ABSTRACT
The way that contractility varies between beats—the force-frequency relationship—was determined for rabbit papillary muscles held at different lengths. When the maximum rate of rise of tension in a contraction, $F_{\text{max}}$, was used as a measure of contractility, the way contractility changed between contractions was independent of length. That is, when the values of $F_{\text{max}}$ obtained at one length were multiplied by an appropriate scaling factor, the force-frequency relationship determined at that length was indistinguishable from the force-frequency relationship determined at another length. If, however, the peak tension in a contraction was used as a measure of contractility, the force-frequency relationship generally was not independent of muscle length. Therefore, it is proposed that the ratio of two values of $F_{\text{max}}$ obtained by perturbing the rate of stimulation at any given length should be tried as a length-independent index of the inotropic state of the muscle.

KEY WORDS maximum rate of rise of tension peak tension rabbit Frank-Starling law inotropy stress relaxation force-frequency ratio

None of the indexes of cardiac contractility so far proposed are independent of muscle fiber length. The peak tension and the maximum rate of rise of tension in isometric contractions of isolated papillary muscle (1-3) and the peak intraventricular pressure and the maximum rate of development of ventricular pressure in the intact heart (4-7) change dramatically with changes in muscle fiber length according to the Frank-Starling law.

A quantity shown to be essentially constant over a wide range of sarcomere lengths (3.10-1.95μ) in skeletal muscle is the velocity of shortening at zero load, $V_{\text{max}}$ (8). The velocity obtained by extrapolating the load-velocity curve of isolated cardiac muscle to zero load (so-called $V_{\text{max}}$) is independent of muscle length and dependent on the inotropic state of the muscle (3, 9), seeming to qualify this $V_{\text{max}}$ as the desired index of cardiac contractility. However, much effort has been expended to measure $V_{\text{max}}$ in the intact hearts of animals and man as a means of evaluating myocardial performance or ventricular function (10-13). The length independence of this $V_{\text{max}}$ is surprising since, in cardiac muscle, the load is partly borne by a parallel elastic element. Therefore, the simplest mechanical representation of cardiac muscle is a three-element model rather than the two-element model that is appropriate for skeletal muscle, so that $V_{\text{max}}$ of the overall muscle and $V_{\text{max}}$ of the contractile element cannot be simultaneously independent of muscle length (14). Indeed, recent experiments (15-17) with isolated cardiac muscle have shown that $V_{\text{max}}$ of the overall muscle does, in general, depend on muscle length. Moreover, $V_{\text{max}}$ of the contractile element calculated from assumed mechanical models also depends on muscle length (17).

The concept has been, then, that changes in contractility are of two kinds, one resulting from changes in fiber or sarcomere length and the other resulting from other changes in the muscle which, collectively, represent changes in an inotropic state of the muscle. Moreover, it has been further presumed that a quantity could be found which would measure the inotropic changes alone. Failure to find such a quantity could mean either that the two kinds of changes are inextricably interdependent or that no measurable quantity exists (or can be found) which is purely a measure of changes of the inotropic kind. Rather than be driven to these conclusions and give up the search entirely, one should perhaps give up the search for a single
quantity inherently independent of length and, instead, search for a ratio of two length-dependent quantities, the value of which can be demonstrated to be independent of length. For example, such a ratio would be independent of length if a change in length merely multiplied the quantities by the same factor. In the present paper we exploit the force-frequency relationship to obtain such a ratio.

Methods
Rabbits at least 6 weeks old were killed by a sharp blow to the back of the neck. Hearts were excised from the rabbits within 30 seconds and placed immediately into Krebs-Henseleit solution (18) aerated with a gas mixture of 95% O\textsubscript{2}-5% CO\textsubscript{2} at 38°C. Papillary muscles were excised from the right ventricle and mounted in a thermostatically controlled tissue bath at 38°C. The tissue bath was a channel, 1 ml in volume, milled into one face (2 cm x 4.5 cm) of a rectangular block of silver (2 cm x 4.5 cm x 1.5 cm). The channel was closed with a Lucite lid in which a strip of silver (1 cm x 0.3 cm) was embedded. This silver strip together with the walls and floor of the bath formed the two stimulating electrodes. The muscle was stimulated with a 1-msec pulse that was 25% greater than threshold. The bathing solution passed (5 ml/min) through a heat exchanger, formed by a series of interconnecting tunnels, 0.15 cm in diameter and 25 cm in length, drilled into the walls of the bath. The block was heated with a thin Nichrome ribbon heating element wrapped around and thinly insulated, electrically, from the silver, and the temperature of the block was thermostatically controlled to within 0.01°C. The chorda tendina end of the papillary muscle was tied with 4-0 silk thread using a sheet bend knot; the chorda was the bight of the knot. The thread was tied at one end of the chamber to a stainless steel hook attached to a semiconductor strain-gauge force transducer (resonant frequency approximately 250 Hz, compliance 10^{-4} cm/dyne). The base of the papillary muscle was tied firmly to a silver post at the other end of the bath. The two strain gauges of the transducer formed two arms of the bridge of a Tektronix carrier amplifier unit, the output of which was differentiated using an operational amplifier as a differentiator (high frequency cutoff 200 Hz, gain constant 30 m/sec, rise time 4 m/sec). Both the force signal and its time derivative were displayed and photographed on a Tektronix storage oscilloscope. The force transducer was mounted on a worm screw with a vernier so that the muscle length could be controlled and measured.

Results
AN INOTROPIC INDEX: RATIONALE
Any of a variety of indicators of contractility, e.g., tension, intraventricular pressure, or their first derivatives, show that the contractility of cardiac muscle changes with muscle length. Moreover, at any given length, contractility can be made to change by a number of inotropic maneuvers, e.g., changes in rate and pattern of stimulation, changes in ionic environment, and exposure of the tissue to drugs. Therefore, the contractility, C, is a function of the muscle length, l, and some inotropic function, ψ:

\[ C = f(l, \psi). \]  (1)

Conceivably, the inotropic function appropriate to any particular measure of contractility could depend on the length of the muscle. In this case, Eq. 1 would be

\[ C = f[l, \psi(l)]. \]  (2)

In other words, the way in which contractility is changed by an inotropic agent could itself depend on the length of the muscle. For example, the slope of the dose-response curve (log active tension vs. log concentration) for a drug could depend on muscle length. Were this the case, it would be impossible to distinguish the effects of changes in l from changes in ψ. Only if some measure of contractility can be found for which ψ is independent of l, would it be possible to distinguish changes in l from changes in ψ. In such a case, C, would take one of the following two forms:

\[ C = h(l) + g(\psi) \]  (3)

or

\[ C = h(l) \cdot g(\psi). \]  (4)

To determine whether the contractility, C, takes either of these two forms, two or more values of contractility must be measured at each of two lengths. These values could be the inotropic response to two doses of a drug or they could be chosen from the innumerable values that can be obtained from exploiting the force-frequency relationship.

If Eq. 3 is correct, then the difference between the contractilities for the two values of inotropy, e.g., the two doses of a drug, would be the same at both lengths. If, on the other hand, Eq. 4 is correct, then the ratio of the contractilities for the two values of inotropy would be the same at both lengths. If neither the difference nor the ratio of the contractilities is independent of muscle length, then it must be concluded that ψ is inherently a function of l, i.e., Eq. 2 holds.

The many functions that make up the entire inotropic function fall into two classes. One class—the force-frequency relationship—encompasses the complete dependency of contractility on the intervals between contractions in the past. The other class includes a family of, what are in essence,
dose-response curves, where the dose might be the temperature or the concentration of ions, gases, metabolites, or drugs. Our first choice of inotropic function is the force-frequency relationship rather than any member of the second class, because a dose-response curve is a single-valued function, whereas the force-frequency relationship is a complex of many interrelated functions. Not only is the force-frequency relationship in practice more easily determinable and less subject to secondary variations due to extracardiac factors, but also, because of its complexity, it is potentially a much more sensitive indicator of inotropic changes than is a single dose-response curve.

THE FORCE-FREQUENCY RELATIONSHIP

Changes in contractility as measured by the maximum rate of rise of tension in a contraction, $F_{\text{max}}$, following a sudden change in rate of stimulation occur in two phases. In the first or rapid phase, the changes in $F_{\text{max}}$ from contraction to contraction are large; in the subsequent or slow phase, the changes are small, becoming less and less until a new steady value of $F_{\text{max}}$ is reached. The initial rapidly equilibrating changes (exemplified by postextrasystolic potentiation and negative and positive staircase) and the subsequent slowly equilibrating changes have been observed in the heart in vivo (19–21) and in the isolated preparation (2, 22). In the in vivo heart, the slow changes of contractility that follow a sudden change in rate almost obliterate the contribution of the rapid changes that occur in the first few beats (20, 21). Because our intention was to develop an index of contractility that ultimately can be used in vivo, it would have been foolish to choose the steady-state contractility at different rates of stimulation to obtain the different values for the inotropic function.

The rapidly equilibrating changes in contractility were analyzed by the method of Johnson et al. (23) with their two-stage experiment. The first stage determines the way contractility changes between contractions at a constant rate. The muscle is stimulated at a low constant rate (20/min) and a test stimulus is applied regularly but infrequently, e.g., after every fifth regular stimulus, so that the effect of the contraction in response to the test stimulus has time to pass away before the next test stimulus is applied. The time between the test stimulus and the preceding regular stimulus, the test interval, is varied, and $F_{\text{max}}$ of the contraction in response to the test stimulus is plotted as a function of the test interval. The second stage of the experiment determines the effect of an extra contraction on the time course of contractility between regular contractions. This stage is identical to the first stage except that the test stimulus is applied after an extra stimulus positioned at a fixed time after the preceding stimulus at the regular rate. As before, $F_{\text{max}}$ of the test contraction is plotted as a function of the test interval. An example of the results from such an experiment is shown in Figure 1.

Normally, the second stage of the experiment is repeated for several fixed positions of the extra stimulus relative to the preceding regular stimulus. For the purpose of the present study, however, only one position was considered to be necessary, since one position provided enough data to establish the way the force-frequency relationship depended on length, and the time saved reduced the amount of deterioration in contractility due to aging of the muscle during the experiment.

An abridged version of this experiment was used to monitor the time-dependent changes in the force-frequency relationship that occur following a change in length and to detect a change in the relationship with length. As in the second stage of the above experiment, an extra stimulus was introduced at a fixed interval following every fifth regular contraction, but unlike the above experiment, the test interval (e.g., 2.5 seconds) was kept constant. Thus the same pattern of stimulation was continually repeated; with each repetition, the ratio of $F_{\text{max}}$ for the test and the regular contractions updated the value of the same, single, force-frequency ratio, allowing the effect of a length change on the ratio to be followed.
EFFECTS OF LENGTH CHANGE

The muscle was stretched close to the length at which \( F_{\text{max}} \) was maximum, and, after waiting 20–30 minutes for \( F_{\text{max}} \) to reach reasonably steady values, the two-stage experiment described above was performed. The muscle was then shortened to a length at which the active tension was very small, and after 20–30 minutes the two-stage experiment was again repeated. Figure 2 shows typical results of such experiments from one of six muscles. Except for a scaling factor, the way \( F_{\text{max}} \) changed between contractions was the same at the two lengths. It is reasonable to assume from the results of this experiment that the inotropic function describing the force-frequency relationship is independent of length and that Eq. 4 rather than Eqs. 2 or 3 describes the dependence of \( F_{\text{max}} \) on muscle length.

Selecting the right measure of contractility is important: if peak tension had been used as a measure of contractility, the force-frequency relationship so obtained would not have been independent of length. The findings in Figure 3 not only illustrate this point but warn us that the use of a single force-frequency ratio to establish the length independence would be insufficient, since at some points in the relationship in Figure 3 the force-frequency ratio happens to be the same at two lengths. If, fortuitously, such a point had been chosen to evaluate the force-frequency ratio, it could have been concluded, erroneously, that the ratio was in general independent of length and that peak tension was an appropriate index of contractility.

The transient changes in \( F_{\text{max}} \) that occur after a change in length were monitored in 20 muscles.
using the abridged experiment described above. These changes occurred in two phases. In the initial phase, the ratio declined regardless of the direction of the length change and returned to its original value within 0.5–10 minutes (Fig. 4). However, during the next 20–40 minutes, the ratio remained constant, but $F_{\text{max}}$ continued to increase or to decline, depending on the direction of the length change, to a new stable value (Fig. 5). The nature of these changes is unknown, but the initial transient might be related to catecholamine release, since the magnitude and the duration of the change in the ratio was reduced by exposure of the preparation to propranolol ($10^{-7}$ g/ml); the later phase of changes in $F_{\text{max}}$ remained unchanged. The most plausible explanation for the later phase is that it is a consequence of changes in muscle fiber length associated with a slow stress relaxation of some mechanical element in the muscle.

Neglecting the initial transient phase, the force-frequency ratio is independent of length. This finding is true throughout the ascending and into the descending limb of the Frank-Starling relationship. In Figure 6 the magnitudes of two values of $F_{\text{max}}$ and their ratio are plotted at five muscle lengths. Although the magnitudes of $F_{\text{max}}$ cover a wide range, the ratios remain essentially constant.

**Discussion**

We have found that the force-frequency ratio meets the requirements of a length-independent inotropic index, i.e., the ratio of two values of contractility obtained by perturbing the rate of stimulation at one length is exactly the same as the ratio obtained from the same perturbation at any other length. Could such an index be shown to exist in the intact animal and man? And, if so, do changes in contractility change this index? These two questions need to be answered.

Although there is no question that changes in contractility in the intact heart can be produced by a perturbation in rate, it is by no means certain which quantity, when used as a measure of contractility, will yield a ratio that is independent of fiber length. For example, in papillary muscle, had peak tension been used as a measure of contractility rather than the maximum rate of rise of tension, the force-frequency ratio would not have been independent of length. The intraventricular pressure, wall tension, or their first derivatives are among the likely candidates that should be investigated.

With regard to the second question, all changes in contractility could conceivably occur with no change in the force-frequency ratio; then this index

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**FIGURE 4**

Changes in a force-frequency ratio following a 20% increase in muscle length: results of an abridged experiment. The ratio of $F_{\text{max}}$ of a test contraction following an extra contraction (TS) to $F_{\text{max}}$ of the previous regular contraction (R) is plotted as a function of time following the change in length. The arrow indicates the moment of stretch. Fixed values for the test interval and the extra stimulus interval were 2.5 seconds and 0.22 seconds, respectively.

**FIGURE 5**

Changes in $F_{\text{max}}$ and force-frequency ratio following a 20% increase in muscle length. $F_{\text{max}}$ of the preceding regular and test contractions and their ratio are plotted as a function of time following the change in length. Solid squares = $F_{\text{max}}$ of the test contraction, open squares = $F_{\text{max}}$ of the regular contraction, and X = the ratio of $F_{\text{max}}$ of the test contraction to $F_{\text{max}}$ of the regular contraction. Note that the force-frequency ratio remains constant throughout the changes in $F_{\text{max}}$. Same experiment as that considered in Figure 4.
would be useless. Although there is evidence that this situation is not the case for a variety of inotropic agents (24), it is by no means clear that the inotropic actions of these agents can be distinguished from one another by their effects on the force-frequency relationships. Clearly, such a study is needed.

We hope that, ultimately, the force-frequency ratio can be used to evaluate myocardial performance and to quantify the degree of cardiac failure. To achieve this goal one must begin with the isolated preparation in which the entire force-frequency relationship can be determined. This complex, multivalued function is a particularly sensitive and exacting indicator of a change in inotropy since the complete function encompasses a multiplicity of force-frequency ratios, some of which may change with a change in contractility while others may not. Exhaustive examination of all possible force-frequency ratios not only establishes whether any change in contractility coincides with changes in any force-frequency ratio, but also pinpoints the force-frequency ratio that is most sensitive to a given change in inotropy, in this case the changes in inotropy that we call heart failure.

This advantage of the isolated preparation must be weighed against the considerable uncertainty as to the fidelity with which artificially induced states of negative inotropy mimic naturally occurring failure in the intact heart. For this reason the investigation should expand to include the whole animal in which more realistic failure states may be induced. The limited ability to examine the force-frequency relationship under these circumstances should be more than offset by previous insight gained from more detailed studies in the isolated preparation.

In the final adoption to the clinical state in man, we foresee the use of two or three, if not a single, force-frequency ratio determined at points in the force-frequency relationship that have been found to be the most sensitive to the suspected change in inotropy.

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Force-Frequency Relationship: A Basis for a New Index of Cardiac Contractility?

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Circ Res. 1973;33:665-671
doi: 10.1161/01.RES.33.6.665

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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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:
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