AN EXPERIMENTAL STUDY OF MUSCLE GROWTH IN THE RABBIT

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Descriptions of the growth of voluntary muscle are based almost entirely on the examination of histological sections. The greater part of a growing muscle fibre has the characteristic striated structure, but at its extremities there is a region of undifferentiated sarcoplasm where rapid nuclear proliferation occurs. This is presumably accompanied by formation of new sarcoplasm which later becomes differentiated to form the structure of the striated muscle fibre. Longitudinal growth is thus generally believed to take place mainly, if not exclusively, at the end of the fibre; and such a view has been supported by Riedel (1874), Froriep (1878), Felix (1889), Mingazzini (1889), Morpurgo (1899), Heidenhain (1911), Schmidt (1927) and Carr (1931). Since in a strap muscle or a fusiform muscle many fibres terminate at the ends of the muscle belly, it might be supposed that the growth of the muscle as a whole would occur mainly here. This supposition has not been proved, however, and there is in fact very little *direct* evidence concerning longitudinal growth of the muscle belly. Perhaps the most satisfactory is that of Speidel (1938), who, after prolonged observation of the myomeres in the tails of living tadpoles, concluded that growth actually does take place in the way described.

In a study on tendon growth Crawford (1950) described a method of placing small indian ink marks at intervals along the tendons in young rabbits. The distance between the marks was measured during the operation and again after an interval, and the site of growth was thus determined. This method has now been applied to the muscle belly, where it proved equally informative. An additional method was devised for marking the deep part of the muscle substance by inserting at intervals thin pieces of wire into the muscle belly and measuring the distance between them on radiographs taken before and after a period of growth. These experiments provided confirmation of the results obtained by the indian ink technique.

It is widely believed that the form of a muscle belly is highly adapted to its function; this has been stressed, for example, by Frohse and Fränkel (1908). Haines (1932) suggested that a muscle belly usually contracts only by a certain proportion of the length of its fibres, and that the length of the muscle belly is correlated with the distance it is required to contract to cause full movement of the joint on which it acts. It may be possible to deduce how the length of the muscle belly is adapted to its function, but the immediate factors that determine the extent of its growth before and after birth are quite unknown. An experiment was therefore devised to alter the distance through which a muscle had to contract in order to produce its normal degree of movement, with the intention of determining whether this leads to any alteration in the extent of its ultimate growth.

EXPERIMENTAL MATERIAL

Rabbits were used for the experiments and were operated on when about five weeks old. At this age they are growing rapidly and they stand the experimental procedure well. The muscle tibialis anterior was investigated because it is readily accessible, of convenient length and relatively simple in structure, and because its function can be easily modified.

THE GROWTH OF A MUSCLE AS DETERMINED BY INDIAN INK MARKING

Technique—In each of five experiments the rabbit was anaesthetised with nembutal and placed with the leg fully extended at the knee and the foot fully plantar-flexed. The muscle

belly of tibialis anterior was exposed, and small marks were made at intervals of four to seven millimetres along its anterior surface with a fine needle, the point of which had been dipped in indian ink. Much of the ink remained on the surface of the muscle but some was deposited within its substance. The diameter of each mark was about one millimetre. The distance between the marks was measured with fine dividers and a ruler graduated to 0.5 millimetres. Because of the disposition of the leg during the operation the distance between the marks was measured while the muscle belly was fully extended. The most proximal mark was usually from three to five millimetres distal to the tibial tuberosity. The most distal mark was three millimetres proximal to the muscle-tendon junction in rabbits 1 and 2, whereas in the other three it was on the junction.

From fifteen to twenty-two weeks later the muscle belly was again exposed under anaesthesia and the distances between the marks were again measured, with the muscle fully extended. By this means the longitudinal growth that had occurred in each of the marked segments of the muscle belly was determined. The indian ink marks survived well (Fig. 1), but each mark tended to spread slightly so the measurements were always made from their centre.

In all cases the experimental tibialis anterior never differed from its normal counterpart of the opposite leg in appearance, in length of belly (within 1 millimetre) or in extent of contraction on stimulation. There were no adhesions limiting movement of the muscle belly, and its fascial sheath was invariably intact. Except for a very slight and localised fibrous tissue reaction around some of the ink marks on the surface of the muscle, but never within it, the experimental muscle tissue and the intact side were indistinguishable histologically.

Results—The results are shown in Figure 2. The total amount of growth in the muscle belly varied considerably from animal to animal. In every case, however, the distance between the adjacent marks increased, thus indicating that longitudinal growth had occurred throughout the length of the muscle belly. The amount of growth in the various segments can be more readily compared in Figure 3. Here the increase in length of each segment, expressed as a percentage of its initial length, is plotted against the distance of the segment from the muscle-tendon junction. The amount of growth apparently varies to some extent from segment to segment. However, since the total amount of growth in each segment was always quite small, an error of measurement of only 0.5 millimetres might give rise to a difference of 10 per cent in the growth illustrated in Figure 3. Many more experiments would be required to show whether there is any significant difference.

FIG. 1

Indian ink marks made on the muscle belly of tibialis anterior at an operation performed twenty-two weeks before the rabbit was killed.

THE GROWTH OF A MUSCLE AS DETERMINED BY MARKING WITH WIRE

Technique—The tibialis anterior in each of four rabbits was exposed as before with the leg arranged so that the muscle belly was fully extended. Tantalum wire, 0.08 millimetre thick and contained in glass capillary tubes, was inserted through the middle of the muscle belly. The capillary tube was then withdrawn and the wire left threaded through the muscle about half-way between its anterior and posterior surfaces (Fig. 4). The free ends of the wire were brought across the front of the muscle and tied without constricting it. Preliminary experiments had shown that unless the wire was tied in place it almost invariably worked loose in two or three days. Five or six wires were thus inserted through the muscle belly at intervals along its length, and the distance between them was measured. The distance

FIG. 2

Diagram illustrating the longitudinal growth of the muscle bellies of tibialis anterior, which had been marked with indian ink in the growing rabbits. The vertical lines are in pairs; the left line of each pair represents the muscle belly, extended by plantar flexion of the foot, at the time of the operation, and the right member of the pair represents the same muscle belly at the end of the experimental period, again with the foot plantar-flexed. (The upper part of each vertical line represents the proximal part of the muscle belly.) Each of the five pairs of lines represents one of the experimental animals. The oblique lines join the position of the same ink marks at the beginning and the end of the experiment. The diagram is drawn to scale and hence shows the amount which each marked segment of the muscle belly has grown.

FIG. 3

Diagram illustrating the amount of growth in the various segments defined by the marks in the muscles of Figure 2. The numbers against the curves correspond with those in Figure 2.

of the most proximal piece of wire from the tibial tuberosity was five to eight millimetres, and the most distal wire from the muscle-tendon junction three to four millimetres.

Within forty-eight hours of the operation a radiograph of the leg was taken, with the leg fully extended at the knee and the foot fully plantar-flexed. The anterior surface of the leg was pressed up against the x-ray plate; the x-ray tube was at 72 centimetres distance. The distances between the middle of those parts of the wire situated within the muscle belly were measured on the radiograph. The distance between the wires was measured during the operation only to serve as a check and it was found that these measurements never differed by more than half a millimetre from those taken from the radiographs. After from seventeen to twenty-eight weeks the fully extended leg was radiographed with the plate and x-ray tube in the same position as before. The distance between the wires was again measured on the radiograph, and the growth of the segments was thus determined.

FIG. 4

FIG. 5

Figure 4—Method of inserting wires. Figure 5—Wires inserted twenty weeks before.

The rabbit was then killed and the experimental tibialis anterior examined and compared with its normal fellow. The wires were buried in the muscle and were often difficult to find. The dissection needed to reveal the wires altered their position, and their distance apart after dissection was therefore not measured. The experimental muscle belly never differed from its normal fellow by more than two millimetres in length, and contracted equally when stimulated. There were no adhesions limiting movement. The experimental muscle was histologically normal except for a localised fibrosis around the wire.

Results—The results are shown diagrammatically in Figure 6, all measurements having been taken from the radiographs. Since the distance of the most distal piece of wire from the muscle-tendon junction could not be measured on the final radiograph, a small region of the most distal part of the muscle belly is not represented in the diagram. For the same reason, the growth of the most proximally lying segment of the muscle was not recorded.

The diagram shows that all the marked segments have elongated, and that longitudinal growth has occurred throughout the length of the muscle belly. Figure 7 shows the percentage increase in length of each segment which does not appear to vary significantly between the segments. These results agree well with those obtained from marking the surface of the muscle belly with indian ink.

THE EFFECT OF TRANSFERRING THE TENDON OF TIBIALIS ANTERIOR IN FRONT OF THE CRURAL LIGAMENT

Experiments were devised to ascertain whether the ultimate growth in length of the muscle belly of tibialis anterior is determined to any extent by the distance through which it needs to contract in order to dorsiflex the foot to the normal extent.

FIG. 6

As in Figure 2, with wire markers instead of ink. The bottom end of each vertical line represents the muscle-tendon junction.

FIG. 7

Diagram illustrating the amount of growth in the various segments of the muscles of Figure 6.

Technique—In eight rabbits the crural ligament and the adjacent fascia overlying the tibialis anterior were divided. The connective tissue underlying about the most distal centimetre of the muscle belly and the most proximal centimetre of the tendon of tibialis

anterior was cut through. This part of the tibialis anterior was thus mobilised and brought forward anterior to the crural ligament, and the latter was then sutured so that it lay deep to the tibialis anterior, but superficial to the remaining extensor tendons (Fig. 8). Thus the restraining influence of the crural ligament, which normally keeps tibialis anterior approximated to the front of the ankle joint, was eliminated. The operation did not appear to interfere significantly with the blood supply of the muscle or tendon.

The effect of this experiment is shown diagrammatically in Figure 9. Normally the length which tibialis anterior must contract in order to dorsiflex the foot fully is represented by the difference between 1) the length from the crural ligament to the point of insertion of the tendon when the foot is fully plantar-flexed and 2) the similar distance when the foot is fully dorsiflexed. On the other hand, under the conditions imposed by the operation, the distance which the muscle needs to contract is represented theoretically by the difference between 1) the shortest distance from the origin to the insertion of the tibialis anterior when the foot is fully plantar-flexed, and 2) the similar distance when the foot is fully dorsiflexed. It will be seen from the diagram that the distance which the muscle must contract to effect the same movement is increased by the operation. In practice the difference is not

FIG. 8

Diagram illustrating (left) the normal position of tibialis anterior and (right) the tibialis anterior after transfer in front of the crural ligament.

FIG. 9

Diagram illustrating the course which tibialis anterior normally takes while the foot is plantar-flexed and dorsiflexed, and the course theoretically taken after its transference in front of the crural ligament. (Not to scale.)

as great as this suggests, because the restraining function of the crural ligament is partly taken on by skin and fascia. If the growth in length of the muscle belly is to any extent determined by the distance which it needs to contract in order to effect full movement at

the joint on which it acts, it might be expected that in this experiment the muscle belly of tibialis anterior on the side of operation would ultimately be longer than normal. It was in order to test this hypothesis that these experiments were carried out.

After twelve months, when the rabbits were fully grown, the length of the muscle belly was measured in the normal and experimental leg. The junction between the muscle belly and tendon, and the insertion of the tendon into the base of the first metatarsal, are well defined so that measurement of the length of the tendon is easy. The origin of the belly, however, is quite extensive, for its fibres are attached to the lateral condyle of the tibia, the lateral surface of the tibial tuberosity, and the upper third of the antero-lateral surface of the tibia. A well defined point was therefore selected at the distal border of the tibial tuberosity—that is, at approximately the mid-point of the origin of the muscle, and the distance between this and the muscle-tendon junction was taken to represent the length of the muscle belly.

TABLE I
COMPARATIVE LENGTHS OF NORMAL AND EXPERIMENTAL TIBIALIS ANTERIOR ONE YEAR AFTER TRANSFER OF ONE MUSCLE IN FRONT OF THE CRURAL LIGAMENT

Rabbit number		Length of tendon (millimetres)	Length of muscle belly (millimetres)		Excursion of muscle belly expressed as percentage of fully extended length	Length of muscle belly (millimetres) under tension of:			
			With foot fully plantar-flexed	With foot fully dorsiflexed		20 gm.	50 gm.	100 gm.	200 gm.
1	Normal . . .	49	46	—	—	—	—	—	—
	Experimental	32	63	—	—	—	—	—	—
2	Normal . . .	47	47	29.5	38	—	—	—	—
	Experimental	37	56.5	35	38	—	—	—	—
3	Normal . . .	51	63	37	41	54.5	57.5	59	60
	Experimental	33	79	45	43	70	73	77	77
4	Normal . . .	56	60	30	50	56.5	57.5	59	61
	Experimental	48	67	30.5	54	61	63.5	67	70
5	Normal . . .	49	60	33	45	54	57.5	59.5	62
	Experimental	38.5	70	37	47	64	67	69	71
6	Normal . . .	54	54	31.5	42	50	52	55	56
	Experimental	48	59	33	44	51.5	55	56.5	57
7	Normal . . .	51	54	32.5	40	50	51	53.5	56
	Experimental	33	70	39	44	63	65	66	69
8	Normal . . .	50	55	29	47	53	55	57	59
	Experimental	41	63.5	33	48	60	63	64.5	66

The length of the muscles was measured in the same way in the normal and experimental legs. The rabbit was anaesthetised and the tibia was firmly held by being transfixed at either end with drills mounted in clamps. The flexor tendons on the posterior aspect of the tibia were cut. Sufficient fascia and connective tissue covering the muscle were also removed to afford a good view of the muscle-tendon junction, but the fascia was disturbed to the minimal extent in order to observe and measure the contraction of the muscle belly under as normal conditions as possible. The lengths of tendon and belly of tibialis anterior were measured with the foot fully plantar-flexed. The muscle belly was then subjected to faradic stimulation strong enough to dorsiflex the foot as far as possible, and was again measured. Finally the sciatic nerves were divided, the tendon of the normal and of the experimental tibialis anterior was cut at its insertion, tethered to a weighted cotton thread and passed over a pulley. Increasing tensions from 20 grammes to 200 grammes were then applied, and the lengths at different tensions were recorded.

Results—The results of these experiments are shown in Table I. In the first rabbit only the lengths of the tendon and of the muscle belly in full extension were recorded, and in the second the lengths of the muscle belly under known tensions were not ascertained.

Tendon—It will be observed that the tendon in the experimental leg was always considerably shorter than the normal (Fig. 10); the reason will be discussed below.

Muscle belly—In plantar flexion, when the muscle was fully extended in situ, the length of the experimental muscle belly was always greater than the normal. The sum of the lengths of the extended muscle belly and the tendon represents the total length of tibialis anterior. In all cases this was about the same in the normal and experimental leg. The reason for this is that in full plantar flexion the foot and the tibia lie in about the same straight line, and the distance between the origin and insertion of tibialis anterior must therefore be the same on the two sides. When the tibialis anterior was stimulated to contract maximally the normal and experimental muscle bellies shortened by about the same proportion of their extended lengths. This was in both cases sufficient to cause full dorsiflexion of the foot. When fully contracted in this way the length of the experimental muscle belly was, in all but one case, longer than the normal.

The farther the tibialis anterior is able to start forward from the ankle during contraction the shorter will be its course from origin to insertion during dorsiflexion of the foot, and the greater therefore will be the distance it must contract in order to effect this movement. The difference in length between the normal and experimental muscles (and tendons) in the adult rabbits varied, probably with the effectiveness with which the restraining effect of the crural ligament was replaced by the overlying fascia and skin during growth. But the important feature of the experimental results is that in every case the experimental muscle belly was longer than its normal fellow, often considerably.

It remains possible that the primary difference between the normal and experimental tibialis anterior might be the shortness of the tendon on the abnormal side. The difference in the length of the course of the normal and experimental tibialis anterior during full dorsiflexion of the foot would then be compensated for by a corresponding shortness of the tendon in the experimental leg. In fact, however, it can be calculated from the figures given that the tendon, in every case but one, is shorter than would be expected on this hypothesis and that the muscle belly is correspondingly longer. It seems unlikely that this can be explained except by the functioning of the muscle belly during dorsiflexion.

Finally, when tibialis anterior was subjected to varying tensions it was found that the experimental muscle belly was longer than its normal counterpart (Table I). The increase in length of the muscle on the side of operation was of the same order as the increase found to be present with the muscle in situ and the foot fully plantar-flexed.

FIG. 10

On the left is shown the adult tibialis anterior muscle and tendon, which had been transferred in front of the crural ligament at an operation performed when the rabbit was five weeks old. On the right is the normal adult tibialis anterior from the opposite leg of the same animal. The measurements of these structures are given in Table I (Rabbit 1).

DISCUSSION

The results of measuring the growth of the tibialis anterior muscle in the rabbit, by marking with indian ink or with inserted wires, are in good accord. Both methods demonstrate that longitudinal growth occurs fairly evenly throughout the length of the muscle belly. It remains uncertain, however, whether this applies to the individual fibres of a muscle. These probably only rarely extend the whole length of a muscle, and there is no evidence that they do so in the tibialis anterior; its interstitial growth could therefore result from growth at the ends of the component fibres and some rearrangement of their position. The histological evidence previously mentioned suggests that the individual muscle fibre grows predominantly, if not exclusively, at its extremities and that interstitial growth throughout its length does not occur.

In young rabbits the muscle belly of the tibialis anterior which has been transferred in front of the crural ligament grows to a somewhat greater length than the normal. In the adult animal the final lengths of the experimental muscle belly and the normal of the intact limb appear to be such that contraction by the same percentage of their extended lengths causes approximately the same degree of dorsiflexion in the foot. This supports the widely held assumption that there is a relationship between the length of a muscle belly and the distance through which it must contract in order to produce full movement of the joint on which it acts. The immediate cause of the increased longitudinal growth of the displaced muscle belly is unknown; but, possibly, changes in the tensions to which the muscle and tendon are subjected play a part. Thus, for a time immediately after transfer of the tibialis anterior in front of the crural ligament, while the belly was still of normal length, its diminished course in dorsiflexion of the foot might imply diminished tension. This might explain the *initial* retardation in growth of the tendon. As growth failure appears to be greater than would suffice to compensate for the decreased course of the tibialis anterior, there is an actual (and not merely a relative) increase in the growth rate of the muscle belly, which requires further explanation. Possibly a muscle shortened by more than a certain proportion of its extended length does not exert enough tension to stimulate tendon growth. If so, realignment of the tibialis anterior might necessitate such an increased excursion of the belly during dorsiflexion as to diminish growth of the tendon. On plantar flexion the abnormally short tendon might in turn subject the muscle belly to increased tension, and this may be what leads to an increase in the growth rate of the muscle belly. On this hypothesis, muscle growth would in fact continue until in contracting by the percentage of its length necessary to cause dorsiflexion, the belly would also exert sufficient tension to stimulate tendon growth.

SUMMARY

1. In young rabbits the muscle belly of the tibialis anterior was marked at intervals, either on its surface with indian ink, or in its substance by wires. The intervals between ink marks were measured directly, and those between wires by radiography. After four to seven months the measurements were repeated and the amount and site of longitudinal growth determined. The experiments showed that it occurred fairly evenly throughout the length of the muscle belly.
2. By transfer of the tibialis anterior in front of the crural ligament in young rabbits its course was reduced and the extent of contraction necessary to dorsiflex the foot was increased. The rabbits were killed when fully grown and the lengths of the tendons and muscle bellies of the tibialis anterior of the normal and experimental legs were compared. It was found that in every case the tendon of the experimental muscle was shortened and its belly lengthened in comparison with the normal. It is suggested that the increased length of the muscle belly was determined by the increased distance which it had to contract in order to dorsiflex the foot.

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