J. Physiol. (1952) 116, 129–136

MUSCLE PERFORMANCE IN RATS, NORMAL AND TREATED WITH GROWTH HORMONE

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(*Received* 30 *April* 1951)

Lee & Schaffer (1934) demonstrated that rats, treated with crude pituitary growth hormone extracts, gained significantly more weight than untreated control rats, even when the food intake of the two groups of animals was the same. Analysis of the tissues from these rats revealed that the gain in weight could be accounted for by the increase in protein and water content of the carcass. Similar studies by Young (1945) and by Li, Simpson & Evans (1948), who used purified growth hormone, have confirmed these findings, although no attempt was made to localize the site of new protein deposition.

The first attempt to identify the site of new growth was made by Bartoli, Reed & Struck (1937), who investigated changes in the weight of the quadriceps muscle in rats treated with pituitary extracts. They found a small but definite increase in the weight of this muscle from treated rats as compared with untreated controls. This finding has been confirmed and extended by Greenbaum & Young (1950), who reported that the rate of growth of the tissues was not uniform throughout the body. The differences of the rates of growth of the muscles were particularly striking; some, for example, gastrocnemius, soleus, tibialis anterior, grew isometrically with respect to body weight, whereas others, for example, quadriceps, diaphragm, usually grew at a greater rate.

It was of particular interest to measure the physiological properties of these hypertrophied muscles to establish whether, in fact, the possession of a larger muscle confers any advantage. Such measurements were made by Plattener & Reed (1939), who examined the twitch tension of the gastrocnemius muscle during long continued stimulation and found that the hypertrophied muscle did not develop more tension than did the muscles from control rats. Their work is, however, open to criticism on several counts. No attempt was made to pair their experimental and control rats with respect to initial weight; their animals were chosen at random from large groups of treated and untreated rats. It frequently happened, therefore, that the controls were actually heavier than the experimental animals. The use of large groups precluded the

use of the paired-feeding technique, and an increase in the size of the muscles of treated animals may thus have been secondary to the larger food intake which anterior pituitary extracts always stimulate. All their experiments were performed on the gastrocnemius, a muscle in which the increase in weight under growth hormone treatment is relatively small (Greenbaum & Young, 1950). The animals were killed before the commencement of the experiment so that the twitch tension and the rate at which it declined was probably due to the sensitivity of the preparation to asphyxia. Twitch tension is not a particularly sensitive index of functional changes in a muscle.

For these reasons it seemed desirable to repeat the observations of Plattener & Reed using a muscle, the quadriceps, which responds to growth hormone more strikingly than does the gastrocnemius, with more careful control over the selection and pretreatment of the animals, and with methods which might be expected to reveal relatively small changes in functional activity.

The opportunity was also taken to amass some information on the normal behaviour of rat muscles with a view to using them for other neuromuscular myographic studies.

The result of this investigation has been to confirm the finding of Plattener & Reed that the hypertrophied limb muscles of rats treated with anterior pituitary growth hormone are not capable of any greater tension development than those from untreated control rats.

METHODS

Full-grown female hooded Norway rats, aged 6–9 months, were used. They were selected in pairs so that the weight difference was usually less than 5 g and never exceeded 10 g. One member of each pair received a daily subcutaneous injection of 0·5 mg of 'pure' growth hormone in 0·5 ml. water adjusted to pH 9·0, for 21 days. The second member of the pair received 0·5 ml. of water adjusted to pH 9·0. The growth hormone preparation used was the 'fraction B' of Wilhelmi, Fishman & Russell (1948). Throughout the period of injection the food intake of the treated rats was restricted to that of their controls. Fifteen pairs of rats were used.

The rats were anaesthetized with 0·6 ml./100 g of a 2·5 % solution of bromethol (Avertin) by intraperitoneal injection. A further 0·3 ml./100 g was given after 5 min if necessary. This produced full anaesthesia for about 20 min. The trachea was cannulated, both carotids were tied and the rat was decerebrated through an opening in the left parietal bone. We found that this preparation remained in good condition for more than 8 hr.

Myographic technique. A longitudinal incision was made through the ventral aspect of the skin of the thigh. The patellar tendon was tied and detached from the tibia, together with a fragment of bone. The tensor fasciae latae was transected and removed so as to leave the quadriceps quite free. The femoral nerve was dissected and ligated as high up as possible, leaving about 1½ cm of nerve exposed. The femur was immobilized by means of fine drills inserted into the trochanter and the distal end. The drills were clamped rigidly to a myograph table, so arranged that the tension on the muscle could be altered with a screw adjustment. The table also carried an optical isometric myograph of spring steel, giving a magnification of 300–600 and allowing about 0·2 mm movement of the tendon for a full-scale deflexion on the recording camera. It was calibrated by attaching weights to it. The nerve was stimulated with voltage pulses derived from an Attree (1950) stimulator and applied to the nerve through fine platinum wire electrodes.

To keep the temperature constant the edge of the skin round the muscle was drawn up by cotton

threads to a flexible metal ring. The cavity so formed was filled with paraffin. The temperature was recorded by a copper-constantan thermocouple, one end of which was placed in melting ice, while the other, held within a hypodermic needle, was inserted into an adjacent muscle in the paraffin bath. Alteration of temperature was brought about by switching on either an infra-red heating lamp or an electric fan.

At the beginning of each experiment a base-line was recorded with no tension on the muscle, so that the resting tension on each subsequent record could be measured directly.

Histological technique. The increase in weight of the muscles resulting from growth hormone treatment led us to expect a corresponding change in fibre diameter which might in turn be correlated with the change in function if any should occur. At the end of the experiment the muscle was weighed and a slice cut from it by means of two razor blades clamped 1 mm apart. The incision was always made in the same part of the muscle in such a way that the muscle fibres were cut as transversely as possible. The slice was then mounted on a freezing microtome and sections cut about 30 μ thick. These were mounted on a slide in glycerine, magnified 200 times, and photographed. All prints in which the fibres were not cut quite transversely were discarded. The cross-section area of 100 fibres was measured with a planimeter.

RESULTS

After 21 days the injected animals were 25–50 g heavier than their controls, an increase of about 20 %. The average weights of the quadriceps obtained from these animals were respectively, control 1·77 g $\pm$ 0·12 g (S.E.), treated 2·21 $\pm$ 0·15 g and the mean of the percentage differences calculated from each pair 24·30 $\pm$ 2·90. We were thus able largely to confirm Greenbaum & Young's (1950) observation that this muscle grows at a greater rate than the rest of the body when treated with growth hormone, although some anomalies were found.

Histological comparison of the treated muscles with those from the untreated animals showed that an increase in muscle weight of 20–30 % (mean 26·2 $\pm$ 1·54 %) was associated with an increase of fibre cross-sectional area of 6–12 %, although in two exceptional animals there was as much as 20 % increase (mean 12·44 $\pm$ 1·513).

The figures quoted in the following sections are mean values obtained from all muscles regardless of the treatment they have received except where otherwise stated. They therefore lie between the figures given separately for the treated and control muscles in Table 1.

Single twitches. The strength of stimulus necessary for maximality was found for each preparation. With shocks about twice this strength single twitches were recorded, keeping the resting tension constant at about 50 g. The average tension produced by such twitches in all muscles was 415·3 $\pm$ 44·3 g.

Tension-length curves were not constructed because it is difficult to estimate satisfactorily the resting length of such a complex muscle as quadriceps. We therefore decided to investigate the effect of alteration of the resting tension on twitch tension. In all experiments it was found that if the resting tension was increased from 20 to 80 g the twitch tension increased by between 100 and 250 g. With greater increases of initial tension the twitch tension

of some muscles slowly declined, whereas with other muscles it remained constant, or continued to increase slightly up to a maximal value at 400–500 g initial tension, above which it declined. Typical results are shown in Fig. 1.

In some experiments the temperature of the muscle was varied and a series of twitches recorded at each temperature. When the temperature was lowered from 35 to 25° C the tension was increased by 100–200 g, i.e. 30–50 % (Fig. 2). This change is somewhat less than that found by Walker (1949) for a similar change of temperature. He, however, was working on male rats which appear to have exerted greater tensions per g muscle than ours.

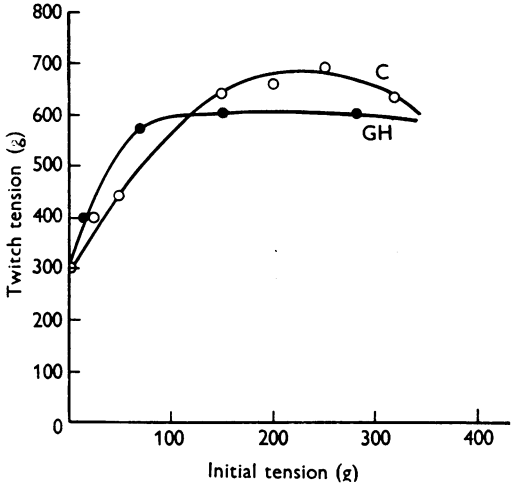

Fig. 1. Twitch tension responses to changes of initial tension. In this and all subsequent figures the solid dots represent results obtained from a growth hormone-treated muscle (GH) and the open rings those from its control (C).

Summation. The muscular summation curve was plotted by introducing a second stimulus of 10× maximal strength at varying time intervals after the first supramaximal stimulus. In almost every experiment the shortest interval of time between the two stimuli which resulted in an increase of twitch tension was constant at a value of 0·8 msec, the absolute refractory period. A few muscles were found in which the absolute refractory period was as short as 0·6 msec or as long as 1·0 msec. Maximum summation occurred at about 10 msec separation and sometimes lasted until the two stimuli were about 30 msec apart. With greater intervals the degree of summation declined.

As with single twitches, the maximum summated tension was variable from one muscle to the next. The mean for twenty experiments was 748·43 ± 37·70 g tension, showing an increase of 80·2 % over the average tension developed in the single twitches. The results obtained from one pair of rats are shown in Fig. 3.

Tetanus/twitch tension ratio. The nerve was stimulated with repetitive shocks at increasing frequencies. Stimulation lasted for not more than 3 sec, and the preparation was allowed to rest for at least 2 min between recordings. The linearity of the muscle tension in relation to the frequency of the tetanizing stimulus up to fusion frequency was demonstrated (cf. Adrian & Bronk, 1929).

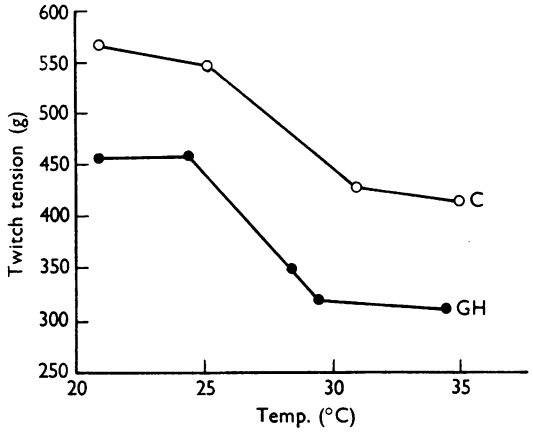

Fig. 2. Effect of changes of temperature on twitch tension.

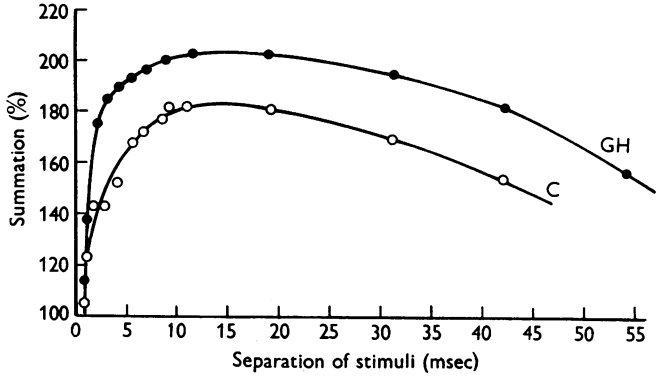

Fig. 3. Two summation curves relating the tension developed by the muscle to the interval between two successive stimuli. Tensions developed are plotted as percentages of the maximal twitch tensions which are regarded as 100 % in each case.

Fusion frequency was usually reached at some value between 30–50/sec and resulted in a tetanus of 1200–3000 g tension (mean $1660 \pm 74{\cdot}1$) (Fig. 4). At higher frequencies the tension developed was usually smaller. The tetanus/twitch ratios obtained from these experiments were usually about 4, varying between the limits of 2·8–5·0.

Fatigue. The nerve was stimulated at a frequency of 100/sec. This value was high enough to produce a fully fused tetanus without being so high that a reduction of maximum tetanic tension resulted. Stimulation was continued

for 10 sec, after which the preparation was allowed to rest for 15 min to recover. The results were plotted as percentage of the maximum tetanic tension. The tension fell to 70 % of its maximum value after 3·5–6 sec and to 40–60 % after 10 sec.

Effect of growth hormone

All these experiments were performed on both rats of several pairs and the results given are the mean values obtained. In Table 1 the figures from the growth hormone treated animals and their controls are shown separately.

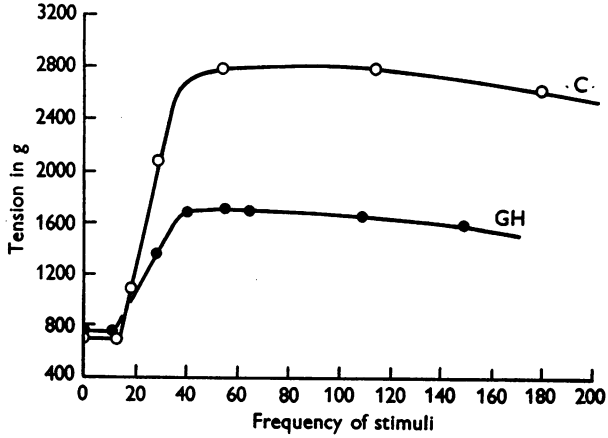

Fig. 4. Two typical tension responses to variation of stimulating frequency.

TABLE 1. Muscle tensions, normal and after treatment with growth hormone

	Mean treated (GH)	Mean control (C)	Difference of means (GH−C)	t	Degrees of freedom	Significance
Twitch tension in g	410·1 ±44·9	420·0 ± 32·3	− 10·75	1·637	149	neg.
Twitch tension in g/g muscle	169·2 ±14·0	223·1 ± 14·3	− 53·9	2·952	149	+ (1 %)
Summation tension in g	721 ±53·0	775 ± 54·0	− 53·9	0·582	10	neg.
Summation tension in g/g muscle	298 ±20·6	389 ± 21·6	− 91·0	2·731	10	+ (5 %)
Tetanic tension in g	1490 ±80·8	1918 ±169·8	−427·9	3·248	26	+ (1 %)
Fatigue						
(1) Time to fall by 30 %	4·22 ± 0·129	4·79 ± 0·356	− 0·57	1·173	11	neg.
(2) % fall after 9 sec	41·73 ± 0·28	44·0 ± 4·80	− 2·27	0·176	14	neg.

The results given are mean values of each group together with standard errors of the mean values. t tests were calculated treating the results as matched pairs and weighting them according to the number of observations obtained from each muscle.

It can be seen that although the treated muscles were 15–40 % heavier, and had fibres 6–20 % greater in cross-sectional areas than their corresponding controls, the tensions they produced were no greater. The tetanic tensions developed by the control muscles were, indeed, significantly greater than those of the treated. When the results were expressed as g tension/g muscle those from the controls were significantly higher than those from the treated animals in all cases. No correlation could be obtained between the percentage increase

in weight when treated with growth hormone, and the reduction in g tension/g muscle.

DISCUSSION

In none of the experiments we have described have we been able to detect any increase in the performance of the hypertrophied muscle. Indeed, when expressed as g tension/g muscle the figures obtained from the treated muscle were significantly lower than those from their control counterparts. We considered that if any change had occurred as a result of growth hormone treatment, it would have appeared in at least one of the properties we have investigated. The fact that the control muscles produced a significantly greater tetanic tension, even when uncorrected for muscle weight, than did the treated muscles suggests that the new substance laid down was not itself contractile material and actually impaired the efficiency of the contractile mechanism. We were admittedly unable to record the contractions of muscles stimulated directly, since it is not practicable to excite maximally and to record from such a large muscle as quadriceps lying *in situ* with an intact blood supply. The deficit in tension found in the hypertrophic muscles might have been due to defects in transmission at the neuromuscular junction or to changes in the propagation of the contraction wave from its origin at the motor end-plate. This point could only be settled by using a muscle suitable for comparing the effects of excitation through its nerve with those produced by direct stimulation at one end and 'all-over' with multiple electrodes. Some preliminary experiments which have been made with the isolated diaphragm have given results suggesting that the hypertrophic muscles may, with direct stimulation, develop greater tensions than do control muscles similarly stimulated. Whatever may be the outcome of these experiments, our present findings provide adequate evidence that the hypertrophy resulting from treatment with growth hormone does not confer any functional advantage on those muscles of the limb affected by it.

SUMMARY

1. Rats were injected with growth hormone and each was pair fed with its untreated control.

2. From the quadriceps muscle of each rat isometric records of single twitches, summated twitches, tetanus and fatigue were taken.

3. In spite of differences in weight of up to 40 % between the two types of muscle, the treated muscles gave less g tension/g muscle weight than the controls.

4. Measurements of muscle fibre cross-section area revealed a 6–12 % increase in those from the treated animals.

We are greatly indebted to Dr A. L. Greenbaum of the Department of Biochemistry, University College, London, for providing us with the rats treated with the growth hormone which he made for this purpose.

Our thanks are also due to Mr N. W. Please of the Statistics Department, University College, London, for his assistance in the statistical analyses and in the computation of the table.

We wish to express our gratitude to Prof. F. G. Young of the Biochemistry Department, Cambridge, for interesting us in this problem in the first place.

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