PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 1050–1053 (1974)

Variation in Serum Hormone Concentrations in Different Rat Strains (38244)

HENRY J. ESBER, FLORIAN F. MENNINGER, JR., AND ARTHUR E. BOGDEN
(Introduced by R. R. Gala)
Department of Immunobiology, Mason Research Institute, Worcester, Massachusetts 01608

Knowledge of hormones in blood has greatly expanded in the past 10 years due particularly to the wide use of specific immunological methods for their quantitation. Different investigators have studied various factors such as age, sex, pregnancy, lactation, and anesthesia on growth hormone regulation (1, 2). Others have investigated the effect of age and chronic cold exposure on the variation of serum immunoreactive insulin levels (3, 4). However, no systematic comparison of circulating hormone concentrations in the blood of different rat strains has been conducted. The purpose of this investigation was to determine and compare the levels of three nonsex related hormones (insulin, growth hormone and thyroxine) in six different rat strains commonly employed in endocrinology and biomedical research.

Materials and Methods. Both inbred and outbred stock female rats were studied. The inbred strains were the Fischer 344/CRBL, Wistar–Furth/ARS, Wistar–Lewis/MAI, Copenhagen/CRBL and ACI 9935/MAI. ARS-SD:SPF(SD) was the only outbred stock rat. All animals were 56–58 days old at time of sacrifice. They were housed 2 animals/cage, allowed to acclimatize to the laboratory environment for a period of 10 days and were maintained on Purina Lab chow and water *ad libitum*. On the evening prior to the day of sacrifice, at 1700 hr, the rat chow was removed from all the cages and the following day, at 1000 hr, all the animals were exsanguinated following light ether anesthesia. Blood was collected from the dorsal aorta, allowed to clot at 4° for 2 hr, centrifuged under refrigeration and the serum was separated and stored immediately at −20° for later testing.

Purified rat growth hormone (NIAMD-Rat-GH-I-1) for iodination, RGH reference preparation (NIAMD-Rat-GH-RP-1) and monkey anti-RGH (NIAMD-Anti-Rat GH Serum-1) were obtained from the Rat Pituitary Hormone Distribution Program, National Institute of Arthritis and Metabolic Diseases, NIH. Plasma RGH was measured by a modification of the double antibody radioimmunoassay method of Birge, *et al.* (1). RGH was iodinated with ^{125}I (Cambridge Nuclear, Cambridge, MA) according to the method of Greenwood *et al.* (5). The specific activity obtained was usually in the order of 100 mCi/mg. Removal of the immunologically inactive components of ^{125}I-RGH was accomplished by gel filtration in Sephadex G-75. Separation of antibody-bound from free hormone was performed by precipitation of antibody-bound hormone with rabbit anti-monkey γ-globulins (Antibodies Incorporated, Davis, CA). After washing and centrifugation, the precipitates were counted on a Packard Gamma Spectrometer Model 3002 with an efficiency of 50% for ^{125}I.

Radioimmunoassay of insulin was determined by a modification of the method reported by Hales and Randle (6) using porcine insulin standards (Schwarz/Mann, Orangeburg, NY).

A commercial competitive protein-binding assay kit was employed for rat thyroxine

(RT_4) measurements (Nuclear Medical Laboratories, Inc., Houston, TX). The reason for choosing this kit is its high degree of sensitivity in the range of 10–60 ng/ml. The procedure was followed in its entirety but with one exception: 10, 20, 30, 40 and 50 ng/ml standards were added to the standard curve in addition to the standards recommended in the kit (0, 60, 120 and 180 ng/ml) to avoid excessive extrapolation. The lower standards were prepared by dilution using the zero standards as the diluent.

All samples were tested in duplicates using two different dilutions in one assay run to eliminate interassay variations in the GH, insulin and T_4 levels.

Results and Discussion. The results of the growth hormone, insulin and thyroxine are illustrated in Table I. The RGH levels in the ACI 9935/MAI, Copenhagen/CRBL, Wistar–Furth/ARS, Fischer 344/CRBL are essentially the same, differences being statistically insignificant ($P < 0.1$). Higher RGH levels were observed in the ARS-SD:SPF(SD) stock and Wistar–Furth/ARS rats, and the values were significantly different from the other rat strains ($P < 0.01$). However, no statistical difference was noted between the ARS-SD:SPF (SD) stock and Wistar–Furth/ARS RGH levels.

Immunoreactive rat insulin levels also varied from one strain to another but not to the same degree as RGH. The Wistar–Furth/ARS strain exhibited the highest serum insulin levels, the difference in mean levels for the strain being statistically significant when compared with all other groups ($P < 0.01$). The Wistar–Furth/ARS strain demonstrated slightly higher insulin levels but the mean level was statistically significant ($P < 0.05$) only from the Fischer 344/CRBL strain. An average insulin value of 37 μU/ml was obtained with the remaining rat strains.

A substance showing a reaction of identity behaves in a manner indistinguishable from that of the antigen itself in an antigen–antibody reaction. Figures 1 and 2 show dose-response curves for rat GH and insulin. The curves produced by the addition of increasing increments of serum samples from different rat strains have the same slope as that of RGH and insulin. This parallelism between serum samples and RGH or insulin standards is suggestive of immunologic identity, but not proof of identity.

All rat strains demonstrated low concentrations of serum thyroxine. The thyroxine levels obtained were different among the various strains and in agreement with the values reported by Refetoff *et al.* (7). The RT_4 concentrations in the ARS-SD:SPF(SD) stock, Copenhagen/CRBL and ACI 9935/MAI were approximately in the range of 20 ng/ml whereas the remaining strains exhibited a higher range of RT_4, approximately 40 ng/ml.

It has been reported that hyperinsulinemia in rats and humans frequently accompanies the genetical characteristic of obesity (8, 9). Of the rat strains tested, the Wistar–Lewis/MAI and the ARS-SD:SPF(SD) rats were in the range of 160–175 and 185–192 g in body wt respectively when sacrificed at 58 days of age, as compared to 130 ± 5 g for the other strains (see Table

TABLE I. Variations in Serum Growth Hormone, Insulin and Thyroxine in Different Rat Strains.[a]

Animal strain or stock	Growth hormone (ng/ml)	Insulin (μU/ml)	Thyroxine (ng/ml)
ACI 9935/MAI	25.6 ± 4.6	37.5 ± 6.6	21.2 ± 9.3
Copenhagen/CRBL	21.7 ± 8.8	37.1 ± 7.4	23.9 ± 12.9
Wistar–Furth/ARS	21.3 ± 3.2	42.6 ± 5.9	39.5 ± 14.2
Fischer 344/CRBL	22.0 ± 2.9	36.8 ± 3.3	32.6 ± 8.4
ARS-AD:SPF (SD)	78.8 ± 24.7	38.8 ± 6.8	19.1 ± 14.4
Wistar–Lewis/MAI	66.7 ± 11.5	53.2 ± 6.1	44.2 ± 16.6

[a] Results are expressed as the average ±SD on 15 rats/group.

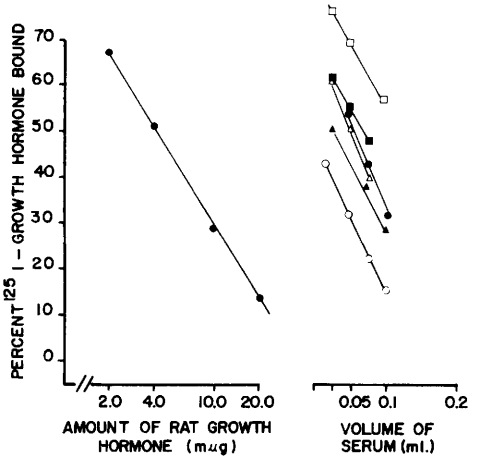

FIG. 1. Dose-response curves for growth hormone in different rat strains. ○——○ ARS-SD:SPF(SD); △——△ Fischer 344/CRBL; □——□ Copenhagen/CRBL; ⬡——⬡ ACI 9935/MAI; ⬡——⬡ Wistar–Furth/ARS; ■——■ Wistar–Lewis/MAI and ●——● NIAMD-Rat-GH-RP-1 reference standards.

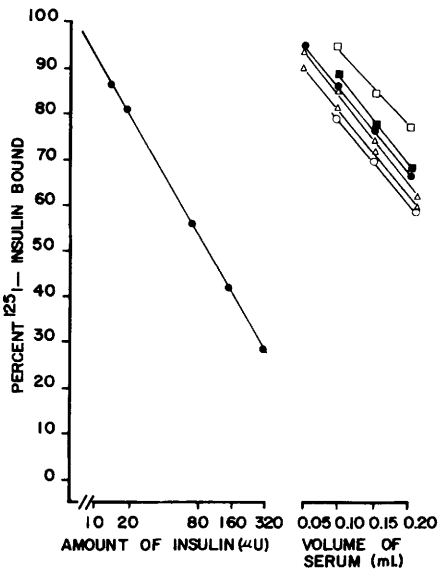

FIG. 2. Dose-response curve for insulin in different rat strains. ○——○ ARS-SD:SPF(SD); △——△ Fischer 344/CRBL; □——□ Copenhagen/CRBL; ⬡——⬡ ACI 9935/MAI; ⬡——⬡ Wistar–Furth/ARS; ■——■ Wistar–Lewis/MAI and ●——● porcine insulin standards.

II). However, of the 2 strains showing increased amounts of body wt, only the Wistar–Lewis/MAI rats demonstrated the highest serum insulin levels, which was significantly different from the other 5 rat strains ($P < 0.01$). Similarly serum growth hormone levels were significantly higher in the ARS-SD:SPF(SD) stock rat and the Wistar–Lewis/MAI rat than the other strains. It should also be pointed out that since both growth hormone and insulin secretion are affected by food intake, the differences in circulating hormonal concentrations may not be related to basal secretions and clearance rates, but rather to strain differences in response to food withdrawal.

Although all strains have relatively low serum thyroxine levels, the Wistar–Lewis and the Wistar–Furth rats have shown significantly higher levels ($P < 0.01$) than the other strains. This appears to parallel with the higher serum immunoreactive insulin levels in the same rat strains. Whether the increased insulin and thyroxine levels in the Wistar–Furth/ARS and the Wistar–Lewis/MAI rats are physiologically related can-

TABLE II. Range of Puberty Age and Body Weights in Different Rat Strains.[a]

Animal strain or stock	Age in days	Puberty age in days	Body wt g
ACI 9935/MAI	50–60	42–50	145–155
Copenhagen/CRBL	56–60	42–50	130–140
Wistar–Furth/ARS	56–60	42–50	135–150
Fischer 344/CRBL	57–63	56	116–130
ARS-SD:SPF (SD)	53–60	42–50	182–192
Wistar–Lewis/MAI	52–62	56	151–175

[a] Puberty age and body wt were furnished by the different breeding laboratories.

not be fully assessed in this investigation. There appears to be no correlation between serum thyroxine levels and growth hormones in the different rat strains.

Summary. Serum insulin, growth hormone and thyroxine levels were determined in rats of five inbred strains and one outbred stock. Insulin levels were higher in the Wistar–Lewis/MAI than all other rat strains. Growth hormone was significantly increased in ARS-SD:SPF(SD) and Wistar–Lewis/MAI rats. All rats demonstrated low serum thyroxine levels in the range of 19.1–44.2 ng/ml. Higher insulin and growth hormone levels were attributed to the increase in body wt.

This investigation was supported by Public Health contracts Nos. PH 43-65-6 and NIH 70-2204 from the National Cancer Institute.

We are grateful to Susan Flemming and Beth Marker for their excellent technical assistance and to Patricia Staples for her aid in preparing this manuscript.

The growth hormone reagents were a gift of the National Institute of Arthritis and Metabolic Diseases, Rat Pituitary Hormone Distribution Program.

1. Birge, C. A., Peake, G. T., Mariz, I. K., and Danhady, W. H., Endocrinology 81, 195 (1967).

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

3. Berdanier, C. D., Marshall, M. W., and Moser, P., Life Sci. 10, 105 (1971).

4. Belk, L. V., Zaharko, D. S., and Kalser, S. C., Life Sci. 6, 1501 (1967).

5. Greenwood, F. C., Hunter, W. M., and Glover, J. S., Biochem. J. 89, 114 (1963).

6. Hales, C. N., and Randall, P. J., Biochem. J. 88, 137 (1963).

7. Refetoff, S., Robin, N. I., and Fang, V. S., Endocrinology 86, 793 (1970).

8. Gemuth, S. M., Endocrinology 84, 386 (1969).

9. Zucker, L. M., and Antoniades, H. M., Fed. Proc. 29, 379 (1970).

Received Jan. 28, 1974. P.S.E.B.M., 1974, Vol. 146.