# SUPRAVITAL EXCITABILITY OF SKELETAL MUSCLE OF RATS AND BULLFROGS TO ELECTRICAL STIMULI

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#### Abstract

Supravital excitability to electrical stimuli (for convenience, supravital electrocontractility (S.Ec)) of the gastrocnemius muscle of rats and bullfrogs was examined after "somatic death" and the time during which S.Ec could be detected (S.Ec duration) was measured. S.Ec of the gastrocnemius muscle of rats and bullfrogs depended on temperature. The maximum S.Ec duration of the muscle of rats and bullfrogs were 110 min and 96 hr, respectively, at a low temperature (5°), and 60 min and 9 hr, respectively, at a high temperature (30°).

The time course of rigor mortis at 5° was slow and it was rapid at 30°. In rats, the S.Ec disappeared completely before the onset of rigor mortis, and in bullfrogs, S.Ec existed at progressive stages of rigor mortis and it disappeared completely when rigor mortis reached about the maximum.

#### Introduction

Experimental investigations on supravital excitability to electrical stimuli (for convenience, supravital electrocontractility (S.Ec)) of human skeletal muscles have been carried out by Popwassilew and Palm,<sup>1)</sup> Prokop,<sup>2)</sup> Schleyer.<sup>3)</sup> Furuya<sup>4–7)</sup> reported that S.Ec depended on the kind of animals and also the way of killing animals.

In the present experiment, the changes of S.Ec of gastrocnemius muscle of rats and bullfrogs after "somatic death" and the relation between S.Ec and temperature or rigor mortis of these muscles were investigated, in order to examine the process from "somatic death" to "cell death."

## MATERIALS AND METHODS

Materials: Gastrocnemius muscles of male Wistar rats (body weight, 200–250 g)

and bullfrogs (body weight, 250–300 g) were used

Methods: Both rats and bullfrogs were killed by decapitation; the spinal cord of bullfrogs was destroyed by a wire. Experiments were carried out at low temperature  $(5^{\circ})$ , room temperature  $(20^{\circ})$ , and high temperature (30°). Electric stimuli were given to gastrocnemius muscles by an electronic stimulator (MSE-20, Nihon Kohden).4) The intensity of S.Ec was observed with naked eye and the intensity was graded from - to +++; -, none; +, slight; ++, moderate; +++, intense S.Ec. Postmortem physical changes (extensibility) was measured by mean of a mechanical transducer according to the method of Shintaku.8,9) Muscles were loaded with 500 g tension, and the value of extensibility measured immediately after death was taken as 100%.

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Temp. (°C)	Postmortem lapse of time (min)											
remp. (C)	40	50	60	70	80	90	100	110	120			
5	+++(3)	+++(3)	+++(3)	+ (2) - (1)		-(2) + (1)		. ,	<b>—</b> (3)			
20	+++(3)	+++(3)		+++(2) + +(1)	,	,	٠,	<b>—</b> (3)				
30	+++(3)	+++(2) + (1)	+ (2) $- (1)$	— (3)								

Table 1. Postmortem changes of the supravital excitability of gastrocnemius muscle of rats to electrical stimuli (at 5°, 20°, and 30°)

Supravital excitability to electrical stimuli (S.Ec); - none, + slight, ++ moderate, +++ intense. Figures in parentheses indicate the number of experimental samples.

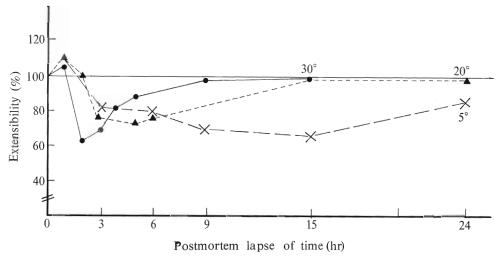


Fig. 1. Postmortem changes of the extensibility of gastrocnemius muscle of rats (at  $5^{\circ}$ ,  $20^{\circ}$ , and  $30^{\circ}$ ).

### RESULTS AND DISCUSSION

## a) Gastrocnemius muscle of rats

Postmortem changes of S.Ec are summarized in Table 1. After rats were killed, electric stimulus was given to gastrocnemius muscle in order to measure the contraction of these muscles (S.Ec). S.Ec decreased gradually after "somatic death", and finally disappeared in "cell death". The maximum S.Ec durations were 110, 100, and 60 min

at 5°, 20°, and 30°, respectively.

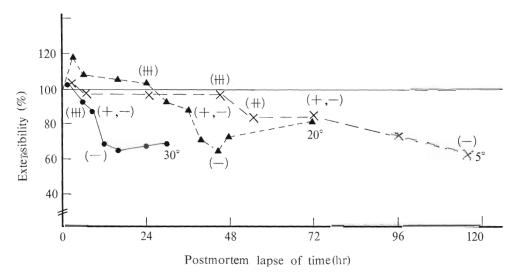
The time course of rigor mortis (at 5°, 20°, and 30°) are summarized in Fig. 1. At each temperature, the primary flaccidity of muscles was observed at 1 hr postmortem. The maximum rigor mortis appeared at 15, 5, and 2 hr postmortem at 5°, 20°, and 30°, respectively. Secondary flaccidity occured after maximum rigor mortis.

b) Gastrocnemius muscle of bullfrogs Postmortem changes of S.Ec is sum-

Temp.		Postmortem lapse of time (hr)												
	3	6	9	12	16	24	30	36	40	45	54	72	96	116
5	## (5)	## (4)				## (4)				## (6)	++ (4)		-(5) + (1)	<u></u> – (6)
20	## (4)	## (6)			## (6)	## (6)	# (6) — (2)		<b>-</b> (8)	<u>— (8)</u>	<b>—</b> (8)			
30	## (4)		# (4) - (2)	<b>-</b> (4)	<u>— (4)</u>	<b>—</b> (4)								

Table 2. Postmortem changes of the supravital excitability of gastrocnemius muscle of bullfrogs to electrical stimuli (at  $5^{\circ}$ ,  $20^{\circ}$ , and  $30^{\circ}$ )

Supravital excitability to electrical stimuli (S.Ec); — none, + slight, # moderate, ## intense. Figures in parentheses indicate the number of experimental samples. Underlined figures show maximum of rigor mortis.



Degree of S.Ec is indicaued in parentheses

Fig. 2. Porstmortem changes of the extensibility and supravital excitability (S.Ec) of gastrocnemius muscle of bullfrogs (at 5°, 20°, and 30°).

marized in Table 2. S.Ec of gastrocnemius muscle of bullfrogs was examined by the same method as in rats. Responses to the stimuli lasted for much longer periods; the maximum S.Ec time was 96, 36, and 9 hr at 5°, 20°, and 30°, respectively.

The time course of rigor mortis observed at 5°, 20°, and 30° is summarized in Fig. 2. The primary flaccidity of muscles was ob-

served 3 hr postmortem at 5° and 20°, and 1 hr postmortem at 30°. Rigor mortis was observed to develope slowly thereafter. The maximum rigor mortis was observed 115, 45, and 16 hr postmortem at 5°, 20°, and 30°, respectively. The secondary flaccidity occured after the maximum rigor mortis.

From the above results, it is thought that in rats, muscle fibers keep alive for 60–110

min after "somatic death" and, thereafter, "cell death" and rigor mortis develope.

In bullfrogs, S.Ec existed even during the progressive stage of rigor mortis and it disappeared completely when rigor mortis reached about the maximum. Also, even in the state of progress of irreversible deformation (rigor mortis), the fact that S.Ec exists and progressively weakened in the muscle shows that muscle contained both living and dead muscle fiber.

It is thought that the muscle fibers in the dead are in progress of rigor mortis and the muscle fibers in a living organism have S.Ec in it. At 5°, this state remained for a longer period of time.

Rigor mortis progressed as +/- ratio of S.Ec of muscle fibers became smaller, and S.Ec disappeared when the rigor mortis reached the maximum. It is belived that all muscle fibers reached death at this point.

This way of thinking will not be inconsistent with presence of S.Ec even in the state of progress of rigor mortis.

From above results, it is thought that the muscle fiber of rats reach death earlier and that the muscle fiber of bullfrogs live for a longer period of time.

## CONCLUSION

In the present experiment, the changes of S.Ec of gastrocnemius muscle of rats and bullfrogs after "somatic death" were examined, and the relation between S.Ec and temperature or rigor mortis of these muscles was investigated. The findings were as follows:

- 1) After "somatic death", S.Ec decreased gradually and finally disappears, arriving at "cell death".
- 2) When S.Ec and the time course of rigor mortis were compared in bullfrogs, S.Ec existed at progressive stages of rigor

mortis and disappeared completely when the rigor mortis became about maximum. In rats, S.Ec disappeared completely at an early postmortem stage. This indicated that the muscle fibers of bullfrogs live for longer period of time than those of rats.

3) S.Ec of gastrocnemius muscle of rats and bullfrogs depended on temperature. At low temperature, S.Ec remained for a longer period of time.

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