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A STUDY OF THE CONTRACTURES INDUCED IN FROG ATRIAL TRABECULAE BY A REDUCTION OF THE BATHING SODIUM CONCENTRATION

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SUMMARY

1. The relationship between the $[Ca]_o$, the $[Na]_o$ and the strength of the contracture evoked when the $[Na]_o$ is reduced has been investigated in isolated frog atrial trabeculae.

2. The strength of the contracture varies by the $[Ca]_o^2$ and by $\sqrt[4]{([Na]_o)}$ over the lower tension range.

3. The contracture induced by reduction of $[Na]_o$ is not sustained, but relaxes spontaneously. The rate of this relaxation is only dependent on the $[Na]_o$ in the presence of strophanthidin.

4. After the spontaneous relaxation of an Na-free contracture, the ability of the trabecula to develop tension upon a second challenge with Na-free solution returns in about 3 min if the muscle is perfused with Na-containing fluid. This recovery process is slowed if the $[Na]_o$ is low during the recovery period, but the recovery is hastened by electrical stimulation of the preparation or by perfusion with K-free or strophanthidin containing sodium-Ringer.

5. It is suggested that the influx of Ca^{2+} which induces the Na-free contracture depends on the presence of Na^+ inside the cells. When the intracellular Na concentration falls, the Ca influx falls, and the muscle relaxes as a result of the activity of an intracellular relaxing structure.

INTRODUCTION

When the Na concentration in the medium bathing frog heart is reduced, the strength of the heart beat increases in much the same way as when the Ca concentration is raised (Daly & Clarke, 1921). Quantitative studies of this increase have shown that the contractile strength varies with the ratio of $[Ca]_o/[Na]_o^2$ (Wilbrandt & Koller, 1948; Lüttgau & Niedergerke, 1958). When the Na is replaced, a strong contracture develops, associated with a large increase in the influx of calcium into the

heart cells (Niedergerke, 1963). The hypothesis, adopted to account for the effect of these ions in the external fluid, assumes that a receptor molecule within the cell membrane can carry either one calcium or two Na ions into the sarcoplasm (Lüttgau & Niedergerke, 1958).

Kinetic studies of the changes of the twitch responses associated with a rapid alteration of the bathing Ca or Na concentration, and the relationship between the strength of the K contracture and the concentration of these ions, suggest that the Ca ions that finally activate contraction are derived from more than one source (Chapman & Niedergerke, 1970*a*; Chapman & Tunstall, 1971; Chapman, 1971*a*). This implies that the competition between Ca and Na ions may not be exclusive to the outer surface of the muscle cells. The present work investigates the factors that influence the strength of the contracture that develops when the bathing Na concentration is suddenly reduced. It shows, as far as its effects on the contraction of the muscle is concerned, that the competition between Na and Ca not only occurs at the outer surface of the muscle cells, and that under some circumstances the intracellular concentration of Na can also have profound effects on the contractility of the heart.

METHODS

The technique of isolating, mounting and perfusing the auricular trabeculae, and the method of recording the isometric tension they generate and the membrane potential of the muscle cells, has been described in detail elsewhere (Chapman & Tunstall, 1971; Chapman, 1973). The constituents of the experimental solutions are shown in Table 1. The Na in the Ringer solution has been replaced by Tris HCl, choline chloride (with 10^{-5} M atropine), sucrose or LiCl. Ca was added as 1 M-$CaCl_2$ volumetric solution (B.D.H.) Strophanthidin (Sigma) was added as 10 mM solution in absolute ethanol. In experiments where this drug was used, an equal concentration of ethanol was added to the other solutions that perfused the heart. All other chemicals were analytical grade where possible.

To avoid the complications of the staircase response upon the results, the isolated trabecula was stimulated electrically at a rate between 4 and 24 min^{-1}. In some experiments stimulation was interrupted as part of the experimental procedure, and the heart was not electrically stimulated during exposure to Na-depleted solutions.

The experimental scheme was to induce a series of contractures with the various experimental changes. Between contractures, the heart was stimulated electrically in normal Ringer solution. After a series of experimental manoeuvres were completed, they were then repeated in the reverse order, and a second set of results obtained. This 'mirror' procedure was adopted whenever possible so that any unidirectional changes in the sensitivity of the heart were recognized, and allowed for.

Where appropriate, the results were subjected to regression analysis using a Wang 700 C programmable desk calculator to give details of the slopes, intercepts, standard error of the line and coefficient of correlation.

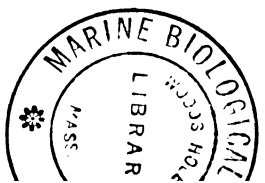
The preparations were taken from the hearts of healthy frogs of the species *Rana pipiens*, first killed by pithing and central nervous system. The experiments were performed in a cooled room, temperature range 14–21° C, with a variation during a single experiment of less than 1°.

TABLE 1. Constituents of solutions used

Solution	NaCl (mM)	KCl (mM)	Na_2HPO_4 (mM)	NaH_2PO_4 (mM)	Glucose (mM)	pH
Sodium Ringer	114·5	3·0	0·8	0·2	5·0	7·3
High-potassium Ringer	114·5	100·0	0·8	0·2	5·0	7·3

Sodium-free Ringer	KCl (mM)	LiCl (mM)	Tris HCl (mM)	Choline Cl (mM)	Sucrose (mM)	K_2HPO_4 (mM)	KH_2PO_4 (mM)	Glucose (mM)	pH
Tris Ringer	3·0	—	129·2	—	—	—	—	5·0	7·3
Choline Ringer	1·2	—	—	122·5	—	0·8	0·2	5·0	7·3
Lithium Ringer	3·0	114·5	2·0	—	—	—	—	5·0	7·3
Sucrose Ringer	1·2	—	—	—	205·0	0·8	0·2	5·0	7·3

Sodium-depleted solutions were made up by mixing the appropriate amounts of Na-free and Na-Ringers.



RESULTS

The form of the low-Na contracture

Perfusion of a trabecula with solutions in which much of the Na has been replaced by Tris HCl, choline chloride (with atropine), sucrose or LiCl, results in the rapid development of a strong contracture ($t_{\frac{1}{2}} \simeq 3$ sec). Even though perfusion by the Na-depleted solution is continued, this contracture subsides spontaneously after a plateau phase, becoming completely relaxed after about 5 min (Fig. 1*a*). In a few preparations, following this spontaneous relaxation, quite large spontaneous

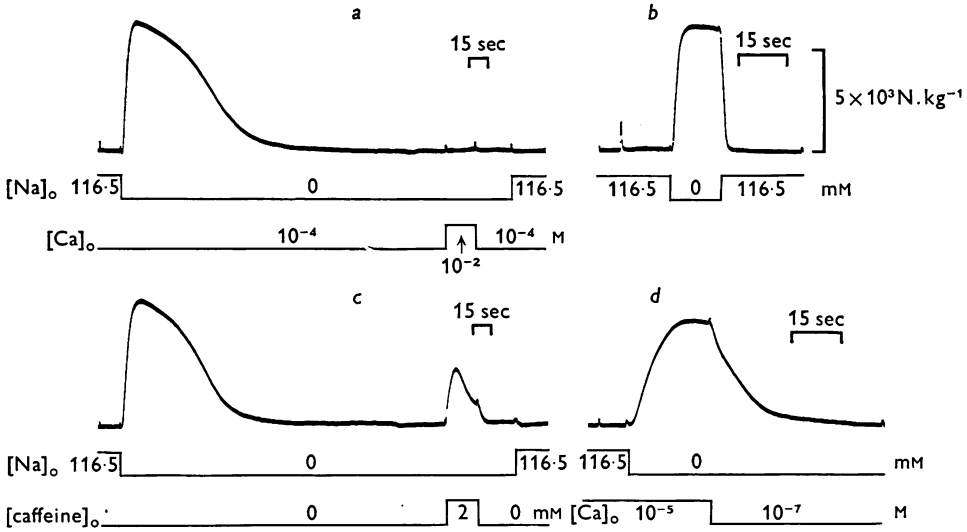


Fig. 1. Traces of contractures evoked by perfusion with Na-free solutions.

a, The Na-free contracture shows spontaneous relaxation. If the Ca concentration is raised, by as much as 100 times after the spontaneous relaxation, it fails to induce any change in the tension generated by the trabecula.

b, The contraction initiated by withdrawal of the Na ions from the bathing solution is rapidly abolished if the Na is returned to the solution: 1 mM-Ca.

c, After the spontaneous relaxation of a Na-free contracture, addition of 2 mM caffeine induces a large redevelopment of tension: 0·1 mM-Ca.

d, A Na-free contracture develops when the $[Ca]_o$ is as low as 10^{-5} M, but on the addition of 0·4 mM Tris EGTA (to give a free Ca concentration of less than 10^{-7} M) the trabecula relaxes. This relaxation is slower than that seen when Na ions are added to the bathing medium.

All records are from the same experiment, 20·5° C. The tensions generated by the muscle are expressed in terms of the wet weight of the trabecula. The wet weight of the preparations used was generally between 0·02 and 0·08 mg. All subsequent Figures use the same convention.

oscillations of tension occur, resembling those reported in frog ventricle strips (Winegrad, 1972).

Raising the $[Ca]_o$ has little effect on the contracture evoked by Na-free perfusion. When this contracture has been allowed to relax spontaneously, raising the Ca concentration by as much as 100 times fails to induce a further development of tension (Fig. 1*a*). Following the spontaneous relaxation of the Na-free contracture, however, the addition of millimolar concentrations of caffeine induces a large but again transient contracture (Fig. 1*c*). If the normal high-Na concentration is re-established at any time

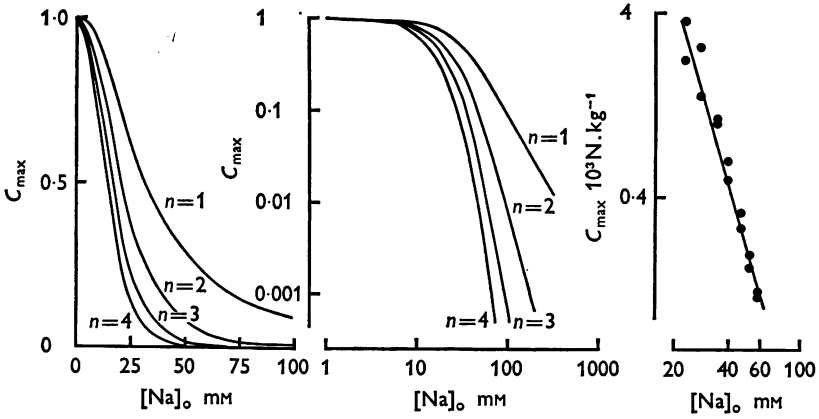


Fig. 2. The theoretical values of the contracture tension derived from eqn. (1) with the constants K_{Ca}, ω and α, set to unity and n varying from 1 to 4, plotted against the bathing Na concentration, at a constant Ca concentration, (*a*) on linear and (*b*) on logarithmic co-ordinates, (*c*) the results of a typical experiment; where contractures are evoked to various reductions of the $[Na]_o$, Tris HCl replacing NaCl, plotted on logarithmic co-ordinates. The continuous line is a regression line of slope −3·78 (co-efficient of correlation, 0·98, $P \ll 0{\cdot}001$). 19·0° C; 1 mM-Ca.

during a low-Na contracture, any remaining tension is rapidly lost ($t_{\frac{1}{2}} \simeq 0{\cdot}5$ sec) (Fig. 1*b*), and after a few seconds, normal twitches can be evoked by electrical stimulation. This relaxation, of the Na-free contracture, is always faster than the contraction, as noted by Chapman & Tunstall (1968). The tension developed on perfusion with Na-free solutions relaxes on the reduction of the free-Ca concentration to less than 10^{-7} M by the addition of EGTA (Fig. 1*d*), but rather more slowly than when Na ions are added. If the heart is perfused for as little as 15 sec in Ca-free Na-Ringer, before the Na is withdrawn, a contracture fails to develop.

The relationship between the $[Na]_o$ *and the tension developed during the contracture*

$$\text{Contracture tension } (C_{max}) = \omega\left[\frac{\alpha[Ca]_o K_{Ca}}{[Ca]_o K_{Ca} + [Na]_o^2}\right]^n \quad (1)$$

where $[Ca]_o$ and $[Na]_o$ are the Ca and Na concentrations in the bathing fluid and K_{Ca} is the equilibrium constant of the reaction

$$nCa + nNa_2R \rightleftharpoons nCaR + 2nNa,$$

and ω and α are proportionality constants, while n is the number of Ca ions involved in the complex Ca_nR or the number of CaR required to generate tension at the unit level.

Eqn. (1) reasonably predicts the effects of varying either or both the $[Na]_o$ or $[Ca]_o$ on the strength of the K contracture (Chapman & Tunstall, 1971). Fig. 2 shows theoretical plots of this equation when only the sodium concentration is reduced, on linear and logarithmic co-ordinates, when the value of n is varied from 1 to 4. The slope of the logarithmic lines approaches the value of $-2n$ at high Na concentrations and hence small contracture tensions, because eqn. (1) can be simplified under these conditions of constant $[Ca]_o$ to eqn. (2).

$$C_{max} \propto (1/[Na]_o^2)^n. \quad (2)$$

This means that by evoking contractures by partial withdrawal of Na ions from the bathing medium, n can be estimated experimentally. The maximum slope of the theoretical line on log-log co-ordinates is, however, somewhat less than $-2n$, e.g. for $n = 2$ the slope of the line in Fig. 2*b* is $-3{\cdot}57$, while experimental results, over the range 25–60 mM-Na, have a mean slope of $-3{\cdot}78$ (S.D. 0·49) (Fig. 2*c*). This value is larger than that expected if $n = 2$, but is really not large enough to suggest that $n = 3$. The transience of the low-Na contracture could mean that the peak tension of the smaller contractures are underestimated making the measured slope of the log-log plot too steep. This seems likely, because if the maximum rate of rise of the contractures (determined by electronic differentiation) is used to assess the contractility, then the slope of the plot of the log of the maximum rate of tension development against the log of the $[Na]_o$, for the same series of experiments, is $-3{\cdot}30$, S.D. 0·55. There is the possibility that the results may be complicated by a change in the membrane potential of the cells in Na-free media as described by Goto, Kimoto & Suetsugu (1972).

The effect of the Na concentration on the resting potential

Lüttgau & Niedergerke (1958) and Vassort (1973) found little evidence to suggest that even the total replacement of the Na in the bathing fluid

causes much change in the resting potential of frog heart muscle cells, while Goto *et al.* (1972) observed up to 15 mV hyperpolarization on replacement of the external Na by sucrose or Tris HCl.

In a series of measurements on twenty-two cells, rapid replacement of all of the NaCl by Tris HCl in the solution perfusing frog ventricle or auricle preparations produced a hyperpolarization, mean value 10·0 mV (S.E. 0·9 mV). This hyperpolarization developed slowly on removal of the sodium ($t_{\frac{1}{2}}$ = 10·2, S.D., 2·0 sec) and recovered rapidly if the sodium was restored ($t_{\frac{1}{2}}$ = 4·6, S.D. 0·4 sec) as shown in Fig. 3*b*. The hyperpolarization is not maintained, but subsides with a half-time of between 3 and 10 min (Fig. 3*a*), a value similar to that reported for the loss of Na from frog ventricle,

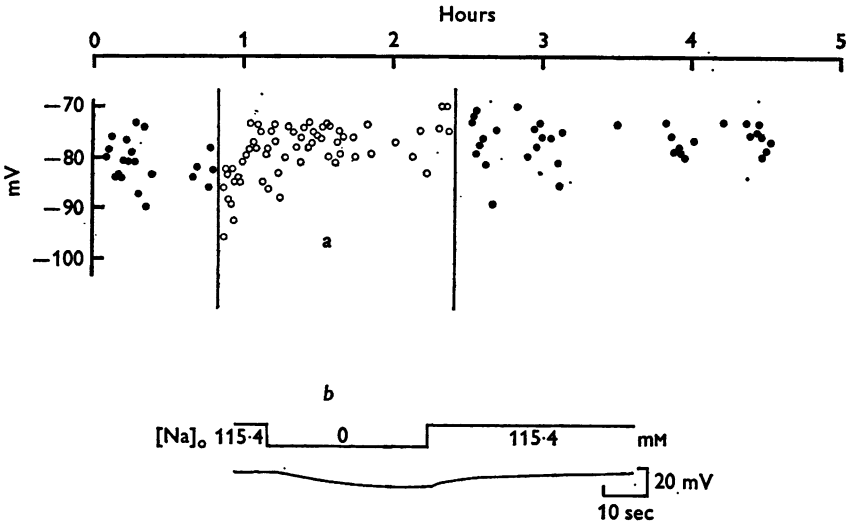


Fig. 3*a*. The resting membrane potentials of cells within a ventricular trabecula, represented by the filled circles, are plotted against the time of the experiment. At the first line, all the NaCl in the bathing fluid was replaced by Tris HCl, the membrane potentials in this solution are shown by the open circles. At the second line the trabecula was returned to Na Ringer, 20·0° C; 1 mM-Ca.

b, A trace of the change in resting potential of another trabecula (lower trace) when the Na was removed and then returned to the bathing medium as represented by the upper trace. 20·5° C; 1 mM-Ca.

but under rather different conditions (Keenan & Niedergerke, 1967). When the $[Na]_o$ is reduced to 20 mM there is little or no change in the membrane potential, this means that the contracture tensions can be investigated over a range of sodium concentrations where there is no complicating change in the resting potential of the muscle cells.

The relationship between $[Ca]_o$ *and the tension developed in Na-depleted solutions*

When the heart has been perfused with Ringer containing a testing Ca concentration, for at least 10 min so that the regularly evoked twitches are of steady amplitude, the contractures evoked by the sudden reduction of the bathing Na concentration to 25, 30 or 35 mM show a sigmoidal relationship to the bathing Ca concentration. When the tensions developed are less than 10% of the maximum tension, a logarithmic plot of results yields a straight line relationship, the slope of which should be approximately equal to n in eqn. (1). In these experiments, the mean slope was +1·79, S.D. 0·26 suggesting that $n = 2$. In the terms of the hypothesis that underlies eqn. (1), the low-Na contracture would seem to be elicited by activator Ca released from two sites or, on the other hand, two Ca ions are required to activate contraction at the unit level.

If the strength of the contracture, in Na-depleted solutions, can be described by eqn. (1) when $n = 2$, then a plot of $1/\sqrt{C_{max}}$ against $1/[Ca]_o$, should be a straight line intercepting the reciprocal concentration axis at the value of K_{Ca} (it is in fact analogous to a Lineweaver–Burke plot). Plotted this way, the results of contractures evoked by different Na concentrations should have a common intercept with the reciprocal tension axis, and the ratio of the equilibrium constants should be as given in eqn. (3).

$$\frac{K'_{Ca}}{K_{Ca}} = \frac{[Na']_o^2}{[Na]_o^2} \tag{3}$$

where $[Na']_o$ = one reduced [Na] contracture fluid, $[Na]_o$ another; K'_{Ca} and K_{Ca} their respective equilibrium constants as expressed in eqn. (1).

The results of experiments of this type have straight regression lines, with small standard errors, only when the tensions generated by the muscle are in the lower part of the tension range (unlike the potassium contractures, Chapman & Tunstall, 1971). Measurements over this range have common intercepts with the $1/\sqrt{}$(tension) axis, but these intercepts correspond to a maximum contracture tension of 1·5–2·0 times the value found experimentally (Fig. 4*b*). In comparison to a value expected from eqn. (3) of 0·69, the ratio of the experimentally determined equilibrium constants for 25 mM-Na and 30 mM-Na was 0·66, S.D. 0·05.

When eqn. (1) is fitted to the data from a wide range of Na concentrations (continuous line in Fig. 5), the saturation phenomenon is apparent, a good fit is achieved only over the lower range of tensions. A similar result has been found in experiments on fish heart, where the contractures induced by Na-free solutions are smaller than those evoked in solutions containing a small amount of sodium (Busselen & Carmeliet, 1973).

Comparison of the contractures initiated by high-K and low-Na solutions

The different dependence of the potassium contracture and the low-Na contractures on the $[Ca]_o$ has been already noted. In experiments made to compare the variation of the contracture strength in high-K (100 mM-K) and Na-depleted solutions over the same range of $[Ca]_o$, the K contracture shows a steeper dependence on the bathing Ca concentration than the contracture evoked by Na-depleted fluids. At $[Ca]_o$ below 1 mM, the difference between the slopes of the log-log curves for the two types of contractures is 0·26. The slope for low-Na contractures, in these experiments, is relatively large, i.e. very near to 2·0, while the slope for the high-K contractures, although greater than 2·0 is not near 3·0. It is possible that the slope of the relationship between the low-Na contracture and the $[Ca]_o$ is exaggerated by a change in $[Na]_i$ under these conditions.

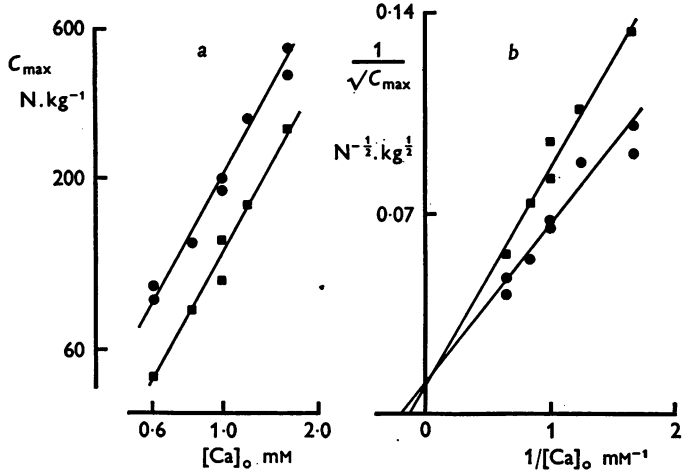


Fig. 4*a*. A logarithmic plot of the contracture tension evoked by Ringer containing 25 mM-Na (●—●) and by one containing 30 mM-Na (■—■) over a range of $[Ca]_o$. The $[Ca]_o$ had been established for at least 10 min before the Na withdrawal contracture was elicited. The lines are regression lines, the slopes are +1·75 for the contractures evoked by 25 mM-Na, and +1·92 for those in 30 mM-Na.

b, A double reciprocal plot of the same experiment as *a*, where $1/\sqrt{(C_{max})}$ is plotted against $1/[Ca]_o$ for the contractures evoked at the two Na concentrations. The continuous lines are regression lines which intersect close to the $1/\sqrt{(C_{max})}$ axis. The K_{Ca} for the 25 mM-Na contracture is 4·71 mM and at 30 mM-Na is 7·06 mM, which gives a ratio of 0·67 compared to the value of 0·69 expected from eqn. (3). 14·0° C.

The restoration of the Na-free contracture, and it's dependence on the bathing Na concentration

For a variety of muscles the transient form of the contracture, that develops during sustained depolarization, has been explained assuming that the process releasing Ca into the sarcoplasm becomes progressively inactivated, while the Ca already released is taken up by some intracellular

structure (Hodgkin & Horowizc, 1960; Beeler & Reuter, 1970; Chapman, 1973). As the low-Na contractures of frog atrial muscle relax spontaneously it is possible that a similar mechanism is operative.

In an experimental procedure, where the muscle is allowed to undergo the full spontaneous relaxation of a Na-free contracture and is then returned to normal Na Ringer, the ability of the muscle to develop a second contracture is complete after 3–5 min at room temperature. If, however, the relaxed muscle is returned to a reduced outside sodium concentration, the rate of recovery of the Na-free contracture is slowed.

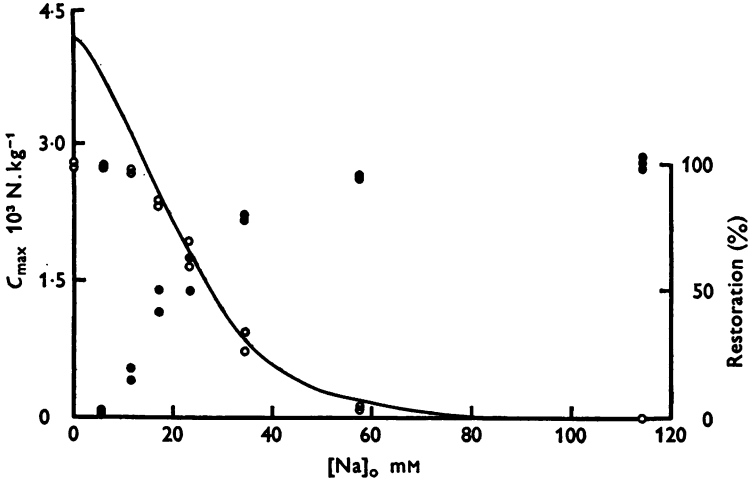


Fig. 5. The strength of the contracture evoked by reducing the $[Na]_o$ to various levels, at 1 mM-Ca (○—○), is compared to a line derived from eqn. (1). This line is calculated by fitting eqn. (1) to the results obtained in $[Na]_o$ above 20 mM, i.e. where the equation approximates to the results and where K_{Ca} can be obtained by a double reciprocal plot (as in Fig. 4).

When a trabecula has developed tension in Na-free solution, this tension subsides spontaneously. If the muscle is returned to Na Ringer, the ability of the muscle to generate a subsequent Na-free contracture recovers to be complete in 3 min in this preparation. The degree of recovery achieved in this time is reduced if the muscle is perfused by Na-depleted fluids between contractures. A graph of the contracture tension, evoked by a test application of Na-free Ringer, when plotted against the preceding Na concentration shows the dependence of the recovery of the $[Na]_o$ (●—●). 1 mM-Ca; 20·0° C.

The relationship between the Na concentration, perfused over the muscle for a fixed period, and the degree of recovery of the Na-free contracture is shown in Fig. 5. This relationship is compared to the dose–response curve of the contracture initiated by reducing the bathing Na concentration (Fig. 5). The dependence of the recovery of a Na-free contracture on

the outside Na concentration, suggests that the release of Ca, into the sarcoplasm from the bathing medium, is progressively inhibited when Na is removed and that this inhibition is reversed on exposure of the muscle to Na ions.

The effects of stimulation, strophanthidin and K-free media on the recovery of the Na-free contracture

The recovery of the Na-free contracture follows an exponential time course. The time constant for the recovery estimated from a semilogarithmic plot, has a mean value of 36·9, S.D. 8·5 sec when the preparation is not stimulated in the Na-containing fluid. When the preparation is stimulated at 24 min^{-1} the time constant is reduced to 19·1, S.D. 4·9 sec (Fig. 6). The rate of recovery is still enhanced if the preparation is stimulated in Ca-free Ringer, in which twitches do not develop. Therefore, the increased rate of

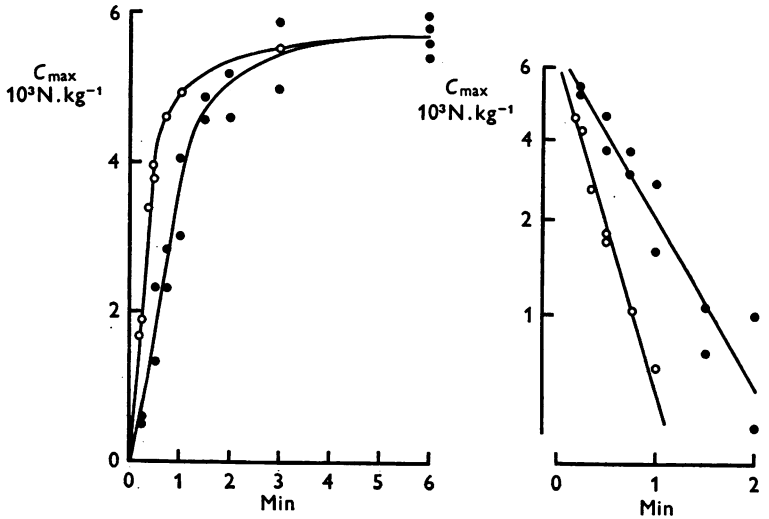


Fig. 6. After the spontaneous relaxation of the tension developed in Na-free media a period of exposure to Na-containing Ringer is necessary before another full sized contracture can be elicited. The time course of this recovery can be followed by exposing the muscle to different pre-treatment times in the sodium Ringer.

a, Shows the recovery of the Na-free contracture, represented by plotting the amplitude of the Na-free contracture against the preceding period in Na Ringer, when the preparation was not stimulated (●—●) and when it was stimulated at 24 min^{-1} (○—○).

b, A semilogarithmic plot of the same results as *a*. The continuous lines are drawn to the equation, contracture $tension_{at\ time\ t}$ = maximum contracture $tension_{at\ time\ t=\infty}$ $(1-\exp^{t/\tau})$. The time constant, τ, is obtained by exponential regression analysis, and was 42·5 sec for the unstimulated recovery and 22·9 sec for the stimulated recovery. 20·5° C; 1 mM-Ca.

recovery is not due to the accumulation of intracellular Ca associated with the twitch responses. Furthermore, large twitches can be elicited when the Tris-Ringer is replaced by Li-Ringer, but on return to Tris-Ringer little or no contracture develops. Exposure of trabeculae to either K-free Ringer or to one containing 10^{-6}–10^{-5} M strophanthidin also

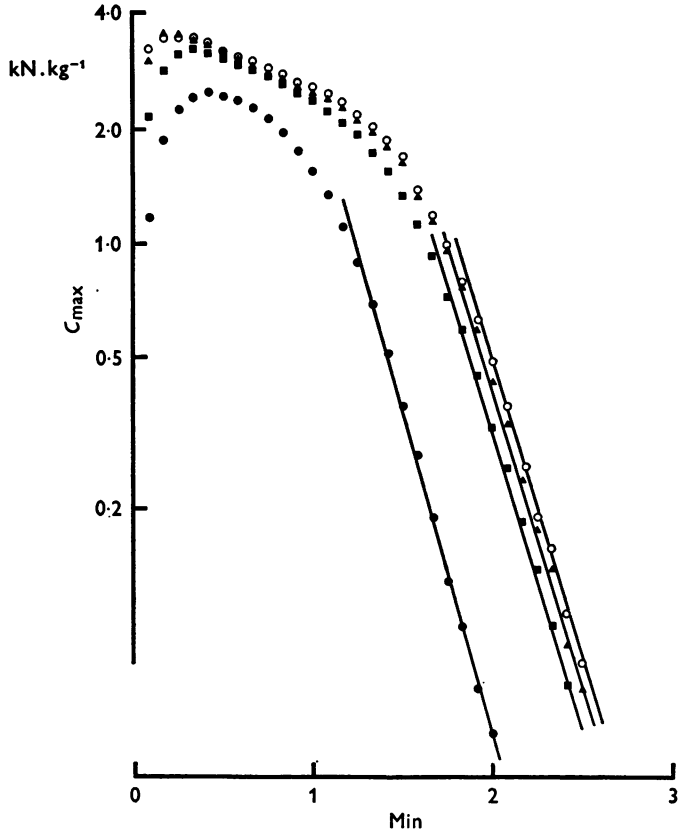


Fig. 7. A semilogarithmic plot of the full time course of contractures evoked by 0 mM-Na (○—○); 5 mM-Na (▲—▲); 10 mM-Na (■—■) and 20 mM-Na (●—●); showing the initial plateau and the final exponential phase of the spontaneous relaxation. Tris HCl replaced NaCl. The continuous regression lines show that the exponential phase is not affected by the $[Na]_o$. 2 mM-Ca; 20·0° C.

enhances the rate of recovery of the Na-free contracture. However, because K-free solutions induce a rather erratic spontaneous activity which further increases the rate of recovery and as long exposures to strophanthidin slow the spontaneous relaxation of a subsequent Na-free contracture, these results cannot be expressed in the same way as the stimulation

experiments. All these experimental procedures will be expected to increase the intracellular Na concentration, and the results support the idea that the accumulation of Na ions within the muscle cells is necessary for the recovery of the contractile processes.

The spontaneous relaxation of the contracture induced by Na-depleted solutions; the effects of strophanthidin

The sudden reduction of the bathing Na concentration causes a contracture that develops at a rate dependent on the final Na concentration.

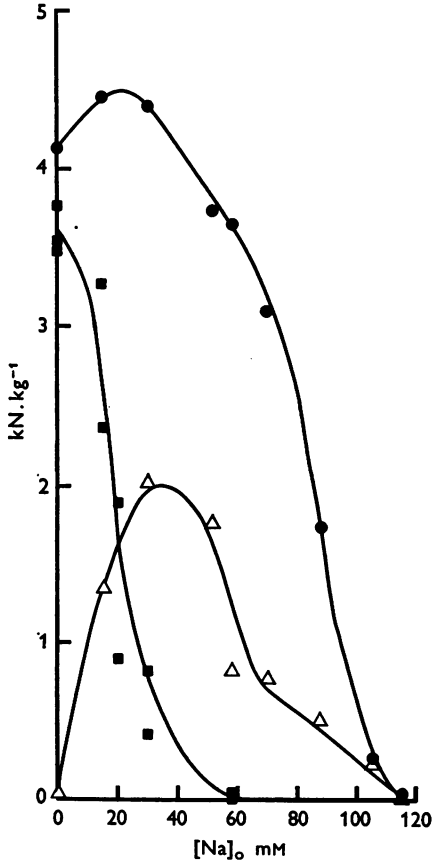


Fig. 8. The effects of 10^{-5} M strophanthidin on the contractures evoked by various Na-depleted solutions, with Tris HCl replacing NaCl.

The relationship between the strength of the contracture and the final $[Na]_o$ before (■—■) and during (●—●) exposure to the glycoside. After the effects of strophanthidin have stabilized, the strength of the low-Na contractures is much increased and these contractures do not relax completely. The residual tension, under these conditions, is also dependent on the $[Na]_o$, (△—△). The lines are drawn by eye. 1 mM-Ca: 20·2° C.

The spontaneous relaxation of this contracture shows two phases; the first, a relatively slow phase of relaxation forms a plateau, the duration of which depends on the Na concentration, being longest in Na-free solutions and almost absent in 20 mM-Na; the second, is an exponential phase that is independent of the bathing Na-concentration (Fig. 7). Both phases of the spontaneous relaxation are influenced by cardiac glycosides. When the effects of 10^{-6}–10^{-5} M strophanthidin have reached their full intensity, as judged by the amplitude and shape of the twitch responses, exposure of the heart to Na-free solutions (also containing the glycoside) evokes a prolonged contracture, which still relaxes completely in the Na-free medium. The initial plateau phase is lengthened and the late exponential phase is slowed, the time constant increasing from a mean value of 12·7, S.E. 1·2 sec to a value of 23·6, S.E. 4·2 sec in 10^{-5} M strophanthidin (five experiments).

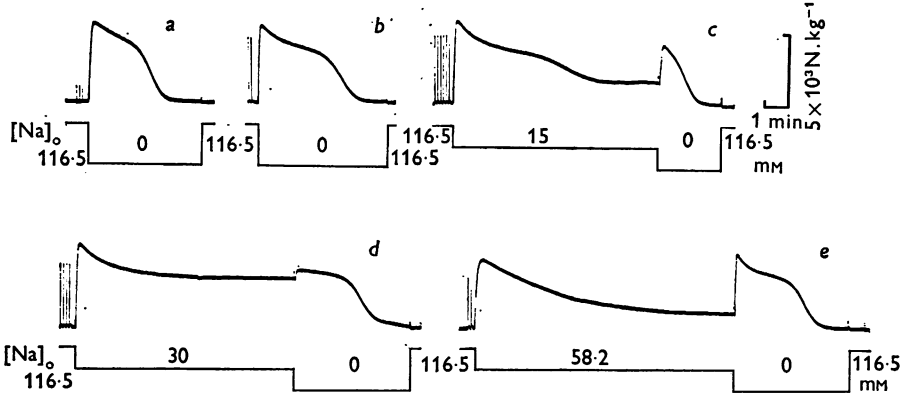


Fig. 9. Traces of the contractures evoked by Na-free and Na-depleted solutions, in the presence of 10^{-5} M strophanthidin. 1 mM-Ca; 19·9° C. *a*, a Na-free contracture elicted before application of the glycoside. *b*, a Na-free contracture in the presence of strophanthidin. *c*, a 15 mM-Na contracture. *d*, a 30 mM-Na contracture. *e*, a 58·2 mM-Na contracture. (*b*) to (*e*), all evoked in the presence of 10^{-5} M strophanthidin.

The Na-depleted contractures fail to show complete spontaneous relaxation of tension. However, withdrawal of all the Na from the bathing medium results in full spontaneous relaxation, after an initial contraction.

The glycoside potentiates the contractures evoked by Na-depleted solutions, so that the tension–Na concentration curve is shifted to lower Na concentrations by an amount equivalent to about 60 mM-Na (Fig. 8). The maximum tension developed by the muscle in Na-depleted solutions, in the presence of strophanthidin, is 1·2–1·5 times larger than the maximum force generated in Na-free fluids in the absence of strophanthidin (Fig. 8). This observation may well be relevant to the difference between

the maximum tension predicted by fitting eqn. (1) to the results of the type shown in Fig. 5, and that value obtained experimentally. Perhaps the hyperpolarization of the membrane, or even the loss of intracellular Na, causes a depression of the tension generated in solutions without Na. An increase in the strength of the contraction is also seen with K contracture in the presence of cardiac glycosides (Otsuka & Nonomura, 1963).

The spontaneous relaxation of the contractures evoked by Na-depleted solutions, in the presence of strophanthidin, is incomplete so that a steady tension remains after a period of relaxation. The amplitude of the steady tension is greatest at sodium concentrations between 30 and 40 mM (Fig. 8). The failure to relax completely is dependent upon the presence of Na ions in the bathing medium, for when a contracture of this type has stabilized at a steady tension and all the Na is suddenly removed, the muscle initially contracts and then subsequently relaxes completely (Fig. 9). This tension change shows a plateau phase which is longer when the Na-concentration in the original contracture fluid is high (Fig. 9).

DISCUSSION

The relationship between the tension induced by replacement of Na, and either the Ca concentration, or the final Na concentration bathing frog atrial muscle, can be fitted by an equation that assumes a competition between Na and Ca ions. The results suggest that the Ca released into the sarcoplasm to activate contraction originates at two sites where Na and Ca compete. The Ca contained at each of these sites (they could be on the same carrier molecule) is in equilibrium with the bathing solution. A similar hypothesis proposed for the depolarization-induced contractures of this muscle considers three sources of activator Ca necessary to account for the quantitative relationship between $[Ca]_o$ and the contracture tension (Chapman & Tunstall, 1971; Chapman, 1971*a*). This difference, however, would appear to be genuine, because the Na-free contracture is abolished in the presence of Mn ions, while the K contracture still persists (Chapman & Ochi, 1972). It could be that the concentration of activator calcium contained in, or released by one of the compartments revealed by the K contracture experiments changes too slowly, when the composition of the bathing fluid is altered, to influence the strength of the Na contracture. The two more rapidly exchanging compartments would be expected to be found at the outer region of the cell (Chapman, 1971*a*), and the third (not concerned in the Na withdrawal contracture) should correspond to the slowest compartment described by Chapman & Niedergerke (1970*a*, *b*).

There is a large influx of Ca into frog heart, when the outside Na con-

centration is low (Niedergerke, 1963), and this influx would seem to be important in the initiation of the contracture, for an outside Ca concentration greater than 10^{-6} M is required if tension is to develop. The spontaneous relaxation of the Na-free contracture shows that this model is inadequate, because eqn. (1) predicts a sustained contracture. The influx of Ca into the muscle cells must therefore be transient, and it must reach a very low value, for when the spontaneous relaxation is complete, a 100-fold increase in the bathing Ca concentration fails to induce any further tension (Fig. 1).

In Na-containing media, the rate of recovery of the Na-free contracture, which is increased by stimulation of the preparation or by K-free or strophanthidin containing Ringer, is too slow for Na ions to be having an extracellular effect. The exchange time of the extracellular space has been found to be 3 sec (Chapman, 1971*b*). Therefore, it would seem that the recovery process is in some way related to a build up of intracellular Na. This means that the intracellular Na could be regulating the influx of Ca into the cell. If this is true, an exchange of Ca^{2+} for Na^{+} across the cell membrane of the type proposed by Reuter & Seitz (1968) and by Baker, Blaustein, Hodgkin & Steinhardt (1969) is possible. The entry of Ca and the exit of Na, under the conditions of the present experiments, would result in a transient influx of Ca, because the intracellular Na-concentration is low (Keenan & Niedergerke, 1967), and during perfusion with Na-free solutions this concentration should fall, so that there is progressively less intracellular Na to exchange for Ca, resulting in a reducing influx of Ca. The recovery of the Na-free contracture, in Na-containing solutions would then become the consequence of the restoration of the original intracellular Na level, because recovery is hastened by increasing the rate of accumulation of intracellular Na. If the intracellular Na-concentration is kept high, by evoking contractures by partial replacement of the bathing Na when the active extrusion of Na is blocked by strophanthidin, the spontaneous relaxation is slowed and incomplete and the contracture tension is increased. This suggests that the influx of Ca is sustained or even augmented under these conditions.

If this exchange, of extracellular Ca for intracellular Na, is by means of a carrier in the cell membrane, then the quantitative relationship between the strength of the contraction and the bathing concentrations of Na and Ca will fit in this hypothesis and into eqn. (1); if the carrier has four binding sites for Na ions or if it has two such sites and the exchanges at the inside and the outside of the membrane are coupled. The spontaneous relaxation of the Na-free contracture would result from the transience of the influx of Ca due to the fall of the intracellular Na concentration. The Ca already released would, however, have to be removed from the sarco-

plasm for full relaxation to occur. The development of caffeine or cooling contractures, in the spontaneously relaxed muscle in the virtual absence of extracellular Ca, would suggest that the sarcoplasmic reticulum is involved (Chapman & Miller, 1972; Chapman & Ellis, 1973). A competition between intracellular Na and Ca ions at the relaxing structure may also operate, so that a reduced intracellular Na level would favour the uptake of Ca, in much the same way as described by Palmer & Posey (1967) for isolated cardiac sarcoplasmic reticulum. Such a mechanism could be responsible for the slowed rate of spontaneous relaxation of the Na-free contracture in the presence of strophanthidin.

The conclusions drawn from these experiments suggest a mechanism for the action of cardiac glycosides on the beating heart, where the intracellular Na concentration controls the influx of Ca from the bathing medium. Similar schemes, in which changes in the intracellular Na concentration have an effect on the intracellular Ca concentration, have been devised to explain the reduction of contracture tension induced in frog ventricle by adrenaline (Graham & Lamb, 1968) and for the potentiation of endplate potentials caused by digoxin (Birks & Cohen, 1968). However, these conclusions differ from those of Gadesby, Niedergerke & Page (1971) who deduced that the strength of the twitch response of frog ventricle did not vary in a consistent way with the intracellular Na concentration. As for the most part, they were studying increases in the intracellular Na content, it may be that the levels of intracellular Na in their experiments were always above those in the present experiments, and may have therefore been above the level where intracellular Na has an effect on the influx of Ca. This suggestion is not consistent with the appearance of a slowly developing contracture in K-free Ringer (Thomas, 1960; R. A. Chapman, unpublished), or with the large increase in the contracture tension evoked by Na-depleted solution, caused by exposure to cardiac glycosides. It may be, as suggested by Delahayes & Bozler (1972) and by Langer (1973), that another interpretation of the results of Gadesby *et al.* (1971) is necessary.

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REFERENCES

BAKER, P. E., BLAUSTEIN, M. P., HODGKIN, A. L. & STEINHARDT, R. A. (1969). The influence of calcium on sodium efflux in squid axons. *J. Physiol.* **200**, 431–458.

BEELER, G. W. & REUTER, H. (1970). The relation between membrane potential, membrane currents and activation of contraction in ventricular myocardial fibres. *J. Physiol.* **207**, 211–229.

BIRKS, R. I. & COHEN, M. W. (1968). The influence of internal sodium on the behaviour of motor nerve endings. *Proc. R. Soc.* B **170**, 401–421.

BUSSELEN, P. & CARMELIET, E. (1973). Protagonistic effects of Na and Ca on tension development in cardiac muscle in low extracellular Na concentrations. *Nature, New Biol.* **243**, 57–58.

CHAPMAN, R. A. (1971*a*). Experimental alteration of the relationship between the external calcium concentration and the contractile force generated by auricular trabeculae isolated from the heart of the frog *Rana pipiens*. *J. Physiol.* **218**, 147–161.

CHAPMAN, R. A. (1971*b*). Is there a T-system in frog atrial muscle cells? *J. Physiol.* **215**, 48–49*P*.

CHAPMAN, R. A. (1973). The ionic dependence of the strength and spontaneous relaxation of the potassium contracture induced in the heart of the frog *Rana pipiens*. *J. Physiol.* **231**, 209–232.

CHAPMAN, R. A. & ELLIS, D. (1973). Synergistic effects of cooling and caffeine on the contraction of the frog's heart. *J. Physiol.* **232**, 101–102*P*.

CHAPMAN, R. A. & MILLER, D. J. (1972). Caffeine contractures induced in frog auricular trabeculae in the absence of external calcium. *J. Physiol.* **225**, 52–54*P*.

CHAPMAN, R. A. & NIEDERGERKE, R. (1970*a*). Effects of calcium on the contractions of the hypodynamic frog heart. *J. Physiol.* **211**, 389–421.

CHAPMAN, R. A. & NIEDERGERKE, R. (1970*b*). Interaction between heart rate and calcium concentration in control of contractile strength of the frog heart. *J. Physiol.* **211**, 423–443.

CHAPMAN, R. A. & OCHI, R. (1972). The effects of manganese ions on the contractile responses of isolated frog atrial trabeculae. *J. Physiol.* **222**, 56–58*P*.

CHAPMAN, R. A. & TUNSTALL, J. (1968). Evidence for the site of Na/Ca antagonism in cardiac muscle of the frog *Rana pipiens*. *J. Physiol.* **201**, 9–11*P*.

CHAPMAN, R. A. & TUNSTALL, J. (1971). The dependence of the contractile force generated by frog auricular trabeculae on the external calcium concentration. *J. Physiol.* **215**, 139–162.

DALY, E. DE B. & CLARKE, A. J. (1921). The action of ions upon the frog's heart. *J. Physiol.* **54**, 367–383.

DELAHAYES, J. F. & BOZLER, E. (1972). Effects of drugs and stimulation on ^{45}Ca movements in frog ventricle. *Proc. soc. exp. Biol. Med.* **141**, 423–430.

GADESBY, D. C., NIEDERGERKE, R. & PAGE, S. (1971). Do intracellular concentrations of potassium or sodium regulate the strength of the heart beat? *Nature, New Biol.* **232**, 651–653.

GRAHAM, J. A. & LAMB, J. F. (1968). The effect of adrenaline on the tension development in contractures and twitches of the ventricle of the frog. *J. Physiol.* **197**, 479–509.

GOTO, M., KIMOTO, Y. & SUETSUGU, Y. (1972). Membrane currents responsible for contraction and relaxation of the bull frog ventricle. *Jap. J. Physiol.* **22**, 315–331.

HODGKIN, A. L. & HOROWIZC, P. (1960). Potassium contractures in single muscle fibres. *J. Physiol.* **153**, 386–403.

KEENAN, M. J. & NIEDERGERKE, R. (1967). Intracellular sodium concentration and resting sodium fluxes of the frog heart ventricle. *J. Physiol.* **188**, 235–260.

LANGER, G. A. (1973). Heart: Excitation-contraction coupling. *Rev. Physiol.* **35**, 55–86.

LÜTTGAU, H. C. & NIEDERGERKE, R. (1958). The antagonism between Ca and Na ions on the frog's heart. *J. Physiol.* **143**, 486–505.

NIEDERGERKE, R. (1963). Movements of Ca in frog heart ventricles at rest and during contractures. *J. Physiol.* **167**, 515–550.

OTSUKA, M. & NONOMURA, Y. (1963). The influence of ouabain on the relation between membrane potential and tension in frog heart muscle. *J. Pharmac. exp. Ther.* **141**, 1–5.

PALMER, R. F. & POSEY, V. (1967). Ion effects on calcium accumulation by cardiac sarcoplasmic reticulum. *J. gen. Physiol.* **50**, 2085–2095.

REUTER, H. & SEITZ, N. (1968). The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. *J. Physiol.* **195**, 451–470.

THOMAS, L. J. (1960). Increase in labelled calcium uptake in heart muscle during potassium lack contracture. *J. gen. Physiol.* **339**, 225–240.

VASSORT, G. (1973). Influence of sodium ions on the regulation of frog myocardial contractility. *Pflügers Arch. ges. Physiol.* **339**, 225–240.

WILBRANDT, W. VON & KOLLER, H. (1948). Die Calciumwirkung am Froschherzen als Funktion des Ionengleichgewichts zwischen Zellmembren und Umgebung. *Helv. physiol. pharmac. Acta* **6**, 208–221.

WINEGRAD, S. (1972). Spontaneous mechanical activity in depolarized frog ventricle. *J. Physiol.* **224**, 52–53*P*.