TAIL GROWTH IN THE THYROXINE-TREATED HYPOPHYSECTOMIZED RAT AS A SENSITIVE CRITERION FOR GROWTH HORMONE ACTIVITY

By

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ABSTRACT

Tail measurements were performed on hypophysectomized rats sensitized to growth hormone by simultaneous treatment with thyroxine; three weeks of daily injections suffice to differentiate the standard (continuous) thyroxine growth from the additional growth caused by growth hormone. It was found necessary to select the rats according to tail length.

Skeletal growth is stimulated by growth hormone as well as by thyroxine: bone length and bone width increase in hypophysectomized rats by growth hormone injections; thyroxine enlarges merely bone length in these animals (Boeré & Gaarenstroom 1943).

Bone growth is apparently stimulated by each hormone in a different way: thyroxine accelerates metabolic processes in the body; thus most effects caused by thyroxine and, consequently, bone growth as well, might be accounted for by this catalysing action. Growth hormone on the other hand, causes bone growth by acting on the epiphyseal cartilage plate. The fact that growth hormone enlarges the epiphyseal width in hypophysectomized rats was the basis for the well-known tibia test.

A potentiation should therefore result from the interaction of the two hormones. Thus it might be assumed that in normal bone growth, thyroxine accelerates bone formation, which is made possible by the constant renewal of cartilage induced by growth hormone, up to complete maturity. Hence, thyroxine should merely have a short-lasting bone growth promoting effect after hypophysectomy because of the ensuing lack of cartilage; as a matter of fact, the tail at first continues to grow spontaneously in a hypophysectomized rat,
and this has been explained by the decreasing activity of the still functioning thyroid gland (Simpson et al. 1950).

However, not all the known facts are in agreement with this point of view. Over quite a number of years, Evans, Li and their co-workers have studied many aspects of hormonal action on bone growth. They found in various experimental conditions that thyroxine differentiates (i.e. »ages«) bone, while growth hormone furthers cartilage formation (i.e. gives an appearance of immaturity to the bone). Of special interest in this respect are four recent publications of Baume et al. (1957 a, b; 1958 a, b). Apart from the main influence of »ageing«, thyroxine seems to exert a persistent, though slight, stimulating effect on cartilage proliferation. Growth hormone, on the other hand, chiefly stimulates chondrogenesis and primary osteogenesis, with but little effect on secondary bone transformation by erosion.

In fact, optimal bone growth in hypophysectomized rats can only be obtained by a combination of thyroxine and growth hormone. Simpson et al. (1950) have shown that suitable quantities of both hormones can accelerate bone growth even above the normal. The epiphyseal cartilage of the tibia has also been shown to react to a combination of thyroxine and growth hormone more than to growth hormone by itself (Geschwind & Li 1952).

It was decided to try and let this synergistic action serve as a means of measuring growth hormone activity in hypophysectomized rats by their tail lengths. It was hoped that differentiation of growth hormone activity would be possible, if the conditions were kept optimal. For instance, the rats should not be too young: since the younger the rat, the more chance there is of a considerable spontaneous tail growth after hypophysectomy which obscures early hormonal effects. It was felt that the rat tail length is easier to measure than the width of the epiphyseal cartilage of the tibia; there is no need for histological embedding, sectioning and the use of dyes, nor for microscopic estimations with their inherent inaccuracy.

The tail is unique in having successive centres of chondrogenesis, osteogenesis and maturation in each vertebra; hence it is the only organ with sufficient total growth changes to permit macroscopic measurement.

METHODS

Almost mature female albino rats were used: the body weight was approximately 80 g and the tail length 120-135 mm (in full-grown rats the tail length amounts to about 180 mm). Hypophysectomy was carried out by the parapharyngeal method under ether anaesthesia. The hormone injections were started the day after hypophysectomy. Thyroxine\(^6\) was administered subcutaneously in two daily doses of 3 \(\mu g\) in 0.1 ml (on holidays one dose of 3 \(\mu g\)). This design was chosen after initial experiments with

\(^6\) Thyroxine: Sigma.

424
TSH, which gave increasing tolerance, and with higher amounts of thyroxine, which caused death. Growth hormone** was administered subcutaneously in various concentrations (see Results) once a day, within 90 minutes after preparing the solution. A number of rats (mentioned in each experiment) had no thyroxine: here all treatment was withheld, or consisted of doses of growth hormone only.

Tail measurements were carried out every two days (the rats were then also weighed), except during the last experiment, in which it was done once a week. The tails were measured with the aid of a transparent tail-fitting cylinder, complete with millimeter scale. At the end of each experiment, completeness of hypophysectomy was checked in all rats by means of a binocular dissecting-microscope. In doubtful cases serial sections of the sella turcica were prepared for histological examination.

RESULTS

Experiment no. 1.

Groups of 11 rats each, were injected with:

a) 150 µg growth hormone/day.
b) 6 µg thyroxine/day.
c) both hormones combined.

At the end of the experiment, i.e. 5 weeks of injections, 9, 7 and 7 rats respectively had survived. Tail length increase from the day of hypophysectomy is given in Fig. 1. The figure only deals with the surviving animals.

Experiment no. 2.

Groups of 9 rats each, were injected with:

a) 6 µg thyroxine/day.
b) " + 50 µg growth hormone/day.
c) " + 100 µg growth hormone/day.
d) " + 150 µg growth hormone/day.

At the end of the experiment 8, 7, 6 and 4 rats respectively had survived; again the figure only deals with the surviving animals and (Fig. 2) with the tail length increase from the day of hypophysectomy onwards.

In this experiment bone growth seems to be much less than in the preceding series. It has to be kept in mind, however, that the mean tail length must vary when experimental animals have died. The average tail length at the beginning of the experiment is equal in all experimental groups, but the individual tail lengths differ: variation is caused when a rat dies or has to be discarded in the case of a pituitary remnant. The results of experiments 1 and 2 are presented again, but now as »absolute« tail length in Fig. 3: here, tail growth from the first two experiments is given as total length (minus 120 mm). From this it is now evident that a short tail at the start of the experiment tends to show a larger thyroxine growth than a long one; probably some spontaneous

** Growth hormone: Armour.

425
growth is left and enhanced by thyroxine. Of even more interest is the fact that 150 and 100 µg growth hormone show a maximal growth while this is not the case with 50 µg.

**Experiment no. 3.**

In view of the submaximal growth with 50 µg of growth hormone (+ thyroxine), doses around 50 µg (viz. 20, 40 and 60 µg of growth hormone) were used in the next experiment; these failed to produce any significant differences in growth, although dose and growth were related to each other.

**Experiment no. 4.**

Groups of 10 rats each were either not injected at all (to assess spontaneous tail growth) or injected with:
At the end of the experiment 10, 10, 9, 10 and 10 rats respectively survived; one rat in the total number of 50 died, an exceptional survival rate. In Fig. 4 the »absolute« tail length is shown. Here it is obvious that we have the optimal area of growth hormone activity: tail growth differs enough among the various groups to make it possible to differentiate between the doses of growth hormone; three weeks of injections is sufficient to obtain a clear-cut difference. Thyroxine itself induces a constant, by no means negligible growth which far exceeds the spontaneous growth of the tail after hypophysectomy; moreover, this growth shows no signs of cessation even after five weeks.
Experiment no. 5.

Finally, since it became clear that differentiation between low doses of growth hormone (in ratio of 1:2:4) is possible with thyroxine, we wanted to compare the effects of the standard dose of thyroxine and that of a low dose of growth hormone with the combined effect of both hormones. Since we could not expect such favourable conditions as in experiment 3, we tried to find a compromise by keeping the mean starting tail length equal in the three groups. Thus, when there were deaths in one group, rats with a corresponding starting tail length were discarded from the two other groups, so that the number as well as the mean starting tail length were kept constant in all three groups. This of course, is an expensive way of doing experiments: it can however be avoided if the rat stock is sufficiently large so that all rats with exactly the same tail length can be chosen at the time of hypophysectomy.

Groups of 14 rats each were injected with:
a) 10 µg growth hormone.
b) 6 µg thyroxine.
c) both hormones combined.

At the end of the experiments the groups consisted of 10 rats; the handling of the animals was kept to a minimum by measuring the tail once a week. Tail length increases are shown in Fig. 5. With a suboptimal average of starting tail length (about 122.5 mm; up to 130 mm a fair amount of spontaneous growth can be expected) it can still be seen that the thyroxine growth surpasses the effect of the low dose of growth hormone, while the combination easily surpasses that of thyroxine alone and presents a picture of sustained growth.

**DISCUSSION**

A very simple method for detecting small doses of growth hormone arises from the synergistic action of thyroxine, provided certain conditions are fulfilled.
Ideally, a sufficient number of rats with a tail length of 128–130 mm should be available in the store rooms; if not, a selection as made in the last experiment is expedient. Measurements should preferably be done once a week; more often is unnecessary (perhaps too much handling is even harmful to the animals); even less often might suffice, but gives no clear picture of the growth differentiation. The period of three weeks of injections is sufficient to distinguish between growth by the hormone combination and the continuous effect of thyroxine by itself. The latter effect was not quite as expected. From most of the papers mentioned a safe prediction for a short-lasting growth stimulation by thyroxine seemed certain. However, previous work from this laboratory (Boere & Gaarenstroom 1943), with rats given thyroid powder orally, already indicated a long-lasting growth stimulation.

The method is usable because of the proportionally enlarged increase by growth hormone of a standard tail growth by thyroxine. If the determination of growth hormone activity is wanted in an impure pituitary extract, the
presence of TSH must be allowed for. Unchecked TSH-activity might magnify a growth hormone effect by the release of endogenous thyroxine, since we know from our preliminary experiments that a less cautious dosage scheme of thyroxine (plus growth hormone) gives rise to a far greater tail growth. Hence, with impure material it is also necessary to inject a thiouracil preparation, in order to limit the thyroxine activity to that of the standard dose. Other pituitary hormones have no important stimulating effect on bone growth, except perhaps prolactin (Marx et al. 1944), but even very large doses of prolactin give at most a minimal response of the tibia cartilage. The growth hormone-like action of prolactin on $^{35}$S fixation in costal cartilage is also limited (Denko 1959). ACTH, on the other hand, might mask growth hormone effects, since Maassen (1952 a, b and c) has shown that adrenocortical steroids inhibit bone growth. To avoid interference of gonadotrophic hormones, castrated animals should be used.

If the above-mentioned criterion should be extended to a definite assay method for the quantitative determination of biologically active substances, two main factors would have to be analysed, i.e. the sensitivity and the accuracy of the procedure. As far as the sensitivity is concerned: it has been demonstrated in these experiments that the addition of thyroxine allows for growth hormone effects in the range of 5 to 20 $\mu$g. We have not gone deeply into the accuracy of the test, but have shown that it is possible to obtain significant differences in small experimental groups with doses differing from each other by 100%.

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