A QUANTITATIVE DESCRIPTION OF THE RELATIONSHIP BETWEEN THE AREA OF RABBIT VENTRICULAR ACTION POTENTIALS AND THE PATTERN OF STIMULATION

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ABSTRACT Intracellular microelectrodes were used to record action potentials from fibres of the isolated rabbit right ventricle and the areas of the action potentials were measured. The action potential area was found to depend in a reproducible way on the preceding pattern of stimulation. A mathematical model reproducing all the observed changes in the action potential area was developed. In the model the action potential area is taken as a linear function of the product of two time and stimulation dependent variables, M and N. The behaviour of each variable between action potentials is described by the solution of a second order differential equation. During each action potential the variables are assumed to change discontinuously, the magnitudes of the discontinuous changes being given by a set of subsidiary equations. It was found that the behaviour of all the fibres tested was described by the same set of equations, each single fibre being characterized by a set of ten independent constants.

INTRODUCTION

The voltage–time course or "shape" of the repolarization phase of action potentials recorded from cardiac muscle is known to depend on the animal species and the kind of tissue, and can vary from fibre to fibre in the same tissue. In any particular fibre the repolarization phase is remarkably sensitive to changes in environmental conditions, *e.g.* temperature and oxygen tension, and to metabolic inhibitors and drugs (Coraboeuf, 1960). However, with all other experimental conditions held constant the voltage–time course of the repolarization phase depends in a remarkably reproducible way on the frequency and pattern of stimulation. Since a knowledge of this dependence should provide an insight into the nature of the mechanism which generates the prolonged repolarization phase of the cardiac action potential, we have formulated a quantitative description of this dependence. We have selected as the measured quantity the action potential area (see Definitions) in preference to the duration. The area is easily and accurately determined

and is a more sensitive measure of changes in the repolarization phase than the duration.

This paper is divided into two parts. In the first part a number of experimental results describing the relationship between action potential area and the frequency and pattern of stimulation are presented. The second part is devoted to the development of a mathematical model which describes the dynamic behaviour of the action potential area. The model gives a detailed description of this behaviour; it predicts the area of all action potentials in response to various patterns of stimulation.

In the model, action potential area is made a function of two abstract variables and the way in which the magnitudes of these variables change with time and stimulation is described by a set of differential equations. The final model which satisfactorily describes the experimental results can be considered as a logical argument for the existence of the abstract quantities it contains. The dynamic behaviour of these quantities provides a clue towards their real nature and the kind of chemical and physical system in which they take part.

The two basic patterns of stimulation used were designed not only to provide the information necessary for the formulation of the model but were chosen in such a way that almost any pattern of stimulation can be reduced to a combination of the two basic types. It must be emphasized that the purpose of this paper is to describe the model and the inductive process leading to it. Hence, the determination of the effects of environmental changes on the model's applicability lies outside the scope of this paper. However, it should be noted that the applicability of the model does not seem to be limited to the tissue used; *i.e.*, the rabbit right ventricle. Action potential areas from the rabbit atrium appear to behave in the same way, and results from guinea pig ventricle suggest that at least certain aspects of the model are also applicable.

METHODS

Details, other than those described below, of the apparatus and methods, and the preparation of the isolated rabbit right ventricle have been given previously (Johnson and Tille, 1961). Single barrelled microelectrodes were used to record transmembrane action potentials.

Integrator. The area of the action potential (see Definitions) was obtained by electronic integration of the action potential wave-form derived from the output of the floating grid electrometer. To subtract the resting potential from this signal during integration, one input of a differential amplifier was clamped, on command, to the value of membrane potential existing just prior to the upstroke of the action potential, whilst the other grid was alowed to follow the subsequent action potential. The output of this differential amplifier was applied to a feed-back integrator, the time of integration of which could be preset to include one or more action potentials. The integration error over the times and voltages met with in the experiments was less than 1 per cent.

Stimulation. The patterns of stimulation were generated by appropriate use of

a 1:4 or 1:8 scaler, two Tektronix type 162 wave-form generators, two 161 pulse generators, and a Grass stimulator. The action potential and integral signals were displayed on a Tektronix type 532 oscilloscope, equipped with a type 53/54C beam-splitting unit. The traces were photographed when required with a Shackman MK 3 auto camera. Measurements of the integral were made by projecting the film on a calibration grid.

Times. The timing of the film exposures was obtained by recording continuously throughout the experiments the sound of the opening and closing of the camera shutter on a magnetic tape recorder. The tape was replayed and the time intervals measured with the aid of a stop-watch. When required, the time intervals between individual stimuli were either derived from the readings of calibrated controls on the Grass stimulator or by reading from the projected film.

RESULTS

The results described in this section of the paper demonstrate the characteristic features of the dependence of the action potential area on the preceding pattern of stimulation. These results establish the basic experimental evidence for the formulation of the mathematical model which is developed in the next part of this paper. Since the importance of many aspects of the results becomes apparent only after some consideration has been given to the model, these aspects will be described at such a time when they can be usefully included in the argument. In formulating the model 88 hearts were used and data were obtained from 117 fibres.

Definitions. 1. To determine a relationship between the shape of an action potential and the preceding pattern of stimulation it is necessary to select some measurable quantity which will reflect changes in the shapes of action potentials. Such a quantity is the *action potential area* defined by $\int_{t_1}^{t_2} (V - V_m)dt$; where V_m is the membrane resting potential, V the membrane potential at any time, and the limits t_1 and t_2 are such as to include the whole of the action potential so that $V = V_m$ when $t = t_1$ and when $t = t_2$.

The value of the area is expressed in arbitrary units throughout the paper. The scale employed is not always the same in results from different cells.

2. Action potentials initiated at a constant rate will be called *normal* action potentials. Action potentials interposed between the action potentials occurring at a constant rate will be called *extra* action potentials.

Action Potential Area at Constant Rates of Stimulation. The preparation was stimulated at a number of constant rates and the areas of action potentials obtained from a single fibre were recorded. After several minutes of stimulation at any given constant rate all action potentials became identical in shape and in area. In all fibres the steady-state value of area was found to depend only on the existing rate of stimulation and to be independent of the stimulus pattern preceding the initiation of the existing rate. Thus in every fibre each constant rate of stimulation is associated with a unique steady-state value of the action

potential area. The steady-state area has a maximum value at a rate of about 2 sec.$^{-1}$ and declines both at higher and at lower rates (Gibbs and Johnson, 1961). This is shown in Fig. 1.

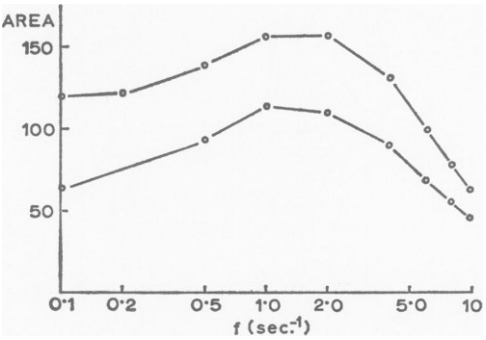


FIGURE 1 Two examples of the relationship between the steady-state area and the rate of stimulation, *f*. Note the decrease in area at low rates.

Changes in Action Potential Area after a Change to a New Rate of Stimulation. The changes in action potential area which occur when the rate of stimulation is suddenly altered to a new constant rate were examined in the following way:

The muscle was stimulated at a constant rate until the action potential area reached a steady value. The rate of stimulation was then altered and the areas of action potentials were recorded at frequent intervals and plotted against time. An increase in the rate of stimulation produced in all cases a rapid increase in area (within a few action potentials) to a maximum value after which the area declined much more slowly with an approximately exponential time course to its stable value. A decrease in the rate of stimulation produced in most cases a rapid decrease in area followed in all cases by a slow, approximately exponential, increase to the stable value.

Some characteristic graphs of action potential area against time are shown in Fig. 2. Examples of the changes in shape which take place during these experiments are illustrated in Fig. 3.

The Effect of an Extra Action Potential. The tissue was stimulated at a low constant rate (in most cases about 0.3 sec.$^{-1}$) and an extra stimulus was applied after every fourth stimulus at the constant rate. The controlled variables in these experiments were the rate of stimulation and the *stimulus interval.* This was the interval between the extra stimulus and the *preceding normal* stimulus. For each extra stimulus applied, the areas of three action potentials were measured: the

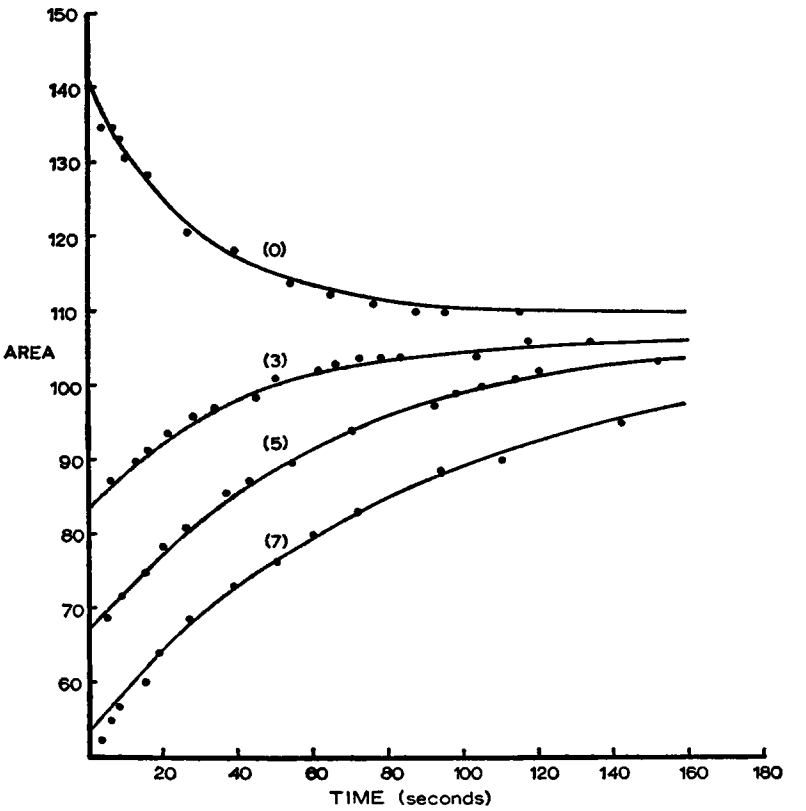


FIGURE 2 The recovery of the action potential area after a change in the rate of stimulation. The experimental points (●) are fitted with exponential curves of the form: Area $= P + Qe^{-t/r}$ (solid lines). All the points shown were obtained at a stimulation rate of 1.5 sec.$^{-1}$. Prior to $t = 0$ the tissue was stimulated for 2 to 3 minutes at the rate shown by the numeral in parenthesis above each curve. The time constants of the fitted curves are plotted in Fig. 10. The steady-state area was 85 at 3 sec.$^{-1}$, 67 at 5 sec.$^{-1}$, and 51 at 7 sec.$^{-1}$.

area of the normal action potential preceding the extra stimulus, the area of the action potential in response to the extra stimulus (the extra action potential), and the area of the following normal action potential. The areas of the three action potentials were then plotted against the stimulus interval as in Fig. 4. An example of a record of the three action potentials together with the integral trace is shown in Fig. 5.

The area of an extra action potential was always greater than the area of the preceding normal action potential; it was greatest at the shortest stimulus interval and decreased as the stimulus interval was increased.

The area of the following normal action potential was also affected by the stimulus interval; it was least at the shortest stimulus interval and increased steadily as the

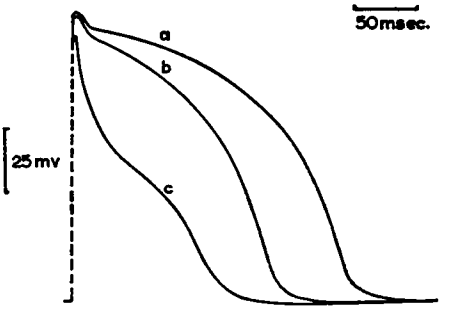


FIGURE 3 Three differently shaped action potentials obtained from one fibre by commencing stimulation at a rate of 1.5 sec.$^{-1}$ after a 2 minute period of rest. *c* is the first action potential initiated after the period of rest, *a* is the action potential with the maximum value of area (in this case the fourth action potential initiated after rest), and *b* is the normal steady-state action potential at a rate of 1.5 sec.$^{-1}$.

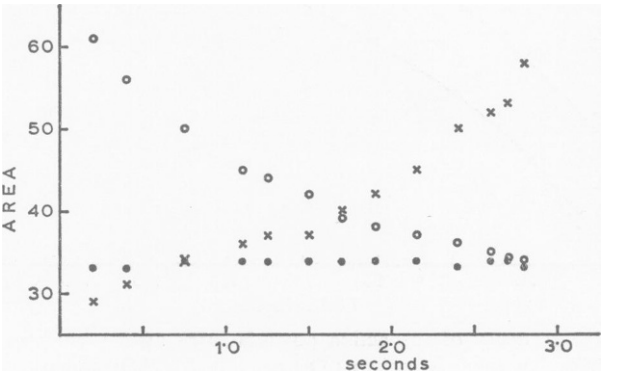


FIGURE 4 The relationship between the areas of the extra action potential (○), the following normal action potential (×), and the stimulus interval. The value of the area of the preceding normal action potential (●) was recorded as a control. Each vertical set of three points corresponds to values obtained from three consecutive action potentials photographed on one oscilloscope sweep as in Fig. 6. The stimulation rate was 0.31 sec.$^{-1}$.

stimulus interval was increased. At the greatest possible stimulus interval, *i.e.* with the extra action potential initiated just before it, the area of the following normal action potential approached the maximum area attainable by the extra action potential. It is clear that in this situation, *i.e.* with the greatest stimulus interval, the extra action potential approximates a normal action potential while the following normal action potential approximates an extra action potential. When the stimulus interval was very short, the area of the following normal action potential was often smaller than the area of the preceding normal action potential. This effect was most noticeable at the lowest rates of stimulation.

The Behaviour of the Second Extra Action Potential. The previous ex-

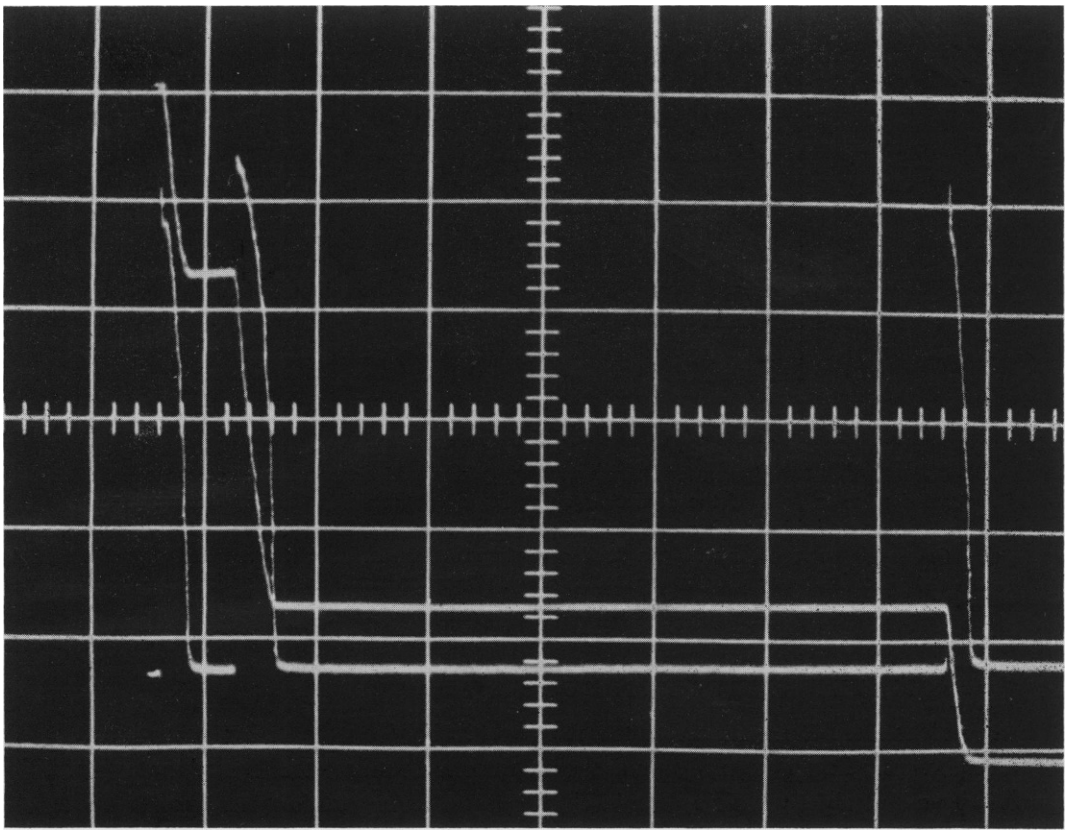


FIGURE 5 A typical experimental record of three action potentials together with their integrals. The trace beginning in the lower left-hand corner shows a normal, an extra, and a following normal action potential (1 large division = 25 mv). The upstrokes of the action potentials are not visible. The trace beginning in the upper left-hand corner and ending as the lowest trace in the lower right-hand corner shows the integrals of the action potentials; the value of the area of each action potential is proportional to the vertical displacement of this trace produced over the duration of that action potential. Time, 1 large division = 500 msec. The rate of stimulation is 0.286 $sec.^{-1}$

periment was extended by the introduction of a second extra stimulus. The stimuli were arranged into a repeating pattern consisting of one normal, two extra, and three normal stimuli, in that order.

The following experimental procedure was employed. After carrying out the experiment with one extra action potential as described in the previous section, the extra action potential was initiated at a fixed stimulus interval and a second extra action potential was introduced between the first extra and the following normal action potentials. The position of the second extra action potential was then varied and its area recorded and plotted against time as in Fig. 6.

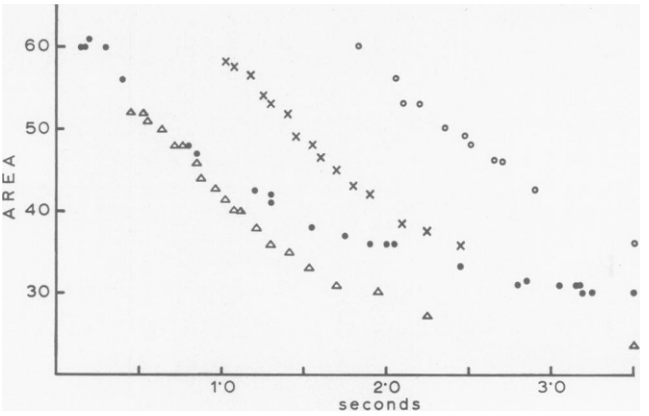


FIGURE 6 The relationship between the areas of the first and second extra action potentials and the time after the upstroke of the preceding normal action potential. The decline in area of the second extra action potential when the first extra action potential was initiated after a stimulus interval of 210 (△), 750 (×), and 1600 msec. (○) is shown together with the decline of the area of the first extra action potential (●). The tissue was stimulated at 0.286 sec.$^{-1}$. The value of the normal action potential area was 30.

MATHEMATICAL MODEL

It is clear from the results that the relationship between the action potential area and the preceding pattern of stimulation appears to be more complicated in the fibres of the rabbit right ventricle than in similar fibres from certain other species where the predominant effect is the shortening of the action potential at high rates of stimulation (Coraboeuf, 1960). The two most apparent differences are the behaviour of the area of the extra action potential and the decline in the steady-state area at low rates of stimulation. These facts lead to the hypothesis that in rabbit ventricular fibres there exists an additional process, the effects of which are most apparent at low rates of stimulation, operating simultaneously with the process which causes the shortening of the action potential at high rates of stimulation.

The aim of this part of the paper is to establish some model capable of describing the area of any action potential in terms of the previous pattern of stimulation. As the results suggest the existence of two processes, the first step is to consider the area as a function of two variables dependent on time and stimulation. The decline in area at both low and high rates of stimulation is interpreted by assuming that one of these variables decreases when the rate is decreased and that the other variable decreases when the rate is increased. The simplest suitable equation expressing the area as a function of two variables M and N is:

$$\text{Area} = A + BNM \tag{1}$$

in which A and B are constants.

Equation (1) leads to considerable simplification in the interpretation of the experimental results. Since the process which shortens the action potential at high rates can be considered to be inoperative at low rates of stimulation, it is assumed that one of the variables (M) tends to a limiting constant value as the rate of stimulation is decreased and, therefore, the area becomes a linear function of the second variable (N). Thus, the results obtained at very low rates of stimulation can be used to determine the behaviour of N.

Behaviour of the Action Potential Area at Low Rates of Stimulation. Assuming that at any low rate of stimulation the variable M has a value not significantly different from its limiting maximum value, say M_{max}, we shall describe the area of the action potential at low rates of stimulation or, more strictly, when the number of stimuli is not greater than say, five in any 10 second interval, as a linear function of a single variable N, and attempt to formulate some differential equation relating the rate of change of N to time and stimulation. Thus equation (1) becomes:

$$\text{Area} = A + CN, C = BM_{max} \tag{2}$$

where N is the solution of a differential equation. To avoid ambiguity we let equation (2) relate the area of an action potential to the value of N existing just *prior* to the upstroke.

From this definition of N it follows that a measure of the value of N existing at any time can be obtained by initiating an action potential at that time and measuring its area. However, to follow the time course of the change in N, say between two action potentials initiated at a constant rate, a more cautious procedure has to be adopted because each action potential obviously modifies the following time course of N. For this reason only the area of the first extra action potential can be used for the determination of the unmodified time course of N between the two normal action potentials.

A method for the determination of the changes in N between two normal action potentials can be found from a consideration of the following facts. When the tissue is stimulated at a low constant rate all action potentials become identical in area after a few action potentials. If the constant rate is replaced by some rhythmic figure which is repeated at regular intervals, the action potentials comprising each rhythmic figure will, in general, differ in area. However, if the rhythmic figure is repeated a sufficient number of times, the areas of action potentials in corresponding positions in all the figures become identical. Consider now the situation where the tissue is stimulated at a constant rate and an extra stimulus is applied after, say, every fourth stimulus at the constant rate. If the time interval between the extra stimulus and the preceding normal stimulus, *the stimulus interval,* is kept constant, the result is a repeated rhythmic figure consisting of five action potentials. We define this rhythmic figure such that the extra action potential is always the second action potential in each figure.

It was found that when the stimulus interval was varied, the areas of the extra action potential and of the following normal action potential were affected by the stimulus interval, but the area of the first action potential of the next figure was not affected. This means that when the first action potential of each figure is initiated, the fibre is always in the same state and that this state is not affected by a change in the stimulus interval in the preceding figure. In other words, the three intervening normal action potentials are sufficient to abolish the effect of the variation of the stimulus interval in the preceding figure.

Thus under these conditions the area of each extra action potential is a measure of the value of N existing at the time of its upstroke. The variation of N with time between two normal action potentials is obtained by varying the stimulus interval and plotting the resulting area of the extra action potentials against the stimulus interval as in Fig. 4.

Three important facts can be selected from the results obtained at *low* rates of stimulation:

(a) The system rapidly attains a state of dynamic equilibrium when stimulated at a constant rate or by a regularly repeated rhythmic figure. At a constant rate equilibrium is reached after two or three action potentials. In the case of a rhythmic figure containing at least four action potentials the results obtained from all figures except the first are identical.

(b) The area of any action potential depends on the time interval separating it from the preceding action potential. The area decreases steadily as this interval is increased. (see Figs. 4 and 6).

(c) If an extra action potential is initiated shortly after a normal one, the area of the following normal action potential is often smaller than the normal area. This can be seen in Figs. 4 and 9.

From (b) it follows that N decreases steadily between action potentials and therefore, to satisfy (a), it must increase by some amount, say ΔN, at some time during the action potentials. The value of N existing at the beginning of the upstroke of an action potential (*i.e.* before ΔN has been added) will determine the area of that action potential according to equation (2). The steady decrease of N between action potentials is to be described by the solution of some differential equation. The effect of stimulation will be simulated by increasing N by an amount ΔN every time an action potential is initiated. For the sake of simplicity it is assumed that N increases instantaneously by the amount ΔN during the upstroke of each action potential.

Result (c) can now be interpreted in the following way: When an extra action potential is initiated shortly after a normal one, N is increased by a further ΔN in the extra action potential. The fact that the following normal action potential is often smaller than normal means that N, which in this case starts from an initial

value which is higher than normal, must decrease at such a rate as to have a value lower than normal by the time the next normal action potential is initiated. This is shown schematically in Fig. 7.

This last fact means that the rate of decline of N cannot be governed by a single first order differential equation. We shall therefore attempt to describe the behaviour of N between stimuli by the following two equations:

$$\frac{dN}{dt} = f(\nu, N) \tag{3}$$

$$\frac{d\nu}{dt} = g(\nu, N) \tag{4}$$

in which ν is another variable governing the rate of decline of N. The first problem is to find the form of the functions f and g such that the solutions $N(t)$ of equations

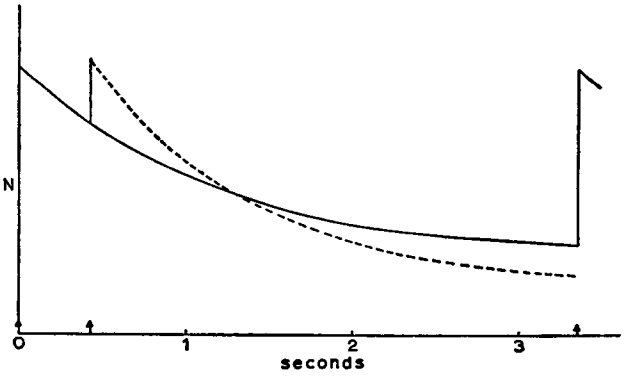


FIGURE 7 A graphic description of the behaviour of the variable N. The solid line represents the variation of N occurring at a constant rate of stimulation. The positions of the normal action potentials are indicated by the first and third arrows on the abscissa. The broken line represents the decline of N after an extra action potential initiated at the time indicated by the second arrow.

(3) and (4) will reproduce the shapes of the experimental curves. The second problem is to find the dependence of ΔN on the other variables.

Some properties of $f(\nu, N)$ can be deduced from the experimental results. Result (b) indicates that dN/dt is negative at all times (except during the upstroke when ΔN is being added) and therefore $f(\nu, N)$ must always be negative. To avoid the possibility of obtaining a negative area in equation (2), N must be always positive and therefore dN/dt must tend to zero as N tends to zero. A simple function satisfying these conditions is:

$$\frac{dN}{dt} = -a_N \nu N, \qquad a_N > 0 \tag{5}$$

The next step is to determine the form of the function $g(\nu, N)$. To satisfy result

(*b*) and keep dN/dt negative, ν must be positive at all times. Result (*c*) is represented in Fig. 7 where the full line represents the hypothetical time course of N at a constant rate of stimulation and the broken line the time course of N following an extra action potential. To satisfy result (*c*), ν must also increase when N is increased by an action potential.

The variable ν can be considered to be continuous during the upstroke of the action potential. Several forms of the function g of equation (4) together with equation (5) were solved on a digital computer. ΔN was kept constant and was added at appropriate times to simulate experimental conditions. The simplest form of g which would yield solutions in close agreement with the results was:

$$\frac{d\nu}{dt} = g = b(cN^2 - \nu)\, e^{d(cN^2-\nu)} \tag{6}$$

The complexity of this function made us abandon the attempt to keep ν continuous during the upstroke, for not only is the solution of the simultaneous equations (5) and (6) difficult, but also the physical meaning of g in equation (6) is obscure. On the other hand, when ν is allowed to change discontinuously a very simple form of $d\nu/dt$ leads to solutions of dN/dt which represent the results with excellent accuracy:

$$\frac{d\nu}{dt} = -b_N\nu, \qquad \nu_0 > 0, \qquad b_N > 0 \tag{7}$$

where ν_0 is the value of ν at $t = 0$. This equation governs the changes in ν between the upstrokes and ν increases by an amount $\Delta\nu$ during the upstroke. The solution, $N(t)$, of equations (5) and (7) is:

$$N = N_0 \exp\left[-\frac{a_N}{b_N}\nu_0(1 - e^{-b_N t})\right] \tag{8}$$

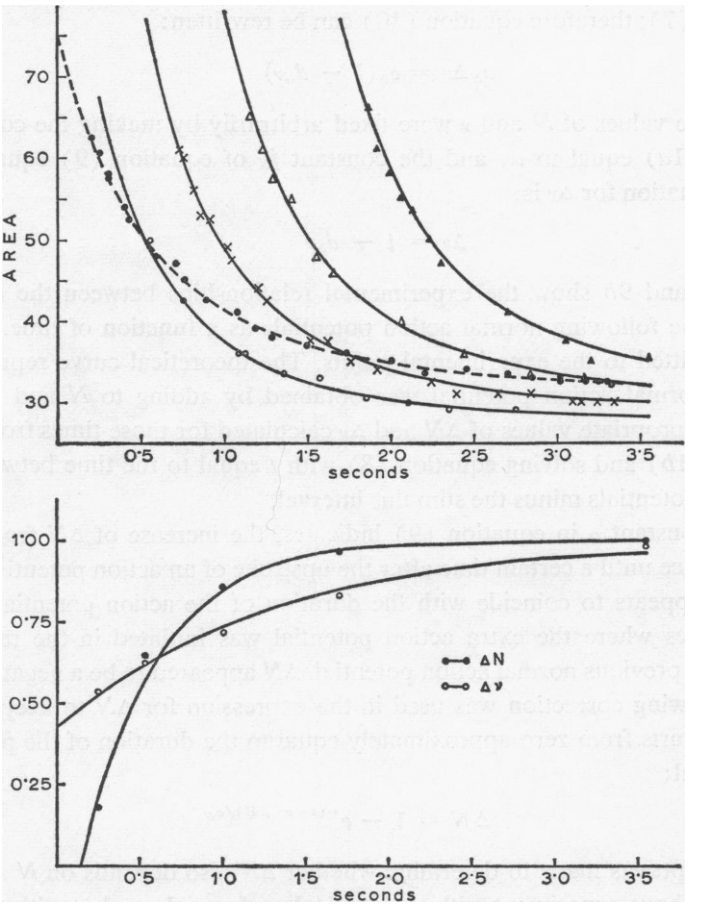
in which N_0 and ν_0 are the values at $t = 0$ (i.e. just after the upstroke) of N and ν respectively.

The remaining problem is to find the dependence of the quantities ΔN and $\Delta\nu$ on time, N and ν. This dependence is to be determined from experiments using two and three extra action potentials.

The variation of ΔN between two action potentials initiated at a constant rate was determined in the following way. First, the decline of N between the normal action potentials was determined as above. Then, keeping the time of occurrence of the extra action potential fixed, the decline of N after the extra action potential was determined by introducing a second extra action potential and recording the variation in its area with the interval between the two extra action potentials. The variation in N after the first extra action potential was then extrapolated back to the time of the upstroke of that action potential. The difference between the extrapolated value and the value to which N had declined after the first normal action potential (as determined in the first experiment) was taken as the value of ΔN at the time

of the upstroke of that action potential. Fig. 8 illustrates the results of such an experiment.

The mathematical procedure was as follows: First, the values of the constants A and C of equation (2) and $a_{N\nu_0}$, b_N, and N_0 of equation (8) were determined by fitting the decline of N between two normal action potentials with the expression of equation (8). Then keeping A, C, and b_N constant, the new values of N_0 and $a_{n\nu_0}$ were found by fitting the relationship of equation (8) to the experimental relationships obtained with the second extra action potential.



FIGURES 8*a* and 8*b* The determination of the time dependence of ΔN and $\Delta \nu$. In Fig. 8*a* the broken line and the solid lines are plots of the equation $N = N_0 \exp [-1.93 \nu_0(1 - e^{-0.789t})]$, with Area $= 24.8 + 42.5N$ fitted to the experimental points. The experimental points show the decline of N after a normal action potential (●) and after extra action potentials initiated after a stimulus interval of 250 (○), 530 (×), 1000 (△), and 1700 msec. (▲). The basic stimulation rate was 0.282 sec.$^{-1}$. Fig. 8*b* shows the relationship between ΔN and $\Delta \nu$ and time obtained from Fig. 8*a* by the method described in the text. The full lines are plots of $\Delta N = 1 - e^{-(t0.15)/.45}$ and $\Delta \nu = 1 - 0.579\, e^{-0.789t}$.

It was found that both ΔN and the quantity $a_N \Delta v$ (which was derived by a method analogous to that for ΔN) were exponential functions of time of the forms:

$$\Delta N = f_N(1 - e^{-(t-\alpha)/c_N}) \tag{9}$$

$$a_N \Delta v = \gamma + \delta(1 - e^{-\epsilon t}) \tag{10}$$

in which t is the time interval from the upstroke of the preceding action potential and c_N, f_N, α, γ, δ, and ϵ are constants. The constant ϵ of equation (10) was found in every case to be equal (within the accuracy of the experiment) to the constant b_N of equation (7); therefore equation (10) can be rewritten:

$$a_N \Delta v = e_N(1 - d_N v) \tag{11a}$$

The absolute values of N and v were fixed arbitrarily by making the constant e_N of equation (11*a*) equal to a_N and the constant f_N of equation (9) equal to 1. The resulting equation for Δv is:

$$\Delta v = 1 - d_N v \tag{11b}$$

Figs. 9*a* and 9*b* show the experimental relationships between the areas of the extra and the following normal action potentials as a function of time. Theoretical curves are fitted to the experimental points. The theoretical curve representing the following normal action potential was obtained by adding to N and v at various times the appropriate values of ΔN and Δv calculated for those times from equations (9) and (11*b*) and solving equation (8) with t equal to the time between the normal action potentials minus the stimulus interval.

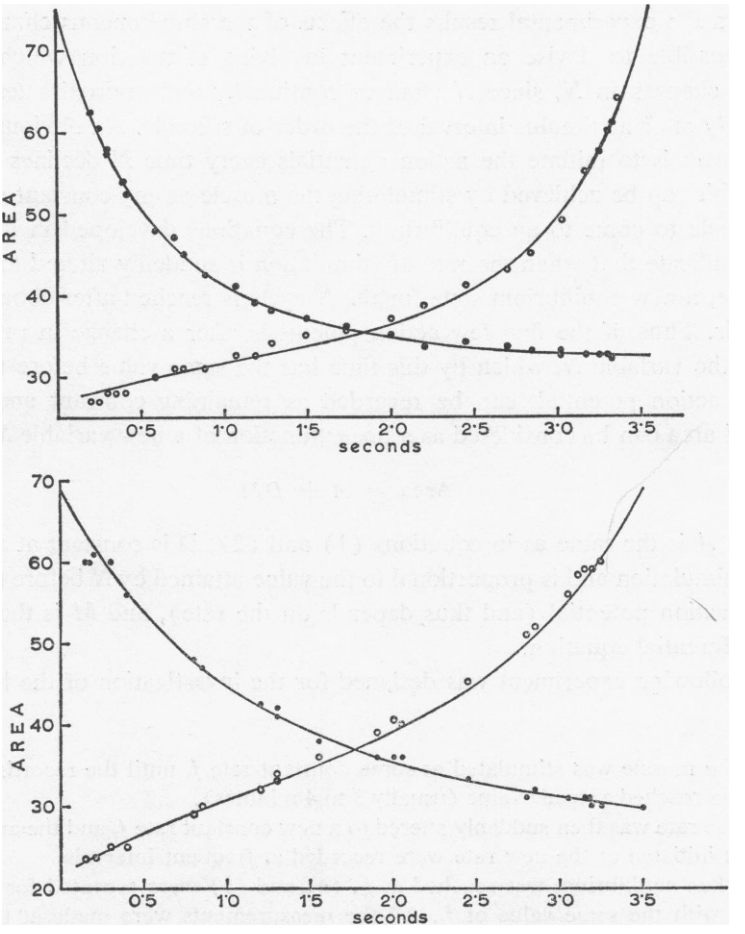
As the constant α in equation (9) indicates, the increase of ΔN from zero does not commence until a certain time after the upstroke of an action potential (Fig. 8*b*). This time appears to coincide with the duration of the action potential; indeed, in the few cases where the extra action potential was initiated in the repolarization phase of the previous normal action potential, ΔN appeared to be a negative quantity.

The following correction was used in the expression for ΔN to keep the time at which ΔN starts from zero approximately equal to the duration of the preceding action potential:

$$\Delta N = 1 - e^{-(t-\alpha-\beta M)/c_N} \tag{12}$$

An attempt was made to determine whether ΔN also depends on N and v by following the above experiment with an identical performed one but with the first normal action potential in each figure replaced by two closely spaced action potentials. However, this is a lengthy and difficult experiment and no conclusive results were obtained.

In summary, the behaviour of the action potential area at low rates of stimulation can be described by the variation of a single variable N which decreases between action potentials in accordance with equation (5) and which increases during the



FIGURES 9*a* and 9*b* The areas of the extra action potential (●) and the following normal action potential (○) as functions of the stimulus interval in two different fibres. The solid lines are the theoretical curves. In Fig. 9*a* the theoretical curve representing the following normal action potential was constructed by the method described in the text from the values of constants obtained in Fig. 8. In Fig. 9*b* the area of the extra action potential is fitted by: Area $= 4.3 + 39\ N$, where $N = 1.66 \exp [-1.075\ (1 - e^{-0.571t})]$. The curve representing the area of the following normal action potential was obtained using the values $a_N\nu_0 = 0.614$ sec.$^{-1}$, $b_N = 0.571$ sec.$^{-1}$, $c_N = 0.75$ sec., $d_N = 0.336$, and $(\alpha + \beta M) = 0.13$ sec., from data also shown in Fig. 6.

upstroke of each action potential by an amount ΔN given by equation (12). The variable ν which influences the rate of decrease of N behaves in a similar way; it decreases between action potentials according to equation (7) and increases during the upstroke of each action potential by an amount $\Delta\nu$ given by equation (11).

Action Potential Area at Constant Rates of Stimulation. To be able to examine the behaviour of the second variable, M, it is essential to eliminate in some

way from the experimental results the effects of the simultaneous changes in N. It is not possible to devise an experiment involving stimulation which would not produce changes in N, since N changes continually and approximates a constant value only after a stimulus interval of the order of seconds. An obvious solution to the problem is to initiate the action potentials every time N declines to a certain value. This can be achieved by stimulating the muscle at any constant rate to allow the N cycle to come to an equilibrium. The equations developed in the preceding section indicate that when the rate of stimulation is suddenly altered to a new constant rate, a new equilibrium state for the N cycle is reached after about five action potentials. Thus, if the first few action potentials after a change in rate are disregarded, the variable N, which by this time has the same value before the upstroke of each action potential, can be regarded as remaining constant and the action potential area can be considered as a linear function of a new variable M:

$$\text{Area} = A + DM \tag{13}$$

in which A is the same as in equations (1) and (2), D is constant at any constant rate of stimulation and is proportional to the value attained by N before the upstroke of each action potential (and thus depends on the rate), and M is the solution of some differential equation.

The following experiment was designed for the investigation of the behaviour of M:

(a) The muscle was stimulated at some constant rate f_1 until the recorded action potential area reached a steady value (usually 3 to 4 minutes).

(b) The rate was then suddenly altered to a new constant rate f_2 and the areas of action potentials initiated at the new rate were recorded at frequent intervals.

(c) When equilibrium was reached at f_2, (a) and (b) were repeated for other values of f_1 but with the same value of f_2. All the measurements were made at the rate f_2 so that throughout the experiment the value of N could be regarded as being constant. (a) and (b) were carried out usually for four values of f_1 and each run was repeated.

If the whole experiment was carried out without dislocation of the microlectrode from the cell, it was repeated with a different value of f_2.

The following approach was adopted in the attempt to represent these results in a mathematical form: The *basic assumption* is, as in the case of the variable N, that each action potential produces a sudden change, in this case a decrease in the variable M, whereas in between stimuli M increases steadily and asymptotically to some constant value, M_{max}. There is, however, an important distinction between the two variables M and N which necessitates a different approach to each problem. The changes in N brought about by each action potential are sufficiently great and the subsequent decline in N sufficiently rapid to reveal directly the relationship between N and time. In the case of M, however, more than a hundred action potentials must occur before the area reaches a steady value after any appreciable change in the

rate of stimulation. This indicates that the change in M produced by any single action potential is very small compared with the value of M. Because of this, the changes in M cannot be measured directly (as can the changes in N); in addition to being small, the changes in M are completely obscured by the simultaneous changes in N.

Since it is not possible to examine directly the regeneration of M during the resting state, the regeneration of M and its depletion by the action potentials will be considered together as a single system. Because the sudden changes in M caused by the action potentials, say ΔM, are small compared with the value of M, it is a useful approximation to consider M as being used up at a continuous rate proportional to the rate of stimulation rather than in discrete amounts. The differential equations which are to describe the composite system of depletion and regeneration can then be formulated with the rate of stimulation as a parameter.

In the experiments described above the time course of the changes in area are very closely approximated by exponential curves (see Fig. 2). Although subsequent analysis shows that the curves are not in fact exponential, the difference is slight; we have as a first approximation considered them to be exponential and estimated the constants P, Q, and τ by fitting the experimental points with the equation:

$$\text{Area} = P + Qe^{-t/\tau}$$

The constant P which is the steady-state area was estimated directly from the last few points of each curve, and the constant Q and the time constant τ were estimated by fitting an exponential curve to the experimental points by minimizing the squares of the deviations of the time coordinate.

Although the results were obtained from the relationship between area and time, they can be discussed in terms of M since changes rather than absolute values are of interest. Several features of the results are of importance:

(*a*) The system attains equilibrium: If the muscle is stimulated at any given constant rate for a sufficient length of time, all action potentials (recorded from any one cell) have identical areas characteristic of that rate (Fig. 1).

(*b*) The steady state area is always approached asymptotically; equilibrium is never attained in an oscillatory manner.

(*c*) The relationship between M and time after a change in rate is very closely approximated by exponential curves (Fig. 2).

(*d*) The slope, dM/dt, at any one rate f, is not uniquely related to the value of M. This can be seen in Fig. 2 if a horizontal line, say Area = 90, is drawn and the three values of the slope are compared.

(*e*) As M_1, the steady-state value of M at the rate f_1, is decreased (by increasing f_1) the value of τ rises, at first slowly and then very rapidly (Fig. 10).

(*f*) For experiments in which $f_1 > f_2$ (*i.e.* $M_1 < M_2$) the initial value of the slope, $(dM/dt)_0$, (*i.e.* just after the change in rate to f_2) first increases as f_1 is increased,

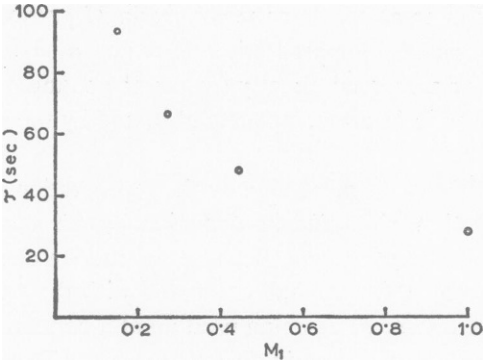


FIGURE 10 Relationship between the time constant of exponential curves fitted to the recovery of the action potential area after a change in the rate of stimulation, and the initial value of M, M_1. The recovery curves were obtained at a rate of stimulation of 1.5 sec.$^{-1}$ (Fig. 2).

but when f_1 is increased beyond a certain value ($\gtrsim 7$ sec.$^{-1}$) the initial slope begins to decrease again.

(g) The relationship between the rate f and M_f, the steady-state value of M, was obtained at a constant value of N by extrapolating the exponential curves obtained at f_2 back to $t = 0$. The extrapolated values of M were taken as M_f (see Discussion). When this relationship is plotted as in Fig. 11, it is found that it can be fitted about

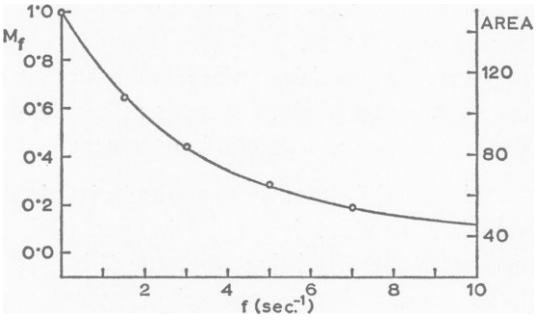


FIGURE 11 The relationship between the steady-state value of M, M_f, and the rate of stimulation, f. The open circles (○) are the mean values of experimental points obtained from the same cell as in Fig. 2 with Area $= 32 + 118\ M$. The solid line is the plot of $M_f = 1/(1 + .28f + .05f^2)$.

equally well by either of the following two equations:

$$M_f = \frac{\alpha}{\alpha + f} \tag{14}$$

$$M_f = e^{-\beta f} \tag{15}$$

in which α and β are constants. To simplify calculations the constant D in equation (13) was chosen for each fibre to make $M_{max} = 1$.

From these observations certain deductions can be made about the differential equations which govern the behaviour of M. Result (d) indicates that at least a second order differential equation is necessary to describe the behaviour of M. Therefore, taking into account the basic assumption and approximating the depletion of M by a continuous process, a suitable expression for dM/dt is:

$$\frac{dM}{dt} = g(f, M, \mu) - h(f, M, \mu) \tag{16a}$$

where the behaviour of μ is defined by:

$$\frac{d\mu}{dt} = \phi(f, M, \mu) \tag{16b}$$

The function g corresponds to the regeneration of M and h to the depletion of M by the action potentials. To satisfy (c) the solutions of these equations must either be exponential curves or if they are not they must not depart too far in shape from exponential curves. Result (a) means that for all experimental values of f, dM/dt tends to zero after long times and therefore in the steady state $g = h$. To satisfy (g) the solutions for M_f of:

$$g(f, M_f, \mu_f) = h(f, M_f, \mu_f)$$

in which M_f and μ_f are the steady-state values of M and μ at the rate f, should preferably lie between the two relationships given by equations (14) and (15). The significance of results (e) and (f) will be discussed later.

To limit the number of possible forms that equations (16a and 16b) could take, one further assumption was made. The assumption is that the functions g and h in equation (16a) are independent of each other, except through the value of M. This is not only plausible, as it is merely assumed that the two processes g and h do not interfere with each other, but it also leads to a considerable simplification of the two functions and gives them a more precise meaning. The assumption has the following consequences: Since h, the rate of depletion by the action potentials is obviously proportional to the rate f, g must be independent of f; and the dependence of μ in equations (16a and 16b) can be assigned arbitrarily to either g or h, but a more meaningful system is obtained if g is made to depend on μ and h is made independent of it. Equations (16a and 16b) now simplify to:

$$\frac{dM}{dt} = g(M, \mu) - f \cdot h(M) \tag{17a}$$

$$\frac{d\mu}{dt} = \phi(f, M, \mu) \tag{17b}$$

These equations describe the following system: The variable M is being regen-

erated at a rate dependent on the magnitude of M and μ. The amount of M used up per action potential depends only on M.

It is now possible to find the form of the function $h(M)$. Since at constant rates $dM/dt = 0$:

$$g(M_f, \mu_f) = f \cdot h(M_f)$$

in which M_f and μ_f are the steady-state values of M and μ at the rate f. Consider the situation in which the system is at equilibrium at the rate f_1 and the rate is changed suddenly to f_2. Just prior to the change in rate:

$$g(M_1, \mu_1) = f_1 \cdot h(M_1) \tag{18}$$

When the rate is changed to f_2 the initial value of dM/dt, say $(dM/dt)_0$, is given by (since none of the variables except f have had time to change):

$$\left(\frac{dM}{dt}\right)_0 = g(M_1, \mu_1) - f_2 \cdot h(M_1)$$

Substituting for g from equation (18):

$$\left(\frac{dM}{dt}\right)_0 = f_1 \cdot h(M_1) - f_2 \cdot h(M_1)$$

or

$$h(M_1) = \frac{\left(\frac{dM}{dt}\right)_0}{f_1 - f_2} \tag{19}$$

in which all the quantities on the right-hand side are known. Thus by repeating the experiment for different values of f_1 the relationship between $h(M)$ and M can be obtained (Fig. 12). The form of the relationship appears to be one of propor-

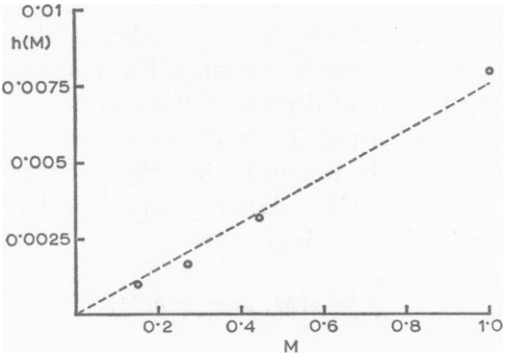


FIGURE 12 The relationship between $h(M)$ and M. The open circles are values calculated from the results shown in Fig. 2 using equation (19). The slope of the broken line is equal to 0.0076.

tionality; $h(M) = b_M M$, where b_M is a constant. Thus:

$$\frac{dM}{dt} = g(M, \mu) - fb_M M$$

Since the value of M is to approach 1 when $f = 0$ a reasonable form of dM/dt would be:

$$\frac{dM}{dt} = a_M(1 - M)\psi(\mu) - fb_M M$$

If $\psi(\mu)$ were a constant the solution of this equation corresponding to the experiments would be exponential curves with a time constant equal to $(a_M\psi + b_M f_2)$ (*i.e.* independent of f_1) and M_f would equal $(a_M\psi)/(a_M\psi + b_M f)$. Also, if f_1 were increased, M would start off from lower and lower values and the initial value of dM/dt would always increase. Thus to satisfy the results (e) and (f) the steady-state value of $\psi(\mu)$ must decrease as f is increased in order to slow down the recovery of M after high rates of stimulation.

The final form of equation $(17a)$ was obtained by letting $\psi(\mu) = \mu$:

$$\frac{dM}{dt} = a_M\mu(1 - M) - fb_M M \tag{20}$$

Finally, the form of $\phi(f, M, \mu)$ in equation $(17b)$ must be determined. From the experimental results it is clear that μ must behave in a manner similar to that of M. It must decrease when the rate is increased and increase when the rate is decreased.

Four forms of equation $(17b)$ were tried. Each one was solved together with equation (20) on a digital computer and the solutions were compared with the experimental results. In each case a suitable set of constants was chosen; the steady-state values of M and μ were calculated for several rates of stimulation by solving the equations $dM/dt = 0$ and $d\mu/dt = 0$, and with each pair of values (M_f and μ_f) as the initial conditions, the equations were solved taking f (this would correspond to f_2 in the experiments) as 1 sec.$^{-1}$ or 1.5 sec.$^{-1}$. This procedure simulated the experiments described at the beginning of this section.

The equations were:

$$\frac{d\mu}{dt} = c_M(1 - \mu) - fd_M\mu$$

$$\frac{d\mu}{dt} = c_M M(1 - \mu) - fd_M\mu$$

$$\frac{d\mu}{dt} = \mu[c_M(1 - \mu) - fd_M\mu]$$

$$\frac{d\mu}{dt} = M[c_M(1 - \mu) - fd_M\mu] \tag{21}$$

All of the above equations except equation (21) failed to reproduce some of the

aspects of the results. The solutions, $M(t)$, of equations (20 and 21) are nearly exponential; the relationship between M_f and f is of a suitable form:

$$M_f = \frac{a_M c_M}{a_M c_M + b_M c_M f + b_M d_M f^2} \tag{22}$$

which lies between the two forms given in equations (14 and 15) and the "time constant" varies in the correct manner with M_1. Fig. 13 shows experimental results fitted with the solutions of equations (20 and 21).

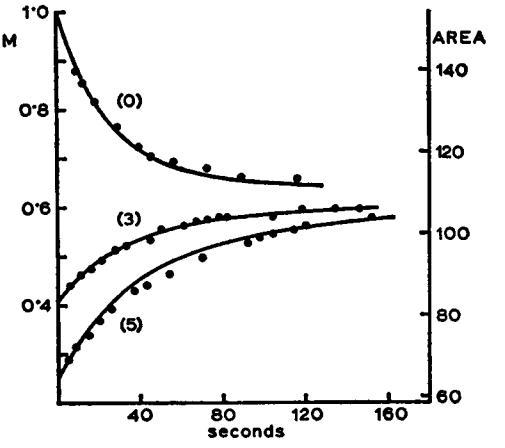


FIGURE 13 The recovery of the action potential area after a change in the rate of stimulation. The experimental points (●) (obtained in the same cell as those in Fig. 3) are fitted with the solutions, $M(t)$, (solid lines) of the two simultaneous equations (20 and 21) with $a_M = 0.03$ sec.$^{-1}$, $b_M = 0.01$, $c_M = 0.01$ sec.$^{-1}$, $d_M = 0.0017$, and $f = 1.5$ sec.$^{-1}$. All the points were obtained at the stimulation rate of 1.5 sec.$^{-1}$. The abscissa gives the time after the change in the rate of stimulation. Prior to $t = 0$ the tissue was stimulated for 2 to 3 minutes at the rate shown by the numeral in parenthesis situated above each curve.

The following procedure was adopted in determining the values of the constants: The experimental relationship between the steady-state area and the rate of stimulation was fitted by the expression given in equation (22). From this expression the values of the ratios a_M/b_M and c_M/d_M were obtained. The value of b_M was obtained from the relationship between $h(M)$ and M. Suitable values for c_M and d_M were then chosen and the equations (20 and 21) were solved. The solutions were compared with the experimental results and the values of the constants were altered if the comparison was not satisfactory. Note that when the experimental relationship is fitted in this way, the initial values of M and dM/dt are slightly different from those obtained by fitting exponential curves.

In summary, the behaviour of the action potential area at constant rates of stimulation is adequately described by the variation of a single variable, M, which increases

between action potentials, the rate of increase being given by:

$$\frac{dM}{dt} = a_M\mu(1 - M) \tag{23}$$

and which decreases during each action potential by an amount ΔM which depends only on M:

$$\Delta M = -b_M M \tag{24}$$

The variable μ which influences the rate of increase of M behaves in a similar way: between action potentials it increases in accordance with:

$$\frac{d\mu}{dt} = c_M M(1 - \mu) \tag{25}$$

and during the action potential it decreases by an amount $\Delta\mu$:

$$\Delta\mu = -d_M\mu M \tag{26}$$

The description of M by equations (23 to 26) is approximated by equations (20 and 21) in accordance with the explanation on page 449 and in the Discussion.

The Relationship between the Steady-State Area and the Rate of Stimulation. The steady-state values of M and N were obtained for various rates of stimulation using for M the same values of the constants as in Fig. 13 and for N the same values of the constants as in Fig. 8. This theoretical relationship is compared with an experimental one (from a different fibre) in Fig. 14.

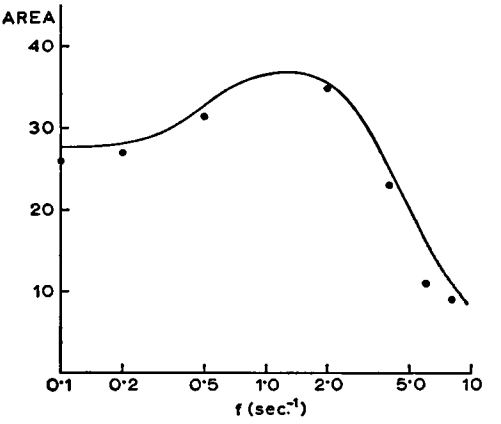


FIGURE 14 The relationship between the steady-state area and the rate of stimulation, f. The full circles (●) are experimental points obtained from one fibre. The solid line is the plot of Area $= 6 + 130\ MN$. The steady-state values of M and N were calculated using the following values of constants: $a_N = 1.52$ sec.$^{-1}$, $b_N = 0.789$ sec.$^{-1}$, $c_N = 0.45$ sec., $d_N = 0.289$, $a_M = 0.03$ sec.$^{-1}$, $b_M = 0.01$, $c_M = 0.01$ sec.$^{-1}$, $d_M = 0.0017$; the constants α and β of equation (12) have the values 0.05 sec. and 0.10 sec. respectively. No attempt was made in this case to reproduce exactly the results obtained from this fibre but the figure demonstrates that the general shape of the relationship is well accounted for by the theory. Compare also with Fig. 1.

The relationship is a good over-all test of the model since it involves all the differential equations and all the equations determining the Δ's. Unfortunately the time required for an experiment in which the values of all the constants could be determined in the one fibre makes such an experiment a practical impossibility. Therefore, it was necessary to use the constants from two different fibres and to compare the steady-state areas calculated with these constants with the steady-state areas recorded from a third fibre. Nevertheless, the general shape of the relationship is well reproduced by the model.

The Number of Independent Constants. Since neither of the variables M and N can be measured independently of the product BMN occurring in equation (1), their absolute values have no meaning at the present time; only their variation is of interest. Similarly, the variables μ and ν only appear in the equations describing the behaviour of M and N as the products $a_M\mu$ and $a_N\nu$. For these reasons several of the constants occurring in the equations can be eliminated.

This elimination has been carried out in the case of the M equations by making the values of M_{max} and μ_{max} equal to 1 and in the case of N making the constant e_N of equation (11) equal to a_N and f_N of equation (9) equal to 1.

Thus the behaviour of the action potential area of fibres from the rabbit right ventricle can be described by the nine equations (1, 5, 7, 11, 12, 23, 24, 25, and 26) containing the ten independent constants A, B, a_M, b_M, c_M, d_M, a_N, b_N, c_N, and d_N. The two constants α and β of equation (12) appear to be only indirectly connected with the dynamic behaviour of the variables.

DISCUSSION

To compare our results with those of other investigators is difficult, particularly because they measure the duration and not the area of the action potential. Carmeliet (1955, 1958) has described in detail the relationship between the steady-state duration of the frog ventricular action potential and the frequency of stimulation and has expressed this relationship by the equation $A = A_\infty (1 - e^{-\alpha T})$, where A is the duration of the action potential, A_∞ and α are constants, and T is the stimulus interval. Comparison can only be approximate since it is necessary to assume that in the frog ventricle the area of the action potential in a linear function of the duration. With this assumption, Carmeliet's finding would mean that N is constant in the frog heart. The duration of the frog action potential would then become a linear function of M in which case the present model would closely predict Carmeliet's relationship between the frequency of stimulation and the steady-state duration.

Interference of One Variable in Experiments Performed on the Other Variable. It is clear from the model that the changes in area in any one experiment can never be completely ascribed to only one of the two variables M and N. However, since N varies rapidly and attains a state of dynamic equilibrium after only a few

action potentials at any constant rate and since the decrements in M for each response are small and equilibrium in M is attained slowly, after hundreds of action potentials, one can perform experiments in which the value of one or the other of the variables can be considered to remain sufficiently constant to admit a simple and meaningful interpretation of the results.

The simple assumption, made at the beginning of the section describing the behaviour of N, that at low rates of stimulation M has its maximum value can be seen to be not strictly correct; small changes in the level of M can be expected even when the rate of stimulation is changed, say, from 0.2 sec.$^{-1}$ to 0.4 sec.$^{-1}$. However, the changes in M produced by single action potentials are small enough to allow us to consider M to be constant when stimulating with a repeating rhythmic figure. This means that the theoretical values of N should agree exactly with the experimental ones for any stimulation arrangement as long as the number of action potentials in successive 10 second intervals is kept constant (and as long as they are not too unequally spaced, *e.g.* in a single burst followed by a pause), but that the constant C in equation (2) has to be changed slightly when the number of action potentials in the 10 second intervals is altered.

In the experiments designed to reveal the behaviour of M the effect of N is twofold: (*a*) because of the complex changes in N after a sudden change in the constant rate of stimulation, the first few action potentials in each run must be disregarded; (*b*) although at any constant rate of stimulation N will have the same value just prior to the upstroke of each action potential, this value will be different for different constant rates of stimulation. Thus the constant D in equation (13) will differ in the same way, and although this does not effect the estimation of the time constant of the curves relating the area of the action potential to the time after a sudden change in rate, it does however make it impossible to directly correlate the magnitudes and changes in the level of N obtained at different rates of stimulation. On the other hand, if all experiments are carried out at the one rate f_2, N can be considered to have the one constant value and equation (13) will hold for all times at which measurements are made. Thus to determine the relationship between f and the steady state value of M, results obtained at only one rate of stimulation must somehow be used. This was done by performing the experiments at the one rate as described earlier and extrapolating the curves back to $t = 0$, ignoring the first few responses during the rapid changes in N. These extrapolated values can then be plotted against f, yielding the required relationship according to equation (13) in which D is now constant.

Behaviour of the Variables during an Action Potential. It has been assumed that all four of the variables, M, μ, N, and ν change discontinuously during the upstroke of the action potential. The real system is likely to behave in one of three different ways (for the case of N and ν the words regeneration and depletion must be interchanged): (*a*) The regeneration process goes on continuously all the time,

while the depletion is superimposed on it approximately instantaneously at some time during the action potential; (*b*) the regeneration process behaves as in (*a*) but the superimposed depletion occurs during a definite time interval (*e.g.*, for the whole duration of the action potential); (*c*) the two processes are mutually exclusive (*i.e.*, the regeneration stops while the depletion takes place).

The present equations are based on (*a*). The third possibility (*c*) was discounted as it would result in large discrepancies between the predicted and observed results at higher rates of stimulation. On the other hand, (*a*) and (*b*) are both acceptable and it must be made clear what modifications (*b*) would produce in the model.

In the case of M and μ the depletion is taken as going on all the time; this approximation is justified by the smallness of the quantities ΔM and $\Delta\mu$ which are two orders of magnitude smaller than the values of M and μ. The error resulting from this approximation was calculated and found to be negligible. The approximation clearly encompasses both possibilities (*a*) and (*b*) and, therefore, both (*a*) and (*b*) can be regarded as possibilities with indistinguishable effects. In the case of N and ν the two possibilities (*a*) and (*b*) would result in slightly different time courses of N between action potentials, but the effect would be smaller than the present experimental error. Thus both possibilities should be kept in mind in any future interpretations of the model.

Assumptions. As far as the mathematical description of the results is concerned, the model contains no assumptions. All the assumptions made earlier were only tentative; this mathematical model could not have been constructed on purely deductive reasoning. At the present time the only physical quantity which is represented by the model is the action potential area. Its dynamic behaviour is adequately described in terms of the behaviour of the two variables M and N. It is only when correlation of the underlying differential equations with physical processes is attempted that some of the statements made in the paper will have to be regarded as assumptions.

We are grateful to Dr. J. M. Bennett and to the staff of the digital computer SILLIAC for carrying out some of the computations, and in particular to Dr. E. P. George for his kind and patient help with the computer work without which this paper could not have been finished.

We wish to thank the Life Insurance and Medical Research Fund of Australia and New Zealand for a grant in aid of this work.

Received for publication, October 19, 1962.

REFERENCES

CARMELIET, E., 1955, *Arch. Intern. Physiol. et Biochim.*, **63**, 222.
CARMELIET, E., 1958, *J. Physiol.*, Paris, **50**, 204.
CARMELIET, E., and BOULPAEP, E., 1958, *Arch. Intern. Physiol. et Biochim.*, **66**, 87.
CORABOEUF, E., 1960, *J. Physiol.*, Paris, **52**, 323.
GIBBS, C. L., and JOHNSON, E. A., 1961, *Circulation Research*, **9**, 165.
JOHNSON, E. A., and TILLE, J., 1961, *J. Gen. Physiol.*, **44**, 443.