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Shrinkage of muscle fibres during the fixation of cadaveric tissue

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INTRODUCTION

Whenever the properties of biological tissue are studied, work often has to be carried out on tissue that has been preserved in some way. This investigation was undertaken in conjunction with work on the sarcomere lengths of human muscle (Cutts, 1988). Whilst it is reasonable to accept that a muscle brought to a position and held there by rigor mortis has the same sarcomere length as that when the muscle has been brought to that position by an active contraction (Bendall, 1973), it is quite possible that a major change in muscle length may have taken place during fixing. There appears to be no documentation as to how much, if any, muscle shrinkage occurs when cadavers are fixed in embalming fluid. The purpose of this investigation was to determine whether or not a correction factor needs to be applied to the sarcomere length of human embalmed tissue in order to extrapolate the results to living tissue. Dimery (1985), in a study of sarcomere length during rabbit locomotion, eliminated the possibility of the influence of shrinkage on her results by stitching the muscle fibres to a matchstick whilst fixation was taking place, but such a procedure would not be possible with human cadaveric tissue, which is only available when already fixed. The positions of the muscles relative to the skeleton in the study of human sarcomere length means that the skeleton cannot be relied upon to preserve the lengths, as was the case in an investigation of bird flight muscle sarcomere lengths (Cutts, 1986). In this case, the majority of the muscle length was attached to the skeleton, making shrinkage virtually impossible.

MATERIAL AND METHODS

The following muscles were dissected from a freshly amputated human leg, removed because of gangrene in the foot: extensor digitorum longus, extensor hallucis longus, peroneus longus, tibialis anterior, soleus, gastrocnemius. The lengths of the contractile portions of the muscles were measured, then the muscles were immersed in standard embalming solution, comprising 17 parts industrial methylated spirit, 4 parts liquid phenol, 2 parts glycerine and 1 part 40% formalin. The muscles were injected with this solution to ensure thorough distribution through the tissue, then left for three days, the length of time that the cadavers used in the sarcomere length investigation were allowed to fix. The muscles were then removed from the embalming fluid and measured again, before being stored in airtight bags under refrigeration at 5 °C, simulating the conditions of storage of whole cadavers. A standard cadaver used for dissection may legally be stored for up to two years, but most are used within a few months. The possibility of further shrinkage during the storage period was investigated by measuring the muscle lengths again after one and two months storage.

To determine whether the shrinkage varied between muscles fixed intact on the skeleton and those fixed in isolation, the hind quarters of four laboratory rats (*Rattus norvegicus*) were obtained. The animals were painlessly destroyed to obtain material for another investigation by a colleague, who donated the hind quarters before disposal of the bodies. It was intended to investigate the effects of removing the muscles from the skeleton prior to fixation by comparing muscle lengths from the right and left hind limbs of the same animal after the muscles of one leg had been fixed *in situ* and the muscles of the other leg in isolation. A preliminary investigation was carried out to determine if there was any significant difference between the muscle lengths of the left and right hind limbs. Four muscles were dissected from each hind limb of two of the rats; the lengths of these were measured and compared statistically.

Once it had thus been established that a comparison between two methods of fixation could be made by using a different method on each hind limb, the corresponding four muscles were removed from the right leg of the two remaining rats and immersed in standard embalming fluid for 3 days. The remaining parts of the hind quarters of the two rats, including the musculature of the left leg, were also immersed in the same embalming fluid. After 3 days, the isolated muscles were removed from the fixative and their lengths measured. The remaining parts of the hind quarters were removed from the fixative after the same period of time; the muscles were dissected from them and the muscle lengths measured. The muscle lengths after fixing on and off the skeleton were then compared statistically.

RESULTS

The muscle lengths before and after fixation and after the various storage periods are shown in Table 1. These values refer to the contractile portion of the muscles rather than the entire muscle, including the tendon. The mean shrinkage of the muscles during the initial fixing period is 2·22 %. A statistical comparison using the paired t test between the muscle lengths before and after fixation shows a significant loss in length ($P(t \geqslant 2{\cdot}905$ with 5 D.F.$) < 0{\cdot}02$). To determine if further significant shrinkage took place during storage after the initial fixation period, an analysis of variance was carried out using the muscle lengths after fixation and at the various storage periods as reported in Table 1. This indicated no further significant change in length throughout storage ($F = 0{\cdot}029$, $P > F$).

Table 2 shows the lengths of the muscles of the left and right limbs of two laboratory rats. A paired t test carried out on the combined data from both animals gave $0{\cdot}15 < P(t \geqslant 1{\cdot}254$ with 7 D.F.$) < 0{\cdot}10$, indicating no significant difference between the muscle length in one limb compared to the other in the same animal.

Table 3 shows the muscle lengths of the right hind limbs of two rats before fixing and after they had been fixed in isolation from the skeleton. The lengths of the left hind limb muscles are shown after fixing on the skeleton. Before fixation these are assumed to be the same, or not significantly different from, the prefixing lengths of the corresponding muscles from the right hind limb. Student's t test shows that there is a significant decrease in muscle length if the muscles are fixed in isolation from the skeleton ($P(t \geqslant 2{\cdot}627$ with 7 D.F.$) < 0{\cdot}027$), with a mean shortening of 1·06 %. If the muscles are fixed whilst still *in situ* on the skeleton the muscle shrinkage is obviously not significant. A statistical analysis of this situation is precluded by the cases where (*length before fixing* − *length after fixing*) yields a positive value. These must be due to a slight difference in muscle length between the left and right legs.

Table 1. *Human muscle lengths before and after fixing*

Muscle	Length of contractile portion (mm)			
	Before fixing	After 3 days	After 1 month	After 3 months
Extensor digitorum longus	325	315	314	311
Extensor hallucis longus	240	230	230	227
Peroneus longus	295	295	292	292
Tibialis anterior	265	265	260	260
Soleus	355	350	348	348
Gastrocnemius	215	205	203	203

Table 2. *Muscle lengths in right and left legs of two rats*

Muscle	Length of contractile portion of muscle (mm)			
	Rat 1		Rat 2	
	Left leg	Right leg	Left leg	Right leg
Rectus femoris	31·4	31·5	30·8	30·6
Gastrocnemius	26·2	26·3	26·3	26·4
Soleus	24·8	24·7	24·6	24·8
Tibialis anterior	24·1	24·2	24·4	24·6

Table 3. *Rat muscle lengths before and after fixing*

Muscle	Length of contractile portion of muscle (mm)		
	Before fixing	After fixing off the skeleton	After fixing on the skeleton
Gastrocnemius (1)	25·6	25·0	25·5
Soleus (1)	23·5	23·0	23·5
Tibialis anterior (1)	22·0	22·0	22·0
Rectus femoris (1)	23·5	23·5	23·5
Gastrocnemius (2)	26·0	25·5	26·5
Soleus (2)	23·0	23·0	23·0
Tibialis anterior (2)	23·0	22·5	23·0
Rectus femoris (2)	22·0	22·0	23·0

DISCUSSION

When isolated muscles of both the rat (*R. norvegicus*) and the human hind limbs were fixed in a standard embalming fluid, a small but significant decrease in their length was observed. In the rat, a further experiment showed that if the muscles are fixed whilst still intact on the skeleton, the degree of shrinkage is non-significant. Since the amount of shrinkage in both rat and human muscle when fixed in isolation from

the skeleton is of the same magnitude (1·06% and 2·22% in the two species respectively), it is reasonable to assume that human muscle does not shrink significantly during fixation whilst on the skeleton. Since the investigation for which this information was required was carried out on cadaveric tissue which had been fixed *in situ* on the skeleton, it was concluded that it was not necessary to use any correction factor for shrinkage when extrapolating data from cadavers to living tissue.

SUMMARY

It has been shown that a small but significant loss in length occurs in human muscles which are fixed after removal from the skeleton. A comparison was made between the loss in muscle length when muscles were fixed in isolation from, and whilst still attached to, the skeleton in the rat. The conclusion was that no significant loss of length occurs when the muscles were fixed intact on the skeleton. Since the length loss when muscles are fixed independently of the skeleton in both the rat and the human is of the same order, it is reasonable to assume that when human muscles are fixed on the skeleton, no significant loss in length occurs. Since all human cadaveric tissue is fixed whilst on the skeleton, we may assume that shrinkage of the muscles in such specimens is negligible.

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