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Why does rigor mortis progress downwards?

-Masahiko Kobayashi Department of Forensic Medicine Saitama Medical School JAPAN Fax: +81-49-294-9713 mkob@saitamamed.ac.jp

Abstract

Determinants of the downward sequence of rigor mortis are not clear, although rigor mortis is an important postmortem change referred to by forensic pathologists in estimating the postmortem interval. We determined postmortem changes in the tension of some muscles mounted in liquid paraffin at 37°C or 25°C, and found that the course of rigor mortis was affected by the proportion of muscle fiber types in the muscles and by temperature. These factors could be causes of the downward progress of rigor mortis.

Key Words

Rigor mortis, Muscle fiber type, Temperature

Introduction

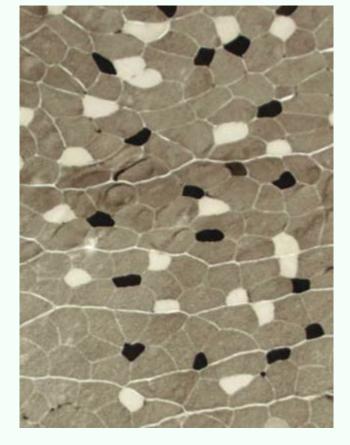


Fig.1: Frozen section of extensor digitorum longus muscle of rat stained with ATPase enzyme histochemistry (pH 4.35-4.45). Muscle fibers are identified as Type I, IIA, or IIB by dark, light, or intermediate staining, respectively.

Rigor mortis is one of the important postmortem changes which forensic pathologists refer to in estimating postmortem interval ¹⁻³. Nysten first reported the downward progress of rigor mortis in human cadavers in 1812 ⁴. Many forensic pathologists have verified this tendency, although it may not be applicable to all cadavers. Some hypotheses about the determinants of the downward progress of rigor mortis have been proposed. The most famous hypothesis among these was Shapiro's theory in 1950 ^{5,6}. He suggested that rigor mortis would occur much more rapidly in small muscles than in large muscles because rigor mortis progressed similarly in all muscles. Therefore, he thought that a relatively small joint, such as the temporomandibular joint, surrounded by a moderate amount of muscle would become immobilized sooner than a large joint surrounded by

a relatively large mass of muscle. When he proposed this hypothesis, little

was known about muscle. Therefore his persuasive hypothesis was

completely based on his own experiences and imagination. Since Shapiro's

proposal, mechanisms of muscle contraction have been progressively

clarified. For example, depletion of adenosine triphosphate (ATP) is now regarded as the main cause of rigor mortis. However, no one has experimentally tested Shapiro's hypothesis. We studied the sequence of rigor mortis ⁷⁻¹⁰. In this paper, we review our findings and propose possible determinants of the sequence.

First, we examined levels of adenosine nucleotides and lactic acid in rat five kinds of muscles removed immediately after death from one side of rats, and 2 hours after death from the other side of the same rats, to investigate whether the postmortem biochemical changes in the muscle would progress similarly in all the muscles as Shapiro's theory suggested. The results indicated that the postmortem changes in rat muscle were different between muscles. Next, we investigated determinants of the difference in postmortem changes between muscles, thinking that the proportion of muscle fiber types might influence rigor mortis.

Muscle fibers are divided into several types, mainly by enzyme histochemistry. Each type has its own metabolic, morphological, and physiological features ^{11, 12} as Table 1 shows. Muscles consist of a mosaic of these types of fibers as shown in Fig. 1 (above and to the left). In rats, red muscles consist mainly of Type I or IIA muscle fibers. Type IIB fibers predominate in white muscles. The proportion of fiber types differs according to the kind or part of the muscle ¹³⁻¹⁷. However, the proportion in a kind or

Fiber type	Type I	Type IIA	Type IIB			
Energy metabolism	Oxidative phosphorylation	Both	Glycolysis			
Myoglobin content	High	High	Low			
Mitochondria	Many	Many	Few			
Glycogen content	Low	High	High			
Table 1: Properties of skeletal muscle fiber						

part of muscle does not differ very much between individuals. For example, soleus is a representative of red muscles, in which Type I muscle fibers predominate, for almost all individuals.

In order to compare postmortem changes of several muscles, we examined muscles from rat cadavers. To do this we developed an experimental model of postmortem skeletal muscle which was soaked in liquid paraffin to prevent drying and diffusion across the muscle surface. Postmortem changes in tension, one index of the dynamic changes in rigor mortis, of the muscle samples were determined at 37°C or 25°C.

Materials and methods

Muscle samples

Nine-month-old male Sprague-Dawley rats were used in the experiments. They were injected with a total of 200-250 mg/kg mephenesin intraperitoneally and movement of all skeletal muscles except those used in breathing was stopped for 30 min to exclude the influence of antemortem muscle exercise ¹⁸. The rats were then killed by deep anaesthesia with diethyl ether. Red gastrocnemius (RG), white gastrocnemius (WG), soleus (SO), and erector spinae (ES) muscles were used. RG and WG are the deepest and most superficial portions of the medial head of the gastrocnemius muscle, respectively. The proportions of muscle fiber types in the muscles were determined with ATPase staining ¹⁹⁻²¹.

The muscles were removed immediately after the rats were killed. Strip sections 1.0-1.2 cm long and 0.4 cm thick were cut parallel to the muscle fibers and mounted in liquid paraffin.

Muscle	Type I	Type IIA	Type IIB
Red gastrocnemius (RG)	38.2 ± 17.5	43.1 ± 16.1	18.8 ± 12.0
White gastrocnemius (WG)	0.0 ± 0.0	0.1 ± 0.4	99.9 ± 0.4
Soleus (SO)	82.4 ± 6.8	13.3 ± 5.9	4.3 ± 2.6
Erector spinae (ES)	6.6 ± 5.7	10.5 ± 2.6	82.9 ± 6.1

Table 2: Average areal proportion of fiber types (± standard deviation) in red gastrocnemius (RG), white gastrocnemius (WG), soleus (SO), and erector spinae (ES) muscles of male Sprague-Dawley rats.

Measurement of rigor mortis

The preparations were mounted vertically in a 20 ml water-jacketed tissue bath, containing liquid paraffin (Wako, Osaka, Japan), and the temperature was kept at 37°C or 25°C. ES was tested only at 37°C. The lower end of the preparation was attached to the bottom of the bath, the other end was connected to an isometric sensor (Star Medical, IM-20BS, Tokyo, Japan) by silk threads. The isometric tension was recorded (Graphtec, Thermal arraycorder WR 7300, Yokohama, Japan) using a preamplifier (Star Medical PA-001, Tokyo, Japan). The first 10 min after death were used to prepare the samples and to set up the apparatus. No measurements were made during this time. At the start of the measurements, the muscle was stretched by 50 mN to tense the thread and enable sensitive measurement of the tension. Change of tension was measured

types.

and recorded for 8 h for each sample.

Statistical analysis

There were four samples of each muscle at each temperature. For each sample the measured tension was expressed relative to the maximum tension found during the 8 h of measurement. Factorial analysis of variance (ANOVA) was used to determine significant differences in the postmortem interval at which the tension reached its peak. In addition, we analyzed the times at which the tension increased to 10%, 50% and 100% of the maximum and the times in which the tension decreased from 100% to 70% and 50% at 37° C. Spearman's correlation coefficients (two-tailed) between the times and the average proportion of Type IIB muscle fibers in the muscles were calculated.

Results

RG and SO were regarded as red muscles in which Type I or IIA red muscle fibers predominated, and WG and ES were white muscles, as shown in Table 2. The tension was recorded as demonstrated in Fig. 2. In all muscle samples, tension increased and then decreased over the 8 h postmortem period.

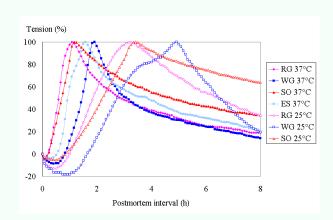


Fig. 2. Average tension as a percentage of the maximum tension in the course of rigor mortis in red gastrocnemius (RG), white gastrocnemius (WG), soleus (SO), and erector spinae (ES) muscles of male Sprague-Dawley rats at $37^{\circ}\mathrm{C}$ and $25^{\circ}\mathrm{C}$ (Click figure to enlarge)

The tension reached a peak sooner in RG and SO than in ES, and earlier in ES than in WG. The time course of the progress of rigor mortis was faster in red muscles than in white muscles at 37 °C as the significant positive

correlation between the postmortem intervals in which rigor tension reached 10%, 50% and 100% of the maximum and the areal proportion of Type IIB white muscle fiber demonstrated (Table 3). However, resolution of rigor mortis was faster in white muscles than in red muscles as the significant negative correlation indicated (Table 3).

The progress of rigor mortis was slower at 25°C than at 37°C. Tension reached a peak sooner in RG than in SO, and earlier in SO than in WG at 25°C, and the resolution was fast in WG at 25°C. Thus, at 25°C the tendency that rigor mortis in red muscles progressed fast and resolved slowly was also seen at 37°C.

Discussion

The effect of muscle fiber types and temperature on rigor mortis

Rigor mortis progressed more rapidly in red muscles than in white muscles as the positive correlation between time for rigor mortis to progress and areal proportion of Type IIB white fibers demonstrates. Differences in the rigor mortis between these muscles are reflected by differences in rigor mortis between the predominant muscle fibers, but the cause of rapid progress of rigor mortis in red muscle fiber is unknown. Depletion of ATP, which facilitates rigor mortis, would be faster in red muscle than in white muscle. It is possible that postmortem production of ATP would be less in red muscle than in white muscle because red muscle fibers contain less

glycogen than white muscle fibers.

	Progress			Resolution	
	0-10%	0-50%	0- 100%	100- 70%	100- 50%
Spearman's correlation coefficient	0.839**	0.802**	0.809**	0.814**	0.747**

Table 3. Spearman's correlation coefficients (two-tailed) between the average proportion of Type IIB muscle fibers and the times in which tension increased to 10%, 50% and 100% of the maximum and in which tension decreased from 100% to 70% and 50% at 37°C.

** p<0.01

Resolution of rigor mortis was faster in white muscles, in which rigor mortis progressed slowly, than in red muscles. Rigor mortis could be resolved by proteases which would be activated by high levels of intracellular Ca²⁺. However, it is not known whether postmortem levels of Ca²⁺ are different between red and white muscle. More experiments are needed to investigate this. It is well-known that temperature influences the progress of rigor mortis. This was also demonstrated in our experiments where rigor mortis progressed and resolved much faster at 37°C than at 25°C.

Rigor mortis in human cadavers

Type I red muscle fibers in human masticatory muscles are unique and different from ones in other muscles ²²⁻²⁴. Type I muscle fibers have about twice the diameter of Type II white fibers in human masticatory muscles. This feature is not seen in the other human muscles or rat masticatory muscles. The areal proportion of Type I muscle fibers in human masticatory muscles is high in spite of their low proportion numerically. Therefore, rigor mortis in human masticatory muscles would progress faster than in the other muscles. This would lead to early fixing of the temporomandibular joint in human cadavers.

Distal extremities cool fast after death. This would result in the relatively slow progress of rigor mortis in the distal extremities. Therefore, the effect of temperature might be one of the determinants in the sequence of rigor mortis.

Moreover, we think that differences in morphological and dynamic features between joints might strongly affect the speed of progress of rigor mortis. In particular, the temporomandibular joint has a special form. The distance between the joint and the point in the mandible where the masseter or temporalis muscle is attached is relatively long compared with the length of the bone. This characteristic of the temporomandibular joint will result in early fixing of the joint by rigor mortis. However, we have not examined in detail the morphological differences between joints, and this must be analyzed to know whether this is one of the determinants of the sequence of rigor mortis.

We know that Nysten's law is not applicable to all human cadavers, but it is possible to say that rigor mortis tends to progress downwards. Especially, the

What is already known on this topic

Downward progress of rigor mortis - Nysten's law - is usually valid in human cadavers in spite of some exceptional cases. Although this is an important characteristic of rigor mortis which is used to determine postmortem interval, its causes or determinants are unknown.

In 1950, Shapiro proposed the most well known and important hypothesis to explain it (see text). He regarded muscle mass as the determinant of Nysten's law and proposed that rigor mortis progressed more rapidly in small muscles than in large ones. The presupposition of this hypothesis was that rigor mortis progressed simultaneously in all the muscles. However, it is difficult to understand Shapiro's hypothesis scientifically and clearly.

onset of rigor mortis in the temporomandibular joint is usually earlier than in the other joints of the extremities. The determinants of this tendency would be the differences in the morphological characteristics in the joints, the proportion of muscle fiber types, and postmortem changes in temperature between muscles. Through scientific evidence we must clearly demonstrate these factors as being the significant determinants. Moreover, we investigated only postmortem changes in the tension of muscles, but this is only one of the indexes of rigor mortis. Synthetic analysis of rigor mortis is under investigation through determination of postmortem changes in stiffness, another index, and phosphates including ATP and creatine phosphate.

Conclusion

Rigor mortis progressed more rapidly in red muscles than in white muscles, and more rapidly at 37 °C than at 25 °C. The proportion of muscle fiber types and temperature might be determinants of the downward progress of rigor mortis in the human cadaver but more scientific evidence or experiments are needed for further clarification.

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Currently, rigor mortis has been studied mainly in animal husbandry in connection with the quality of meat. The quality of fish meat is of particular interest in Japan because of the fact it is often eaten raw. Fishermen have reported that progression of rigor mortis in fish can be delayed by destruction of the spinal cord, and attempts to verify this experimentally are now being conducted in fisheries. In humans, the distances between the brain and the various muscles were also thought to be a determinant of Nysten's law, but as yet, there is no experimental or scientific proof of this.

What This study adds

The present study revealed that the proportion of muscle fiber types in muscles is related to the progression of rigor mortis. Rigor mortis progressed more rapidly in red than in white muscles, and this study was the first to demonstrate such a difference between muscle types. Consequently, it may be necessary to reconsider Shapiro's hypothesis that rigor mortis progresses in all muscles simultaneously.

In human masticatory muscles, such as the masseter and temporalis, there are special red muscle fibers whose diameter is twice that of white muscle fibers. This feature would lead to rapid progression of rigor mortis in masticatory muscles. The relationship between rigor mortis progression and temperature has been pointed out previously, and this was confirmed again in the present study. The author thinks that rapid postmortem cooling of the comparatively thin forearms and crura would delay rigor mortis in these parts of the body.

In conclusion, it is considered that the results of this study can explain the rapid progression of rigor mortis in the temporomandibular joint and slow progression in the forearms or crura. The present findings suggest that differences in the proportions of muscle fiber types and postmortem differences in the rate of temperature change between muscles are some of the determinants of Nysten's law.

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Masahiko Kobayashi,

38 Morohongo, Moroyama, Saitama 350-0495, Japan

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