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Plateauing and Intermittent Treatment with Growth Hormone Extract (33609)

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Treatment of adult Norway rats with anterior pituitary growth hormone (GH) or GH-containing extracts initially produces a major renewal of growth, followed by reduced growth and eventual growth stasis (plateauing) while on treatment at the same GH dosage per rat or per unit weight (1–3). Plateauing on GH treatment is not due to aging (2), diabetes (1), altered steroid metabolism (4, 5), increased diameter of skeletal muscle fibers or decreased nuclear density (6, 7), or change in cardiac index (8). Attempted detection of GH-inactivating plasma antibodies in plateaued rats (9) was unsuccessful by the method used. Plateaued GH-treated rats show a major weight loss and cachexia when taken off treatment (3, 10), demonstrating continued effect of the hormone. This investigation attempts to establish the response to re-institution of GH treatment in rats showing the above weight loss when taken off treatment after plateauing at nearly twice normal adult size.

Methods. Normal intact 7-month-old female Long-Evans rats, averaging 260 gm in weight, were divided into 3 groups by split-litter technique. The GH extract, containing all anterior pituitary extractives soluble in 0.15 *M* NaCl at pH 10.5, was prepared in a single batch by a method previously described (1), designed to produce minimal denaturation. The extract, stored frozen in injection vials, and thawed just prior to injection, was assayed before and after the present investigation by the 10-day normal rat assay (11) and found not to have changed in potency. The daily dose per rat contained the pH 10.5 extractives of 100 mg (wet weight) bovine anterior lobe tissue and was equivalent by the above assay to 3.2 mg crystalline GH. Injections were given once daily s.c., under aseptic conditions, at systematically

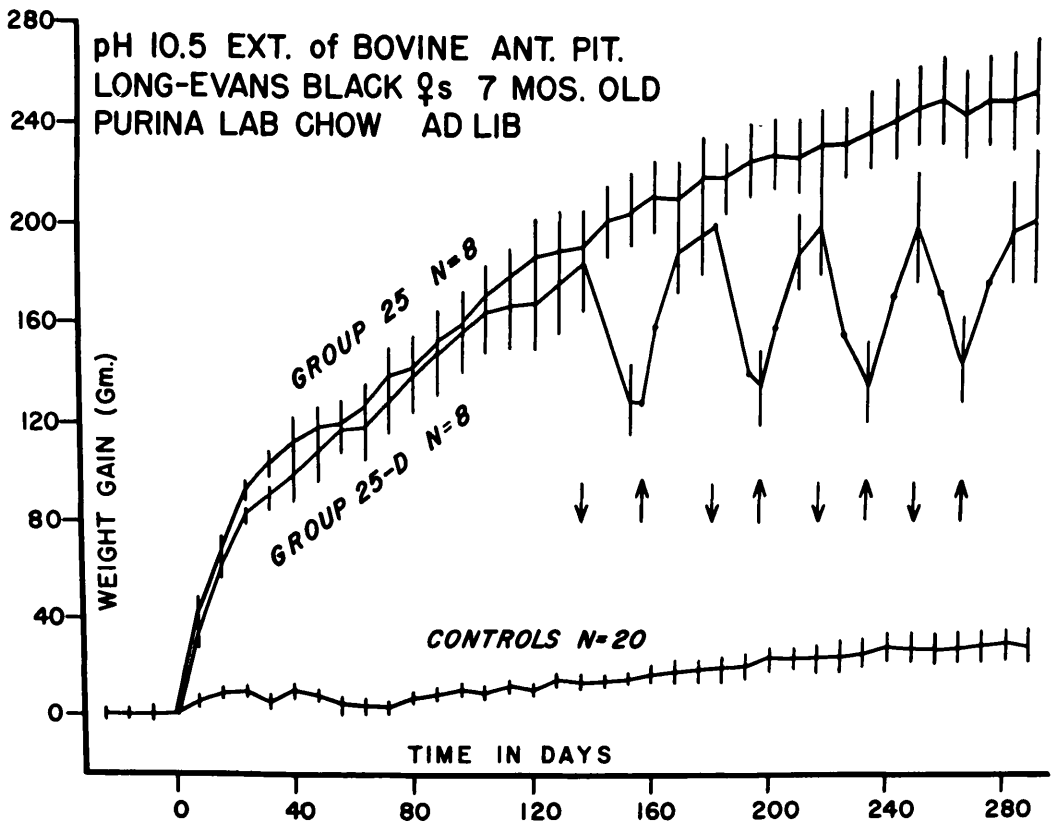

FIG. 1. All rats in treated groups 25 and 25D received daily injections equivalent to 3.2 mg pure GH/rat/day. Injection of group 25D was suspended at day marked by each downward arrow and re-instituted at each upward arrow. Vertical bars extend 1 standard error of mean above and 1 standard error of mean below the mean at each point.

varied sites along the dorsal surface, followed by a reward of 0.5 ml imitation maple syrup orally. Controls received syrup but no injection, as previous work (3) demonstrated no growth effect from daily injection of pH 10.5 normal saline. The experiments were performed in a semiisolation room maintained at 22–24°, in which the rats were raised from birth.

Results. After 136 days on treatment, plateauing had occurred in both treated groups (Fig. 1). Comparison of weight gain in Group 25 on days 1–16 of treatment versus days 120–136 (Table I) gives a t value of 10.2 ($p<10^{-10}$). Similar comparison in Group 25D gives a t of 11.8 ($p<10^{-10}$). Group 25D was next taken off treatment for 16 days, then placed back on treatment at the same dosage until the lost weight had been regained. This procedure was continued until treatment had been withdrawn and re-instituted four times. The weight gain of Group 25D during the first 16 days of re-treatment in each of the four cases failed to differ significantly from that of the first 16 days of treatment of either Group 25 or Group 25D (Table I). The rats in Group 25D had a mean body weight of 389 gm (mean gain of 129 gm) at the beginning of the first re-treatment period (day 156). They initially reached a mean weight of 389 gm on day 72. The mean gain between day 72 and day 88 was 20.5 ± 3.3 gm compared to 62.0 ± 7.4 gm between day 156 and day 172 ($t = 5.1, p<0.0001$).

Discussion. If plateauing were due to an immunological response it would be anticipated that the response during the 16-day

TABLE I. Continuous and Intermittent Treatment with pH 10.5 Growth Hormone Extract.

		Growth (g) during 16-day periods (mean ± SE)					
Days:		1–16	120–136	156–172	196–212	232–248	266–282
				Re-treatment period			
Group	No. rats			1st	2nd	3rd	4th
Continuous treatment[a] (Group 25)	8	68.4 ± 2.2	4.1 ± 4.2	10.2 ± 2.7	5.2 ± 2.6	8.5 ± 3.3	6.4 ± 2.6
Intermittent treatment[a] (Group 25D)	8	63.9 ± 1.7	5.5 ± 4.7	62.0 ± 7.4	57.1 ± 5.6	60.3 ± 6.3	55.0 ±11.8
Control (Group 23)	20	8.0 ± 1.4	3.4 ± 1.0	3.0 ± 1.3	−0.5 ± 0.9	1.2 ± 0.9	−1.9 ± 1.3

[a] Daily dose/rat equivalent to 3.2 mg GH.

re-treatment periods would have been much less than during the first 16 days of treatment. If body size were imposing a parametric limit, the response on 16-day re-treatment periods should have been comparable to the response when the same body size was initially achieved. The data in this investigation do not appear to support either of these possibilities.

Summary. Two groups of 7-month-old female Long-Evans rats, having a mean weight of 260 gm, were injected daily with a growth hormone containing extract of bovine anterior pituitaries at a dose level corresponding to 3.2 mg growth hormone/rat/day. After 136 days of treatment, growth was significantly less than initially ($p<10^{-10}$ in both groups). Treatment was then suspended for 16 to 20 days on 4 different occasions in one group. This suspension led to cachexia with a mean loss of approximately 60 gm body weight each time. When daily injections were re-instituted the mean gain during the first 16 days of re-treatment ranged from 55.0 ± 11.8 to 62.0 ± 7.4 gm (mean ± standard error) compared to 63.9 ± 1.7 gm during the initial 16 days of treatment. Re-treatment growth was significantly greater in each case than during the first 16 days of treatment after the rats initially reached the same body weight.

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