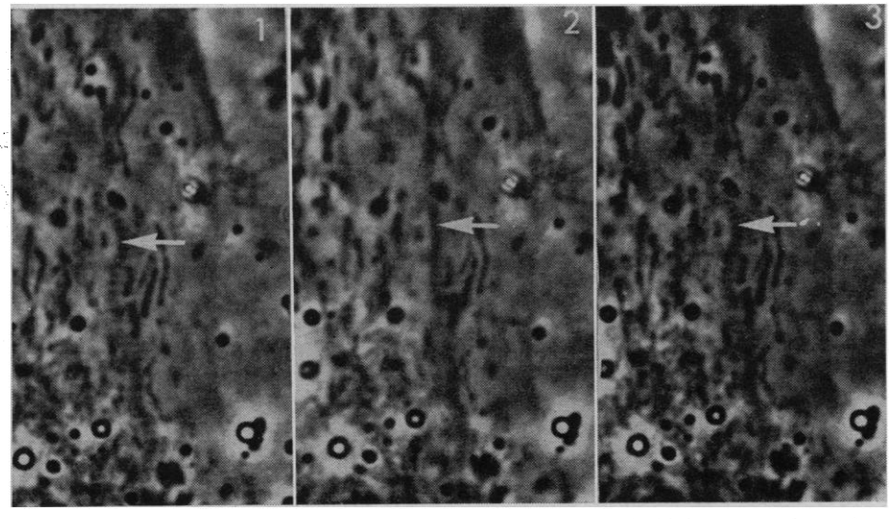
Muscle Relaxation: Evidence for an Intrafibrillar Restoring Force in Vertebrate Striated Muscle

Abstract. *Observations of contracting muscle fibrils in cultured cells indicate that the force which restores the resting length of the sarcomere comes from the contractile elements themselves and not from external elasticity, as is now generally accepted. In light of biochemical studies on the contraction-relaxation cycle, it is postulated that the elongating force is one of internal elasticity in the sarcomere, which arises during contraction from the distortion of bonds between filaments and/or structural proteins. This mechanism of restoration may serve to establish optimal sarcomere length for production of maximum contractile force, and in cardiac muscle this mechanism may be a factor in ventricular filling.*

Most studies dealing with the dynamic properties of muscle have focused upon its contraction or shortening; relatively little attention has been given to the physical mechanism of elongation, that is, relaxation (*1*). Of the various theories regarding the activity of the filament during contraction, the sliding filament mechanism (*2*) seems to receive the most substantial support from present morphological and physiological evidence. How one filament is driven past another remains unknown. Although contraction within individual sarcomeres has been studied (*3*), similar morphological and physiological studies of the events following contraction are lacking. Data from studies of single contracting cells indicate that sarcomere elongation and restoration of the resting length of the fibrils are inherent properties of the contractile elements themselves.

Embryonic chick heart cells cultured by routine methods (*4*) develop striated fibrils which are morphologically similar to those of intact muscle tissue (*5*). These fibrils have spontaneous, rhythmic, contractile activity characteristic of cardiac muscle. Contraction in such fibrils is marked by an abrupt change in banding configuration in which the Z bands become very prominent. Elongation is marked by an equally abrupt reversion to the resting configuration. During the contraction-elongation cycle most fibrils remain straight. However, occasionally fibrils are observed that are straight only during contraction, and which, upon resuming the resting length and the pattern of striations characteristic of relaxation, are folded or kinked. Ciné sequences of a kinking fibril show that, as the fibril shortens during contraction, it straightens, and that during elongation it is thrown into folds (Figs. 1–3). Such fibrils are too long for the space provided in the cultured cell.



Figs. 1–3. A ciné sequence of a contracting myofibril which kinks upon elongation. Our initial timing studies on these preparations show that the speed of elongation is comparable to that of contraction and that at lower temperatures one phase of elongation is more rapid than contraction. During such a contraction the kinking fibrils shorten 15 to 20 percent and during elongation rapidly extend over this same distance to assume the resting sarcomere configuration and length.

Fibrils in cultured myoblasts are essentially isometric fibril preparations firmly attached at either end with a series elastic component included in the fibril-sarcolemma, sarcolemma-glass junction. We have examined the sites of the attachment of the myoblast to the glass using reflection interference microscopy. The sarcolemmas of all the cells examined have numerous peripheral attachment sites in the areas where the myofibrils end. Fibrils in cardiac muscle are anchored to the sarcolemma by structures resembling half-desmosomes (*5*). We have observed identical anchor points in cultured myoblasts. In many cultured cells, the distance between anchor points is equal to the resting length of the fibrils. The series elastic component is responsible for the maintenance of the straight form of the fibrils during the contraction-elongation cycle. However, changes in shape, and hence in attachment sites, are routinely observed in cultured cells. As a result of such changes a contracting fibril frequently must fold or kink if it is to assume its resting length, because the distance between its anchor points is less than the original length of the fibril at rest. In these cells, the series elastic component cannot be the only force which pulls the fibril out to the fully relaxed or elongated state; if it were, the fibrils would be pulled straight and elongation would cease at that point. That elongating fibrils are thrown into folds with each sarcomere fully extended in the resting configuration is evidence for an intrafibrillar restoring force.

There is no morphological evidence to suggest that cytoplasmic structures peripheral to the myofibril in the developing myoblast cause an elongating fibril to kink. Rather, in developing muscle, mitochondria, microtubules, and filaments are aligned parallel to the fibrils, and this arrangement should favor straightening of the fibril rather than kinking. In fact, kinking fibrils displace the surrounding cytoplasm and organelles. In one instance a kinked fibril forced a nucleus laterally. That adjacent pairs of fibrils in the same cell are regularly seen in which only one pair shows any kinking is further evidence that the cytoplasm is not responsible for the folding.

Studies of muscle relaxation date to

at least 1859, but none has focused on the activity at the sarcomere. Kühne (*6*) found that an isolated muscle floating on mercury would not uncoil after contraction. However, Kaiser (*7*) showed that a muscle would relax on mercury if it were first dipped in oil to reduce friction.

Ramsey and Street (*8*) noted that a single fiber does not elongate after contraction if it has shortened to greater than 60 percent of its resting length. They defined irreversible contraction as the delta state and postulated an alteration in the contractile elements. Length-tension measurements made before and after shortening to 60 percent of fiber length showed that excessive shortening had altered the contractile capacity of the fiber, which, in turn, affected relaxation.

A. V. Hill (*9*) has demonstrated gross differences between the resting and relaxation properties of whole muscles and those of single fibrils. Despite this finding, Hill concluded from his work on whole muscles that relaxation was not an active process, and that muscle elongation was due to elastic forces outside the contractile elements (*9*). Translation of experimental data, such as Hill's, from an intact muscle to the sarcomere is not a simple matter, since the mechanics and forces involved in a muscle are more complex than those in a single fiber, and are even more so than in the case of the sarcomeres or a single fibril. The properties of sarcomere relaxation or elongation can be observed directly, eliminating factors in the intact muscle that may obscure or alter important details of the contraction-elongation cycle. Relatively gross measurements or observations of muscle relaxation should be confirmed by direct microscopic observations of the sarcomere to determine its configuration.

In their consideration of active relaxation, Buchthal and Kaiser (*10*) cite instances in which elongation occurs and note that elastic energy may be stored in the sarcolemma or contractile elements during contraction, but they did not find an "unambiguous answer to the problem of active or passive relaxation."

Recently Podolsky and Costantin (*11*) have shown that isolated fiber segments of frog semitendinosus immersed in paraffin will contract upon the direct application of calcium. The entire segment remains contracted until the concentration of calcium is sufficiently decreased, presumably by being pumped into the sarcoplasmic reticulum. The segment then elongates and approaches the initial rest length. Before the calcium is applied, the sarcolemma is stripped from the fibers, thus eliminating it as a possible source of elastic energy for elongation.

Observations on the behavior of cardiac muscle during diastole indicate that the force needed for relaxation is within the muscle itself. Cardiac muscle is unique, for there are no tendons or antagonistic muscles to aid in elongation of its sarcomeres. However, ventricular relaxation is not entirely due to the pressure of blood flow from the auricles. During the initial phase of rapid filling in diastole, the ventricles are empty but relaxed, and the blood actually seems to be sucked into them (*12*). Ventricular relaxation and the suction effect in ventricular filling may be a gross manifestation of the elastic elongating force of the individual sarcomeres.

An intrafibrillar force of elongation seems to be present not only in fibrils of cultured myoblasts but in vertebrate striated muscle in general (*13*). Such a force could use chemical energy directly, but studies of Hill (*14*) and Cain *et al.* (*15*) indicate that contraction is the phase immediately dependent on chemical energy. Our observations favor the idea that, during shortening, elastic energy is stored in the contractile elements by distortion of bonds between the contractile elements and the structural proteins of the sarcomere. This energy is then released at the end of contraction, thus restoring the resting sarcomere length. It seems reasonable to suggest further that, since sarcomere length, that is, the overlap of thick and thin filaments, is mechanically important for the development of tension (*3*), there should be a mechanism within the sarcomere which reestablishes the optimum precontractile configuration in the extended or uncontracted fibril.

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References and Notes

1. In our examination of motile activity of the sarcomere, the term elongation better describes the events following contraction, or shortening, than does relaxation. As a result of elongation a sarcomere assumes the resting configuration (*2*), and a fiber or fibril resumes its resting length.
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Iphita limbata Stal.: Components of Neurosecretory Material

Abstract. *The median neurosecretory cells of the pars intercerebralis of* Iphita limbata *seem to release two demonstrable components, probably representing allatotropin to the corpus allatum and myotropin to the aortic neurohemal site.*

The neurosecretory system is an important endocrine center in *Iphita limbata* Stal. (Pyrrhocoridae, Hemiptera) (*1–3*). Nayar's histophysiological evidence suggests that the cells of the pars intercerebralis of the brain supply neurosecretory materials to the corpus allatum, possibly stimulating the gland to exercise its gonadotropic functions in the female. In *Iphita,* oviposition is delayed until copulation, which usually occupies about 3 weeks, is complete. As oviposition begins, the corpus allatum is denied its neurosecretory supply and neurosecretory colloids are discharged into the blood (*3*).

Using Humberstone's Victoria Blue