A Study of the "Staircase" in Ventricular Muscle and Its Relationship to the Inotropic Activity of Certain Drugs

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ABSTRACT Tension duration curves recorded during isometric contractions of toad ventricular strips were studied under a variety of conditions. Changes recorded in these curves during inotropic responses could be divided into two classes: (*a*) those in which the time required to reach peak tension (t_p) was decreased and the twitch duration shortened. (*b*) those in which t_p was either unaltered or increased and the twitch duration unchanged. The first class is represented by excess Ca^{++}, strophanthin, and the "staircase" phenomenon while the second is represented by noradrenaline, "amine-like" substances liberated by tyramine, cocaine, and reserpine, and reduced K^+. The results suggest that Ca^{++} is a common factor underlying the inotropic response recorded both during the staircase and during glycoside activity. The involvement of amines in the staircase was not demonstrated.

INTRODUCTION

Bowditch, in 1871, first reported a step-like augmentation of the contractile force in isolated hearts following a rest period. This phenomenon he called the "staircase." The original observation, which was made on the frog heart, has subsequently been confirmed in the hearts of many other species, *e.g.*, the perfused dog heart (Woodworth (1902), cat papillary muscle (Cattell and Gold, (1955), and isolated rabbit ventricular muscle (Rosin and Farah (1955)).

Several attempts have been made to explain the fundamental events which result in this staircase phenomenon. Hofmann (1926) suggested that a link between excitation and contraction, firmly established during isochronous repetitive stimulation, becomes weakened during a period of rest. Hajdu and

The expenses of this investigation were defrayed by a grant from the Life Insurance Medical Research Fund of Australia and New Zealand.
Received for publication, April 21, 1960.

Szent-Györgyi (1952) argued that the loss of K^+ which takes place during preceding contractions (Fenn and Cobb (1936)) establishes a favourable condition in which the contractile elements can more easily shorten. Others, including Loewi (1917), Moulin and Wilbrandt (1955), and Niedergerke (1956) maintain that the surface concentration of Ca^{++} rather than intracellular K^+ concentration regulates the strength of contraction. Whalen *et al.* (1958) proposed the hypothesis that "all augmentation phenomena are due

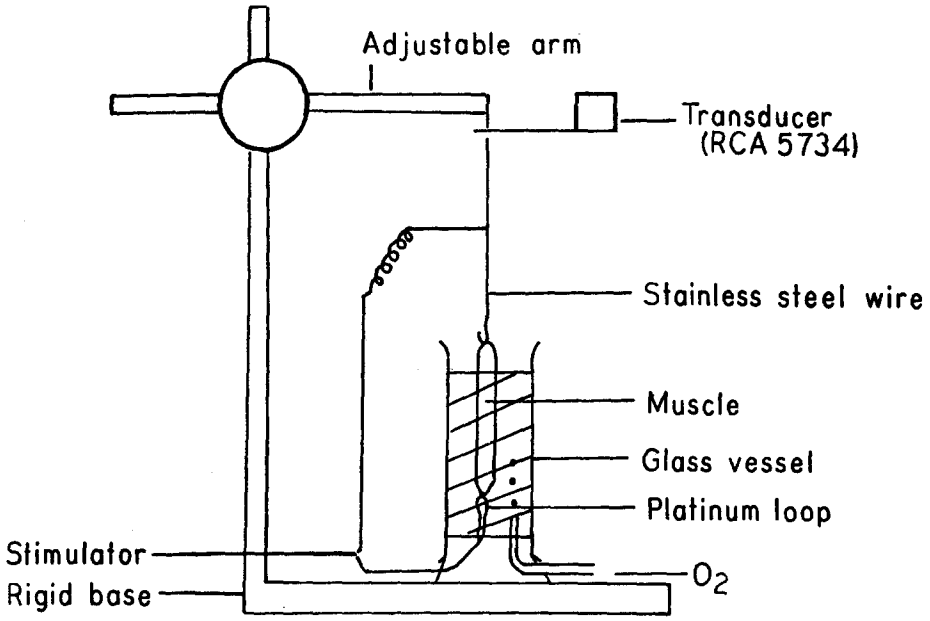

FIGURE 1. Diagram of the apparatus used for the measurement of tension duration curves during the isometric contractions of ventricular muscle strips. The whole assembly was mounted on a rigid platform.

to the intracardiac liberation of noradrenaline during each contraction" so that the amine liberated during preliminary contractions functions as a naturally occurring potentiating substance.

The investigations reported in this paper, which were performed on toad ventricular strips, were designed to reexamine these hypotheses and to determine which most satisfactorily explains the staircase phenomenon. Tension duration curves were recorded from ventricular muscle strips during isometric contraction under a variety of conditions and the changes noted compared with those detected during the staircase. Some general observations on the positive inotropic action of certain drugs on strips undergoing isometric contraction were also made.

METHOD

Strips approximately 1.5 mm. in thickness and 20 mm. in length were dissected from a circular portion of the ventricle in unselected summer toads (*Bufo marinus*) as described by Dale (1932), care being taken to remove all pacemaker tissue. The strips were suspended in the muscle chamber shown in Fig. 1.

Using linen thread previously soaked in the perfusion fluid the lower end of the muscle was firmly attached to the fixed platinum electrode and the upper end to the stainless steel wire leading to the transducer. Connections from the muscle to both the stainless steel and platinum loops were kept at a minimum. The preparations were stimulated at a rate of 30 beats/minute and an equilibration period of at least 40 minutes allowed, during which time the tension was adjusted to maintain isometric conditions. Unless otherwise stated the perfusion fluid, which was continually oxygenated throughout the experiment, had the following composition:

NaCl	115 mM
$NaHCO_3$	20.6 mM
$NaH_2PO_4 \cdot 2H_2O$	3.0 mM
$MgSO_4 \cdot 7H_2O$	1.2 mM
Glucose	16.5 mM
KCl	3.2 mM
$CaCl_2$	1.3 mM

All observations were made at room temperature (19–22°C).

Tensions produced during activity were recorded with an RCA 5734 electromechanical transducer to which the steel wire leading into the muscle chamber was attached (see Fig. 1). Both the stimuli and tension duration curves were displayed on a double beam oscilloscope and recorded photographically.

When required the staircase was elicited by interrupting stimulation for known time intervals and the tension duration curves compared before and after the pause. Measurements of the time to reach peak tension during the staircase refer to that time in the first beat after the pause.

In a series of experiments the effect of variations of $[Ca^{++}]$[1] and $[K^+]$ of the perfusate and of the addition of strophanthin-G,[2] tyramine,[3] noradrenaline,[4] and reserpine[5] on the time course of isometric tension records was investigated and compared with changes noted during the staircase.

[1] [ ] denotes concentration.

[2] Strophanthin-G as ouabain arnaud; Laboratoire Nativelle product.

[3] Tyramine as tyramine HCl; British Drug House product.

[4] Noradrenaline as levophed; Winthrop Laboratory product.

[5] Reserpine as serpasil, Ciba product.

RESULTS

(1) *Staircase*

Changes in the contractile force and twitch duration recorded during the staircase confirmed the results of Abbott and Mommaerts (1959) who noted that the progressive increases in the contractile force recorded during the

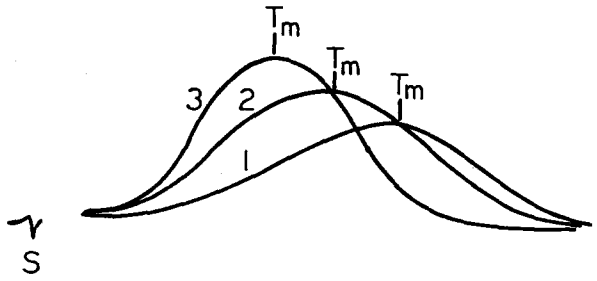

FIGURE 2. Superimposed tracings taken from the photographed record of the first three isometric twitches of a staircase following a rest interval of 12 seconds. Note that as the twitch tension of successive beats increased the t_p (time to reach maximum tension T_m) and the total twitch duration decreased. S = stimulus.

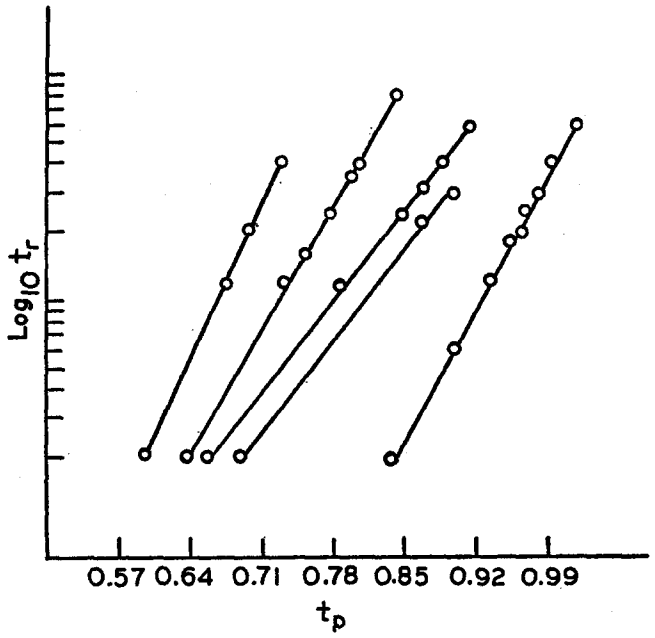

FIGURE 3. Results of five experiments showing the relationship between t_r (the duration in seconds of the pause preceding the staircase) and the time t_p taken to produce maximum tension (T_m) during the first beat of the staircase.

staircase were accompanied by changes in the rate of tension production and of the twitch duration. Comparison of the tension curves before and immediately after the pause revealed reduced twitch tensions associated with increased twitch durations after the pause. From the first three successive tracings of a staircase superimposed in Fig. 2 it is evident that early in the staircase the time (t_p) required to develop maximum tension (T_m) is increased although T_m itself is reduced. Thus, throughout the initial stage of the staircase the increased tensions recorded during successive beats are paralleled by increases in the rates of tension production and reductions in the durations of each

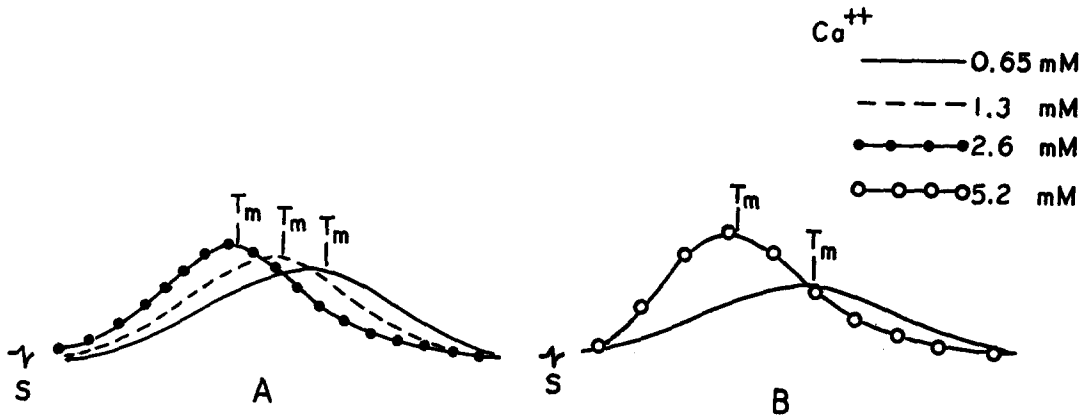

FIGURE 4. Tension duration curves recorded during isometric contractions of muscle strips perfused in Ringer's containing [Ca^{++}] 0.65, 1.3, 2.6, and 5.2 mM. S = stimulus, and T_m = peak tension. Note that the time (t_p) taken to produce maximum tension decreased as the magnitude of T_m increased (curves traced from photographed record).

twitch. Later, when the twitch tension had reached a steady state, the duration of the twitch continued to decline for several beats.

In other experiments the duration of the rest period (t_r) preceding the staircase was varied at random between 2 and 80 seconds. The time which elapsed between stimulation and the production of peak tension in the initial beat after the pause (t_p) was measured from the photographed trace and compared with that of the beat preceding the pause. In Fig. 3, where the mean results of five experiments are displayed on logarithmic scales, it is evident that the t_p interval varied according to the t_r duration.

(2) *Calcium*

In Fig. 4 the changes noted in the tension duration curves following variations in the Ca^{++} content of the perfusate are summarized. The strips were initially equilibrated in Ringer's containing half the normal [Ca^{++}] (0.65 mM) and the

[Ca^{++}] subsequently increased to 1.3, 2.6, and 5.2 mM as required. Ten minutes' equilibration was allowed after each Ca^{++} substitution and the stimulation rate kept constant throughout (30 beats/minute).

Comparison of these results with those shown in Fig. 2 reveals marked similarities. In each instance the increase in contractile force is associated with a decrease in both the t_p interval and the total twitch duration.

(3) *Potassium*

In a similar series the effect of variation in K^+ concentration on the tension duration curves was investigated. K^+ levels used included 0.8, 3.2, 6.4, 9.6, and 12.8 mM. Observations were made at room temperature and the stimula-

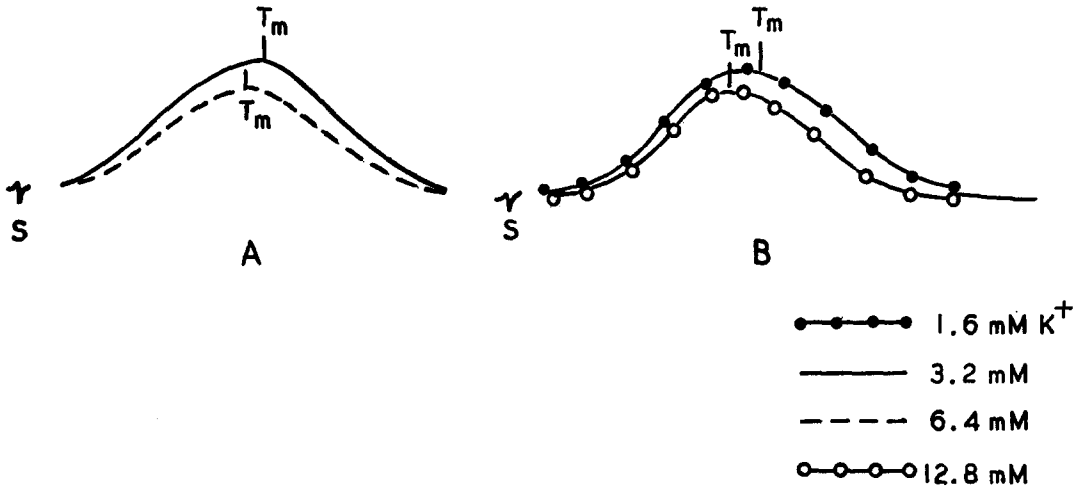

FIGURE 5. Isometric tension records of muscle strips perfused with Ringer's containing [K^+] 1.6, 3.2, 6.4, and 12.8 mM. Note that the time taken to produce peak tension (T_m) decreased as T_m decreased. In Fig. 5*B* note that the twitch duration of the weaker beat was shorter than that of the stronger one (curves traced from photographed record).

tion rate was as above. The results, displayed in Fig. 5, show that increased [K^+] depressed the maximum tension attained in twitches whilst reduced [K^+] enhanced it. These results differ from those recorded during both the staircase and Ca^{++} series. Thus, when the strength of contraction was varied by altering the K^+ concentration of the perfusate the t_p of the weaker contraction was less than that of the stronger one (Fig. 5*A*). Moreover the total twitch duration of the weaker contraction was often less rather than greater than the stronger one (Fig. 5*B*). In some instances the twitch duration remained unchanged.

(4) *Noradrenaline*

The positive inotropic response recorded following the addition of noradrenaline (final concentration 0.1 and 1 μg/ml.) to another series of strips was

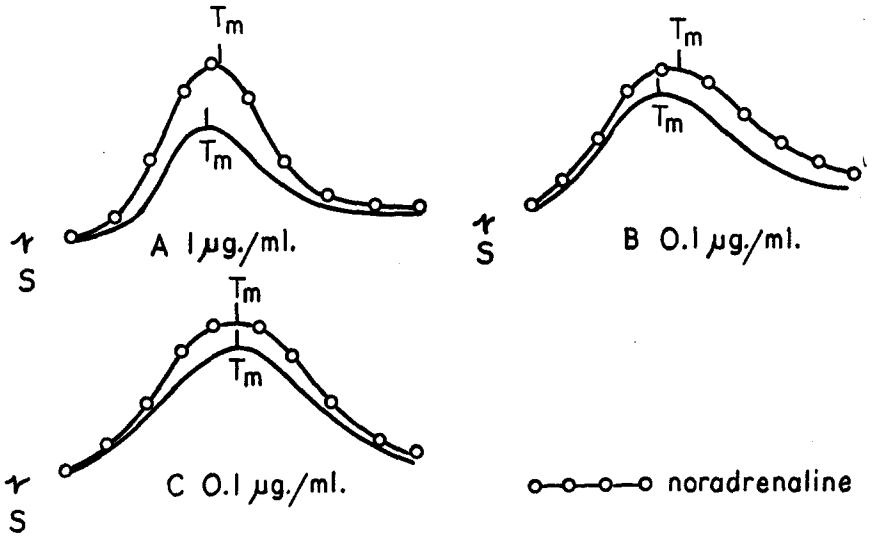

FIGURE 6. Effect of noradrenaline (0.1 and 1.0 μg./ml. final concentration) on the isometric tension duration curves recorded from ventricular muscle strips. S = stimulus, and T_m = peak tension. Note that the time required to produce T_m in the stronger beat was equal to or greater than that of the weaker beat (curves traced from photographed record).

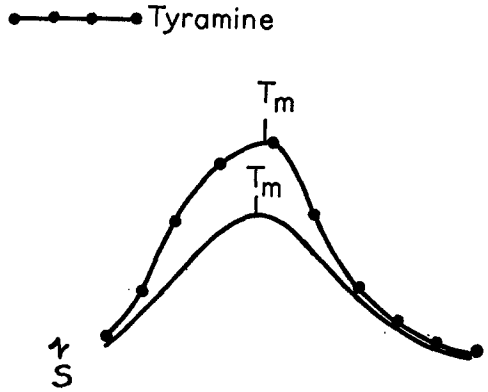

FIGURE 7. Effect of tyramine (10 μg./ml.) on the isometric tension duration curves. Note that the time taken to develop T_m in the stronger beat exceeded that of the weaker one. The twitch duration remained unchanged (curves traced from photographed record).

usually associated with a lengthened t_p period (see Fig. 6). In no instance was a reduced t_p recorded during noradrenaline activity, as was done during the staircase.

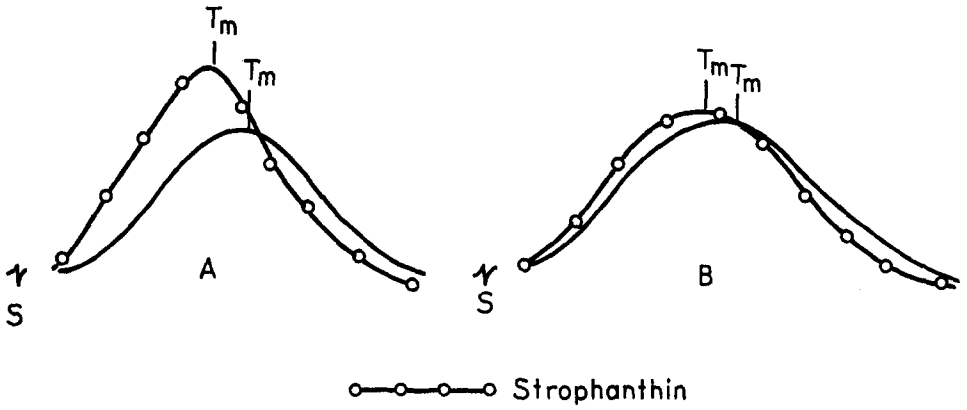

FIGURE 8. Effect of strophanthin (10 μg./ml.) on the isometric tension duration curves of ventricular muscle strips. Note that as T_m increased both the time taken to develop T_m and the total twitch duration decreased (curves traced from photographed record).

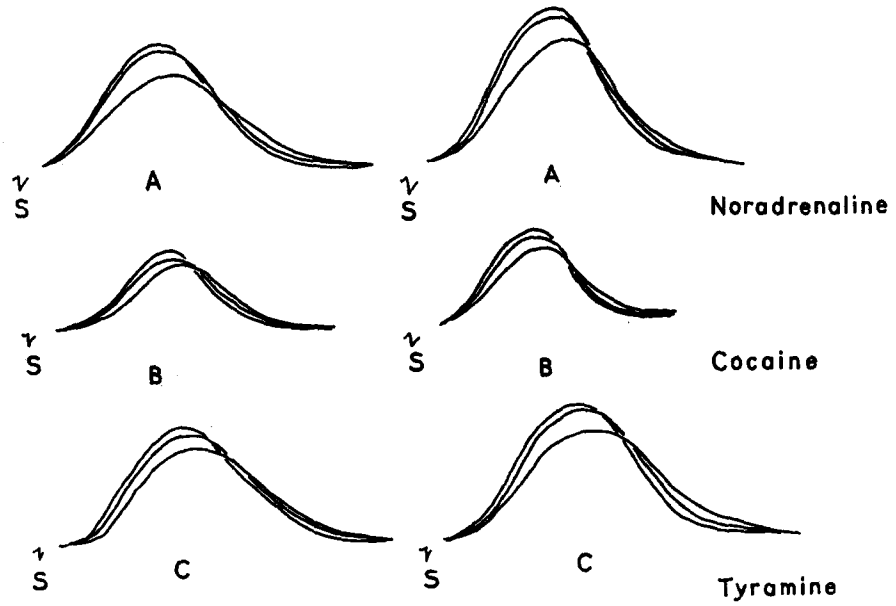

FIGURE 9. *A*, The first three isometric contractions recorded during the staircase before (*A*) and following (*A'*) the administration of noradrenaline (1 μg./ml. final concentration). *B*, Before (*B*) and following (*B'*) the administration of cocaine (100 μg./ml. final concentration). *C*, Before (*C*) and following (*C'*) the administration of tyramine 10 μg./ml. final concentration). In each case the rest pause preceding the staircase was 12 seconds. Note that noradrenaline, cocaine, and tyramine did not modify the staircase.

Tyramine, cocaine, and reserpine, resembled noradrenaline in that they elicited a positive inotropic response associated with an increased or unaltered t_p interval—see Fig. 7 where the response of the strip to 10 μg./ml. (final concentration) tyramine is shown.

(5) *Cardiac Glycoside*

Strophanthin-G (10 μg./ml. final concentration) elicited changes resembling those noted in the Ca^{++} and staircase series. The twitch duration and the time taken to produce maximum tension were both decreased (see Fig. 8).

The reduction in twitch duration occurred even when the positive inotropic response was negligible (Fig. 8*B*).

(6) *Action of Amines on the Staircase*

Noradrenaline (1 to 10 μg./ml. final concentration) and cocaine (10 to 100 μg./ml. final concentration) failed to modify the staircase. See Fig. 9A–C where the first three successive tracings of the staircase recorded in the untreated strip, in the presence of exogenous noradrenaline and following the addition of cocaine, have been superimposed.

Similarly a normal staircase was recorded in ventricular strips removed from those toads depleted of noradrenaline by previous injection with reserpine on 3 consecutive days before use (Nayler (1958)).

DISCUSSION

The results described above may be divided into two distinct classes:

1. Those in which an increase in the contractile force was associated with a shortened twitch duration and a decrease in the time required to produce maximum tension (t_p); *e.g.*, Ca^{++}, strophanthin, and the staircase series.
2. Those in which an increase in contractile force was associated with a delayed or unchanged twitch duration and increased or unchanged t_p interval; *e.g.*, K^+, noradrenaline, tyramine, and reserpine.

The positive inotropic effect recorded during the staircase phenomenon showed little resemblance to that due either to a decreased K^+ concentration of the Ringer's solution or to the activity of exogenous noradrenaline or to the "noradrenaline-like substance" liberated by tyramine, cocaine, and reserpine.

In general these data throw doubt on the validity of the staircase theories both of Whalen *et al.* (1958) and of Hajdu and Szent-Györgyi (1952). The latter workers, following their observation that the staircase was abolished by low K^+ Ringer's, suggested that the K^+ entry into and exit from the myocardium during activity and rest respectively could be used to explain the

staircase. Fenn and Cobb (1936) detected K^+ loss from the myocardium during contraction and Hajdu and Szent-Györgyi postulated that, if beats followed each other in rapid succession, sufficient time would not elapse during diastole to accommodate reentry of all the K^+ lost during the preceding contraction. An over-all and progressive loss of K^+ would thus ensue, accompanied by a positive inotropic response.

If such a K^+ hypothesis is correct, then the tension duration curves recorded during the staircase and during conditions of K^+ loss from the myocardium should be similar. In these experiments this similarity was not recorded. It is interesting to note that Penna and Garb (1956) have also produced evidence against the role of K^+ in potentiation phenomena, since they failed to affect postextrasystolic potentiation in cat papillary muscle by varying the K^+ content of the perfusion fluid.

Alternatively Whalen *et al.* (1958) postulated that the release of noradrenaline or of a "noradrenaline-like" potentiating substance during the normal contraction-relaxation cycle is involved in the staircase effect. Thus during rapid stimulation less time exists between successive beats for the destruction of the released potentiating substance which is then free to elicit the positive inotropic response manifested in the staircase.

It is known that stores of a noradrenaline-like substance do exist within the myocardium, but the data recorded during this study render doubtful the possibility that the release of this substance is vitally involved in the staircase. Firstly, the tension duration curves recorded during amine activity were dissimilar from those in the staircase. In the amine experiments the time required to develop T_m was unchanged or slightly lengthened and the twitch duration not significantly altered, whereas in the staircase experiments marked changes in both twitch durations and t_p intervals were recorded (see Figs 2 and 6). Further the staircase was recorded in strips pretreated with tyramine and reserpine at concentrations sufficient to release the endogenous amine-like substance (Burn and Rand (1958 *a*, *b*); Nayler (1958)). Cocaine, which is known to potentiate the action of noradrenaline, failed to potentiate the staircase, and the presence of exogenous noradrenaline did not modify it in any way (see Fig. 9).

In contrast with the lack of correlation between K^+ and amine activity on the one hand, and the staircase on the other, the involvement of Ca^{++} in the staircase appears far more probable. The marked similarity between the tension duration curves recorded during the staircase and during the inotropic activity of Ca^{++} has already been noted. In both instances the increase in contractile force was associated with increased contraction velocities and reduced twitch durations.

The importance of Ca^{++} in muscular contraction has been appreciated since 1883, when Ringer observed that the strength of contractions in the

isolated frog heart varied according to the Ca^{++} concentration of the perfusion fluid. Clark (1913), Loewi (1917), Heilbrunn (1952), and more recently Moulin and Wilbrandt (1955) and Niedergerke (1956) are among those who postulate that Ca^{++} is involved in the staircase as well as in other positive inotropic phenomena. Many observations in the literature favour the view that Ca^{++} accumulation in or about the vicinity of the membrane regulates the contractile force at least in heart muscle (Niedergerke and Luttgau (1957)). Bianchi and Shanes (1959) support the hypothesis that an increased entry of Ca^{++} associated with depolarization underlies the development of the contractile response. The results reported in the present paper indicate that the accumulation of Ca^{++} during successive beats may be the basis of the potentiation recorded during the staircase. Thus, if Ca^{++} is accumulated during depolarization and early systole and is then released throughout late systole, diastole, and rest, a long rest period between successive beats would lead to a condition of Ca^{++} loss. The first few beats which follow the long pause will, therefore, be weak. Diffusion of Ca^{++} away from the cell during diastole and rest could explain the relationship between the duration of the rest pause and the time taken to reach peak tension in the first beat after the pause (see Fig. 3).

Poststimulation and extrasystolic potentiation can similarly be explained in terms of Ca^{++} gain. In the former case Ca^{++} accumulation will occur during the phase of rapid stimulation since, for a given number of contractions, the time during which Ca^{++} may be lost is reduced. The pause which follows this rapid stimulation and which precedes the enhanced first beats of the poststimulation series may be necessary both for the distribution of the accumulated Ca^{++} throughout the myofibrils and for the recovery of the energy-yielding cycles.

During extrasystolic stimulation Ca^{++} accumulation will take place both during the normal and the extra beat so that a greater Ca^{++} gain will result than would be the case if only the normal beat had occurred. Provided that the next contraction takes place before the additional Ca^{++} associated with the extrasystole is lost, the contraction will be large. Cattell and Gold (1955) using cat papillary muscles recorded extrasystolic potentiation after rest periods exceeding 10 seconds. In contrast to these results extrasystolic potentiation could not be demonstrated in similar experiments using the toad ventricular strips of the present study even when the postextrasystolic rest period was less than 10 seconds. This suggests that the rate at which Ca^{++} is lost from cardiac cells may show marked species variation.

Recently Holland and Sekul (1959) reported that ouabain increased Ca^{++} influx in isolated rabbit atria and it seems probable that this increased Ca^{++} entry underlies the development of the inotropic response. If it is assumed that a changed rate of Ca^{++} flux is the fundamental event in the staircase, it is

not surprising that the changes noted above in the tension duration curves recorded in the staircase and during glycoside and Ca^{++} activity were similar.

These results reported here confirm the observations made recently by Stubbs and Widdas (1959) who, when comparing the effect due to adrenaline and excess Ca^{++} on the E.C.G. of the perfused rabbit heart, noted that adrenaline appeared to prolong the period of systole as measured by the QT interval whereas excess Ca^{++} reduced the QT duration. As was shown in Figs. 4 and 6 calcium reduced and noradrenaline increased the time required to reach peak tension (duration of systole).

I wish to thank Dr. T. E. Lowe for his help and guidance with this project.

REFERENCES

ABBOTT, B. C., and MOMMAERTS, W. F. H. M., *J. Gen. Physiol.*, 1959, **42**, 533.
BIANCHI, C. P., and SHANES, A. M., *J. Gen. Physiol.*, 1959, **42**, 803.
BOWDITCH, H. P., *Ber. Verhandl. sächs Akad. Wissensch., Leipzig*, 1871, **23**, 562.
BURN, J. H., and RAND, M. J., *Brit. Med. J.*, 1958*a*, **1**, 903.
BURN, J. H., and RAND, M. J., *J. Physiol.*, 1958*b*, **144**, 314.
CATTELL, McK., and GOLD, H., *Am. J. Physiol.*, 1955, **182**, 307.
CLARK, A. J., *J. Physiol.*, 1913, **47**, 66.
DALE, A. S., *J. Physiol.*, 1932, **75**, 1.
FENN, W. O., and COBB, D. M., *Am. J. Physiol.*, 1936, **115**, 345.
HAJDU, S., and SZENT-GYÖRGYI, A., *Am. J. Physiol.*, 1952, **168**, 159.
HEILBRUNN, L. V., Outline of General Physiology, 3rd edition, Philadelphia, Saunders, 1952.
HOFMANN, F. B., *Z. ges. exp. Med.*, 1926, **50**, 130.
HOLLAND, W. C., and SEKUL, A. A., *Am. J. Physiol.*, 1959, **197**, 757.
LOEWI, O., *Arch. exp. Path. u. Pharmakol.*, 1917, **83**, 366.
MOULIN, M., and WILBRANDT, W., *Experientia*, 1955, **11**, 72.
NAYLER, W. G., *Australian J. Exp. Biol. and Med. Sc.*, 1958, **36**, 567.
NIEDERGERKE, R., *J. Physiol.*, 1956, **134**, 569.
NIEDERGERKE, R., and LUTTGAU, H. C., *Nature*, 1957, **179**, 1066.
PENNA, M., and GARB, S., *Am. J. Physiol.*, 1956, **184**, 572.
RINGER, S., *J. Physiol.*, 1883, **4**, 29.
ROSIN, H., and FARAH, A., *Am. J. Physiol.*, 1955, **180**, 75.
STUBBS, J., and WIDDAS, W. F., *J. Physiol.*, 1959, **148**, 393.
WHALEN, W. J., RISHMAN, N., and ERICKSON, R., *Am. J. Physiol.*, 1958, **194**, 573.
WOODWORTH, R S., *Am. J. Physiol.*, 1902, **8**, 213.