

## Pituitary and Serum Concentrations of Prolactin and GH in Snell Dwarf Mice<sup>1</sup> (39003)

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(Introduced by W. O. Weigle)

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The mutant dwarf mouse (dw/dw), first described by Snell (1), is endowed with a defective pituitary gland which is lacking in acidophils (2) and thyrotropin producing cells (3). The underdeveloped pituitary has been shown to be deficient in GH (4, 5), prolactin (6, 7), and TSH (8), and lack of these and other hormones in circulation is presumed to be the cause of low growth rate and infertility. This dwarf mouse also has a deficiency of the thymus-dependent immune system (9), which is believed to result from endocrine malfunction. In addition, recent studies indicate that dw/dw mice are deficient in endogenous C-type RNA tumor virus antigen, which can be induced by administration of exogenous prolactin (10). However, no quantitative data on blood levels of prolactin and GH in dwarf mice are available except for that of Garcia and Geschwind (11) who measured GH by a rat GH radioimmunoassay. Therefore, we felt that measurement of prolactin and GH in dw/dw mice by recently developed homologous radioimmunoassays was informative and worthwhile.

**Materials and methods.** Twelve to thirteen dwarf mice (dw/dw) and related normal (+/?) controls of both sexes were obtained at 60 days of age from the Jackson Laboratory, Bar Harbor, Me. Mice were kept in plastic boxes, 10-13 per box, and given Wayne Lab Blox and water *ad libitum*. The animal quarters were lighted from 6:00 AM to 8:00 PM and the room temperature was maintained at  $24 \pm 1^\circ$ .

After about 3 weeks, blood samples were collected by orbital puncture at biweekly intervals on three occasions. Finally, 1 week after the last orbital bleeding, all surviving

mice were decapitated and blood and pituitary glands were recovered. Immediately, each pituitary gland was weighed on an electric Mettler balance and homogenized individually in 0.05 M Na<sub>2</sub>CO<sub>3</sub>-NaHCO<sub>3</sub> buffer, pH 10, as described earlier (12). Prolactin and GH in sera and pituitary extracts were measured by specific homologous radioimmunoassays (13, 14). The biological potency of the mouse prolactin standard was 25 IU/mg and that of mouse GH was 3.1 USP U/mg. The data were analyzed by analysis of variance followed by Duncan's new multiple range test.

**Results.** Body weights of dw/dw mice of both sexes averaged about one-quarter normal (Table I). Their pituitary glands were exceedingly small ( $P < .01$ ) and weighed only about one-fifth of normal. Concentrations of prolactin in the pituitary gland were very low ( $P < .01$ ): dw/dw males possessed only 35 and females 5 ng of prolactin per mg of pituitary tissue, compared to 1.3 and 4.4  $\mu$ g/mg in respective normals. Pituitary GH concentrations fared relatively better: dw/dw males had 9.6 and females 1.8  $\mu$ g/mg ( $P < .01$ ) compared to 67.8 and 37.5  $\mu$ g/mg in respective controls.

Hormone concentrations in sera appeared to be sex-dependent. Overall serum prolactin concentrations in male dwarfs, although lower than normal males ( $P > .05$ ), were only slightly so (13%); in female dwarfs, serum prolactin concentrations were reduced by 82% ( $P < .01$ ). Serum GH concentrations, on the other hand, were reduced ( $P < .01$ ) more in male dwarfs (67%) than in females (56%). Values for prolactin and GH concentrations obtained at the first bleeding were higher ( $P < .01$ ) than at subsequent bleedings, with the values in decapitate sera usually being lowest.

**Discussion.** Treatment of dwarf mice with

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TABLE I. PITUITARY AND SERUM CONCENTRATIONS (mean  $\pm$  SEM) OF PROLACTIN AND GH IN SNELL DWARF MICE MAINTAINED IN THE DW/J STRAIN

Sex	Phenotype	Body weight (g) <sup>a</sup>	Pituitary gland weight (mg)	Pituitary hormone concentration ( $\mu$ g/mg)	Serum hormone concentration (ng/ml) at days					Overall
					85	100	118	125 <sup>b</sup>	125 <sup>b</sup>	
Male	Normal	30 (13)	1.76 $\pm$ 0.07 (11)	1.260 $\pm$ 0.120 (11)	44 $\pm$ 1 (12)	40 $\pm$ 2 (11)	38 $\pm$ 1 (11)	29 $\pm$ 2 (11)	38 $\pm$ 1	
	Dwarf	7 (11)	0.34 $\pm$ 0.01 (5)**	0.035 $\pm$ 0.019 (5)**	34 $\pm$ 2 (11)	36 $\pm$ 5 (10)	39 $\pm$ 3 (9)	19 $\pm$ 1 (5)	33 $\pm$ 2	
Female	Normal	22 (12)	2.00 $\pm$ 0.09 (12)	4.430 $\pm$ 0.430 (12)	235 $\pm$ 37 (12)	174 $\pm$ 28 (12)	129 $\pm$ 23 (12)	55 $\pm$ 9 (12)	148 $\pm$ 16	
	Dwarf	6 (10)	0.31 $\pm$ 0.01 (9)**	0.005 $\pm$ 0.002 (9)**	28 $\pm$ 3 (9)	25 $\pm$ 3 (9)	24 $\pm$ 2 (10)	28 $\pm$ 6 (10) <sup>c</sup>	26 $\pm$ 2**	
Male	Normal	30 (13)	1.76 $\pm$ 0.07 (11)	67.8 $\pm$ 11.3 (11)	42 $\pm$ 18 (13)	35 $\pm$ 8 (12)	18 $\pm$ 4 (11)	10 $\pm$ 1 (11)	27 $\pm$ 6	
	Dwarf	7 (11)	0.34 $\pm$ 0.01 (5)**	9.6 $\pm$ 4.1 (5)**	14 $\pm$ 1 (11)	9 $\pm$ 1 (11)	5 $\pm$ 1 (9)	2 $\pm$ 1 (5)	9 $\pm$ 1**	
Female	Normal	22 (12)	2.00 $\pm$ 0.09 (12)	37.5 $\pm$ 2.7 (12)	38 $\pm$ 11 (12)	30 $\pm$ 10 (12)	13 $\pm$ 2 (12)	10 $\pm$ 1 (12)	23 $\pm$ 4	
	Dwarf	6 (10)	0.31 $\pm$ 0.01 (9)**	1.8 $\pm$ 0.8 (9)**	14 $\pm$ 1 (10)	9 $\pm$ 1 (10)	9 $\pm$ 1 (10)	10 $\pm$ 0.3 (10) <sup>c</sup>	10 $\pm$ 1*	

<sup>a</sup> Body weight at 85 days of age. Values in parentheses indicate number of mice included in the group average.

<sup>b</sup> Blood collected by decapitation at this time; at other intervals blood was collected by orbital puncture.

<sup>c</sup> Due to insufficient quantity, sera from two to three mice were combined into four pools for assays in this group.

\*  $P < .05$ , \*\*  $P < .01$ ; compared to normal controls. Statistical significance of differences in serum hormone concentrations is shown only for the overall averages.

GH produces substantial body growth (15) while treatment with prolactin revives reproductive performance in both males and females (16, 17). The results of our study show that *dw/dw* mice are indeed deficient not only in GH but also in prolactin. We found the pituitary concentrations of GH to be 1/7th to 1/20th normal, in agreement with the results of Lewis *et al.* (4, 5) and Garcia and Geschwind (11). Pituitary prolactin concentrations were reduced even more (1/36th to 1/886th normal). By disc electrophoresis, Cheever *et al.* (7) could observe no prolactin band in the pituitary homogenate of dwarf mice.

However, the decreases in serum prolactin and GH levels were less dramatic. The values for serum prolactin and GH obtained by orbital puncture usually averaged higher in normal and *dw/dw* mice compared to the values obtained by decapitation and may have been influenced by the stress of bleeding (13). A gradual decline with time in prolactin and GH concentration in orbitally obtained blood samples of normal mice was evident, which may represent increasing acclimatization of mice to the bleeding stress. In dwarf mice, prolactin did not seem to be influenced by the bleeding stress as much as did GH, while males displayed this sensitivity more than females.

In any case, *dw/dw* mice did possess measurable amounts of both prolactin and GH in their sera, although the values usually were lower than normal. This contrasts with the observations of Garcia and Geschwind (11) who found no GH in the plasma of *dw/dw* mice. The discrepancy in results may be due to type of assay used. If the decapitation values obtained at 125 days of age are regarded as more realistic estimates of basal levels, only the concentrations of prolactin in females and those of GH in males appeared to be markedly reduced in dwarf mice; serum prolactin concentrations in males and serum GH in females approximated normal levels. This is reminiscent of the situation found in genetically obese *ob/ob* mice (18) and may signify a sex difference in the influence of GH and prolactin on body function. On the other hand, it is conceivable that the GH

and prolactin secreted from the dwarf mouse pituitary and detected in radioimmunoassay may be biologically inactive.

A more likely explanation for the apparent ineffectiveness of prolactin and GH present in the sera of some *dw/dw* mice may be found in the phenomenon of synergism between hormones. Meier *et al.* (19) have recently shown that prolactin, and perhaps other hormones as well, act in temporal synergism with one another to produce a given biological response. Since the *dw/dw* mouse suffers from a generalized endocrine deficiency (5), a proper balance between various hormones may be lacking and thus the resultant failure of their effects. The results of our study provide basis for further investigations into the relationship between the endocrine factors and the metabolic and immunologic disorders of the *dw/dw* mouse.

*Summary.* Secretions of prolactin and GH in dwarf mice were studied using homologous radioimmunoassays. Blood samples from adult male and female *dw/dw* mice were collected by orbital puncture and by decapitation. Compared to related normals (+/?), pituitary concentrations of GH and prolactin were very low in *dw/dw* mice, the concentrations of prolactin being more scarce than those of GH. Prolactin and GH concentrations were also lower in sera of *dw/dw* mice, but the relative differences appeared sex-dependent: serum GH was more reduced in males than in females while serum prolactin was more depressed in females. The data confirm earlier indications of deficiencies in the circulating levels of prolactin and GH in dwarf mice and suggest that the hypoactivity of these hormones may be crucial to some of the anomalies found in this mutant.

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