# SHORT COMMUNICATIONS

## THE USE OF THYROXINE IN THE BIOASSAY OF GROWTH HORMONE*

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Hypophysectomy causes inactivity of the thyroid gland and it has been known for some time that thyroxine augments the body weight and skeletal growth responses to growth hormone in hypophysectomized and hypophysectomized–thyroidectomized rats (Smith 1933; Evans, Simpson, and Pencharz 1939; Scow *et al.* 1949; Li 1953; Asling *et al.* 1954).

This effect has been used by Geschwind and Li (1952) to obtain increased response to growth hormone in the tibial assay by simultaneously giving thyroxine. However, they did not suggest its use as a routine assay procedure.

Bülbring (1938), using increase in body weight in hypophysectomized rats as an index of response to growth hormone, observed that rats given a second series of growth hormone injections did not respond as well as they had to the first series of injections. Evans *et al.* (1938) observed a similar phenomenon and also remarked on the declining sensitivity to growth hormone with increase in age after hypophysectomy.

We ourselves have observed both these effects in the routine assay of growth hormone in hypophysectomized rats. In fact, if our particular strain of rats is used later than about one month after hypophysectomy a large proportion of them are completely refractory. In an attempt to overcome these difficulties and to improve the sensitivity of the assay we have investigated the routine use of thyroxine in growth hormone assays based on the increase in body weight of hypophysectomized rats.

*Methods*

Albino rats were hypophysectomized at 6 weeks of age and kept at 80°F. A pelleted diet and fresh milk were fed *ad lib.* All injections were given intraperitoneally. Rats were weighed daily to the nearest gram.

The regression of body weight on time was calculated and the response to growth hormone treatment expressed as the rate of change in body weight in g per day. The regression coefficient is more accurate than the customary method of taking the difference between the first and final body weights of the treatment period.

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*Results*

*Experiment* 1.—Forty-eight rats hypophysectomized 3–4½ months previously were divided into six groups. All rats were injected with a sheep growth hormone preparation [56·17 (3); IV], half with a dose of 80 μg and half with a dose of 320 μg daily for 10 days. At each growth hormone dosage level, different groups received doses of 0, 2, or 4 μg of sodium L-thyroxine daily. The results are plotted in Figure 1. A positive dose response curve was not obtained in the absence of thyroxine. A dose of 2 μg of thyroxine enhanced the response to increased growth hormone dose as much as a dose of 4 μg.

*Experiment* 2.—Forty-eight rats, hypophysectomized 1½–2 months previously, were divided into four groups. These groups were used to assay two different homogenate preparations (A and B) from sheep pituitary glands. Each preparation yielded a particle extract (P) and a supernatant (S) thus giving four fractions, each of which was assayed at two dosage levels.

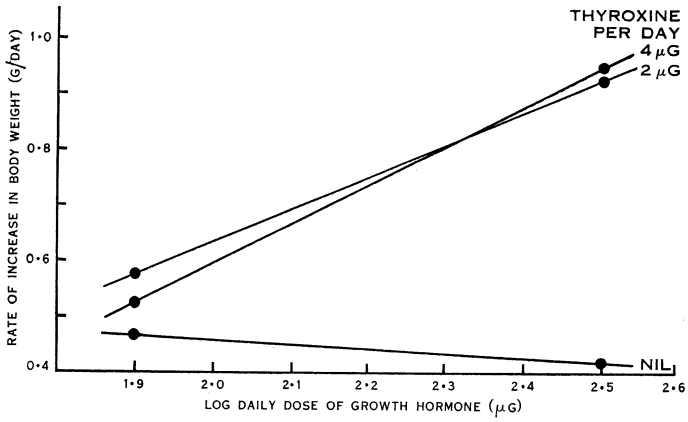


Fig. 1.—Effect of thyroxine on the body weight response to a sheep growth hormone preparation.

Two weeks after the conclusion of this assay the same forty-eight rats were re-randomized and a similar assay was carried out on them, only this time 2 μg of thyroxine was also given daily to each rat. These results are given in Figure 2 and show that consistent dose response curves were obtained only when thyroxine was used. In this experiment when no thyroxine was given 24 of the 48 rats were completely refractory. In the second assay when thyroxine was given only six rats failed to respond. Of these two were obviously sick and declined in body weight throughout treatment, dying shortly after treatment ceased.

*Experiment* 3.—Forty-five rats, hypophysectomized 1–1½ months previously, were used in a third experiment. A growth hormone preparation kindly donated by the Endocrinology Study Section, National Institute of Health (Somar-A, bovine, lot No. R 50109, prepared by the Armour Laboratories, Illinois, U.S.A.), and a sheep pituitary preparation [57·9 (12) 20i] were compared at two dosage levels. There were nine rats at each dosage level, five of which received 2 μg thyroxine daily.

The remaining nine rats were used as a control group and five of these also received 2 $\mu$g thyroxine. The results of this experiment are shown in Figure 3. The dose response curves in the absence of thyroxine were very flat. Thyroxine enhanced the response to both growth hormone preparations. Thyroxine alone did not produce any growth in the hypophysectomized rats.

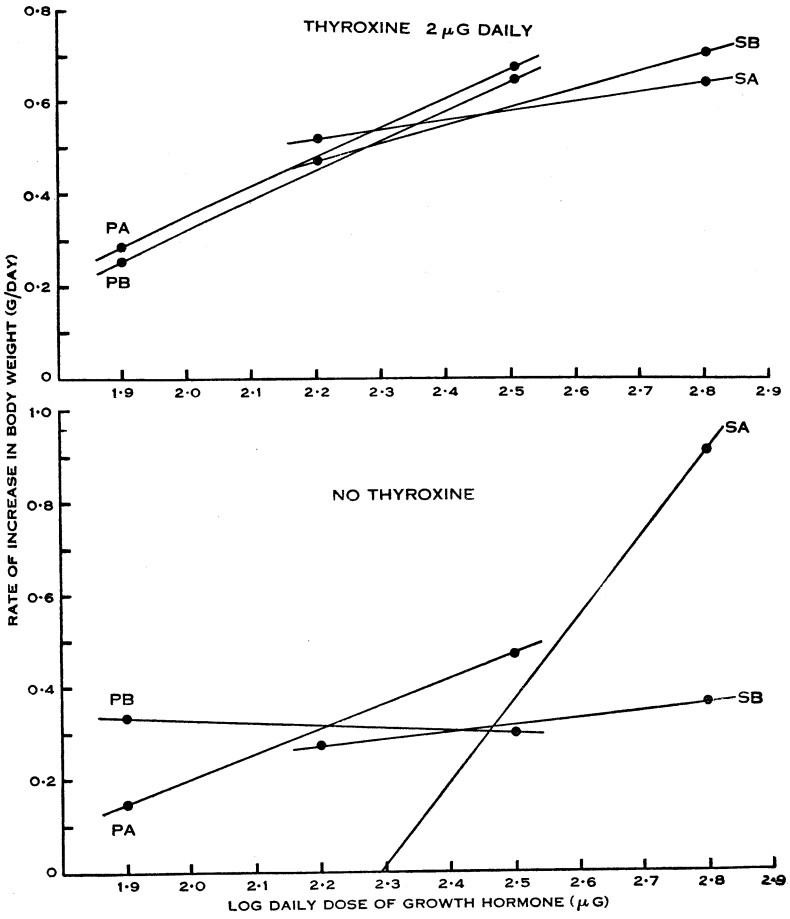


Fig. 2.—Effect of thyroxine on the body weight response to two sheep pituitary fractions. *PA*, *SA*, particle and supernatant fractions of preparation A; *PB*, *SB*, particle and supernatant fractions of preparation B.

*Discussion*

The results clearly show the advantage of injecting thyroxine when our particular strain of rats is used to assay growth hormone by the body-weight-increase method. It is reasonable to assume that thyroxine would be of benefit in reducing variation between individuals in most strains of rats for it is probable that some of this variation is due to differences in residual thyroid activity.

It appears that low thyroid activity is not the only factor responsible for decreased sensitivity to growth hormone for even in groups given thyroxine there were

still a few refractory rats that did not respond at all to growth hormone. It is possible that the degree of residual adrenal function is also important in determining the response to growth hormone. Not much data are available on this point. Geschwind and Li (1954) have shown that deoxycorticosterone acetate, 1 mg/day, in the hypophysectomized–adrenalectomized rat permits a normal tibial response to growth hormone injections.

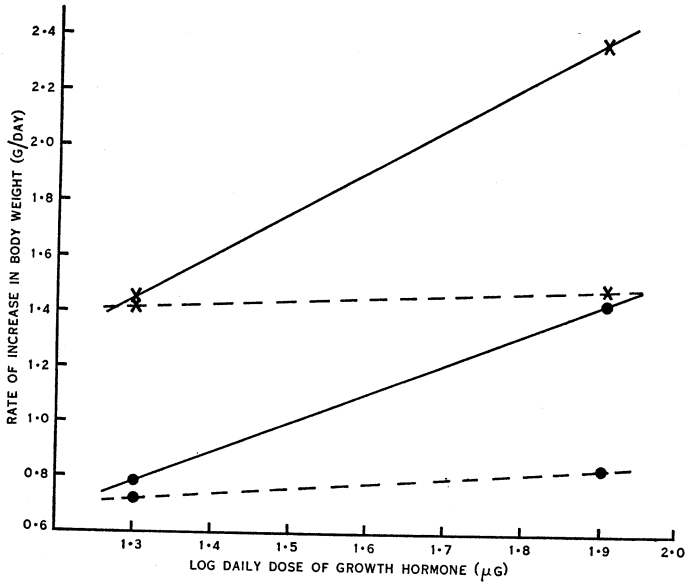


Fig. 3.—Effect of thyroxine on the body weight response to standard ox growth hormone (×) and to a sheep growth hormone (●) preparation. ———— 2 μg thyroxine; – – – – no thyroxine.

One other possible benefit from the use of thyroxine is in preventing an inflated response to growth hormone due to contamination of a preparation with thyroid-stimulating hormone. Geschwind and Li (1954) have suggested that in the tibial assay only very large doses of thyroid-stimulating hormone augment the response to growth hormone, but in assays using body weight gain as an index of growth hormone activity the longer injection period may enable a greater response to thyroid-stimulating hormone.

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