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Effect of Strain and Sex Variation on Growth Hormone in Rabbit Serum[1] (38446)

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(Introduced by H. Meier)

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While studies have been undertaken to define the various interactions between growth hormone (GH) levels and other hormones (1–4), the possibility of interaction between genetic factors and GH levels—analogous to the example of rabbit strain and serum PBI (5)—has not been systematically tested. Growth hormone level as a function of species has been reported in rats (6) and mice (7). In this report we show that, in the rabbit, strain significantly affects GH level.

Materials and Methods. Animals and sera. Rabbits were healthy adults representing the inbred and incipient inbred strains maintained at the Jackson Laboratory on commercial laboratory chow (Purina Rabbit Chow Checkers 20% protein). Ten males and 10 females were chosen randomly for study from each of 12 strains. A vacuum-type bleeder (8) was used to bleed animals from marginal ear veins. Animals were bled between 9 AM and noon to minimize possible contribution of diurnal variation in parameters such as the PBI (9). One male and one female from each strain were bled each day and the order of bleeding was randomized within each day. Bleeding was carried out within a 2-week period. Thirty to 35 cm^3 of blood was taken, allowed to clot at room temperature for approximately 30 min, and then under refrigeration centrifuged for 15 min. Serum was removed, divided into aliquots and stored at −20° until analyzed. In addition to these samples, sera from noninbred rabbits, previously immunized with human thyroidal microsomes, were kindly provided by Dr. David Solomon. Aliquots from these same serum samples had been assayed for serum iron and bilirubin levels (10) and serum PBI, thyroxine-binding prealbumin and dialyzable fraction thyroxine (11).

Plasma growth hormone (GH). GH was measured by a double-antibody radioimmunoassay as previously described (7). Rat growth hormone for radioiodination (Rat-GH-I-1)[2], rat growth hormone reference preparation (Rat-GH-RP-1, with a biological potency of 0.6 IU bovine GH/mg) and monkey anti-rat GH serum (A-Rat GHS-1) were used in these studies. Plasma samples from individual rabbits were assayed in duplicate at each of two different volumes (50 and 100 μl), and the mean GH concentration of the four determinations was used for each estimate. Sensitivity of the assay was such that 0.2 ng GH could be detected. Plasma samples were stored at −20° until assayed. Plasmas were run randomly in four separate assays. Aliquots of pooled rat plasma were run in each assay and the GH concentrations determined on these aliquots were 35.7, 34.2, 34.5 and 34.8 ng/ml, respectively.

Statistical methods. Strain data were analyzed

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[2] All rat growth hormone reagents used in these studies were kindly supplied by the National Institute of Arthritis and Metabolic Diseases Rat Pituitary Hormone Program.

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using a two-way analysis of variance with equal subclass numbers (12). Main effects were sex and strain.

Results. Dilution curves of rabbit pituitaries cross react with rat GH, but do not compete as well as rat GH for binding with antirat GH antibody throughout the entire range of dilutions (Fig. 1). Fairly good parallelism was seen with highly diluted pituitary extracts, *i.e.*, low concentration of rabbit GH, and the rabbit plasmas assayed fell in this low range. Since plasma GH in rabbits was calculated and expressed in terms of rat GH standards, absolute values reported may not be exact; however, values in rabbits of various strains are relative.

Mean level in each sex and strain of circulating GH is presented in Table I, with results of statistical analysis shown in Table II. There is a significant effect of strain on GH level ($P < 0.01$), but no apparent effect of sex × strain interactions.

Discussion. As in the case of serum thyroid hormone levels, measured by PBI (5), levels of growth hormone in these incipient inbred rabbit strains showed significant strain-dependence. In general, genetically related strains are more similar in their serum GH levels than unrelated, *e.g.*, strains III_c, III_{mo}, and III_{vo} all have a common origin in Castle's New Zealand White stock. Strain ACEP was an early segregate from the AC strain because of the *ep* gene for seizure susceptibility. AX_{bubu} has been separated only about five generations from AX. This strain dependence does not appear to be a function of body weight as strains AC, ACEP, ACCR (B), OS, ACCR (Y), and WH are small (Dutch size) in contrast to the rest which are larger, more like a New Zealand White. Representative body weights are given in Laird *et al.* (5). Although AC and ACEP females showed rather high GH

TABLE I. Effect of Strain and Sex on Plasma Growth Hormone in the Rabbit.

Strain	Sex	*N*	Growth hormone (ng/ml)
III_c	F	10	12.11 ± 1.42[a]
	M	10	9.72 ± 1.95
III_{mo}	F	10	10.94 ± 1.77
	M	10	7.65 ± 1.77
AC	F	10	17.45 ± 2.78
	M	10	11.84 ± 2.26
ACEP	F	10	20.38 ± 7.33
	M	10	13.63 ± 2.19
AX	F	10	9.66 ± 1.82
	M	10	8.01 ± 1.89
AX_{bubu}	F	10	8.89 ± 2.64
	M	10	10.03 ± 2.49
ACCR (B)	F	10	10.08 ± 3.16
	M	10	11.81 ± 2.45
C	F	10	16.97 ± 3.60
	M	10	12.92 ± 2.63
OS	F	10	8.95 ± 3.00
	M	10	7.03 ± 1.15
ACCR	F	10	9.60 ± 3.44
	M	10	16.70 ± 3.70
WH	F	10	13.96 ± 2.10
	M	10	16.24 ± 3.08
III_{vo}	F	10	7.80 ± 1.88
	M	10	5.90 ± 0.78

[a] Mean ± SE.

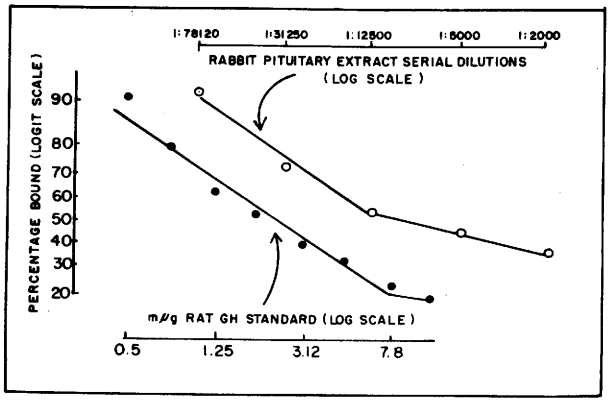

FIG. 1. Dilution response curve for rabbit pituitary extract compared with dilution curve of rat GH standards. Percentage of labeled rat GH bound to antibody is plotted on a logit scale while dose (ng rat GH standard or serial dilution of extract) is plotted on a log scale to make the curves linear. Twenty milligrams of rabbit pituitary were homogenized in 1 ml of phosphosaline buffer, pH 9.0. Serial dilutions were made from this extract.

TABLE II. *F* values from Analysis of Variance of Plasma Growth Hormone.

Source of variation	df	*F* value
Strain	11	2.67[a]
Sex	1	1.21
Sex × Strain	11	0.90
Error	216	

[a] $P = < 0.01$; all other values, $P = > 0.05$.

activity compared with males of the same strain, sex-dependence of GH levels was not demonstrated. Since mutations for various chondrodystrophys (*eg., ac,* achondroplasia, *Da,* dachs; *cd,* chondrodystrophy) are present in some of these strains these data will provide a base line for investigation of the effects of specific dwarfing genes on growth. Because genetic background of these rabbit strains contributes significantly to levels of circulating GH and thyroid hormone (5), it is important in this species to define source, strain, age, and sex in studies which may relate to these endocrine functions.

Summary. 1. Sera from 240 adult rabbits representing ten males and ten females from each of two inbred and 10 incipient inbred strains have been assayed to determine growth hormone levels.

2. Growth hormone (GH) levels in rabbit blood were found to be strain-dependent.

3. No sex difference in GH levels were found.

4. Normal base line values are presented.

1. Glick, S. M., Roth, J., Yalow, R. S., and Berson, S. A., Recent Progr. Horm. Res. **21,** 241 (1965).

2. Meyer, V., and Knobil, E., Endocrinology **80,** 163 (1967).

3. Roth, J., Glick, S. M., Yalow, R. S., and Berson, S. A., Science **140,** 987 (1963).

4. Schalch, D. S., and Reichlin, S., *in* "1st International Symposium on Growth Hormone, Proceedings" (A. Pecile and E. E. Muller, eds.), Vol. 211. Excerpta Medica Foundation, New York (1968).

5. Laird, C. W., Fox, R. R., Schultz, H. S., Mitchell, B. P., and Blau, E. M., Life Sci. **9,** 203 (1970).

6. Schalch, D. S., and Reichlin, S., Endocrinology **79,** 275 (1966).

7. Schindler, W. J., Hutchins, M. O., and Septimus, E. J., Endocrinology **91,** 483 (1972).

8. Hoppe, P. C., Laird, C. W., and Fox, R. R., Lab. Anim. Care **19,** 524 (1969).

9. Laird, C. W., and Fox, R. R., Life Sci. **9,** 191 (1970).

10. Fox, R. R., Laird, C. W., and Kirshenbaum, J., Proc. Soc. Exp. Biol. Med. **145,** 421 (1974).

11. Davis, P. J., Laird, C. W., and Fox, R. R., J. Endocrinol. (in press).

12. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics with Special Reference to the Biological Sciences." McGraw-Hill, New York (1960).

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