Feedback Inhibition of Pituitary Growth Hormone in Rats Infected with *Spirometra mansonoides*[1] (36172)

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(Introduced by David M. Kipnis)
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Hypophysectomized rats infected with spargana of *Spirometra mansonoides* grow much more rapidly than noninfected hypophysectomized rats for a period of 4 to 6 weeks (1, 2). Sera of these actively growing infected hypophysectomized rats do not have radioimmunoassayable rat growth hormone (RGH) (3) or substances which cross-react in a human GH immunoassay (4). In contrast, normal rats infected with spargana show no consistent change in body weight.

Sera from hypophysectomized rats infected with spargana contain somatomedin[3] in ½ to ⅔ the concentration found in normal rat serum (3). Such a concentration of somatomedin correlates well with the growth rate, which is midway between that seen in normal rats and in the hypophysectomized controls (1). In addition, there is a growth hormone-like "worm factor" in sera from infected hypophysectomized rats, which can induce both somatomedin (3) and growth (1, 2) in recipient hypophysectomized rats. This "worm factor" is very potent *in vivo*; as little as 0.1 to 0.5 ml of serum/day can stimulate growth in hypophysectomized rats. Unlike growth hormone, it has a persistent effect for about 2 weeks after the last injection (W. R. Ruegamer, personal communication). The "worm factor" is neutralized by mixing with serum from infected rats whose growth has plateaued, but the *in vitro* effect of somatomedin is not neutralized.

The growth of intact rats is virtually unchanged by infection with *S. mansonoides* (1). To investigate the effect of *S. mansonoides* infection on the pituitary, we have injected the spargana subcutaneously into normal rats. At 3 weeks, we measured pituitary weight and RGH content, serum RGH concentration and the *in vitro* release of RGH from hemipituitaries.

Methods. Animals. Adult male rats weighing about 200 g were obtained from the Charles River Breeding Laboratories, Inc. and were maintained on Purina rat chow and water *ad libitum*, both before and after infection. Spargana of *S. mansonoides* were kindly provided by Professor Justus Mueller. Eight to 10 spargana were injected subcutaneously in 1 ml of 0.15 *M* NaCl and control rats received only the saline. After 3 weeks, the rats were anesthetized with pentobarbital 30 to 45 min before decapitation. The anterior pituitaries were then removed, bisected, and weighed.

Incubations. One-half of each pituitary was incubated in 1 ml of TC 199 (BioQuest) for 45 min (preincubation). Later they were incubated for 1 hr in fresh TC 199 and then fresh TC 199 with 10x potassium.

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[3] Somatomedin has been agreed upon by workers in the field to replace the older terminologies of "sulfation factor" and "thymidine factor" to describe the GH dependent component of plasma which regulates cartilage growth and metabolism (Somatomedin: A proposed designation for the "sulfation factor," Daughaday, W. H., *et al.* Nature (London), in press).

TABLE I. Pituitary Weights (mg).[a]

Control	Infected
10.7	7.2
9.3	4.0
11.0	6.4
8.9	5.0
9.5	7.0
9.6	6.6
8.3	8.2
9.61 ± 0.33	6.34 ± 0.49
$p < .001$	

[a] Last value in each column is the arithmetic mean ± computed standard error of the mean.

Assays. Rat growth hormone was assayed in dilutions of media and of 0.01 *N* NaOH extracts of hemipituitaries by a double antibody radioimmunoassay (5). Results are expressed in terms of potency of rat growth hormone, Wilhelmi standard (R 86567) (6). Serum RGH was determined in a separate immunoassay system by Dr. K. Takahashi.

Results. The pituitary weights were 34% lower ($p < .001$) in the infected rats than in the controls (Table I), while body weight was only slightly less (261 vs 284 g) ($.02 < p < .05$). There was an even greater decrease (68%) in the total pituitary growth hormone content ($p < .001$) in the infected rats (Table II). The mean immunoreactive GH in the sera collected at the time of decapitation (Table III) was somewhat lower for the infected rats (46 vs 53 ng/ml), but the great variance precluded its being statistically significant.

During *in vitro* incubation the smaller hemipituitaries from the infected rats released proportionately less GH (Table IV), both under basal conditions and when stimulated by high potassium. When calculated on a wet weight basis, the decrease appeared to be of borderline significance when incubations were carried out in media with high potassium concentration, but the percentage of the pituitary RGH released was not significantly different in the infected rats.

TABLE II. Pituitary Growth Hormone (μg).

	Control	Infected
	445	214
	559	141
	499	138
	449	126
	375	120
	666	198
	300	115
Mean ± SE	470 ± 45	150 ± 15

Discussion. In rats infected wth *S. mansonoides,* there was a marked decrease in pituitary size and GH content after 3 weeks. Unfortunately, because our supply of spargana was limited, other time periods could not be studied. The means of serum GH concentration for both groups are lower than those reported by Takahashi *et al.* (6), even for nongentled rats given pentobarbital. Since GH serum decreases with stress, it may be that the anesthesia was inadequate to prevent a stress-induced decrease which would mask any intergroup differences due to the worms.

TABLE III. Serum Growth Hormone (ng/ml).

	Control	Infected
	8.0	59.5
	< 2.5	96.0
	38.5	112
	53.0	3.5
	49.5	< 2.5
	106	< 9.0
	116	38.0
Mean ± SE	53.4 ± 15.4	45.8 ± 15.8

The *in vitro* studies showed that the amount of GH released was proportional to the initial GH content of each hemipituitary. High potassium caused a similar increase in GH release in both groups, suggesting that the mechanism for release was not the limiting factor. It seems more likely that the decrease in pituitary GH content was due to altered control of GH synthesis and release by the GH-releasing hormone. This may have resulted from either a decrease in amount of GH-releasing factor or from direct effect on the pituitary.

It seems clear that this suppression of the pituitary GH content is not due to the worms secreting RGH itself, since we have been unable to detect RGH in several samples of

TABLE IV. Growth Hormone Release During Incubation of Hemipituitaries.[a]

	Control		Infected	
	TC 199	High K	TC 199	High K
μg/hemipit.	5.8 ± 0.4	43.6 ± 2.7[b]	3.64 ± 0.68	21.5 ± 2.2[b]
μg/mg of wet wt	1.24 ± 0.10	9.1 ± 0.9[c]	1.17 ± 0.18	7.1 ± 0.6[c]
% GH[c]	0.66 ± 0.09	5.0 ± 0.5[d]	1.11 ± 0.31	6.3 ± 0.9[d]

[a] Each value is the arithmetic mean ± computed standard error of the mean for 7 rats during a 1 hr incubation.

[b] p values (by t test) for differences between means indicated: $p < .01$; [c] $.01 < p < .02$; [d] $p > .20$.

[c] % GH = [RGH (μg) released during 1 hr (100)]/[RGH (μg) in hemipituitary at start].

sera from infected hypophysectomized rats. In rats bearing somatotropin-producing tumors, pituitary RGH concentration is inversly related to serum RGH concentration (7). In those rats, it required serum RGH concentrations in the range of 8 μg/ml to achieve the degree of suppression reported herein. If this amount were present in the serum of our infected normal rats, it would be easily detectable in an assay where values of 10 ng/ml are measurable.

Thus, the most likely cause of the pituitary suppression is the circulating "worm factor." It seems reasonable to postulate that "worm factor" has a growth hormone-like action on the hypothalamus (or pituitary) as it must on the cells that generate sulfation factor, putatively in the liver (8). We cannot exclude the possibility that some of the feedback inhibition is due to somatomedin itself.

Summary. *S. mansonoides* spargana produce a potent "worm factor" that causes both the generation of somatomedin and linear growth in hypophysectomized rats without detectable immunoassayable rat growth hormone (RGH). Implantation of these spargana has no significant effect on the growth of normal rats but did cause a 34% decrease in pituitary weight ($p < .001$) and a 68% decrease ($p < .001$) in pituitary RGH content. Hemipituitaries removed from these rats released a normal proportion of their RGH during a control incubation and responded with an appropriate increase during stimulation by high potassium. Either the "worm factor" has growth hormone-like action on the hypothalamus or pituitary, or somatomedin itself plays an important role in the feedback regulation of pituitary growth hormone.

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