LETTERS TO THE EDITOR

Comments on "Myocyte Growth without Physiological Impairment in Gradually Induced Rat Cardiac Hypertrophy" which appeared in Circ. Res. 49: 1300–1310, 1981

Precise measurement of the contractile properties of cardiac muscle has been hampered by compliant regions at the ends of the isolated preparation. Eliminating the influence of these regions is especially desirable when the contractile behavior of separate specimens is to be compared, as is done in the elegant study by Julian et al. (1981). While a variety of rather sophisticated approaches to this problem have been devised, Julian et al. suggest that application of a fast-setting glue lowers the preparation's compliance, thereby reducing internal shortening to unappreciable levels. It would be very nice if a simple and reliable attachment technique could be devised; the observations they advance in support of this goal, however, only reinforce the importance of using direct observational methods when trying to define active compliance and to compare internal contractile dynamics in isolated heart muscles.

Regardless of any particular attachment technique, it's likely that only a part of the isolated muscle preparation actively shortens (Krueger and Pollack, 1975). The active region may represent only about 40–70% of the preparation's length so that the actual precision with which one can infer true velocities of internal shortening from measurement at the ends of the preparation is accordingly reduced. The use of two markers to delineate a shortening segment within the preparation is one solution, but the accuracy of this approach clearly depends upon the marker's placement fully within the uniformly shortening midregion of the muscle. Moreover, as indicators of internal motion, the suitability of external markers is additionally compromised by the presence of an endocardial sheath. Consequently, external marker motions will probably underestimate internal shortening. Not surprisingly, several groups independently have commented upon the lack of agreement between internal and external motions in an isolated heart muscle preparation (Huntsman et al., 1979; Donald et al., 1980). The additional presence of marker rotation, or "skewing," as observed by Julian et al., indicates not only radial nonuniformity, but also that the true internal shortening was larger than that inferred from marker translation.

Methods for analysis of the preparation's series compliance leave more to uncertainty than the observations of internal shortening. To evaluate shortening in terms of series compliance measurements is inappropriate, since the elastic recoil after a rapid length step represents only a fraction of internal shortening (Pollack and Krueger, 1976). Instead of by elastic recoil, Julian et al. estimate compliance by measuring tension recovery after a partial release. They report the relation between fast phase tension recovery and the length step to be linear in heart muscle, whereas, in skeletal muscle, others find fast tension recovery largely independent of the length step at the same tensions (Ford et al., 1977). (Consequently, the suggestion of a direct analogy between their measurements and the behavior of the stiffer skeletal fiber preparation is difficult to support.) These differences may reflect the fact that a clear deflection in tension recovery is not apparent in their experimental records, in which case the slower phase of tension recovery assumes more influence on these measurements. As long as the mechanism underlying the slower recovery of tension is not understood, it seems unwarranted to imply that either a fast or slow phase of tension recovery can be used as a basis for measuring compliance external to the sarcomere (Ford et al., 1977).

I stress that these comments do not alter the fundamental results of the study by Julian et al. It is premature, however, to assert that their attachment technique (1) significantly reduces the preparation's series compliance and, therefore (2) gives a more reliable indication of internal contractile dynamics. Here, the most direct observational methods are to be preferred, as illustrated by their finding that the glued muscle does not relengthen after unloaded shortening unless a preload is applied. This behavior is not seen in the length of the sarcomere in intact muscle, in unattached single cardiac cells (Krueger et al., 1980), or in careful photographic studies (Figure 1, Julian and Sollins, 1975) which showed that an isolated cardiac muscle preparation buckles if made to relax at short lengths. Clearly, novel attachment techniques cannot substitute for direct observation when a principal aim is precise measurement of contractile shortening.

John W. Krueger
Albert Einstein College of Medicine
Bronx, New York 10461

References
Ford LE, Huxley AF, Simmons RM (1977) Tension responses to sudden length change in stimulated frog muscle fibers near slack length. J Physiol (Lond) 269: 441–515
Krueger JW, Pollack GH (1975) Myocardial sarcomere dynamics in skeletal muscle has been hampered by compliant regions at the ends of the isolated preparation. Eliminating the influence of these regions is especially desirable when the contractile behavior of separate specimens is to be compared, as is done in the elegant study by Julian et al. (1981). While a variety of rather sophisticated approaches to this problem have been devised, Julian et al. suggest that application of a fast-setting glue lowers the preparation's compliance, thereby reducing internal shortening to unappreciable levels. It would be very nice if a simple and reliable attachment technique could be devised; the observations they advance in support of this goal, however, only reinforce the importance of using direct observational methods when trying to define active compliance and to compare internal contractile dynamics in isolated heart muscles.

Regardless of any particular attachment technique, it's likely that only a part of the isolated muscle preparation actively shortens (Krueger and Pollack, 1975). The active region may represent only about 40–70% of the preparation's length so that the actual precision with which one can infer true velocities of internal shortening from measurement at the ends of the preparation is accordingly reduced. The use of two markers to delineate a shortening segment within the preparation is one solution, but the accuracy of this approach clearly depends upon the marker's placement fully within the uniformly shortening midregion of the muscle. Moreover, as indicators of internal motion, the suitability of external markers is additionally compromised by the presence of an endocardial sheath. Consequently, external marker motions will probably underestimate internal shortening. Not surprisingly, several groups independently have commented upon the lack of agreement between internal and external motions in an isolated heart muscle preparation (Huntsman et al., 1979; Donald et al., 1980). The additional presence of marker rotation, or "skewing," as observed by Julian et al., indicates not only radial nonuniformity, but also that the true internal shortening was larger than that inferred from marker translation.

Methods for analysis of the preparation's series compliance leave more to uncertainty than the observations of internal shortening. To evaluate shortening in terms of series compliance measurements is inappropriate, since the elastic recoil after a rapid length step represents only a fraction of internal shortening (Pollack and Krueger, 1976). Instead of by elastic recoil, Julian et al. estimate compliance by measuring tension recovery after a partial release. They report the relation between fast phase tension recovery and the length step to be linear in heart muscle, whereas, in skeletal muscle, others find fast tension recovery largely independent of the length step at the same tensions (Ford et al., 1977). (Consequently, the suggestion of a direct analogy between their measurements and the behavior of the stiffer skeletal fiber preparation is difficult to support.) These differences may reflect the fact that a clear deflection in tension recovery is not apparent in their experimental records, in which case the slower phase of tension recovery assumes more influence on these measurements. As long as the mechanism underlying the slower recovery of tension is not understood, it seems unwarranted to imply that either a fast or slow phase of tension recovery can be used as a basis for measuring compliance external to the sarcomere (Ford et al., 1977).

I stress that these comments do not alter the fundamental results of the study by Julian et al. It is premature, however, to assert that their attachment technique (1) significantly reduces the preparation's series compliance and, therefore (2) gives a more reliable indication of internal contractile dynamics. Here, the most direct observational methods are to be preferred, as illustrated by their finding that the glued muscle does not relengthen after unloaded shortening unless a preload is applied. This behavior is not seen in the length of the sarcomere in intact muscle, in unattached single cardiac cells (Krueger et al., 1980), or in careful photographic studies (Figure 1, Julian and Sollins, 1975) which showed that an isolated cardiac muscle preparation buckles if made to relax at short lengths. Clearly, novel attachment techniques cannot substitute for direct observation when a principal aim is precise measurement of contractile shortening.

John W. Krueger
Albert Einstein College of Medicine
Bronx, New York 10461

References
Ford LE, Huxley AF, Simmons RM (1977) Tension responses to sudden length change in stimulated frog muscle fibers near slack length. J Physiol (Lond) 269: 441–515
Krueger JW, Pollack GH (1975) Myocardial sarcomere dynamics

Downloaded from http://circres.ahajournals.org/ by guest on July 10, 2017
during isometric contraction. J Physiol (Lond) 251: 627-643

Reply to the Preceding Letter

As the author whose prime responsibility was the mechanical measurements, I feel that I should make some reply to Dr. Krueger.

I must first correct the misconception he has about the way in which we measured compliance. Step reductions of muscle length were applied at the peak of a twitch, the size of the length step was plotted against the corresponding fall in tension in the usual manner, and the slope of the resulting plot was used to gauge the compliance. The only unusual feature was that our motor steps were fast enough for the tension record to show a small, fast, (about 1 msec duration) recovery at the end of the step. As the source of this was uncertain (it may correspond to the Huxley and Simmons transients, or it may be viscoelastic), we chose to measure the fall of tension from just before the beginning of the length step to 2 msec later, when the tension was changing much less rapidly than it was at its minimum value. If we had used the usual slower step, the fast recovery would presumably have occurred during the step, as in Ford, et al. (1977), Figures 29 and 30, and not have been visible. The reference in the paper to avoiding this fast recovery apparently led Dr. Krueger to conclude that we measured it, a quite incorrect conclusion. The paper reported a large number of techniques, and was considerably shortened during the editorial process, so that only the departures from standard procedures were described. I would like to apologize to any other reader who may have been misled by the brevity of the description. Dr. Krueger's further comments on the difficulties of gauging compliance from tension recovery, however one would do that, are therefore quite irrelevant, as that is not what we did.

On the more general issues of gauging the quality of muscle attachment, we entirely agree with Dr. Krueger's comments. Our decision to use glued attachments was firmly based on direct observation, both by eye and with a high speed camera, of natural and attached surface markers, of microspheres injected into the perfusion fluid, and of sarcomeres where visible near the edges of muscles. All these measurements showed that we had virtually eliminated the "contraction of a central region—stretching of damaged areas near the ends" type of movement seen by Krueger and Pollack (1975), whose muscles were very crudely clamped between flat jaws at both ends. The success of the glue is that it avoids the damage and deformation of the muscle so apparent with clamps. The compliance measurements were merely a convenient way of checking each muscle in a simple but quantitative manner, to ensure that attachment was uniform between muscles, particularly between operated and control. The decision to use glue, however, was very firmly based on direct observation.

Dr. David L. Morgan
Department of Electrical Engineering
Monash University
Clayton, Victoria
Australia 3168
"Myocyte growth without physiological impairment in gradually induced rat cardiac hypertrophy".

J W Krueger

Circ Res. 1982;51:666-667
doi: 10.1161/01.RES.51.5.666

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/51/5/666.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/