In Vitro Preparation of a Growth Factor from Plerocercoids of the Tapeworm, *Spirometra mansonoides*[1] (37025)

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A growth-stimulating effect in mice infected with plerocercoid (spargana) larvae of the tapeworm, *Spirometra mansonoides* was first demonstrated by Mueller (1). Later work showed that other plerocercoid-infected laboratory animals (rats, hamsters and deer mice) gained weight at an accelerated rate when compared to uninfected controls (2).

The effects of these plerocercoids (spargana) have been compared to the actions of several mammalian hormones: thyroxin (3), insulin (4), and growth hormone (5).

Hypophysectomized (hypox) rats infected with plerocercoids grow considerably faster than noninfected hypox rats (5, 6). In addition, serum from wormy hypox rats when injected into uninfected hypox rats also enhances growth (7).

Steelman *et al.* (5) attempted to develop an *in vitro* source of plerocercoid growth factor (PGF) by incubating plerocercoids for 24–28 hr in Mixture 199 to which was added calf serum and chick embryo extract. They found a growth-promoting factor in the supernatant, and proposed that some protein source, either from calf serum and chick embryo extract or purified plasma proteins, was necessary for the production of PGF by the cultured worms. Isolation of the PGF from such a complex and crude medium would be difficult and the present investigation was undertaken to determine if PGF could be collected after shorter incubation periods in media containing fewer crude materials. If successful, the technique would provide large quantities of relatively high specific activity material for purifying and identifying the active factor.

Methods. Plerocercoids. All stages of the life cycle of *S. mansonoides* were maintained in our laboratory according to the techniques of Mueller (8). Plerocercoids to be incubated were removed from infected mice, rinsed twice in 0.85% saline, and placed in incubation media at a concentration of 100 plerocercoids/10 ml of medium. At the end of each incubation period the plerocercoids were injected subcutaneously back into mice for use at a later time.

Incubations. Two media were employed: one consisted of 0.85% NaCl and 0.04% $NaHCO_3$ in distilled water; the other, 0.85% NaCl, 0.04% $NaHCO_3$ and 0.5% bovine albumin in distilled water.

The plerocercoids were incubated at 30° for either 4 or 8 hr. At the end of each incubation period the supernatant was decanted, centrifuged at 3000 rpm for 10 min, filtered through a 0.22 μm Millipore filter, placed in serum vials and frozen. All operations were carried out at 4° following the incubation step.

Some plerocercoids were incubated for longer periods (up to 24 hr) in each of the two media. The supernatants from each incubation medium were pooled, treated as described and concentrated. The saline medium was flash-evaporated to 20% of the original volume. The saline–albumin medium was lyophilized and reconstituted in distilled water to 20% of the original volume.

Procedure. The PGF preparations were assayed by the hypox rat growth technique. Male Sprague-Dawley hypox rats of approximately 80 g were purchased from Hormone Assay Labs, Chicago, IL, and were fed Wayne Rat Chow and water *ad libitum*. Each animal was weighed daily for 2 wk to determine if hypophysectomy was complete. Only those animals showing a weight gain of less than 5 g over the 2-wk period were used. Each

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TABLE I. Weight Gain in Hypox Rats Injected with Plerocercoid Incubation Media.[a]

Treatment group	Av initial wt (g)	Av gain at 14 days ± SEM (g)	Av % increase over initial wt
Saline Control	82.8	0.8 ± 1.9 (NS)	0.9
4 hr. Saline + Plerocercoids	82.2	11.4 ± 1.4	13.9
8 hr. Saline + Plerocercoids	82.4	9.0 ± 1.8	10.9
Concentrated saline control	94.0	−2.4 + 2.4 (NS)	−2.6[b]
Concentrated saline + Plerocercoids	84.0	10.2 ± 1.2	12.1
Saline-albumin control	84.2	−0.6 ± 1.1 (NS)	−0.7[b]
4 hr. Saline-albumin + Plerocercoids	85.4	10.3 ± 4.5	12.1
8 hr. Saline albumin + Plerocercoids	86.6	14.4 ± 1.1	16.6
Concentrated saline-albumin control	94.0	1.8 ± 4.8 (NS)	1.9
Concentrated saline-albumin + Plerocercoids	81.6	14.8 ± 2.4	18.1

[a] Initial injection 1 ml followed every other day for 10 days by 0.5 ml injections. Five rats in each group. $p < .05$.

[b] Decrease.

experimental group consisted of five rats. The rats were weighed and received subcutaneous injections at the midpoint of a 12-hr photoperiod. Injection schedules were the same for all groups: 1.0 ml of test material on day one followed by 0.5 ml every other day for 10 days (total 3.0 ml). Control groups were injected on the same schedule with 0.85% buf-

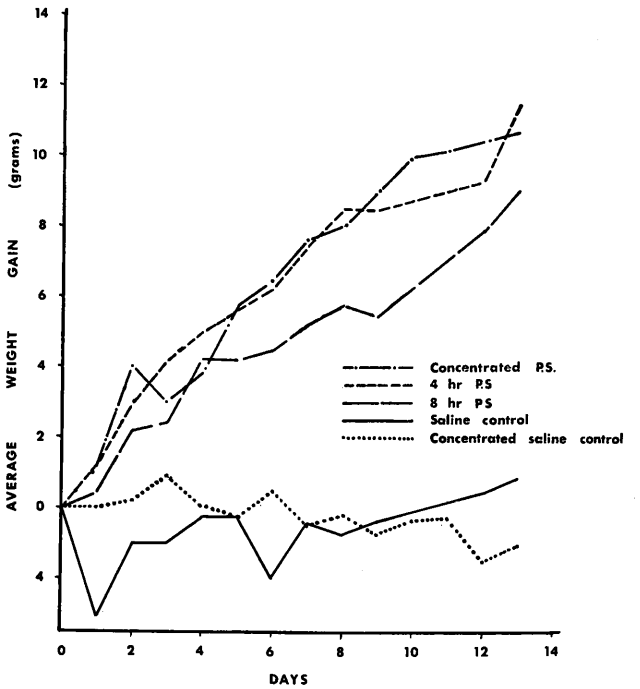

FIG. 1. Weight gain in hypox rats injected with plerocercoid–saline incubation media. Initial injection = 1 ml followed by four (0.5 ml) injections every other day for total injection of 3 ml/animal. $p < .05$.

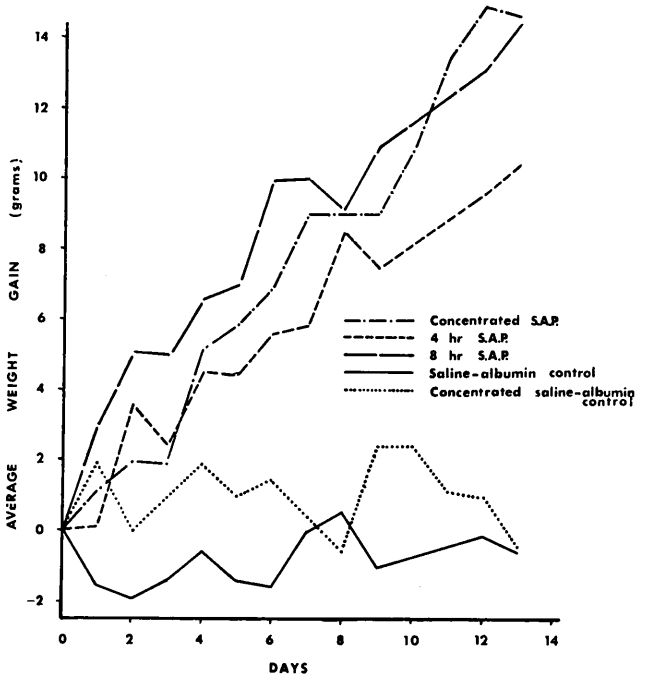

FIG. 2. Weight gain in hypox rats injected with saline–albumin–plerocercoid incubation media. Initial injection = 1 ml followed by four (0.5 ml) injections every other day for total injection of 3 ml/animal. $p < .05$.

fered saline or buffered saline plus albumin. In addition, control groups for the concentrated media received injections of a buffered 4.5% saline solution or a buffered 4.5% saline plus 2.5% albumin solution. Weights were taken daily during the first 14 days. The 95% confidence level using Student's *t* test was accepted as proof of an effect.

Results. All incubation media, concentrated as well as unconcentrated, caused consistent weight gains when injected into hypox rats (Table I). Therefore, PGF was present in the simplest medium, buffered saline, after only 4 hr of incubation. In fact, the 4-hr saline incubation medium produced a greater weight gain response at the end of 2 wk (13.9% increase over initial weight) than did the 8-hr medium (10.9%). Four- and 8-hr saline–albumin incubation media were both more effective than the 8-hr saline media, producing gains of 12.1 and 16.6%, respectively. Concentrated incubation media of both types were effective in producing weight gain responses. The concentrated saline medium produced an average weight gain of 12.1% while the concentrated saline–albumin medium produced the greatest gain of all, 18.1%. The body weights of control animals fluctuated during the same time period but the unconcentrated saline group gained only 0.9% and the unconcentrated saline–albumin group lost slightly (—0.7%). The concentrated saline control group lost 2.6% while the concentrated saline–albumin controls gained slightly (1.9%). The daily body weights of all rats during the 2-wk experimental period are summarized in Figs. 1 and 2. As shown, the growth response to PGF preparations occurs within 2 days after the initial injection, and the body weights continued to rise for the duration of the 2-wk experimental period.

Discussion. Contrary to the conclusions of Steelman *et al.* (5), PGF can be collected after short incubation periods in media containing no protein.

Incubation media containing bovine serum albumin were more active than those containing saline alone, but the mechanism by which albumin enhances PGF activity is not

known. However, the presence of protein material may protect the active factor from rapid degradation.

It is possible that the observed differences in PGF activity were due to variations in the mass of plerocercoids added to the media rather than to variations in chemical composition of the media or to the length of incubation time. As described in Methods, 100 plerocercoids were placed in 10 ml of each incubation medium. The size of individual worms varies and the total worm mass may have varied as much as 25% from group to group. However, the results of these experiments clearly demonstrate that PGF is a product of the worms and can be collected in sufficient quantities to stimulate growth in hypox rats.

As the present data show, it is now possible to obtain the PGF in large quantities by incubating plerocercoids in relatively simple media. The factor is sufficiently stable so that incubation media can be concentrated by conventional methods and stored in the frozen state. These observations should greatly facilitate the ultimate isolation of the PGF in pure form.

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