relatively large amount of material needed for injection since presumably 6-10 animals would be needed for each dose level; (c) the relatively high cost of host animals and materials; and (d) the relatively long period of time (10-20 days) required to obtain a reliable growth response. If a quantitative assay procedure is eventually developed using some measurement other than growth in hypox rats, it will continue to be important to test the PGF preparations qualitatively in hypox rats because of the physiological significance of a growth response in this particular model system.

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Received Jan. 12, 1973. P.S.E.B.M., 1973, Vol. 143.