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## Isometric Twitch Tension of Frog Skeletal Muscle as a Function of Temperature

Current work at this laboratory on the action of high-intensity ultrasound on the twitch contraction of excised frog skeletal muscle makes it necessary to distinguish between temperature and nontemperature effects produced by the sound. Therefore a study (*1*) was undertaken to determine the variation in the magnitude of the maximum isometric tension and the action potential of excised frog biceps muscle at a number of temperatures in the range 2° to 35°C. Unfortunately, in the recent literature there appears little data on frog skeletal muscle twitch tension at temperatures above 25°C. Walker (*2*), working at the two isolated temperatures 14.5° and 27.5°C found different results for *in situ* summer and winter frogs. His experiments on excised muscles produced variable results, with an indication that twitch tension is less at the lower temperature. Hill's well-known results (*3*) are restricted to temperatures below 22°C; Buchthal's results (*4*) on single fibers are limited to temperatures up to 26°C. The work of early investigators, referenced later in this report, yielded variable results.

The following procedure was used in a series of 32 experiments. At an initial temperature of approximately 20°C, the muscle was stimulated once every 10 seconds for a period of 2 minutes, while the action potential and isometric tension were recorded. The Ringer's solution was then siphoned from the bath container, and a solution at a new temperature was added. After an acclimatization period of 3 minutes at this new temperature, the muscle tension and action potential were again recorded. This procedure was repeated at a number of different temperatures, after which the muscle was returned to the initial temperature of 20°C to check reversibility. All muscles were tested at the experimentally determined "rest length" position. Both curarized and noncurarized preparations were studied. An RCA 5734 transducer tube, in conjunction with an amplifier and recorder, was used to measure the muscle tension. Isometric operating conditions were insured by the use of a double-spring system in association with the transducer tube shaft. A copper constantan thermocouple, inserted through the center of some of the muscles, indicated the internal temperature. The action potential was recorded by photographing the trace of an oscilloscope screen.

Electrodes, consisting of silver-plated Nichrome pins inserted completely through the muscle, were used for both stimulation and for picking up electrical responses. These electrodes, originally intended for the ultrasound experiments, were designed as follows. The end of each pin, which is fastened to a flexible lead, is covered by a plastic fitting against which the muscle rests snugly when light pressure is applied by a plastic nut which screws on the free end of a threaded pin after the pin is inserted through the muscle. This design eliminates relative motion between the muscle and each electrode, despite gross movements of the muscle. In the majority of the experiments, wherein both the mechanical and electrical responses were recorded, the stimulating electrodes consisted of a single pair of closely spaced pins rather than a series extending throughout the length of the muscle. In such experiments, the stimulus level used was one yielding maximum amplitude of action potential but not necessarily maximum isometric tension. The results obtained on the mechanical response with such electrodes were checked by a number of experiments with stimulation accomplished by a series of electrodes with an average spacing of 1½ mm along the entire length of the muscle. When this electrode arrangement was used, the stimulus level was graded, at each temperature, from a value just above threshold to one well above that yielding maximum isometric tension.

For temperatures below 20°C, the results of the experiments reported here on maximum isometric twitch tension agree with those found in the literature—that is, as the temperature decreases the amplitude of the tension increases, while the speed of contraction or shortening decreases. However, for temperatures above 20°C the amplitude of the isometric tension again increases, while the speed of contraction and relaxation also increases. The relation between the amplitude of maximum tension and the temperature is indicated in Fig. 1. The changes with temperature, over the range indicated on the graph, are reversible. Similar results were found on both curarized and noncurarized preparations. No indications of heat contractures were obtained at temperatures up to 35°C. Over approximately the same temperature range the isometric *tetanic* tension is, according to A. V. Hill (*5*), a monotonic function of the temperature, rising relatively slowly. [Hajdu's reported results on tetanic tension (*6*) exhibit a maximum within this same temperature range.]

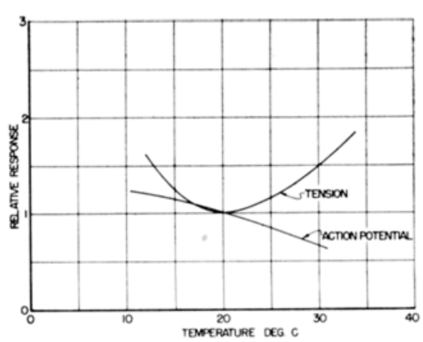


Fig. 1. Amplitude of maximum isometric twitch tension and action potential (macroelectrodes) as a function of temperature for the excised frog biceps muscle.

The electrical measurements show that the amplitude of the muscle action potential, when macroelectrodes are used, decreases monotonically with increasing temperature over the range 2.0° to 32°C. In addition, the "expected" increase in conduction velocity and decrease in duration of electrical response occur with rising temperature. These results agree with those of Sanderson (*7*), who worked with frog sartorius in the range 4° to 20°C, and with those of Welkowitz and Fry (*8*), who worked with frog biceps muscle, in the temperature range 20° to 35°C. The increase in electrical conduction velocity with increasing temperature, determined from our experiments on whole muscle, agrees very closely with the results of Wilska (*9*), who found a factor of 5.4 for the increase in velocity, for single fibers of frog skeletal muscle, as the temperature is raised from 0° to 36°C.

The fact that the action potential versus temperature relation follows a monotonically decreasing curve with rising temperature, while the amplitude of the isometric tension versus temperature goes through a minimum, is of interest, but it does *not* prove that the action potential is unnecessary for initiation of the contractile process. The amplitude of the action potential over the entire temperature range studied may always be much greater than the minimum electrical stimulus required to initiate a mechanical response.

The isometric tension curve of Fig. 1 is of interest in regard to present theories on the variation of the amplitude of twitch tension with temperature. The problem, as formulated up to the present time, has been to explain why the isometric tension decreases with increasing temperature, the highest temperatures generally considered being not much over 20°C. The explanation usually given is that at the higher temperatures the relaxation process overcomes the contraction process before full tension can be reached (*3, 10*). Since the duration of the active state is approximately halved for each 10°C rise in temperature, there is insufficient time for completion of internal shortening before decay of the active state (relaxation) begins (*11*). Theories based on such a hypothesis are inadequate for explaining the increased tension, at temperatures above 20°C, found in the authors' experiments.

Gad and Heymans (*12*), in 1890 reported results similar to those reported here on the variation of maximum twitch tension of excised frog skeletal muscle over the temperature range 0° to 30°C [see also Kaiser 1896 (*13*), Brodie 1898 (*14*), Carvallo and Weiss 1900 (*15*), de Boer 1915 (*16*)]. Unfortunately, Bernstein (*17*) attributed Gad and Heymans' results on increased tension at temperatures above 20°C to a threshold effect. The results of the experiments described here, in which multiple electrodes and supramaximal stimulation were used, definitely indicate that the increased isometric twitch tension at high temperatures is not a threshold effect. Work is now under way on a study of the form (tension as a function of time) of the isometric twitch response at temperatures above 20°C, in order to provide additional information on the behavior of isometric tension with temperature.

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### References and Notes

1. These studies were aided by a contract (NR 119-075) between the Office of Naval Research, Department of the Navy, and the University of Illinois.
2. S. M. Walker, *Am. J. Physiol.* 157, 429 (1949).
3. A. V. Hill, *Proc. Roy. Soc.* B138, 349 (1951).
4. F. Buchthal and E. Kaiser, *The Rheology of the Cross Striated Muscle Fiber* (Royal Academy of Sciences and Letters of Denmark, Copenhagen, 1951).
5. A. V. Hill, *Nature* 167, 377 (1951).
6. S. Hajdu and R. B. O'Sullivan, *Enzymologia* 14, 182 (1952); S. Hajdu, *Enzymologia* 14, 187 (1952); ———, *Arch. Intern. Physiol.* 59, 58 (1951).
7. J. B. Sanderson, *J. Physiol. (London)* 23, 325 (1898).
8. W. Welkowitz and W. J. Fry, *J. Cellular Comp. Physiol.* 48, 435 (1956).
9. A. Wilska and K. Varjoranta, *Scand. Archiv. Physiol.* 83, 88 (1940).
10. T. Buchthal and E. Kaiser, *Acta. Psychiat. Neurol.* 24, 333 (1949).
11. L. Macpherson and D. R. Wilkie, *J. Physiol. (London)* 124, 292 (1954).
12. J. Gad and J. F. Heymans, *Arch. Anat. u. Physiol. (von du Bois-Reymond) Suppl.* 59 (1890).
13. K. Kaiser, *Z. Biol.* 33, 157 (1896).
14. T. G. Brodie, *The Essentials of Experimental Physiology* (Longmans, Green, New York and Bombay, 1898).
15. J. Carvallo and G. Weiss, *J. physiol. et pathol. gen.* 2, 225 (1900).
16. S. de Boer, *Z. Biol.* 65, 239 (1915).
17. J. Bernstein, *Arch. ges. Physiol. Pflüger's* 122, 129 (1908).

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