# The Physiological Basis of Starling's Law of the Heart

Ciba Foundation Symposium 24 (new series)

1974

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*Symposium on The Physiological Basis of Starling's Law of the Heart held at the Ciba Foundation, London, 11–13th September 1973*


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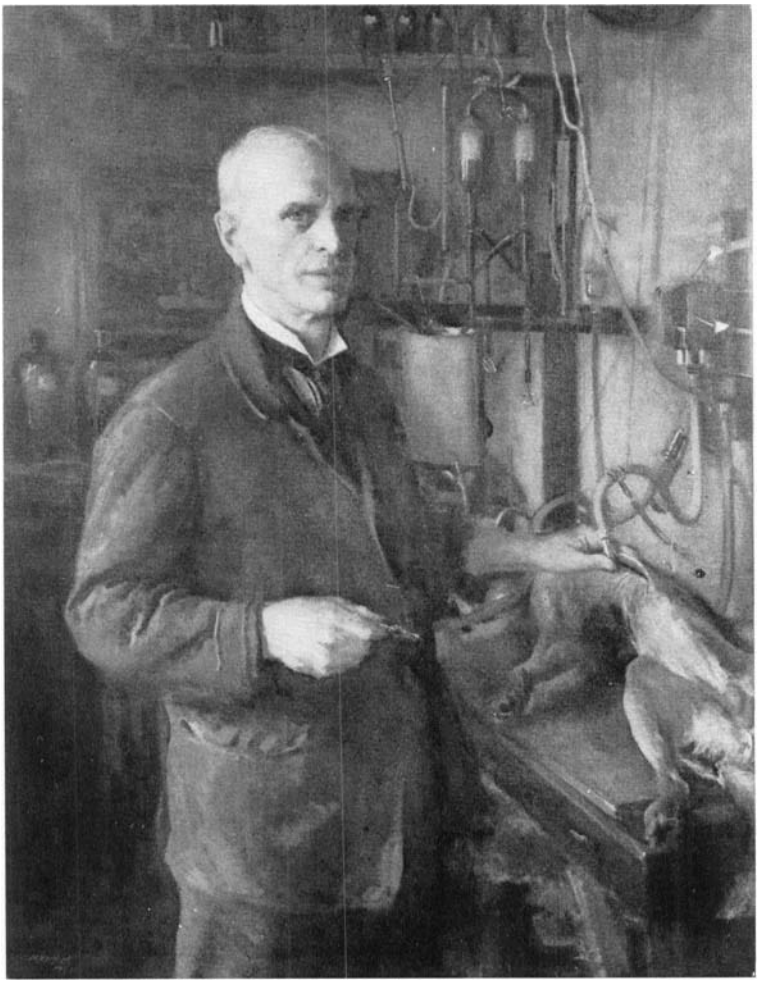

E. H. STARLING

*Portrait painted by W. W. Russell, RA, 1926, reproduced with the kind permission of the Department of Physiology of University College London.*

*(Photograph by Owen & Moroney, London)*

# Chairman's introduction

A. GUZ*

*Department of Medicine, Charing Cross Hospital Medical School, London*

I have been asked why the title of this symposium was not 'The Physiological Basis of the *Frank*–Starling Relation of the Heart'. If we were to give credit in full, we would have to call it the 'Hales–Haller–Müller–Ludwig–Roy–Howell–Donaldson–Frank–Starling relation'. Referring to the measurement of the blood pressure of a mare, Stephen Hales said: 'the violent straining to get loose, did by the acting of most of her muscles, especially the abdominal, impel the blood from all parts of the vena cava, and consequently there was the greater supply for the heart, which must therefore throw out more at each pulsation, and thereby increase the force of the blood in the arteries'. That was in 1740!† Whether he appreciated the import of what he was saying, we have no idea! In 1754, Haller talked about blood irritating the ventricle as it entered and thus causing the chamber to contract more. Müller's text-book of 1844 shows that he was clearly cognisant of the fact that an empty heart beats less strongly than a full heart. Ludwig said the same thing in 1856.

The real link with English physiology (or, dare I say, University College physiology!) is Cohnheim, a student of Ludwig's, who reported the concept to Roy, a great friend of his from Edinburgh. Roy consequently performed some beautiful experiments on the frog heart, making it clear that, if the arterial

* I have leaned heavily on Chapman, C. B. & Mitchell, J. H. (1965) *Starling on the Heart*, Dawsons of Pall Mall, London, for this introduction.

† Professor Donald pointed out that Harvey wrote in 1628: 'It is for this reason that the auricle is needed, that is to say, it has to help to infuse the blood into the ventricle so that the chamber in question may more readily express what has already been set in movement, and may send it on with greater vigour. Just as in a ball-game you will manage to drive the ball harder and farther by hitting it on the rebound than you will by throwing it from rest'. (Harvey, W. [1628] *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus*, translated by K. J. Franklin [1957], p. 104, Blackwell, Oxford). Professor Donald commented that what was most interesting was that Harvey was thinking here not only of heart muscle but of skeletal muscle.

pressure and heart rate are kept constant, then the work can be varied within wide limits by the 'variation in the central distension of the heart' (Roy 1879). That was the first systematic study where the experimental parameters were reasonably well controlled. Subsequently, the famous paper by Roy and Adami in 1892 that Starling quotes is not really a great development of Roy's original paper.

Across the Atlantic, another line of thinking developed at Johns Hopkins, where Howell & Donaldson (1884) constructed the ascending limb of the Starling curve, while working in Martin's Department of Biology. Certainly, a few years before, Martin (1881) had solved the technical problems of working with the isolated heart.

We come, in 1895, to Frank, whose considerable influence on him Starling was never ashamed to admit. Frank worked almost exclusively on the frog heart but nevertheless deduced the fundamental concepts, which he published in 1895. It is intriguing, especially since we do not know what the speed of communication was at that time, that in the Arris & Gale lecture of 1897, Starling, quoting no-one, presented the entire concept of his law, 15 years before he had done any experiments on it. We have a lacuna about this evolutionary period until 1912 when Starling's papers begin to appear (Knowlton & Starling 1912; Patterson & Starling 1914). Starling and his colleagues presented several results, which were primitive compared to those they presented subsequently; they found that as filling pressure goes up the output rises, too—in other words, the ascending limb of the Starling curve (Fig. 1). The first statement of the law of the heart is also in their magnificent paper in 1914 (Patterson *et al.* 1914). (Incidentally, this is a fine example of Anglo-German cooperation just before the first World War broke out. Piper performed some of his experiments in Hügner's laboratory in Berlin and collaborated with Patterson & Starling, but was subsequently killed on the Eastern front in 1917.) Their statement, which therefore makes the law of the heart the same as that of skeletal muscle, is as follows:—

'... the mechanical energy set free on passage from the resting to the contracting state depends on the area of chemically active surfaces, i.e. on the length of muscle fibres'.

The extraordinary relevance of Starling's work to more modern work is revealed in his famous Linacre lecture (Starling 1918)—not his best lecture by any means, but it is the one that is usually quoted. Without any knowledge of ultrastructure, he shows, quite clearly, that a change in surface energy along surfaces disposed longitudinally is likely to be the mechanism responsible for this law. Thereafter, I believe, Starling made a dreadful mistake; he devoted himself to clinical medicine! It was one of his great passions and in some ways

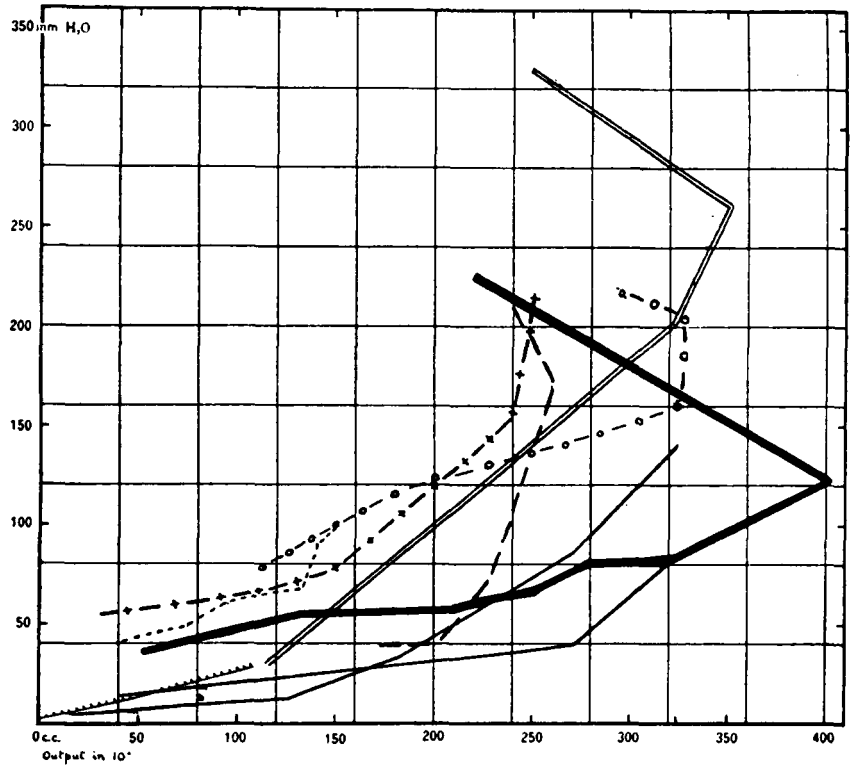


FIG. 1. The original Starling curve. 'The venous pressure in nine different experiments, as measured on the right side of the heart, are plotted against output. It will be seen that as the inflow and output gradually increase, there is a slight rise of venous pressure with each increase. The height of the venous pressure does not, however, rise in a straight line but in a curve, becoming more rapid as the limits of the functional capacity of the heart are reached, until in some cases the heart becomes over distended, is unable to deal with the blood filling its cavities, and the output diminishes although the venous pressure rises to a maximum'. (from Patterson & Starling 1914.)

he was a clinician manqué. During his work at Guy's Hospital, he felt a great compulsion to explain heart failure. (Alas, even in 1974 we still cannot explain 'congestive cardiac failure' and are bogged down in tremendous conflicts.) He tried to explain the effects of exercise on the heart and integrative cardiovascular physiology, claiming that the heart had to enlarge at the beginning of exercise, although radiologists—even at that time—stated that it did not and that Starling's law was irrelevant. So began the arguments which raged for the next 40–50 years about the importance of the intrinsic law of the heart compared with the importance of the nervous control. In the 1950s, there flared up the famous controversy between Sarnoff who expanded the concept of the Starling

curve (Sarnoff & Berglund 1954) and Rushmer (1959) who claimed that the physiology described by the curve did not happen in the intact animal.

Starling was fully aware of the famous work of Blix at the end of the last century in Scandinavia. Although Blix enunciated the concepts of the relation between initial length and force or velocity of contraction in skeletal muscle, it was not until the late 1950s with the striking work of Abbotts & Mommaerts (1959) that the concepts of muscle physiology developed by A. V. Hill were applied to the heart. Subsequently, we have the entire output of Sonnenblick's school which extends this and, in doing so, has created much, very healthy, controversy.

I conclude with an exhortation. Gathered together here are experts, but experts in different fields: for instance, experts in sarcomere length physiology and experts in integrative whole-heart physiology, and the two groups *do* speak different languages. It is vital that we try to make ourselves understood to each other. Therefore, I believe that we must define the concepts we use. Accordingly, I shall start by quoting from A. V. Hill (1965), in the hope that others will follow his advice:–

'One of the difficulties about muscle physiology is that of defining one's quantities in reasonably accurate physical and engineering terms. But we must try'.

The following is a list of definitions which were collected and agreed during the symposium:—

(1) $l_{max}$: The length at which the maximum active tension is found. $P_0$: The ambient developed force at $l_{max}$.

(2) Contractile unit length—this is a term used to avoid the ambiguous phrase 'sarcomere length': the overall length minus the series elastic element extension.

(3) Mechanical refractory period: the shortest period between two stimuli which will just prolong the twitch.

(4) The intensity of the active state (A. V. Hill): the tension that the contractile component can just bear without lengthening or shortening (Hill 1949), i.e. *a tension.* (According to Professor Edman: the capacity of the muscle at any given moment after a stimulus to produce tension, force and motion, measured as shortening velocity at zero load.)

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# Introduction: the changing face of the length–tension relation

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When we were planning this symposium, we decided to start it with a session on the length–tension relation of isolated cardiac muscle, since to many people this *is* the physiological basis of Starling's 'Law of the Heart'.

At the time when Starling was working on the heart–lung preparation, it was well known that muscles contract more forcibly when stretched. This fact seems to have been discovered in the frog gastrocnemius by Schwann in 1835; Dorothy Needham (1971) has commented that 'this experiment caused a great

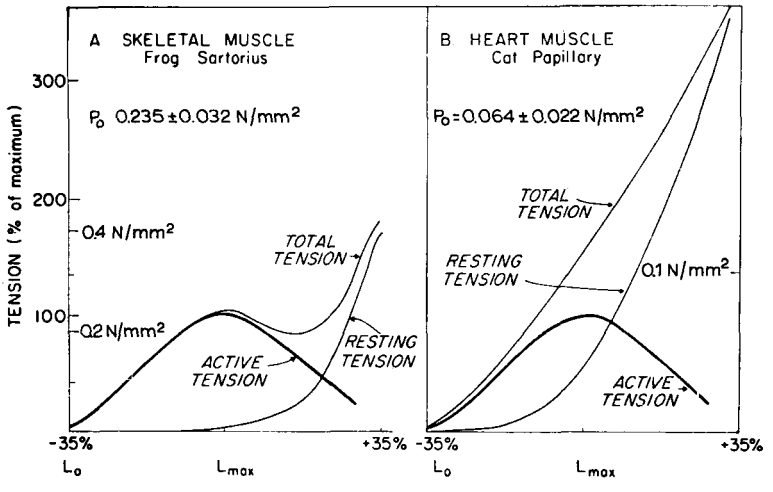


FIG. 1. Typical length–tension relations for preparations of skeletal and cardiac muscle (redrawn from Spiro & Sonnenblick 1964). Muscle length, as a percentage of $l_{max}$ (the length at which the greatest increase in tension occurs on stimulation), is plotted against tension, as a percentage of $P_0$ (the active tension at $l_{max}$). Note the difference in $P_0$ (in N/mm²) for the two preparations (1 N/mm² ≈ 10 kg/cm²).

sensation in Germany as the *fundamentale Versuch* of Schwann'. Schwann's discovery was confirmed in skeletal muscle preparations by various workers during the nineteenth century and also in the frog ventricle by Otto Frank (1895).

Most of the early work on isolated muscle was concerned with what we refer to now as the ascending limb of the length–tension relation, which is well illustrated for prototypes of cardiac and skeletal muscle in Fig. 1 (from Spiro & Sonnenblick 1964). Although these length–tension curves differ in various respects, the point being made in Fig. 1 is that *active tension* (i.e. the increase in tension that occurs on stimulation) shows essentially the same dependence on length in the two preparations. The underlying mechanism may or may not be the same in the two types of muscle.

A few years ago, the underlying mechanism seemed perfectly obvious. Brilliant work from A. F. Huxley's laboratory at University College (Gordon *et al.* 1966) had shown that the form of the length–tension relation of isolated frog muscle fibres could be accounted for in detail by the sliding filament hypothesis, as illustrated in Fig. 2. The key assumption of the hypothesis is that tension is generated by cross-bridges which form in the region of overlap between thick and thin filaments. The descending limb of the length–tension relation (sarcomere lengths above 2.25 μm) was explained by the decreasing overlap between thick and thin filaments as the fibre was stretched. The decline in tension on the ascending limb (sarcomere lengths below 2.0 μm) was attributed to interference with the mechanism of tension generation, due first to double overlap of thin filaments in the middle of the A bands (2.0–1.67 μm) and then to compression of the ends of the thick filaments against the Z lines at shorter sarcomere lengths than this. The short plateau over the range 2.0–2.25 μm was to be expected from the presence of a projection-free zone in the middle of the thick filaments.

This explanation was generally accepted at that time as the basis of the length–tension relation in both cardiac and skeletal muscle, even though no evidence of comparable quality was available from studies of cardiac muscle. This is an example of what my colleague Roger Woledge calls 'intellectual phase lock' (i.e. the assumption, in this instance, that if results obtained from cardiac muscle are consistent with a well established concept from the skeletal muscle field, then this concept must also be true for cardiac muscle). It can be argued that Starling's law of the heart was an early example of this. The danger is that one can live for years phase-locked in a fools' paradise, and what I want to emphasize now is the extent to which the clear picture of 1966 has become blurred by subsequent events.

First, better estimates of filament lengths in living frog muscle (Page 1968) gave a value for the thin filament length that was 0.1 μm shorter than that

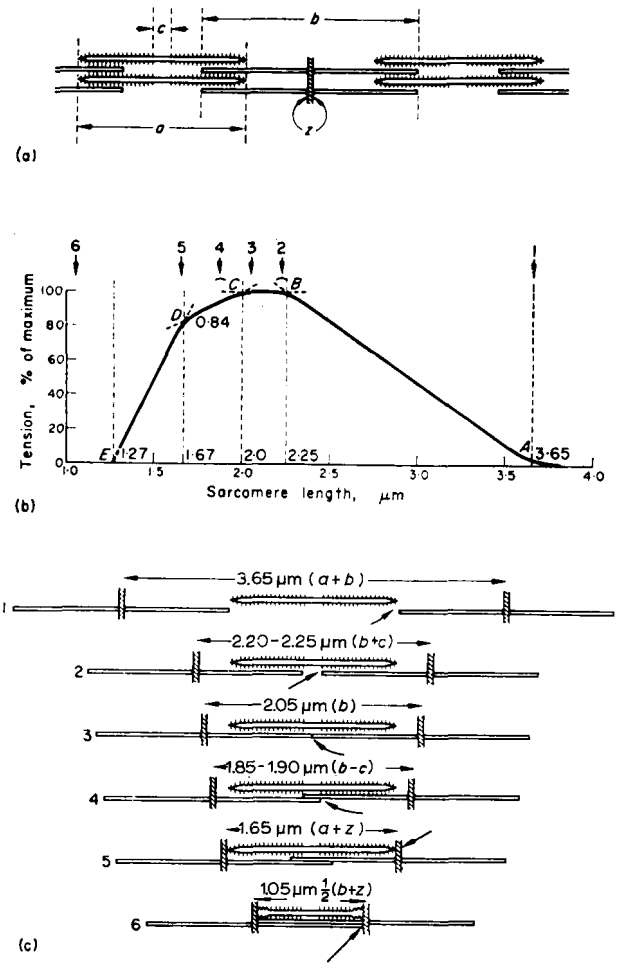


FIG. 2. Sliding filament hypothesis and the length–tension curve (redrawn from Gordon *et al.* 1966). (a) Arrangement of filaments in striated muscle. The following lengths were assumed for the filaments; *a*, thick filament (myosin), 1.60 μm; *b*, thin filament (actin + tropomyosin + troponin) extending through the Z line, 2.05 μm; *c*, projection-free zone in the middle of thick filaments, 0.20–0.25 μm. (b) Length–tension curve for part of a single frog skeletal muscle fibre (schematic summary of results). The numbered arrows show the various critical stages of overlap that are portrayed in (c).

assumed in Fig. 2. This meant that the mechanical data no longer matched the predictions of the sliding filament hypothesis quite so precisely—for example, the plateau of the length–active tension curve should occur between 1.9 and 2.15 μm.

Next, Taylor & Rüdel (1970) found that at short sarcomere lengths frog muscle fibres are not properly activated by electrical stimulation, probably

because of a failure of the excitation–contraction coupling mechanism. They were able to activate fibres more fully by stimulating them in a bathing medium containing low concentrations of caffeine (Rüdel & Taylor 1971); the tensions produced at short sarcomere lengths were then greater than those shown in Fig. 2. This was the first evidence that the form of the length–tension relation in frog muscle might not be solely governed by the sliding filament mechanism.

Close (1972) obtained further evidence that activation processes might play a part in determining the form of the length–tension relation of frog skeletal muscle. He found that at room temperature the length–tension curve for tetanic contractions fell close (Fig. 3*A*) to that obtained by Gordon *et al.* (1966); however, the curve for twitches showed an additional discontinuity at about 2.8 μm (Fig. 3*B*). In muscles of small cross-sectional area the slope of the descending limb changed at this sarcomere length, but in muscles of larger cross-

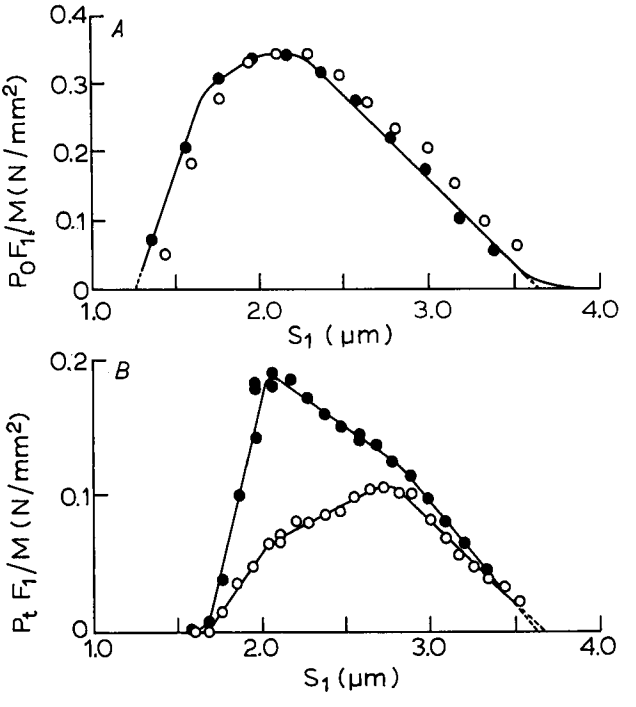


FIG. 3. Length–tension relations for tetanic (*A*) and twitch (*B*) contractions of two frog sartorius muscles (redrawn from Close 1972). The abscissa, $S_1$, is the average sarcomere length in μm. The ordinate is the estimated tension per unit cross-sectional area in N/mm² (note the difference in scale for *A* and *B*): ●, muscle of small cross-sectional area; ○, muscle of larger cross-sectional area. The curve in *A* is the length–tension relation obtained by Gordon *et al.* (1966) (see Fig. 2).

sectional area this was the length at which maximum active tension occurred and the length–tension curve, therefore, had a very long ascending limb. Close considered the latter to be characteristic of muscles that were only partially activated.

Recently, Endo (1973) has presented some extremely interesting results obtained from a skinned fibre preparation, which throw further light on Close's ideas. (The skinned fibre preparation is a single muscle fibre which has had its sarcolemma removed so that the contractile proteins are exposed to the bathing medium.) Endo produced graded activation of the fibres by varying the calcium ion concentration in the bathing medium; the resulting length–tension curves were as shown in Fig. 4. When the fibres were fully activated with a high concentration of $Ca^{2+}$, the descending limb of the curve was of the form shown in Fig. 2, but when the fibres were only partially activated the curves were of the form shown in Fig. 3, with an optimum at about 2.8 μm and a long ascending limb.

After that brief survey of some recent developments in skeletal muscle physiology, we must now turn our attention to cardiac muscle. It would clearly

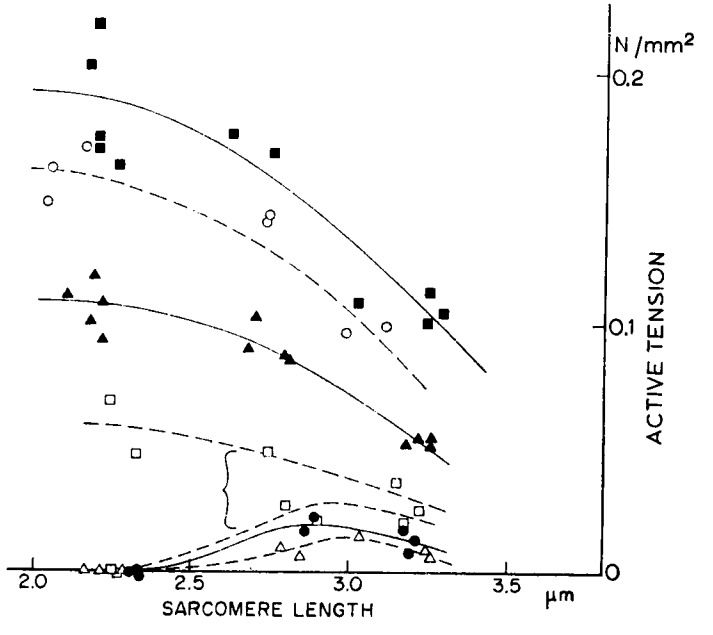


FIG. 4. Length–tension relations of skinned muscle fibres of *Xenopus laevis*, activated by different $Ca^{2+}$ concentrations (redrawn from Endo 1973): ■, 30 μM; ○, 3 μM; ▲, 1.5 μM; □, 1.0 μM; ●, 0.85 μM; △, 0.7 μM. The active tension (in N/mm²) is plotted against sarcomere length.

be most unwise to assume that its length–tension relation is determined simply by the sliding filament system unless other possibilities can definitely be excluded. Length-dependent activation processes strongly influence the form of the curve for skeletal muscle when it is partially activated, and this may also happen in cardiac muscle, which must be regarded as only partially activated under normal conditions. It will be difficult to distinguish between these and other possible mechanisms for the length–tension relation in cardiac muscle, but until we do we shall lack the essential key to understanding the physiological basis of Starling's law of the heart.

ACKNOWLEDGEMENTS

Fig. 1 is redrawn from Fig. 11 of Spiro & Sonnenblick (1964) by permission of the American Heart Association, Inc.; the other figures are published with permission.

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# Measurements of structural parameters in cardiac muscle

SALLY G. PAGE

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*Abstract* Earlier measurements of sarcomere lengths in cardiac tissue are reviewed, including measurements of the range of values in the intact heart and of the value corresponding to $l_{max}$ in isolated preparations. The uncertainties which are inherent in such measurements due to shrinkage of the tissue during preparation for electron microscopy and to other factors are discussed and it is suggested that, because of these sources of error, many published values of sarcomere lengths are as much as 10–12% too low.

Some new measurements of the filament lengths in kitten papillary and frog atrial muscles have been made. The A filaments in both tissues have the same length as in vertebrate skeletal muscles (1.55–1.6 μm). The I filaments are longer than in frog skeletal muscles, being 1.0–1.1 μm in both frog and kitten cardiac muscles. Certain irregularities in the individual I filament lengths within any given sarcomere have been found in both cardiac tissues as well as others in the A filament lattice of the frog atrial muscle.

Two main structural parameters are important in the analysis of the mechanical behaviour of heart tissue. One is the sarcomere length which, as is known from studies on skeletal muscle, decisively determines the contractile response —tension, for example, speed of shortening (Gordon *et al.* 1966) and possibly also the extent of activation (Rüdel & Taylor 1971). In assessing aspects of heart performance, therefore, we should know not only the relation between these physiological properties and sarcomere length but also the range of sarcomere lengths over which the heart normally works.

Secondly, for the interpretation of the effects of various sarcomere lengths, we need to know the lengths of the A and I filaments, because the extent of overlap of these filaments is the important parameter of contractile strength.

I shall review briefly the reported measurements of these parameters, discuss some of the technical difficulties that arise during such measurements and,

finally, present some new data on filament lengths and organization in the mammalian and amphibian heart.

## PROBLEMS OF SHRINKAGE AND OTHER SOURCES OF ERROR

As is widely recognized, attempts to make accurate measurements of either sarcomere or filament lengths from fixed material are beset by sources of errors and uncertainties at almost every stage of the experimental procedure: shrinkage can occur during fixation and dehydration, distortions may arise, either directly or indirectly, from section cutting and problems are associated with calibrating the electron microscope. The last source of error is easily eliminated, at least in principle, provided all relevant factors have been controlled or monitored; unfortunately, published reports rarely give enough information to show whether this has been done adequately.

Another major source of error, the unidirectional compression during the cutting of thin sections, can also be eliminated, and usually is, by choosing the appropriate direction of sectioning, that is, with the cutting edge parallel to the fibre axis. A second, more indirect, distortion in sections not exactly parallel to the fibril axis is less easily avoided. Simple geometrical considerations show that even if this obliquity is sufficiently small for the resulting error to be negligible in fibrils whose band patterns are truly square to the fibril axis, the error can become appreciable and is not easily detected if Z lines and A–I boundaries are also oblique to the fibril axis. However, such obliquity, which is not uncommon in fixed cardiac muscle, should be unlikely to lead to a systematic error in length determination if sampling is adequate, although it should increase the scatter of the results.

Undoubtedly, the major source of error and uncertainty is the shrinkage which occurs during fixation and dehydration. In skeletal muscle, the amount of shrinkage varies, to some extent predictably (Page & Huxley 1963); it depends, for example, on whether the overall length of the muscle is held constant and on the fixative. Also, certain parts of the sarcomere shrink more than others. Further, even after apparently identical and optimal procedures, variations in the amount of shrinkage still occur from one preparation to the next, causing an uncertainty which is difficult to assess precisely.

Originally, we found that glutaraldehyde caused much less shrinkage than osmium tetroxide in skeletal muscle, and many authors since then have used glutaraldehyde in the hope of minimizing shrinkage. But, in contrast to the original study, in which secondary fixation in $OsO_4$ was not used, almost all studies on heart muscle have used $OsO_4$ as well, thereby losing much of the benefit, in this respect, of the use of glutaraldehyde. I should also mention that

more recent work with skeletal muscle showed that even glutaraldehyde alone sometimes produces shrinkage of some 4–7% (unpublished observations).

Table 1 shows the general pattern of shrinkage in heart muscle. As I have not so far found a preparation in which the shrinkage could be assessed, for example, by observing the sarcomere length at every stage of the preparative procedure, as was possible in the skeletal muscle studies, I have instead used the final A band length as a measure of the extent of shrinkage. In other cases, the fine periodicity sometimes seen along the length of the I filament also served as a guide. It is clear that, even in a muscle nominally held at a constant length throughout both fixation and dehydration, the filaments shrink. Depending on the behaviour of non-fibrillar elements in series with the fibrils, this shrinkage need not and does not always reflect a corresponding change in sarcomere length. On the other hand, in the last preparation listed, which was unrestrained throughout most of the time of fixation and dehydration, overall length changes of the fibrils should be possible; as Table 1 shows, the greatest shortening of the filaments was seen in this tissue and this is associated with shortening of the sarcomere. As it is this last method of fixation which most closely follows the preparative procedures used in most other studies of sarcomere lengths in cardiac tissue, it seems likely that up to 10–12% shrinkage could have occurred in these tissues also. Thus, with sarcomeres in the range 1.5–2.5 μm, the possible error, and thus the extent of the uncertainty, should be as much as 0.15–0.3 μm.

TABLE 1
Shrinkage in kitten papillary muscles

| *Fixation* | *A band length* | | *I filament periodicity* | |
|---|---|---|---|---|
| | *(μm)* | *( % of probable* in vivo *values)* | *(nm)* | *( % of probable* in vivo *values)* |
| None; probable *in vivo* values (see text) | 1.6 | 100 | 38.5 | 100 |
| Glutaraldehyde alone | 1.49 ± 0.03 ($n = 39$) | 93 | | |
| Glutaraldehyde + $OsO_4$; restrained from overall shortening (3 different muscles) | 1.46 ± 0.03 ($n = 58$) | 91.5 | | |
| | 1.50 ± 0.03 ($n = 45$) | 94 | | |
| | 1.49 ± 0.04 ($n = 54$) | 93 | 36.2 | 95 |
| Glutaraldehyde + $OsO_4$; unrestrained after first 10 min of fixation | 1.44 ± 0.04 ($n = 27$) | 90 | 33.6 | 88 |

Except for the last measurement, the muscles were held at constant length throughout fixation and dehydration. Sections were cut with the knife edge parallel to the fibril axis.

## SARCOMERE LENGTH AT $l_{max}$

Table 2 lists reported measurements of the sarcomere length at which contractile tension is maximal. The measurements were all made on isolated papillary muscles, fixed at $l_{max}$. I have also estimated the corresponding *in vivo* values—the values corrected for shrinkage on the basis of internal evidence from the papers concerned. The results suggest that the optimum sarcomere length for active tension development is around 2.3–2.35 μm but, as should be obvious from the preceding discussion, this cannot be taken as a precise estimate. Nor does it seem likely that more reliable results from fixed material will be obtainable, although holding the muscle throughout fixation and dehydration would clearly be advantageous. Greater precision requires, to my mind, a different experimental approach, such as might be provided by certain optical studies of living preparations.

One point which I should mention in this context concerns results obtained in a range of short sarcomere lengths (i.e. below $l_{max}$). In resting skeletal muscles, fibrils do not normally shorten below this sarcomere length, and if made to do so become 'wavy' (González-Serratos 1971). In actively shortening muscles, however, I filaments from opposite Z lines can overlap in the middle of the A band, and so lead to short sarcomeres in fibrils which remain straight rather than become 'wavy' as at rest. The question arises whether the same phenomenon also occurs in the heart, which may normally work in a range of fairly short sarcomere lengths. Such published evidence as I have found is conflicting; it is not clear how far the differences reported are due to trivial differences in the techniques employed and how far to something more interesting. Both Gay & Johnson (1967) and Grimm and colleagues (Grimm & Whitehorn 1968; Grimm

TABLE 2
Sarcomere length at $l_{max}$

| *Animal* | *Sarcomere length (μm)* | | *Reference* |
|---|---|---|---|
| | *Measured* | *'Corrected' for shrinkage* | |
| Cat | 2.2 (2.1–2.4) | 2.35[a] | Sonnenblick *et al.* (1963) |
| Cat | 2.2–2.3 | 2.35–2.45[a] | Spiro & Sonnenblick (1964) |
| Cat | 2.2 (2.1–2.3) | 2.4[b] | Fawcett & McNutt (1969) |
| Rat | 2.07 | 2.25–2.3[c] | Grimm & Whitehorn (1968) |
| Rat | 2.03 | 2.2–2.25[c] | Grimm *et al.* (1970) |

All measurements were made on papillary muscles fixed at $l_{max}$ in glutaraldehyde + $OsO_4$ (cat) or formalin (rat). The correction for shrinkage was based on (*a*) the value of the A band length in the fixed tissue (1.5 μm), assuming uniform shrinkage throughout the sarcomere, (*b*) an assumed shrinkage of 10%, as found for this method of fixation in the present study (see Table 1), or (*c*) the estimate given by the authors of the amount of shrinkage.

*et al.* 1970), for example, find that at short sarcomere lengths the fibrils, and perhaps the fibres too, are wavy, whereas Spiro and co-workers (Sonnenblick *et al.* 1963; Spiro & Sonnenblick 1964) show straight fibrils in sarcomeres as short as 1.5 μm. Gay & Johnson find waviness of fibrils in living rabbit strand preparations below about 2.0–2.2 μm, that is, at values not very different from the critical value of about 1.9 μm (corrected for shrinkage) found by Grimm and colleagues in fixed rat papillary muscle. Are these different results due to the fixation process used (glutaraldehyde perhaps causing some contraction and loss of waviness) or, as suggested also by Gay & Johnson, do they arise because only Spiro and colleagues stimulated the muscles at the short lengths before, though not during, fixation? The restoring forces in cardiac muscle leading to sarcomere lengthening may not be as strong as in skeletal fibrils, or there may even be a low level of maintained activation which is insufficient to initiate shortening, but enough to maintain it (cf. Matsubara & Millman 1974).

## RANGE OF SARCOMERE LENGTHS IN SITU

The normal range of sarcomere lengths in the intact heart has been studied in the rat (Grimm & Whitehorn 1968), rabbit (Anversa *et al.* 1969), cat (Spotnitz *et al.* 1966) and dog (Spotnitz *et al.* 1966; Laks *et al.* 1967; Sonnenblick *et al.* 1967; Leyton *et al.* 1971; Yoran *et al.* 1973), mainly in the left ventricle, and usually in the mid-wall region. On the whole, the various results agree, with the exception of rather high values given by Laks *et al.* (1967). Thus, at low end-diastolic or filling pressures, sarcomere lengths in fixed preparations are 1.8–1.9 μm, whereas at about 10–12 mmHg values of 2.0–2.2 μm are reported, and at higher pressures sarcomeres may lengthen up to 2.3–2.4 μm (all values are uncorrected for shrinkage). Although I would like to conclude from these values that, at moderate filling pressures, the heart is working on the ascending limb of the length–tension relation, I feel that owing to the uncertainties due to shrinkage, etc., of 0.2–0.3 μm in the measured sarcomere lengths both at $l_{max}$ and *in situ* this cannot yet be regarded as firmly established.

It seems generally agreed that the sarcomere length differs from region to region in the heart (e.g. across the ventricle wall), but there is lack of agreement about the pattern of this variation. More decisive information is required, therefore, before this potentially interesting phenomenon can be discussed adequately.

## FILAMENT LENGTHS

The A and I filaments in cardiac muscle have lengths similar to those in skeletal muscle, namely, around 1.5 and 1.0 μm for A and I filaments, respect-

ively (Spiro & Sonnenblick 1964; Fawcett & McNutt 1969). However, no great precision was claimed (or had been attempted) in these earlier reports and, indeed, the technical problems of achieving a reasonable degree of accuracy in measurements of this kind are considerable. One reason for this is that errors due to shrinkage are even more complex here than during the measurement of sarcomere lengths since changes in filament lengths can occur without accompanying changes in sarcomere length and the degree of shrinkage may vary in different portions of the same filament. To assess some of these errors we use the fine periodicities present along the length of both A and I filaments as an internal standard. These can sometimes be identified and measured directly on the electron micrographs, but a more sensitive and reliable method is that based on the optical diffraction patterns from the electron micrographs (O'Brien *et al.* 1971). The values of the periodicities obtained by these methods can be compared with the corresponding measurements from X-ray diffraction studies of living muscle (usually skeletal muscle). In correcting for shrinkage in cardiac muscle in this way, it is assumed that the *in vivo* periodicities of the filaments are identical for cardiac and skeletal muscle. This assumption appears to be justified, for the A filament periodicity, by recent X-ray results from mammalian cardiac muscle (Matsubara & Millman 1974).

An additional difficulty which arises in the determination of the I filament length is that in cardiac muscle, as also in certain types of skeletal muscle, the boundary of the H zone is usually ill-defined, if it can be discerned at all. As a consequence, the I filaments have to be measured by a method in which individual filaments can be identified and the position of their ends within the sarcomere determined (see later). I shall now summarize my own attempts to obtain filament lengths in two cardiac tissues, the kitten papillary and the frog auricle muscle.

### *A filaments*

These results, for both A and I filaments, were obtained from muscles held at a constant length throughout fixation and dehydration. The A filament length, determined from a number of A bands, ranged from 1.46 to 1.50 μm in four papillary muscles (Table 1, lines 2–5). As the A filament length in frog skeletal muscle fibres, after precisely the same procedures as those used here, ranged from 1.45 to 1.5 μm, we may safely conclude from the closeness of these results that the *in vivo* length also is probably similar in the two muscles, that is, between 1.55 and 1.6 μm, as obtained for skeletal muscle after allowance for shrinkage (Page & Huxley 1963). A more direct and precise estimate was obtained for one papillary muscle (Table 1, line 5), in which the amount of shrinkage was determined from the results of an optical diffraction study undertaken by