EFFECTS OF SPIROMETRID PLEROCERCIOIDS ON SEVERAL SPECIES OF LOWER VERTEBRATES

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Abstract—1. The effects of the plerocercoid growth factor on several species of lower vertebrates were tested.
2. There was little effect on growth or fattening in either fish or frogs.
3. Plerocercoid infections and to a lesser degree worm homogenate injections markedly stimulated growth but not fattening in lizards.

INTRODUCTION

Perhaps the most enigmatic aspect of the biology of spirometrid tapeworms is the effect their plerocercoids have upon the metabolism of their hosts. Mueller (1963a, b, 1965) noted that mice, rats and hamsters infected with plerocercoids of Spirometra gained weight to a degree that could not be attributed to the weight of the parasite or to any pathologic tissue response to the parasite. It follows that if the metabolism of laboratory rodents is affected, the metabolic activities of vertebrates lower on the phylogenetic tree, and perhaps more closely related to the natural hosts, might also be influenced. Therefore, this study was undertaken to determine the effects of plerocercoid infections or whole worm homogenate injections on several lower vertebrate classes. Weight gain and/or fattening were used as a means of monitoring these effects in the various host species.

MATERIALS AND METHODS

Three different forms of Spirometra were utilized to study the extent of any effect and if the effects might be variable within the genus. The species used were S. mansonoides "A" which had its origin in snakes collected in Louisiana, S. mansonoides "B" from a New York collection and S. mansoni from Colombia, South America. The worms were maintained according to the techniques of Mueller (1966). Plerocercoids derived from these infections were given to experimental hosts either by feeding or by subcutaneous injection.

Homogenates were prepared from plerocercoids quick frozen in an acetone-dry ice-bath then ground (1 g plerocercoids/10 ml physiological saline) with a manual tissue homogenizer and injected subcutaneously into the experimental animals.

Vertebrates used for weight gain experiments were fish, frogs, and lizards. Fresh water killifish, Fundulus chrysotus, were collected near Baton Rouge, Louisiana. Adult leopard frogs, Rana pipiens, were purchased from Carolina Biological Supply, Burlington, North Carolina. The lizards, Anolis carolinensis, were purchased from the Snake Farm, La
Place, Louisiana, or caught by hand on the Louisiana State University Campus. All animals were allowed to feed ad lib. with the exception of the adult frogs which were force-fed three times each week. All were maintained on a photoperiod of 16 hr followed by 8 hr of darkness. The environmental temperature for the experiments was 22°C except for the lizards which were kept at 30°C. In all experiments, the animals were kept under these conditions for a minimum of 2 weeks before the beginning of any experimentation.

RESULTS

Experiment 1. Fish

Mueller (1960) demonstrated that fish could not support an infection of spirometrid plerocercoids. Fish do respond to exogenous prolactin and these reactions were reflected by weight gains and increases in body fat (Meier, 1969). It was considered of interest to know if the plerocercoid growth factor (PGF) might have a hormone-like effect on a species that does not serve as host for plerocercoids, since PGF has been shown to have prolactin (Phares, 1973) and growth hormone-like activity (Steelman et al., 1971). Homogenized plerocercoids rather than live worms were used to test this hypothesis.

Forty animals (F. chrysotus) were divided into four groups of ten fish each. Group A (Control) received 0.01 ml/g body weight of 0.65 per cent saline at each injection. Fish of Group B were injected with 0.01 ml/g body weight of whole worm homogenate (S. mansonoides "A"). Group C (S. mansonoides "B") and Group D (S. mansoni) were injected with like amounts of worm homogenate.

All injections were made at the midpoint of the photoperiod over a period of 7 days. The selection of an injection time was based on the observation of Lee & Meier (1967) that F. chrysotus responds maximally to hormonal stimuli at the midpoint of the photoperiod.

The fish were killed on the eighth day, 24 hr after the final injection. A fresh, dead weight was determined for comparison with pre-test weights. The fish were then dried individually in a vacuum oven and weighed. Body fat was extracted in petroleum ether using a Soxhlet apparatus. The total body fat (dry lipid index, DLI) was calculated and expressed as a percentage of the dry body weight.

Results of this experiment are summarized in Table 1. While all experimental groups had a weight advantage over controls, no gains were of the magnitude described by Lee & Meier (1967) and Meier (1969).

Experiment 2. Frogs

Adult frogs support natural infections of spirometrid plerocercoids (Corkum, 1966) but the effect of the infection on the amphibian host has not been examined. Four groups of ten frogs each were used to ascertain if live plerocercoids had any effect on weight gain or growth. Group A consisted of ten uninfected controls; Groups B, C and D were infected by subcutaneous injection with ten plerocercoids of S. mansonoides "A", S. mansonoides "B", and S. mansoni, respectively. All animals were weighed and toe clipped for later identification. At the end of 1 month, the animals were killed and examined for plerocercoids. Changes in individual weights were determined.
SPIROMETRID PLEROCERCOIDS ON SPECIES OF LOWER VERTEBRATES

Table 1—Average weight gain and fattening in fish injected with plerocercoid homogenate* (0.01 ml/g body wt. per day) for 7 days

<table>
<thead>
<tr>
<th>No. of fish</th>
<th>Avg. wt. gain (mg)</th>
<th>% increase</th>
<th>Dry lipid index†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A‡</td>
<td>10</td>
<td>-69.3</td>
<td>-3.6</td>
</tr>
<tr>
<td>Group B</td>
<td>11</td>
<td>-46.9</td>
<td>-2.1</td>
</tr>
<tr>
<td>Group C</td>
<td>9</td>
<td>11.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Group D</td>
<td>7</td>
<td>-18.4</td>
<td>-1.1</td>
</tr>
</tbody>
</table>

* 1 g plerocercoid/10 ml physiological saline.
† Dry lipid index = % dry body wt. as lipid.

Although some of the Group A (uninfected controls) animals gained weight (Table 2), others lost weight, resulting in a net loss of 7.3 g at the end of the experiment. All of the experimental groups exhibited similar patterns, with some individuals showing a weight gain and others a weight loss. Group B had a net loss of 8.3 g; Group C, 12.2 g; and Group D, 10.2 g.

Table 2—Average weight gain in frogs infected with plerocercoids* for 4 weeks

<table>
<thead>
<tr>
<th>No. of frogs</th>
<th>Avg. wt. gain (g)</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A‡</td>
<td>10</td>
<td>-0.7</td>
</tr>
<tr>
<td>Group B</td>
<td>9</td>
<td>-0.9</td>
</tr>
<tr>
<td>Group C</td>
<td>9</td>
<td>-1.4</td>
</tr>
<tr>
<td>Group D</td>
<td>8</td>
<td>-1.3</td>
</tr>
</tbody>
</table>

* Seven plerocercoids each.

Plerocercoids injected subcutaneously into adult R. pipiens did not move far from the site of introduction and appeared to grow very little during the experimental period. Perhaps this inactivity has some bearing upon the lack of any apparent growth or fat-stimulating effect in frogs.

Experiment 3. Lizards

Reptiles frequently harbor natural infections of spirometrid plerocercoids and the green anole was selected to represent this class.
The work with the lizard consisted of two parts: Experiment 3A involved infected lizards and followed the same protocol outlined in Experiment 2. Experiment 3B differed in that the lizards were injected with a homogenate prepared from whole plerocercoids.

As indicated in Table 3, plerocercoid-infected male anoles produced a definite weight gain (up to 32 per cent) over uninfected controls in a 4-week experiment.

**Table 3—Average weight gain and fattening in male anoles infected with plerocercoids**

<table>
<thead>
<tr>
<th>No. of anoles</th>
<th>Avg. wt. gain (g)</th>
<th>% increase</th>
<th>% body weight as fat bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A†</td>
<td>20</td>
<td>-0.08</td>
<td>-0.2</td>
</tr>
<tr>
<td>Group B</td>
<td>7</td>
<td>0.72</td>
<td>17.8</td>
</tr>
<tr>
<td>Group C</td>
<td>9</td>
<td>1.18</td>
<td>32.8</td>
</tr>
<tr>
<td>Group D</td>
<td>8</td>
<td>0.74</td>
<td>20.3</td>
</tr>
</tbody>
</table>

* Seven plerocercoids each.

Increased growth rate in the infected lizard was not the only phenomenon observed. The plerocercoids grew at a much faster pace in lizards than in other hosts. Only the scolex and a small portion of the body, totalling approximately 5–10 mm, was used to infect the lizards orally. One *S. mansoni* plerocercoid, removed from a lizard infected for only 30 days, measured 113 mm.

In part 3B of this experiment each group of lizards received seven daily injections of 0.1 ml/g body weight of saline or homogenate at the midpoint of the photoperiod and killed 24 hr after the final injection.

Injections of whole worm homogenate produced a weight gain in anoles, but to a lesser degree than infections with plerocercoids. As in the previous experiment involving plerocercoid-infected lizards, the weight gains could not be attributed to an increase in lipid deposits in the fat bodies. All three groups injected with homogenate had less reserve fat than the saline-injected controls. It is apparent, therefore, that increases in body weight were due to overall growth and not to fattening, of the anole. Growth was greatest in lizards infected with live plerocercoids.

**DISCUSSION**

Parasitism is often considered to be a condition in which the parasitic organism thrives at the expense of the host. In many cases the host organism does suffer, or at least is less hardy than the unparasitized members of the same species. In many parasitic infections the host may lose weight. Conversely, the presence of parasites is sometimes associated with increased growth. Tissue hyperplasia or tumor
formations, as in hydatid disease and filarial elephantiasis, can increase the weight of the host. This weight increase, however, is due either to the increasing mass of the parasites or to the defence mechanisms of the host. Increased body weight in various hosts parasitized by spirometrid plerocercoids is due to none of the above, but is a result of stimulated growth in normal tissues by the presence of this tissue invading parasite.

Fisher (1963) stated that in some cases of parasitism of plant and arthropod hosts, the parasite apparently produces substances which are chemically identical or at least mimic the physiological effect of regulatory substances natural to the host. Harlow et al. (1967) described a substance extracted from *S. mansonoides* plerocercoids which behaved in some ways like insulin. This substance retained its stability in hot alkali, a fact which suggests its non-protein character. Mueller & Reed (1968) found that the introduction of spargana in propylthiouracil-treated, growth-arrested rats caused immediate resumption of growth. Mueller (1968) reported striking weight gain in hypophysectomized rats infected with plerocercoids. It is evident from these reports that animals infected with or given extracts of spirometrid plerocercoids are responding in a manner not unlike the activity effected by the introduction of exogenous hormones.

Meier et al. (1966), Lee and Meier (1967) and Meier (1970) found that the golden top-minnow (*F. chrysotus*) gained weight when exogenous prolactin was injected at certain times during a long photoperiod. These weight gains were the result of increased fat stores. In the present experiments with worm homogenate injections, only one group of *F. chrysotus* displayed a weight gain and the lipid content of this group was lower than that of the controls, indicating the weight gain was due to a lean body growth.

When an experimental group increases its initial body weight by more than 32 per cent compared to a net loss of 2 per cent in controls, one must conclude that the presence of plerocercoids stimulates weight gain. Such was the case of plerocercoid infection in lizards. Licht & Jones (1967) demonstrated that anoles injected daily with distilled water lost up to 9 per cent of the initial body weight in a 3 week experiment. Prolactin injected daily over the same period added as much as 15 per cent to initial body weight. They also demonstrated that prolactin in combination with growth hormone could bring about gains of as much as 20 per cent. Licht (1967) concluded that prolactin stimulates weight increases in lizards by augmentation of protein synthesis. In the two separate experiments reported here (Tables 3 and 4), control animals had a higher percentage of fat than did plerocercoid-infected or homogenate-injected animals. Nonetheless, infected animals consistently gained more weight than did controls. These results indicate that the weight gains were not due to fattening but to overall growth of the organisms. A significant difference (*P < 0.01*) was observed between the weight gain of controls and infected lizards but no significant differences were found in the weights of fat bodies.

Results of the lizards experiments indicate that some factor derived from plerocercoids stimulated body growth in green anoles. Bern & Nicoll (1968) list one
of the diverse actions of prolactin in mammals as stimulation of growth and Licht & Jones (1967) found that injections of prolactin played an important role in body growth in anoles. The activities of PGF from spirometrid plerocercoids in this respect resemble the actions of prolactin. In addition, PGF has been shown to possess other prolactin-like activities; stimulation of the pigeon crop sac mucosal epithelium (Phares & Ruegamer, 1973) and inhibition of metamorphic climax in frog tadpoles (Phares, 1973).

PGF is a potent stimulator of growth in several vertebrates and is functionally similar to both prolactin and growth hormone. However, PGF is produced by an organism far down the evolutionary tree and is distinct from mammalian hormones. Studies involving further comparisons of PGF to mammalian growth-regulating hormones as well as the characterization of the PGF molecule are presently underway.

**REFERENCES**


*Key Word Index*—Plerocercoid growth factor; *Spirometra mansonoides*; growth and fattening; sparganum growth factor.