

## Pigeon Crop Sac Stimulation Due to a Factor Produced by Plerocercoids of the Tapeworm, *Spirometra mansonioides*<sup>1</sup> (37273)

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The activities of a factor produced by plerocercoid (spargana) larvae of the tapeworm, *Spirometra mansonioides* have been compared to several mammalian hormones. Harlow *et al.* (1) found that plerocercoids as well as an acetone extract prepared from the plerocercoids exhibited activity similar to that of insulin. Mueller and Reed (2) found that implantation of plerocercoids into propylthiouracil-treated, growth-arrested rats produced a growth response comparable to that of administered thyroxin. Steelman *et al.* (3) compared the plerocercoid growth factor to growth hormone and Garland *et al.* (4) found that spirometrid plerocercoids induced sulfation factor (somatomedin) in hypophysectomized (hypox) rats. However, there is no mention in the literature of possible similarities between the plerocercoid factor and prolactin.

Bern and Nicoll (5) compiled a list of the actions attributed to prolactin in the vertebrates. These actions include: stimulation of larval growth and reduction of lipid deposition in reptiles, and stimulation of growth and lipogenesis in birds and mammals. Later work (6) has shown that prolactin hypersecretion can produce nearly normal growth in human subjects whose level of growth hormone is below normal. It is obvious that prolactin enhances growth in several groups of vertebrates.

One of the most striking effects of prolactin is seen in the Order Columbiformes. Prolactin preparations from many vertebrate species have the ability to stimulate the crop sac of pigeons and doves to produce crop "milk" (5). Crop sac stimulation has subsequently been used as a definitive bioassay for prolactin. In addition, somatotropin has

lactogenic activity (7, 8) and exhibits other actions similar to those attributed to prolactin (5).

The objectives of the experiments described in this paper were to determine: (a) if plerocercoid growth factor (PGF) will stimulate the production of pigeon crop "milk" and (b) if the projected "prolactin-like" activity of PGF on the pigeon crop sac might be used as an assay system for PGF activity.

*Methods. Pigeons.* Six to eight week old Silver King pigeons obtained from the Palmetto Pigeon farm, Sumter, South Carolina, were maintained at 27° on a 12-hr photoperiod followed by 12 hr of dark. The pigeons had free access to water and Kay-Tee pigeon feed and were acclimated for two weeks on this regimen before any experiments were undertaken.

*Plerocercoids.* All stages of the life cycle of *S. mansonioides* were maintained in our laboratory according to the techniques of Mueller (9).

*Assay.* PGF was tested for pigeon crop sac stimulating activity using the local crop sac test of Lyons (10) as modified by Nicoll (11). There were six pigeons in each experimental group, and over a two-day period, each bird was injected with 0.1 ml of test or control material over the right half of the crop sac early and late in the photoperiod (total 4 injections). The birds were killed by decapitation approximately 20 hr after the last injection. The crop sacs were removed and a mucosal area 4 cm in diameter directly under the injection site was carefully scraped, placed in individual marked containers, dried by lyophilization and weighed. The uninjected half of the crop was handled in the same manner and served as an un-

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treated control. One treated hemicrop, as well as the uninjected hemicrop of the same bird, was placed in 10% buffered formalin for histological preparation. Sections of both hemicrops were fixed and stained according to Masson's trichrome method (12).

The 97.5% confidence level using the Student's *t* test was accepted as proof of an effect.

**Results.** Several sources of PGF were assayed in this experiment.

Ruegamer and Mueller (13) found that PGF activity was elicited in uninfected hypox rats by injections of serum from plero-

cercoid-infected hypox rats. Therefore, the effect of "active" serum from plerocercoid-infected hypox rats was tested in Group I. Group II was injected with serum from uninfected hypox rats. Injected wormy serum caused an increase in pigeon crop sac mucosal dry weight (MDW) of 115% over the uninjected controls while serum from uninfected rats produced only a 13.6% increase in MDW over controls (Table I). Microscopic examination of histologically prepared sections of mucosa revealed a considerable lymphocytic infiltration in both groups but wormy serum caused a marked proliferation

TABLE I. Pigeon Crop Sac Stimulation Due to Plerocercoid Growth Factor.<sup>a</sup>

Treatment group	N	Average MDW <sup>b</sup> (mg ± SEM)	Average % increase (experimental over control)
I			
Injected active (wormy) serum	6	12.75 ± 1.7	<i>p</i> < 0.005 115.0
Uninjected control hemicrop	6	5.93 ± .9	
II			
Injected (noninfected) serum	5	11.82 ± 1.2	(NS) 13.6
Uninjected control hemicrop	5	10.40 ± 1.9	
III			
Injected saline + plerocercoid incubation medium	5	11.82 ± .1	<i>p</i> < 0.005 79.6
Uninjected control hemicrop	5	6.58 ± .6	
IV			
Injected buffered saline	5	8.70 ± .9	(NS) -4.4 <sup>c</sup>
Uninjected control hemicrop	5	9.10 ± .2	
V			
Injected saline-bovine albumin + plerocercoid	4	12.70 ± .8	<i>p</i> < 0.025 64.5
Uninjected control hemicrop	4	7.72 ± 1.3	
VI			
Injected saline-bovine albumin	5	9.50 ± 1.1	(NS) 3.2
Uninjected control hemicrop	5	9.20 ± 2.6	
VII			
Implanted Millipore filter chamber contain- ing 5 plerocercoids	6	19.20 ± 1.7	<i>p</i> < 0.005 154.0
Control (no implant) hemicrop	6	7.55 ± .2	
VIII			
Implanted Millipore filter chamber contain- ing saline	5	9.18 ± .8	(NS) -0.2 <sup>c</sup>
Control (no implant) hemicrop	5	9.20 ± 1.1	

<sup>a</sup> Groups I-VI: Two 0.1 ml injections daily over right hemicrop for two days. Groups VII-VIII: Millipore filter chamber implanted for 2 days.

<sup>b</sup> Mucosal dry weight.

<sup>c</sup> Decrease.

of mucosal epithelium.

Phares and Ruegamer (14) showed that PGF could be collected *in vitro* by incubating plerocercoids in simple media. Two incubation media were assayed: one consisted of 0.85% NaCl and 0.04% NaHCO<sub>3</sub> in distilled water; the other, 0.85% NaCl, 0.04% NaHCO<sub>3</sub> and 0.5% bovine albumin in distilled water. Approximately 100 plerocercoids were incubated in each 10 ml of medium at 30° for eight hours. At the end of the incubation period the supernatant was decanted, centrifuged at 1465g for ten minutes, filtered through a 0.22  $\mu$  Millipore filter, placed in serum vials and frozen. All operations were carried out at +4° following the incubation step. There were four groups in this part of the experiment: Group III was injected with buffered saline medium in which plerocercoids were incubated (PS), Group IV was injected with buffered saline and served as a control, Group V was injected with buffered saline-albumin medium in which plerocercoids were incubated (SAP), and Group

VI was injected with buffered saline-albumin medium and served as an additional control. Saline-plerocercoid (PS) incubation medium injected over the crop sac caused a 79.6% increase in MDW (Table I). This difference is quite dramatic when viewed from prepared tissue sections of this group. MDWs of the SAP-injected crops were 64.5% higher than control crops. There was no significant difference in MDWs between injected and uninjected hemicrops in either the saline control group (-4.4%) or the saline-albumin control group (3.2%).

The effect of living plerocercoids on the pigeon crop sac mucosa was also studied. Five plerocercoids were sealed in each of six Millipore filter chambers (pore size 0.22  $\mu$ m). A chamber was surgically implanted directly over the right half of the crop of each of the five experimental birds (Group VII). The chambers remained in place for the same time period as employed for the injected groups of birds (approximately 68 hr from initial injection until decapitation). A group

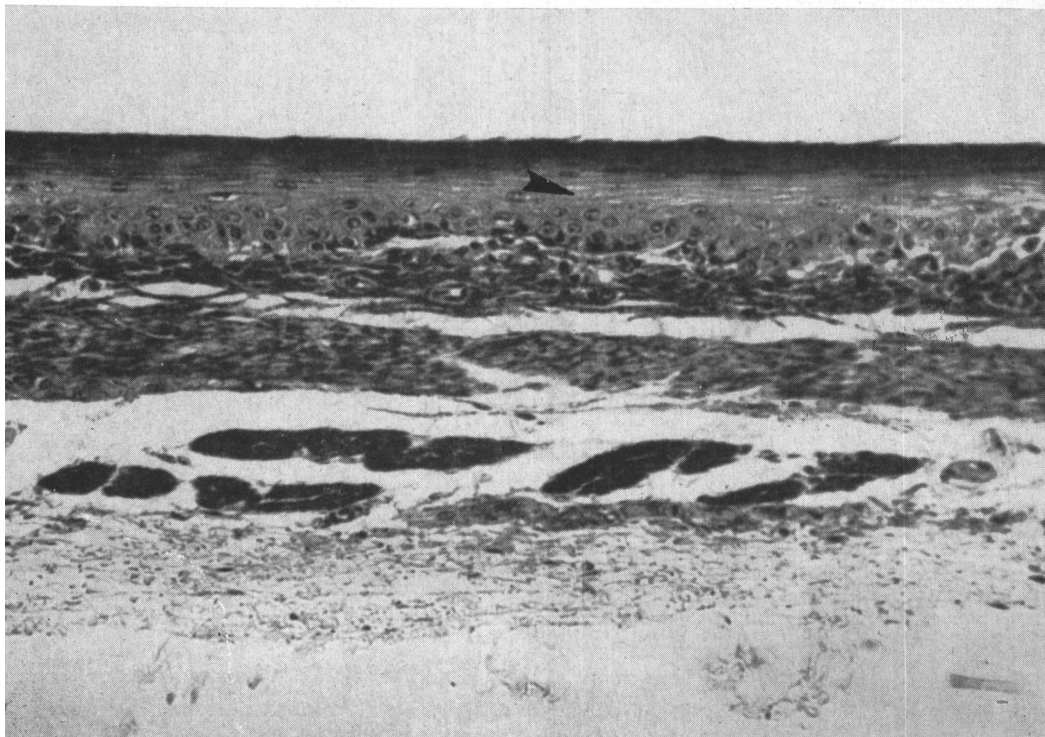


FIG. 1. Effect of Millipore filter chamber without plerocercoids (control) implanted over the crop sac for two days. Mucosal epithelium (arrow) is 5-7 cells thick. (Masson's Trichrome  $\times 150$ ).

of pigeons implanted with Millipore filter chambers without plerocercoids served as control (Group VIII). Millipore filter chambers without worms elicited no response (Fig. 1) in the crop sac ( $-0.2\%$ ) while Millipore filter chambers containing plerocercoids caused the greatest increase in MDW ( $154\%$ ) over controls (Table I). Figure 2 shows the extent to which the mucosal epithelium increased during the 68 hr in which the chambers with worms were implanted.

*Discussion.* The data presented in this communication clearly demonstrate that a factor produced by plerocercoid larvae of *S. mansonioides* has a marked stimulatory effect on the mucosal epithelium of the pigeon crop sac. This stimulatory response suggests the possibility of an assay system which would require less time and less raw material than

the hypophysectomized rat growth assay which has been used for the identification and purification of PGF. While an inflammatory response was seen in some experimental groups, microscopic examination of the prepared crops show that each form of PGF used (serum, incubation media, and living plerocercoids) elicited a true proliferation of the mucosal epithelium. The direct stimulation of the mucosal epithelium due to the presence of living plerocercoids in Millipore filter chambers suggests that the effect produced by the worms is not the result of an induced protein in the pigeon but rather that the active factor is a plerocercoid product.

Somatotropin and prolactin have been shown to functionally overlap in lactogenic activity (7, 8) as well as in growth promotion (5, 6). The activities of PGF have previ-

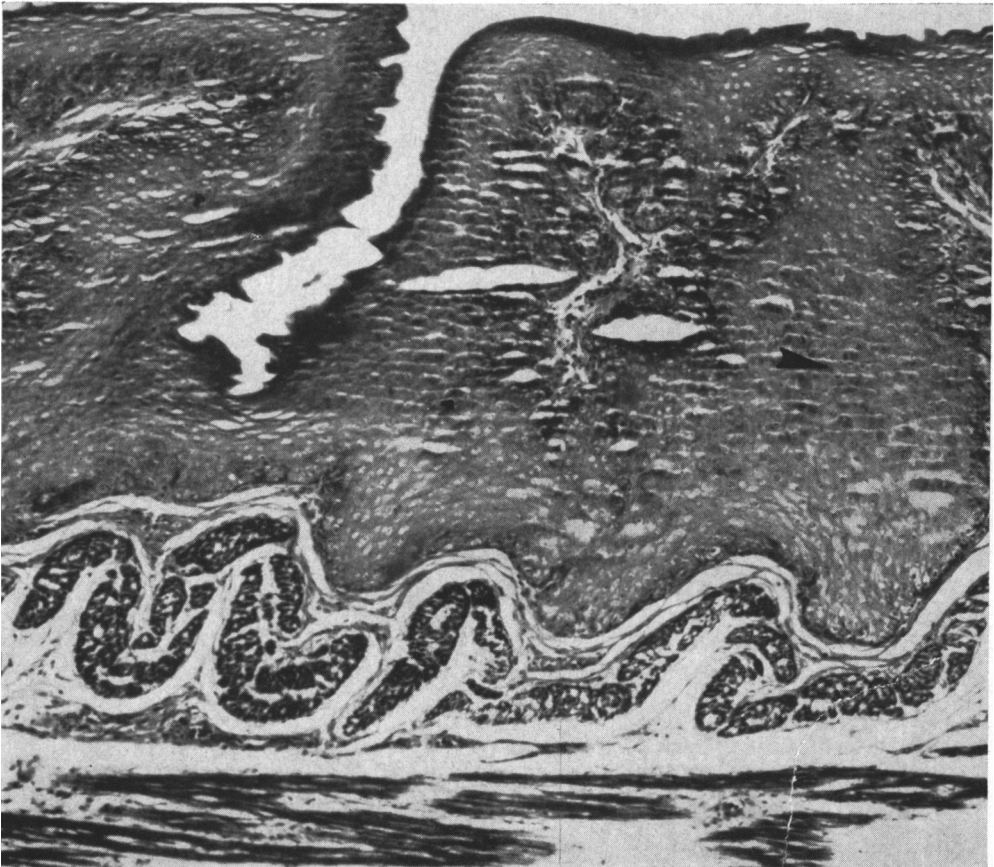


FIG. 2. Effect of a Millipore filter chamber containing five plerocercoids implanted over the crop sac for two days. Even at lower magnification, proliferation of the epithelium is extreme (arrow). (Masson's Trichrome  $\times 63$ ).

ously been shown to be similar to somatotropin in several respects (3). The work presented here shows another similarity between PGF, growth hormone and prolactin in the stimulation of the pigeon crop sac mucosa.

Further work on the identification and characterization of PGF and the extent of its lactogenic activity in other vertebrates is necessary to determine if this factor is more "somatotropin-like" or "prolactin like."

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1. Harlow, D. R., Mertz, W., and Mueller, J. F., *J. Parasitol.* **53**, 449 (1967)
  2. Mueller, J. F., and Reed, P., *J. Parasitol.* **54**, 51 (1968).
  3. Steelman, S. L., Glitzer, M. S., Ostlind, D. A., and Mueller, J. F., *Recent Progr. Hormone*

*Res.* **27**, 97 (1971).

4. Garland, J. T., Ruegamer, W. R., and Daughaday, W. H., *Endocrinology* **88**, 924 (1971).

5. Bern, H. A., and Nicoll, C. S., *Recent Progr. Hormone Res.* **24**, 681 (1968).

6. Weldon, V. V., Jacobs, L. S., Pagliara, A. S., and Daughaday, W. H., *IV Internat. Congr. Endocrinology (Abstr.)* (1972).

7. Nandi, S., *Science* **128**, 772 (1958).

8. Lyons, W. R., Li, C. H., and Johnson, R. E., *Recent Progr. Hormone Res.* **14**, 219 (1958).

9. Mueller, J. F., *J. Parasitol.* **52**, 437 (1966).

10. Lyons, W. R., *Proc. Soc. Exp. Biol. Med.* **35**, 645 (1937).

11. Nicoll, C. S., *Endocrinology* **80**, 641 (1967).

12. Masson, P., *J. Tech. Meth. Bull. Int. Ass. Med. Museum* **12**, 75 (1929).

13. Ruegamer, W. R., and Mueller, J. F., *Progr. Amer. Thyroid Assoc. 45th (Abstr.)* (1969).

14. Phares, C. K., and Ruegamer, W. R., *Proc. Soc. Exp. Biol. Med.* (in press).

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