

## Experimental modification of muscle migration in the rabbit

PHILIP G. GRANT\* AND MICHAEL R. HAWES†

\**Department of Anthropology, University of Texas, Austin, Texas 78712, U.S.A.,*  
and †*School of Physical Education, University of Calgary,*  
*Calgary, Alberta, Canada T2N 1N4*

(Accepted 15 February 1976)

### INTRODUCTION

During growth mammalian long bones increase in length by adding material to their ends through the activity of the epiphyseal growth plates. Tendons, ligaments, and periosteum grow in length both interstitially and by adding material to their ends (Warwick & Wiles, 1934; Crawford, 1950, 1954; Comer, 1956; Hughes, 1956; Videman, 1970*a, b*). Growth may be greater in some parts than in others. Throughout the normal ontogeny of an individual, tendons, ligaments and muscles apparently retain approximately the same relative positions of attachment with regard to the end of the bone. This has not been carefully studied, however, and the exact relationships are not known. Since the attachment of a structure (tendon, ligament or muscle) to a bone is commonly on to the diaphysis, growth in length of the bone tends to leave the structure behind. If the structure did not compensate for this by migrating or creeping toward the growing end of the bone, it would ultimately be attached much more toward the central portion of the shaft than it should be (or in fact is) in the adult (Fig. 1). This aspect of muscle and bone growth has been appreciated for many years, but there has been a curious lack in the literature of attempts to understand how the attachments migrate, and what controls migration. This is a problem of both practical and theoretical significance. In orthopaedics the practice of translocating attachments should be helped by a better knowledge of the control factors involved; while our understanding of the genetic mechanisms responsible for evolutionary changes in the musculoskeletal system could well be illuminated.

The experiment reported here was designed to test the hypothesis that the position of attachment of a structure relative to the end of a bone at least partially controls the direction, rate and amount of migration of the attached structure, that is to say, structures attached near the end of a bone should migrate faster and further than those attached nearer the middle of the shaft. Our hypothesis was based on the following considerations. First, structures exerting tensions in different directions and of different magnitudes at one end of a bone apparently maintain constant relationships with each other during growth. Second, in patients or experimental animals whose muscles became or were made non-functional during early growth stages, the muscles, although atrophied, appear to be located in normal or nearly normal positions in adulthood. Third, if, as appears to be the case, the structures maintain constant relationships to each other during growth, then structures close to the ends must migrate farther than structures nearer the middle portion of the shaft.

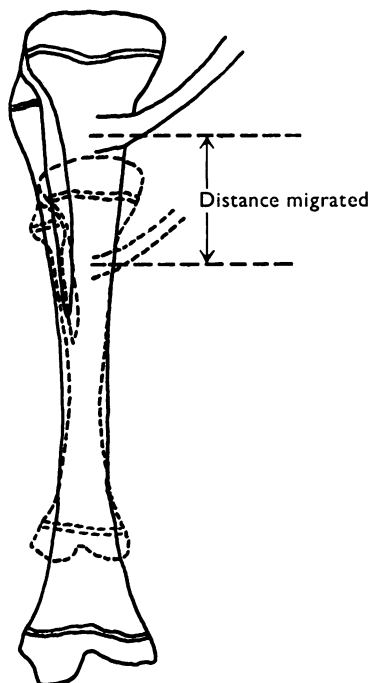


Fig. 1. Migration of the *M. semimembranosus* on a normal rabbit tibia. The dashed outline is the younger bone and the solid outline the older bone. Growth in length occurs only at the growth plates. The distance the muscle must migrate to maintain a normal relationship is indicated on the side.

We expected to find that a structure translocated from its normal position to a new position further from the epiphyseal plate should have a slower rate of migration and should, therefore, migrate less in a given unit of time.

#### MATERIAL AND METHODS

Twenty five female New Zealand White rabbits, obtained from the Evergreen Rabbitry, Eugene, Oregon, were used. Each leg of each rabbit can be considered an independent entity (White, Panjabi & Hardy, 1974). However, one leg of each of six of the animals was used in a different experiment, so there are only 44 experimental limbs in this series. These were divided into five groups as indicated in Table 1. All animals were operated on at approximately eight weeks of age.

Standard aseptic surgical technique was used. Animals were anaesthetized with Innovar-vet (0.22 cc/kg; IM); Metofane was used if needed to increase or maintain anaesthesia for longer periods. In all groups an incision was made through the skin and periosteum of the anterior tibial border, a hole one millimetre in diameter was drilled transversely through the midshaft of the tibia, and a short length of No. 32 stainless steel suture wire tied through the hole and around the anterior half of the tibia to serve as a marker. The distance from the marker to the lower edge of the tendon of the *M. semimembranosus* and the width of that tendon were measured.

Table 1. Numbers of rabbits and of rabbit legs studied in each group

Group	Number in group	Number of each leg in group (R = right leg; L = left leg)
C	11	7R, 11R, 16R, 25R, 29R, 32L, 33L, 34L, 35L, 36L, 37L
0	19	6L, 7L, 8L, 9L, 11L, 12L, 13L, 14L, 16L, 22L, 23L, 24L, 25L, 26L, 27L, 28L, 29L, 30L, 31L
5	4	6R, 14R, 24R, 28R
10	5	8R, 12R, 22R, 26R, 30R,
20	5	9R, 13R, 23R, 27R, 31R

The former distance plus one half of the latter was taken as the original distance of the tendon from the marker. The subsequent experimental procedures are detailed below.

*Group C.* No further operation. These legs were used as controls.

*Group 0.* The tendon of *M. semimembranosus* was detached at its insertion, a 1 mm hole drilled through the base of the tibial tuberosity at the original distance of the tendon from the marker, and the tendon re-attached through this hole, using a modification of the technique of Pulvertaft (1956). Suture thread (Deknatel size 00) was attached to the free end of the tendon with a figure 8 stitch. The ends of the thread were pulled through the tibia from medial to lateral, the tendon drawn into the hole in the medial wall, the thread brought medially over the anterior surface of the tibia and attached to the tendon by a figure 8 stitch. These legs served to indicate how much migration is lost during the time the tendon is not physiologically attached to the bone and thus cannot migrate, as opposed to how much is lost due to the distal displacement of a tendon.

*Group 5.* Same procedure as for Group 0 except that the tendon was re-attached 5 mm distally from the original site of insertion

*Group 10.* Same procedure as for Group 0 except that the tendon was re-attached 10 mm distally from the original site of insertion.

*Group 20.* Same procedure as for Group 0 except that the tendon was re-attached 20 mm distally from the original site of insertion.

All animals were kept in small cages (60 cm by 45 cm) for approximately three weeks, at which time the tendons should have become re-attached (Kernwein, 1942; Forward & Cowan, 1963). They were then transferred to a large outdoor run (30 m by 7 m) for the duration of the experiment. In the outdoor run they could run, jump, and dig holes, so that there should have been no growth disturbances due to close confinement and inability to exercise the limbs. They were fed Purina Rabbit Chow *ad libitum*. A few animals developed mild infections at the site of the operations. These were drained and treated with a local antibiotic (Furacin).

All animals were sacrificed at about 5 months of age. The *Mm Plantaris*, *gastrocnemius*, *gracilis*, *adductor magnus plus semitendinosus* and *semimembranosus* were dissected free and weighed. The length of the tibia, the width of the tendon of

M. semimembranosus, and the distance from the marker to the lower edge of that muscle, were measured to the nearest 0.5 mm with sliding calipers.

The possibility exists that the tendon we measured upon sacrifice may not be the same tendon we measured at the initial operation. Conceivably a new tendon coalesced to join the muscle to the bone, and the original tendon degenerated. Since we did not put markers in the tendon we cannot prove that our interpretation is correct. But in subsequent work we have marked tendons. In most cases at sacrifice there was a well-defined tendon in a new position with the marker still in it. In a few cases there were, in addition to the well-defined tendon with the marker, a few disorganized strands of what appear to be tendon attached further proximally. We interpret these fibres as newly formed fibres, and we only measured the well-defined tendon. The same situation was found in a few of the animals in the experiments described here, and we treated them the same way. Thus we are confident that we are seeing migration, not regeneration.

A two-way analysis of variance, accepting unequal numbers of subjects, and an unweighted-means approach described by Winer (1962) were used to evaluate data on muscle weights and tibial lengths. A one-way analysis of variance was used to analyse the data on muscle migration. The Scheffé *post hoc* test was used to determine significant differences between groups.

#### RESULTS

Analysis of the data on pre- and post-experimental lengths of the tibia, and on weights of the muscles, showed no significant differences between left and right side of the same animal, or between groups. These findings indicate that the experimental effects were localized, and did not significantly alter the growth of the tibia or of the experimental muscles. This was subjectively confirmed by the observation that none of the animals suffered any obvious locomotor deficits as a result of the experimental procedures.

Results on migration distances are shown in Table 2 and Figure 2. Comparing the distances that the M. semimembranosus migrated during the experiment, we found a number of significant differences. One-way analysis tests between the various subgroups of Group 0 (i.e. the left legs of animals whose right legs formed Groups C, 5, 10, 20) showed no significant differences and therefore indicated that we could use this as a group. Significant differences were found between Groups C and 0, 5 and 0, 10 and 0, and between Group 20 and all others. There were no significant differences between Groups 5 and 10 or C and 10. However, it is our opinion that such statements do not adequately reveal the pattern of the results. It would appear that a definite relationship exists between the distance that the tendon was moved distally from its original site of insertion and the distance it subsequently migrated proximally. The further that the tendon is moved, the further proximally it migrates. In fact, a tendon experimentally moved 20 mm distally actually migrates proximally almost twice the distance that a muscle continuously attached throughout the duration does (Fig. 2).

The correlation ( $r$ ) between the distance moved experimentally and the distance migrated is 0.86. Regression analysis gives a regression coefficient of distance migrated on distance moved of  $0.99 \pm 0.10$  ( $Y = 0.99 X + 2.35$ ). Although casual

Table 2. Migration distances

Group	Number	Mean distance migrated	Range	Standard deviation
C	11	11.1	7.8-13.5	2.20
0	19	3.1	0-7.8	2.48
5	4	4.7	3.3-5.8	1.07
10	5	9.7	2.5-18.3	6.44
20	5	23.9	11.7-28.8	7.21

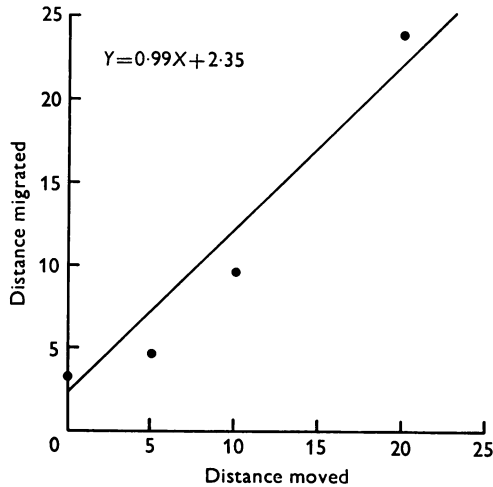


Fig. 2. Regression of the distance migrated by the *M. semimembranosus* in the experimental groups on the distance the muscle was translocated distally. All values in mm. Points are mean values for each group.

examination of Figure 2 suggests the possibility of non-linearity, a test for non-linearity does not support this suggestion. Thus it appears as if the muscle migrates 1 mm proximally for every 1 mm it is transplanted distally.

#### DISCUSSION

The results were not what we had expected. The further distally a muscle was moved experimentally, the greater its proximal migration. But a small distal displacement resulted in less migration than in control animals, while a large displacement resulted in more. Since it is difficult for us to conceive of some local inhibitory factor near the original site of insertion that would affect only the experimental muscles but not the other muscles, we are left with the conclusion that the phenomena described are probably controlled by more than one factor.

Although we were not able to measure the growth of the proximal end of the tibia during the experiment, the average growth of the whole tibia was 26.7 mm (from 83.6 to 110.3 mm). The growth curves of Heikel (1959) show that for his rabbits the

proximal end of the tibia contributed about 58% of the total tibial growth over the period from 50 to 200 days. The growth curves of McCormick, Lowe & Ashworth (1972) show that for their rabbits, from 1000 to 3500 g, which is about the same age range as for Heikel's rabbits, the proximal end of the tibia contributed about 52% of total tibial growth. From these data, and realizing that the breeds and conditions are not identical, we think that as a rough estimate we can say that probably in our rabbits the proximal tibia contributed about 55% of the total growth, i.e. about 14.7 mm. It is interesting to note that this figure is only slightly greater than the average distance migrated by the muscles of the Group C control animals. This relationship is similar enough to the relationship between distance moved experimentally and distance migrated for the suggestion to be made that the growth of each end of each bone may itself in some way influence the direction, amount and timing of migration of the attached structures. But in experimental situations, the muscle may in some way also influence the migration, and the interaction between these multiple factors will probably prove to be quite complex. We are presently engaged in further work on this problem.

#### SUMMARY

The insertion of the *M. semimembranosus* was translocated distally through distances of 0, 5, 10, and 20 mm in 8 weeks old rabbits. Markers were placed in the bone to allow measurements of the distance that each muscle migrated during growth. Rabbits were sacrificed after 3 months. The results showed that the further distally a muscle is moved, the further it will migrate proximally during this time. This may indicate that there are factors other than, or in addition to, the growth of the bone that control migration.

This study was partially supported by Academic Year Research Award, University of Oregon.

#### REFERENCES

- COMER, R. D. (1956). An experimental study of the 'laws' of muscle and tendon growth. *Anatomical Record* **125**, 665-681.
- CRAWFORD, G. N. (1950). An experimental study of tendon growth in the rabbit. *Journal of Bone and Joint Surgery* **32B**, 234-243.
- CRAWFORD, G. N. (1954). An experimental study of muscle growth in the rabbit. *Journal of Bone and Joint Surgery* **36B**, 294-303.
- FORWARD, A. D. & COWAN, J. (1963). Tendon suture to bone. *Journal of Bone and Joint Surgery* **45A**, 807-823.
- HEIKEL, H. V. A. (1959). On ossification and growth of certain bones of the rabbit; with a comparison of the skeletal age in the rabbit and in man. *Acta orthopaedica scandinavica* **29**, 171-184.
- HUGHES, H. (1956). An experimental study of the postnatal growth of tendon. *Anatomischer Anzeiger* **103**, 331-339.
- KERNWEIN, G. A. (1942). A study of tendon implantation into bone. *Surgery, Gynecology and Obstetrics* **75**, 794-796.
- MCCORMICK, J. J., LOWE, P. J. & ASWORTH, M. A. (1972). Analysis of the relative contributions of the proximal and distal epiphyseal plates to the growth in length of the tibia in the New Zealand White rabbit. *Growth* **36**, 133-144.
- PULVERTAFT, R. G. (1956). Tendon grafts for flexor tendon injuries in the fingers and thumb; a study of technique and results. *Journal of Bone and Joint Surgery* **38B**, 175-194.
- VIDEMAN, T. (1970a). An experimental study of the effects of growth on the relationship of tendons and ligaments to bone at the site of diaphyseal insertion. Part II. Determination of growth patterns and inhibition of displacement using metal markers. *Annales chirurgiae et gynaecologiae Fenniae* **59**, 22-34.

- VIDEMAN, T. (1970*b*). An experimental study of the effects of growth on the relationship of tendons and ligaments to bone at the site of diaphyseal insertion. *Acta orthopaedica scandinavica, Supplementum* **131**, 1-22.
- WARWICK, W. T. & WILES, P. (1934). The growth of periosteum in long bones. *British Journal of Surgery* **22**, 169-174.
- WHITE, A. A., PANJABI, M. M. & HARDY, R. J. (1974). Analysis of mechanical symmetry in rabbit long bones. *Acta orthopaedica scandinavica* **45**, 328-336.
- WINER, B. J. (1962). *Statistical Principles in Experimental Design*. San Francisco: McGraw-Hill.