the combined response to SSCG and CSP by propranolol. Heart rate (not illustrated) showed no statistically significant reductions, during CSP, SSCG, or after propranolol. These results show that SSCG alone, and especially in conjunction with an elevated intrasinusal pressure, can produce a systemic depressor response. Propranolol, limited only to the sinus region, further enhanced this depressor response, primarily through the effect on SSCG and not through CSP. This would seem to suggest that an intact sinus innervation is necessary if one wishes to demonstrate an effect of propranolol on the sinus depressor and nerve activity[8].

1 This work was supported by grant No. 22600-1 NIH, Heart, Lung and Blood Institute, Bethesda, MD.
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Changes in the A-band width during contraction in horseshoe crab striated muscle

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Summary. Cinematographic recordings of Ca-activated contraction in glycerinated bundles of myofibrils of the horseshoe crab striated muscle indicated that the A-band width did not change during rapid sarcomere shortening from 11 to 4 μm, while it increased by about 10% in nearly isometric conditions.

Contrary to the general view that contraction in striated muscle results from a relative sliding between the thick and thin filaments[2,3], horseshoe crab striated muscle is known to exhibit marked shortening of the A-band which is accompanied by shortening of the thick filaments under some conditions[4-8], thus suggesting the possibility that the thick filament shortening serves as an additional mechanism producing force and motion. In the above literature, however, the A-band length changes seem not to have been studied under physiological conditions, and it remains to be determined whether or not the thick filament shortening actually takes place in physiological contractions. The present paper deals with changes in the A-band width during the course of Ca-activated contraction in glycerinated horseshoe crab muscle. It will be shown that the A-band shortening may not be involved in physiological contractions.

Material and methods. Adult horseshoe crabs *(Tachypleus tridentatus)* were collected in the vicinity of Fukuoka, Japan, and fed in chilled (18 °C), aerated sea-water in the laboratory until used. Telson depressor muscles[6] were isolated from the animals, and glycerinated by the method of Tanaka et al.[9]. Bundles of myofibrils (20–50 μm in diameter, and about 1 cm in length) were then dissected from the glycerinated muscle fibres in a relaxing solution containing 100 mM KCl, 3 mM EGTA, 5 mM $MgCl_2$, 4 mM ATP and 10 mM histidine (pH 6.8), and placed in a thin layer of the relaxing solution between a slide and a coverslip, both ends being glued to the slide with Aron alpha A adhesive (Sankyo). The preparation was observed under a phase-contrast microscope (Nikon DM40 objective, ×40, n.a. 0.64), and activated with iontophoretically applied Ca ions through a glass pipette (diameter 5–15 μm) filled with 1 M $CaCl_2$[10]. The initial sarcomere length of the preparation mounted in this way was uniform along its length, ranging from 7 to 11 μm. The resulting local contractions were recorded on Kodak 7247 or Fuji Minicopy films with a 16-mm cine-camera (Bolex) at 64 frames/sec (figure 1, a, b and figure 2, a, b). The length changes of a single sarcomere and its A-band at a definite part of the preparation, where the striations were clear, were measured during the course of contraction either directly on the cinefilm with a film motion analyser (Vanguard), or indirectly after microdensitometer (type NLM-D3, Narumi) tracings of the cinefilm with similar results. All experiments were performed at room temperature (18–20 °C).

Results. As shown in figure 1, c, no significant change in the A-band width was observed during the course of sarcomere shortening from 7–11 μm to about 4 μm; the sarcomere shortening was taken up only by the I-band shortening while the A-band width (3–3.5 μm) remained unchanged. When sarcomeres shortened to less than 4 μm, the A-band became invisible because of the contraction band formation[11]. On relaxation, however, the A-band appeared again with sarcomere lengths above 4 μm, and its width remained unchanged until the completion of relaxation.

The above local contractions involved at most 10 sarcomeres while the length of the preparation was about 1 cm, and under such conditions the activated sarcomeres were considered to be shortening against a very small load[12,13]. As a matter of fact, the shortening velocity at the early phase of local contractions (about 1 length/sec at 20 °C) was similar to the maximum shortening velocity determined on intact muscle fibres[14], indicating that the preparation was in a good physiological condition. Thus, the above results indicate that the thick filament shortening mechanism may not be involved in the physiological sarcomere shortening from 11 to 4 μm, since the thick filaments are fairly well aligned within the A-band at sarcomere lengths below 7 μm[6-8] (Suzuki and Sugi, unpublished).

Figure 2 shows the results of experiments in which the distance between the fixed ends of the preparation was made very short (about 100 μm or less), so that all sarcomeres within this short segment were activated fairly

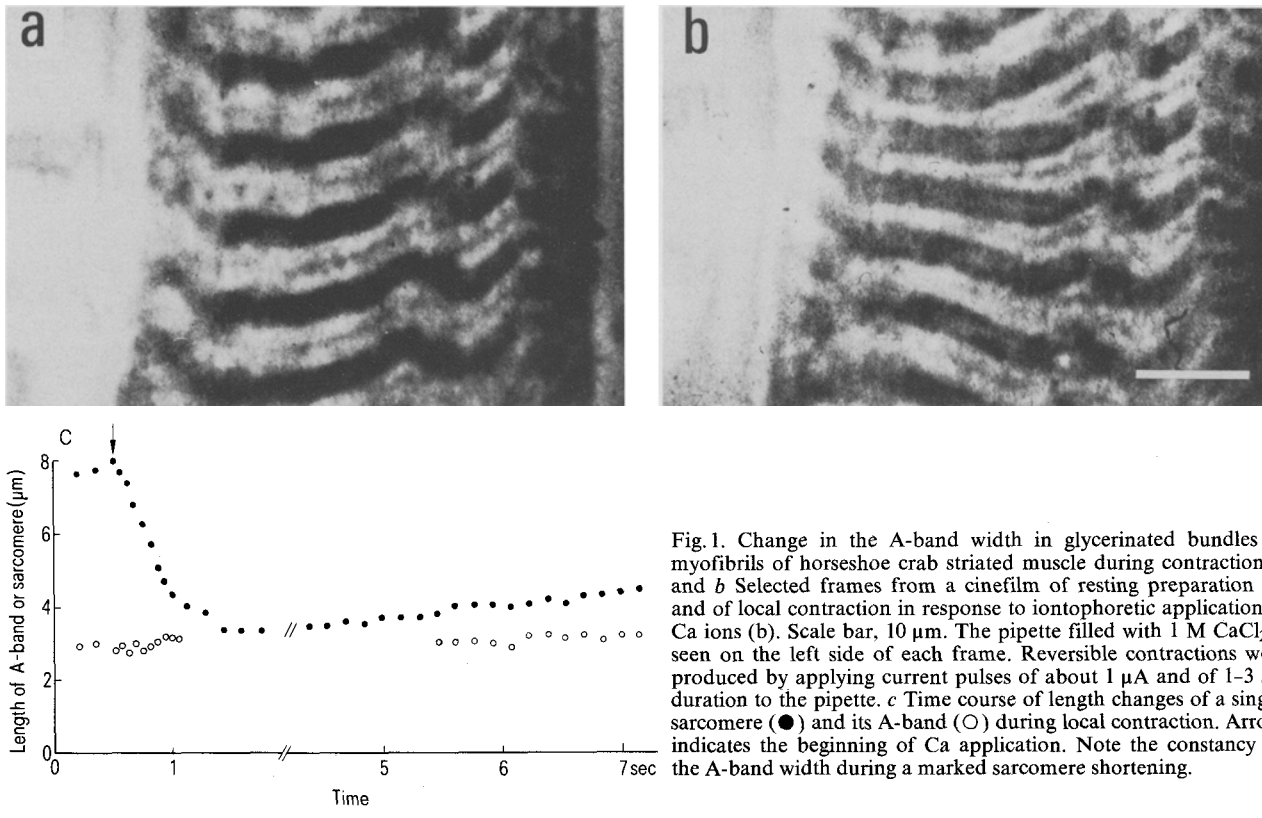

Fig. 1. Change in the A-band width in glycerinated bundles of myofibrils of horseshoe crab striated muscle during contraction. *a* and *b* Selected frames from a cinefilm of resting preparation (a) and of local contraction in response to iontophoretic application of Ca ions (b). Scale bar, 10 µm. The pipette filled with 1 M $CaCl_2$ is seen on the left side of each frame. Reversible contractions were produced by applying current pulses of about 1 µA and of 1–3 sec duration to the pipette. *c* Time course of length changes of a single sarcomere (●) and its A-band (○) during local contraction. Arrow indicates the beginning of Ca application. Note the constancy of the A-band width during a marked sarcomere shortening.

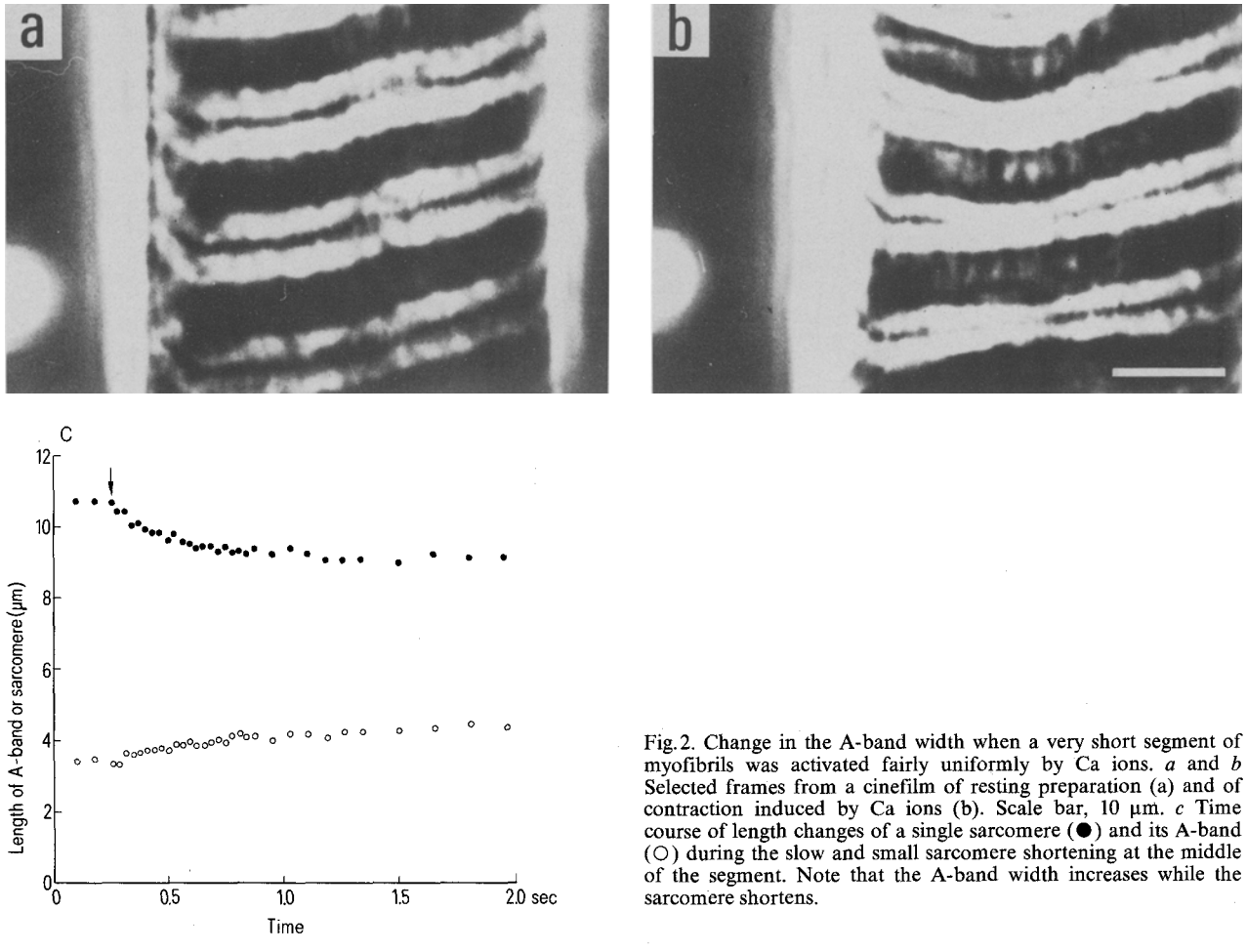

Fig. 2. Change in the A-band width when a very short segment of myofibrils was activated fairly uniformly by Ca ions. *a* and *b* Selected frames from a cinefilm of resting preparation (a) and of contraction induced by Ca ions (b). Scale bar, 10 µm. *c* Time course of length changes of a single sarcomere (●) and its A-band (○) during the slow and small sarcomere shortening at the middle of the segment. Note that the A-band width increases while the sarcomere shortens.

uniformly by iontophoretic application of Ca ions. In this case, both the speed and the extent of shortening at the middle of the preparation were very limited as compared to the experiments shown in figure 1, because there were no non-activated sarcomeres which could be readily stretched by activated ones. As can be seen in figure 2, c, the A-band width was found to increase appreciably (by about 10%) during the course of slow sarcomere shortening, reaching a steady value when the sarcomere shortening at the middle part stopped due to the balance of force between the sarcomeres. This implied the A-band lenghtening during 'isometric' contraction, again indicating that the thick filament shortening does not take place during the generation of physiological force in each sarcomere. It was noticed that the extent of the A-band lengthening in the 'isometric' condition could well account for the elastic extension of the series elasticity at P_0 in intact muscle fibres[14], suggesting that the series elasticity in horseshoe crab striated muscle may mainly reside in the A-band. It is now being investigated whether the increase in A-band width shown in figure 2, c, is attributable to the elastic strain of the thick filaments[15] or to their misalignment as a result of force generation in each sarcomere. The present results, of course, do not exclude the possibility that the thick filament shortening, which has been studied extensively by Dewey and his coworkers[6-8], plays a role in some long-term muscle performance or during extreme sarcomere shortening below 4 μm.

1 We wish to thank T. Taniguchi, M. Kusama and M. Goto for their excellent technical assistance in taking cinematographic recordings.
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Catalase activity in skeletal muscle of varying fibre types

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Summary. Slow oxidative skeletal muscles of rats and hamsters exhibit significantly greater catalase activity than fast oxidative muscles. Furthermore, regions of a single muscle may vary significantly.

Catalases are heme-containing porphyrin enzymes which exhibit ubiquity in cells containing cytochrome systems[1]. They exhibit tissue specificity, which appears to be epigenetic, and are found in highest concentrations in the liver, kidney and erythrocytes[2]. Recently, catalase was found in skeletal muscle[3]. Although catalase has been studied in great detail since it was first described by Thenard[4] in 1818, there is still no agreement as to its physiological role. Furthermore, there is a paucity of information related to the function of this enzyme in skeletal muscle. The data related to muscle that have been published have been faulted by the failure of investigators to report the muscle type which was sampled[5]. It is well known that skeletal muscles vary in the proportion of fibre types which they contain and that the enzyme profile is the principle criterion for the identification of the muscle fiber type. The purpose of our study was to determine the catalase activity in muscles of varying fibre types.

Methods. The data were obtained from 10 male and female Sprague-Dawley derived rats weighing between 250 and 300 g, and 10 male and female Syrian hamsters weighing between 150 and 175 g. The animals were decapitated and exsanguinated. The muscles were rapidly dissected out, rinsed and kept in ice cold Ringer's solution while awaiting homogenization. The muscles were blotted dry, weighed, and minced with scissors on a cold glass plate. A 10% (w/v) homogenate was formed with 15 strokes in a Thomas glass/teflon tissue grinder using deionized glass distilled water. Catalase in the crude tissue preparation was assayed by the oxygen cathode method according to Goldstein[6]. The unit of enzyme activity is the amount of enzyme which releases 1 μmole of O_2 per min at 30 °C, pH 7 and 0.033 M perborate. Protein was determined by the method of Lowry et al.[7].

Results and discussion. These data demonstrate that there is a significant difference between the catalase activity of the

Muscle catalase activity by muscle fibre type (μ/mg protein)

	Rats	Hamsters
Soleus (slow oxidative)	5.75±0.44*	3.60±0.26*
Extensor digitorum longus (EDL) (fast oxidative glycolitic)	2.60±0.41	1.84±0.30
Gastrocnemius		
red	3.63±0.34**	
white	1.42±0.30	

Data are means±SE. * Significantly different from EDL $p \leq 0.05$; ** significantly different from white gastrocnemius $p \leq 0.05$.