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# Dissociation of Catch-Up Growth Control and Neural Control of Growth Hormone Secretion in the Stunted Head-Irradiated Rat

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**ABSTRACT. Male Long-Evans rats were irradiated to the head only at 2 days of age; littermates of the same sex were sham-irradiated. At 40 days of age the irradiated rats were divided into two groups, one of which was fasted 48 h and the other fed a normal diet. The irradiated rats, fasted and nonfasted, were cannulated in the superior vena cava at 48 to 50 days of age. Between 54 and 58 days of age the cannulated undisturbed rats had blood samples withdrawn at 15-min intervals over an 18-h period (9 h light and 9 h dark). Body weight and tail length data showed characteristic stunting following irradiation. The superimposed fast caused transient growth retardation; on refeeding, the fasted rats showed a pattern of catch-up growth limited to the irradiated non-fasted body size. Plasma growth hormone (GH) concentration in the fasted-refed rats as compared with the nonfasted irradiated rats showed no change in the average period of the bursts of GH secretion, the numbers of values in ranges of GH concentration, or the area under the curve of the plasma GH concentration *versus* time. No difference in these parameters was present in light or dark, considered separately. We conclude 1) that the link between the catch-up growth control and neural mechanisms controlling GH secretion is impaired as a consequence of the neonatal head-irradiation and 2) that catch-up growth acceleration is not dependent on increased GH secretion. (*Pediatr Res* 20: 261–264, 1986)**

**Abbreviation**

**GH, pituitary growth hormone**

X-irradiation of only the head of the 2-day-old rat results in stunting of growth of body weight, tail length, and tibial length (1–6). The mechanism of the abscopal growth stunting is unknown, but we have recently reviewed evidence excluding disturbances of some endocrine functions, including GH and thyroxine, as possible causes (7). The pattern of growth in the head-irradiated rat is characterized by lack of spontaneous compensatory (catch-up) growth in males and only a slight degree of catch-up growth in females (3). However, the head-irradiated rats are capable of undergoing brisk catch-up growth after a period of starvation. Under those circumstances, catch-up is limited to the smaller body size resulting from the irradiation (7).

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It is suggested that GH secretion is linked to the catch-up growth mechanism by the finding of increased GH levels in sacrificed rat plasma during recovery after transient growth arrest produced by undernutrition (8, 9), glucocorticoid treatment (9), or hypothyroidism (10) and of increased pulsatile secretion of GH during recovery after fasting (11) and glucocorticoid treatment (12). The stunted head-irradiated rat appears to have a normal pulsatile pattern of plasma GH concentration and normal average period of bursts of GH secretion; however, there is an overall reduction in GH secretion (13). This indicates that either the catch-up growth mechanism is not called into play by the growth stunting of head-irradiation or that the link between the catch-up growth control and the GH releasing mechanism is disturbed as a consequence of effects of irradiation on neural tissue.

The present experiments were designed to determine whether GH secretion is increased in the stunted head-irradiated rat while the rat undergoes catch-up growth acceleration after a period of fasting. The design permitted a test of two hypotheses: 1) the link between the catch-up growth control and the GH releasing system is impaired by head irradiation, and 2) catch-up growth acceleration requires increased GH secretion.

## METHODS

The experiments were carried out on male Long-Evans rats bred from stock obtained from Simonsen Laboratories, Gilroy, CA. The animals were maintained in fresh filtered air, 35–70% relative humidity, at 21.1–23.3° C. The daily light/dark cycle was 14/10 h. Purina Lab Chow (St. Louis, MO) and tap water were provided *ad libitum*. Animal handling and all measurements were carried out by the same individual. Pregnant rats were housed in individual cages and were provided a dustless wood shaving bed from the 14th day of gestation. Litters were reduced to eight pups on the 2nd day postpartum. The 2-day-old rats were X-irradiated with only the head exposed to the beam as described previously (7). Although the present experiments did not require non-irradiated rats, littermates were routinely sham-irradiated, as previously described (7), in order to confirm effectiveness of the irradiation on growth. At weaning the young rats were transferred to individual cages. During the 1st wk after weaning, the irradiated rats were provided a Petri dish containing a mash of powdered Purina Lab Chow and water in addition to Purina Lab Chow pellets; the mash was changed daily. If an irradiated weanling felt cool to touch during the 1st wk, an equivalent size rat was added to provide warmth or gauze squares were placed in the cage to provide a nest during that week only. Mothers were rested 14 days before rebreeding and were discarded after the fourth litter. Breeder males were discarded at 17 months of age. From 2 days of age, measurements were made at intervals of 1 wk or less of body weight to the

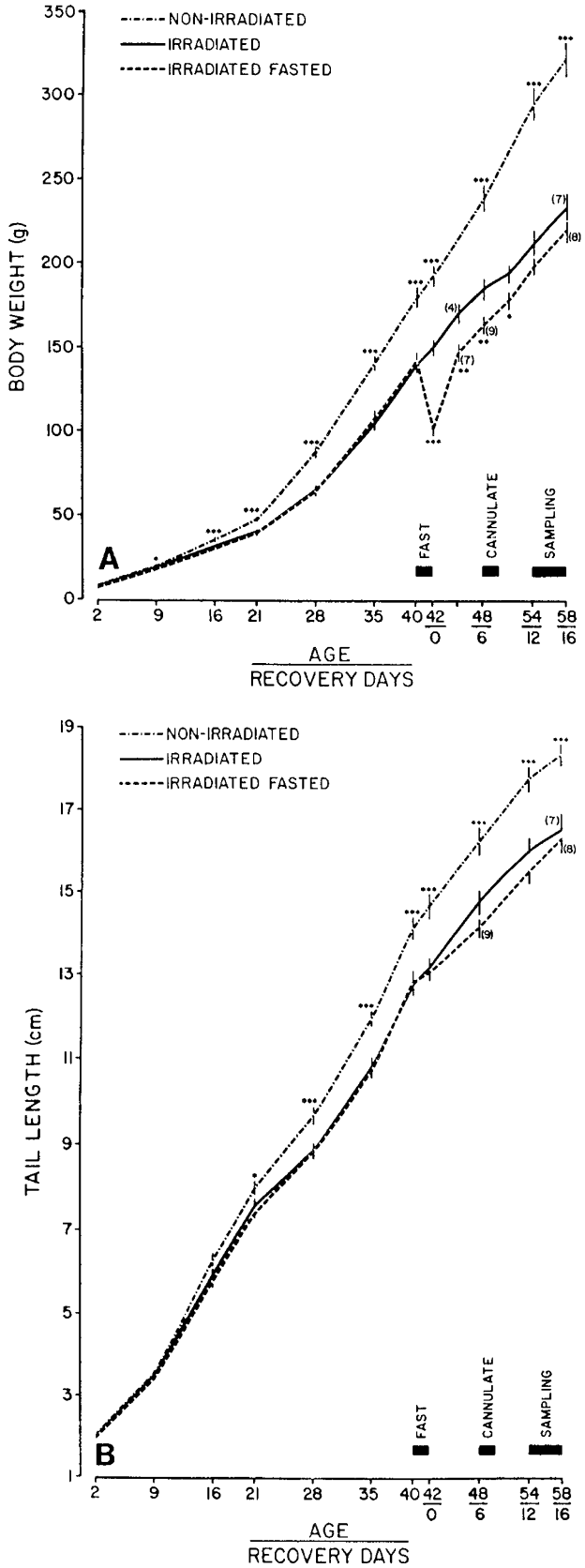


Fig. 1. Growth curves of (*A*) body weight and (*B*) tail length of fasted and nonfasted head-irradiated rats and of nonfasted sham-irradiated rats extending from the time of irradiation at 2 days of age through 58 days of age. In irradiated rats compared with sham-irradiated rats, body weight is significantly lower from 9 days of age and the tail length is significantly shorter from 21 days of age. The fasting period (48 h) is shown by a *bar*; other *bars* indicate the range of age of implantation of the cannulas and

nearest 0.1 g and tail length to the nearest 0.1 cm using the method of De Groot (14).

The experimental groups consisted of 10 irradiated nonfasted rats and 11 irradiated fasted rats. The sham-irradiated group consisted of nine rats. Fasting was carried out by removing food cups for 48 h beginning at 40 days of age. Tap water was provided *ad libitum* through this period. At the start of the fast the irradiated rats were separated into the two groups of approximately equal means and variances of body weight.

At 48 to 50 days of age a catheter was implanted into the superior vena cava (15). On two different days prior to the day of sampling, the rats were habituated for 2 h in insulated chambers (Small Universal Cubical BRS/LVE, Tech Serv Inc., Beltsville, MD) provided with fresh air flow and the routine light/dark cycle. Only one rat was placed in a chamber at a time. Each rat remained in its own storage cage, with food and water, while in the chamber. Actual sampling followed another period of habituation for 3 h. Sampling with replacement of red blood cells from each previous sample was carried out at 15-min intervals. Samples were obtained from 1100 h through 0500 h the following day, for a total of 9 h light and 9 h dark. Each sampling run was usually carried out on two animals simultaneously, one fasted and one nonfasted.

Plasma GH was determined in duplicate by radioimmunoassay (8). The initial assay was carried out with a reference range of 10–250 ng/ml. Samples with values above or below that range were reassayed at a plasma concentration corresponding to ranges of 40–1000 or 1.25–31.25 ng/ml, respectively, in order to determine peak or trough values more precisely. The within assay variation is 4.3% and the between assay variation is 11.5% for the last nine assays. All assay runs included samples of experimental rats and simultaneously sampled controls. The area under the curve of plasma GH concentration plotted against time and the period of GH surges were determined as previously described (13). Differences between means were tested for significance by one-tailed *t* test.

## RESULTS

*Body weight and tail length.* Growth curves from 2 days of age through the completion of the observations are displayed in Figures 1 *A* and *B*. Fasted irradiated rats had a significantly lower weight at the end of the fast than the nonfasted irradiated rats (102.6 ± 3.4 g, mean ± SE, in fasted rats *versus* 150.0 ± 4.5 g in nonfasted rats, $p < 0.005$). During recovery from fasting the mean weights of the fasted irradiated group remained significantly below that of the nonfasted irradiated group at 7 and 14 days; thereafter, no significant differences were observed although body weight in fasted irradiated rats was less than that of nonfasted irradiated rats through that period. Tail length growth appeared to slow in the fasted irradiated group during the 1st wk of recovery. At 7 days recovery the lengths were 14.1 ± 0.2 cm in fasted irradiated rats *versus* 14.7 ± 0.3 in nonfasted irradiated rats, but the level of significance was only $p < 0.1$. Beyond 2 wk of recovery, the number of animals in each group were depleted due to sacrifice following sampling runs. The patterns of growth, however, in the fasted and nonfasted irradiated groups and in the sham-irradiated groups were representative of those established with larger numbers of animals and a longer period of recovery in a previous study (7). There was no mortality during the fast.

*GH.* Both the nonfasted and the fasted irradiated groups displayed normal pulsatile patterns of plasma GH concentration. The periodicity of clustered bursts of GH secretion was not

of blood sampling. The points on the growth curves represent data of nine sham-irradiated rats, 10 irradiated nonfasted rats, and 11 irradiated fasted rats, except as indicated by numbers in parentheses. Levels of significance are shown as follows: $*p < 0.05$; $**p < 0.01$; $***p < 0.005$.

Table 1. *Number of values of GH concentration in plasma occurring within arbitrarily designated ranges in fasted and nonfasted head-irradiated rats (mean ± SE)*

| Groups | Ranges of GH concentration (ng/ml) | | | | |
|---|---|---|---|---|---|
| | 50–99 | 100–199 | 200–499 | 500–999 | >1000 |
| Light and dark | | | | | |
| Nonfasted | 5.2 ± 0.6 | 5.5 ± 1.1 | 3.7 ± 1.1 | 1.3 ± 0.5 | 0.5 ± 0.3 |
| Fasted | 4.5 ± 0.8 | 4.6 ± 0.6 | 4.1 ± 0.8 | 1.3 ± 0.2 | 0.7 ± 0.3 |
| *p* | NS | NS | NS | NS | NS |
| Light only | | | | | |
| Nonfasted | 2.3 ± 0.5 | 2.9 ± 0.7 | 2.3 ± 0.8 | 0.7 ± 0.3 | 0.3 ± 0.2 |
| Fasted | 2.6 ± 0.5 | 1.9 ± 0.3 | 2.0 ± 0.5 | 1.0 ± 0.2 | 0.5 ± 0.2 |
| *p* | NS | <0.1 | NS | NS | NS |
| Dark only | | | | | |
| Nonfasted | 2.9 ± 0.4 | 2.6 ± 0.6 | 1.4 ± 0.3 | 0.6 ± 0.2 | 0.2 ± 0.1 |
| Fasted | 1.9 ± 0.5 | 2.7 ± 0.5 | 2.1 ± 0.4 | 0.3 ± 0.2 | 0.3 ± 0.1 |
| *p* | <0.1 | NS | <0.1 | NS | NS |

Table 2. *The area under the curve of GH concentration vs time in fasted and non-fasted head-irradiated rats expressed as units/interval (mean ± SE)*

| Experimental groups | *n* | area (U/interval) | *p* |
|---|---|---|---|
| Combined light and dark | | | |
| Nonfasted | 10 | 56.4 ± 11.4 | NS |
| Fasted | 11 | 63.7 ± 9.0 | |
| Light | | | |
| Nonfasted | 10 | 62.3 ± 10.7 | NS |
| Fasted | 11 | 72.1 ± 12.1 | |
| Dark | | | |
| Nonfasted | 10 | 51.7 ± 12.8 | NS |
| Fasted | 11 | 54.9 ± 7.7 | |

significantly different between the two groups. The periods were 3.24 ± 0.17 h in nonfasted irradiated rats and 3.25 ± 0.12 h in fasted irradiated rats.

The number of peak values of GH concentration in arbitrarily designated ranges of concentration, 50–99, 100–199, 200–499, 500–999, and 1000 or more ng/ml, were counted. There were no significant differences between means of these ranges for all data (light and dark combined) or for light and dark periods considered separately (Table 1).

The area under the curve of GH concentration plotted against time was not significantly different between the nonfasted irradiated rats and the fasted irradiated rats for light and dark combined or light and dark considered separately (Table 2).

## DISCUSSION

In the present experiments, no difference occurred in the average period of surges of GH secretion, amplitude of GH secretion, and area under the curve of plasma GH concentration *versus* time during the recovery period after a fast in stunted head-irradiated rats as compared with nonfasted irradiated rats. No difference was found when the data from light or dark periods were viewed separately.

Previously we have shown that GH secretion is increased during the recovery period after a fast in intact (nonirradiated) rats suggesting that GH secretion may be linked to the catch-up control and, in addition, may be part of the mechanism responsible for catch-up growth acceleration (8, 9). We have also found increased GH secretion in the glucocorticoid-treated rat (9, 12). Catch-up growth does not occur in the glucocorticoid-treated rat, but this has been attributed to other defects, including long lasting alteration of food efficiency (18) and persistent defects in cartilage structure (19) and function (20) which might interfere with the catch-up growth process in that model. Recently, we have shown that the stunted head-irradiated rat undergoes catch-up growth acceleration during refeeding after a period of starvation. Thus the present results indicate that the link between catch-up growth control and GH release is impaired as a consequence of the irradiation. They also provide evidence that catch-up growth is not dependent on increased GH secretion.

We have previously found that the increased growth hormone secretion in the fasted-refed rat takes place during the diurnal light phase (11) which corresponds to the inactive phase of the rat (21). That no difference occurred in either light or dark in the present study is further evidence for a dissociation between the catch-up control and GH secretion. The data also exclude the possibility that a shift in the total amount of GH secreted might have occurred between the light and dark phases leaving the mean of combined area measurements unchanged.

The present values for area under the curve of GH concentration plotted against time closely match those previously found for stunted head-irradiated rats maintained on stock diet *ad libitum* (13). In that study it was shown that the mean area for the combined data from light and dark periods was significantly less in irradiated rats than in their sham-irradiated littermates (64.9 ± 6.4 *versus* 91.7 ± 10.7 area U/interval, mean ± SE, respectively). Previous experiments have indicated, however, that GH deficiency may not be the cause of the growth failure after neonatal head-irradiation of the rat. Treatment with GH, alone or combined with thyroxine, failed to improve the growth rate in the head-irradiated rat (3). Tibial epiphyseal width in irradiated rats was found to be normal at 70 days of age (7) instead of narrowed as one would expect in GH deficiency (22). We have found no difference in bioassayable somatomedin activity, a GH-dependent factor (23), between irradiated and control rats (Wright JC, Mosier HD Jr, unpublished data). Total cell number in heart, liver, kidney, and skeletal muscles was not significantly reduced in stunted head-irradiated rats at 21–22 days (13); hypopituitarism, on the other hand, would be expected to result in reduced cell number (24). The present evidence that the stunted head-irradiated rat is able to undergo catch-up growth acceleration without increasing GH secretion provides additional evidence that the head-irradiated rat is small for reasons other than a lack of GH.

The stunted head-irradiated rat may be a particularly useful model for the study of the mechanism controlling catch-up growth because in this preparation GH secretion does not appear

to covary with catch-up growth acceleration and yet it has an apparently normal rhythmic secretory pattern. Although the mechanism responsible for catch-up growth is unknown, an attractive conceptual model for catch-up growth involving a central control has been proposed by Prader *et al.* (25) and Tanner (26) involving a sensor for body size, a set-point for normal body size for age, and a mechanism for altering growth rate. On the basis of findings in the head-irradiated rat we have proposed that the putative set-point is reset for a smaller body size and that the remaining elements of the control, *i.e.* sensor of body size and stimulator of growth acceleration, function normally (13).

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