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THE MODE OF ACTION OF QUINIDINE ON ISOLATED RABBIT ATRIA INTERPRETED FROM INTRACELLULAR POTENTIAL RECORDS

BY

E. M. VAUGHAN WILLIAMS

From the Department of Pharmacology, Oxford

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An attempt has been made to show why quinidine, which has long been known not to lengthen the duration of the cardiac action potential, measured with external electrodes, and also not to lengthen, and sometimes to shorten, the absolute refractory period, nevertheless reduces the maximum frequency at which atria can respond to a stimulus. Simultaneous measurements have been made in electrically driven isolated rabbit atria of contractions, conduction velocity and intracellular potentials before and during exposure to a wide range of concentrations of quinidine sulphate. The resting potential remained undiminished, in contrast to the effect of quinidine on Purkinje fibres. In the therapeutic range of doses, up to 10 mg./l., the half-time for repolarization was either shortened or unchanged, thus providing an explanation for the failure of quinidine to prolong the absolute refractory period. In contrast, even at low concentrations of quinidine, conduction velocity and the rate of rise of the action potential were greatly slowed, and the height of the overshoot was reduced. The terminal phase of the action potential was prolonged. It is known that the rate of rise of the action potential is a function of the level of repolarization at which an impulse takes off (the more negative the take-off point, the faster the rate of rise). Normally, a stimulus introduced when repolarization has proceeded to 2/3 of the resting potential evokes a response with a rate of rise fast enough for propagation, so that the duration of the terminal 1/3 of the phase of repolarization has no influence upon the length of the effective refractory period. In the presence of quinidine, however, the rate of rise itself was directly reduced, thus repolarization had to proceed further before the critical take-off point was reached at which the rate of rise was fast enough for propagation, and the duration of the terminal phase of repolarization thus became significant. It has been concluded that quinidine prolongs the effective refractory period by slowing the phase of depolarization, without any change necessarily occurring in the half-time for repolarization, which determines the absolute refractory period. Acetylcholine accelerated the rate of rise of the action potential even in the presence of high concentrations of quinidine.

The importance attached to the various actions of quinidine has to some extent been affected by views about the nature of fibrillation. Lewis, Drury, Iliescu, and Wedd (1921) originally claimed that the absolute refractory period of the atrium of the dog was prolonged by quinidine, and regarded this as evidence in support of the hypothesis that fibrillation consisted of a circular sequence of excitation. When it was shown subsequently by Love (1926) that quinidine did not, in fact, prolong the absolute refractory period, but sometimes shortened it, Lewis and Drury (1926) nevertheless retained the "circus" hypothesis, on the ground that quinidine prolonged what they called the "effective" refractory period; that is, quinidine prolonged the minimal interval at which *propagated* responses could be elicited by stimulation. The evidence relevant to the various hypotheses concerning the nature of fibrillation has been extensively reviewed (Dipalma and Schults, 1950; Prinzmetal, Corday, Brill, Oblath, and Kruger, 1952).

There is no doubt that quinidine reduces the maximum frequency at which atria can respond to stimulation, and the prolongation of the effective refractory period by quinidine has been confirmed by several authors (Dawes, 1946; Gold, 1950; Dawes and Vane, 1956). The paradox remains that quinidine does not prolong the absolute refractory period, that is the shortest

interval after a first stimulus at which a local response can be elicited by a second stimulus. The absolute refractory period of cardiac muscle has long been known to be approximately equal to the duration of the action potential (Adrian, 1921), and the latter also was shown not to be prolonged by quinidine (Wedd, Blair, and Gosselin, 1942). More precise information concerning the action of drugs on the electrical activity of cardiac muscle can now be obtained by means of intracellular electrodes. Studies of the effects of quinidine on intracellular potentials have been made in Purkinje fibres by Weidmann (1955b) and Corabœuf, Boistel, and Distel (1956), and in guinea-pig ventricle by Johnson (1956). The differences between atrial muscle, and ventricular muscle and Purkinje tissue, are considerable. Neither Purkinje tissue nor mammalian ventricle is sensitive to acetylcholine (ACh), and the antagonism between quinidine and ACh has been the subject of much investigation (Dawes, 1946; de Elio, 1948; Rand and Walker, 1958). As the resting potentials and the shapes of the intracellular action potentials are dissimilar, the action of a drug on ventricle or Purkinje tissue cannot be assumed to be the same on atrial tissue. Since antifibrillatory drugs are employed clinically for their effects on atrial muscle, it was thought worth while to investigate the action of quinidine on atria.

Weidmann (1955b) examined the effects of quinidine at one concentration only, 10 mg./l., and in association with a slowing of depolarization there was a large fall in resting potential, from −98 mV. to −72 mV. No such fall has been observed in the present experiments. Corabœuf *et al.* (1956) also examined the effect of quinidine at one concentration only, 100 mg./l., a level which would probably be lethal in man. These authors likewise observed a fall in resting potential, and a flattening of the plateau of the action potential. Since there is no plateau in the atrial action potential, it was clear that the effect of quinidine on atrial muscle itself required investigation.

Clinically the dose required to arrest fibrillation is very critical (Gold, 1950) and the actual serum concentration in 75% of a series of successfully treated patients was found to lie between 4 and 9 mg./l. with a mean of 5.9 mg./l. (Sokolow and Edgar, 1950). It is difficult to estimate the extent to which concentrations bathing an isolated tissue can be regarded as equivalent to therapeutic or toxic serum levels in man. For this reason, in the present experiments measurements of contractions have been made simultaneously with the intracellular records, in order that the toxicity of the various concentrations of quinidine could be independently assessed in relation to their effects on electrical activity.

METHODS

The apparatus has already been described (Vaughan Williams, 1955, 1958). Rabbit atria were placed horizontally in a bath whose temperature was controlled at 31° to within ±0.1°. Contractions were recorded with an RCA 5734 transducer, and displayed on one beam of a Dumont 322 oscilloscope. Intracellular potentials were displayed on the other beam. External potentials, from which conduction velocity was calculated, were recorded from right and left atria with pairs of small platinum bipolar electrodes, and were displayed on a Cossor 1049 oscilloscope. The screens of both oscilloscopes were photographed simultaneously with a Grass camera; the time at which each exposure was made was also recorded to within 0.1 sec. The atria were driven electrically through Ag-AgCl electrodes placed on the tip of the left atrium. All intracellular records were taken from the endocardial surface of the right atrium, some distance from the natural pacemaker. The oscilloscope sweep was started by the stimulus, and at the end of its traverse was itself made to produce a pulse which set in motion the mechanism for shifting one frame in the camera; this in turn operated a shutter to record the time. All records were thus obtained on stationary film, the frames being changed automatically between each heart beat. The concentrations of quinidine have been expressed as w/v of the sulphate.

The observations photographed on the film were subsequently measured on the lined screen of a specially made projector. The rate of rise of the action potential was calculated from direct measurements of the angle of its slope. This method was laborious but permitted the measurement of differences of 3% in the rate of rise, and was preferred to measurement by electrical differentiation which involved greater difficulties in calibration and measurement. The rates of stimulation selected were 10% higher than the natural frequency of the spontaneously beating preparations. The natural pacemaker frequencies had a range of 90 to 160 beats/min.

RESULTS

Effects of Non-toxic Concentrations

Simultaneous records of the contractions, conduction velocity and intracellular potentials of isolated rabbit atria were made in 29 experiments before and during the action of various concentrations of quinidine ranging from 10^{-7} to 6×10^{-5}. Quinidine at a concentration of 10^{-7}

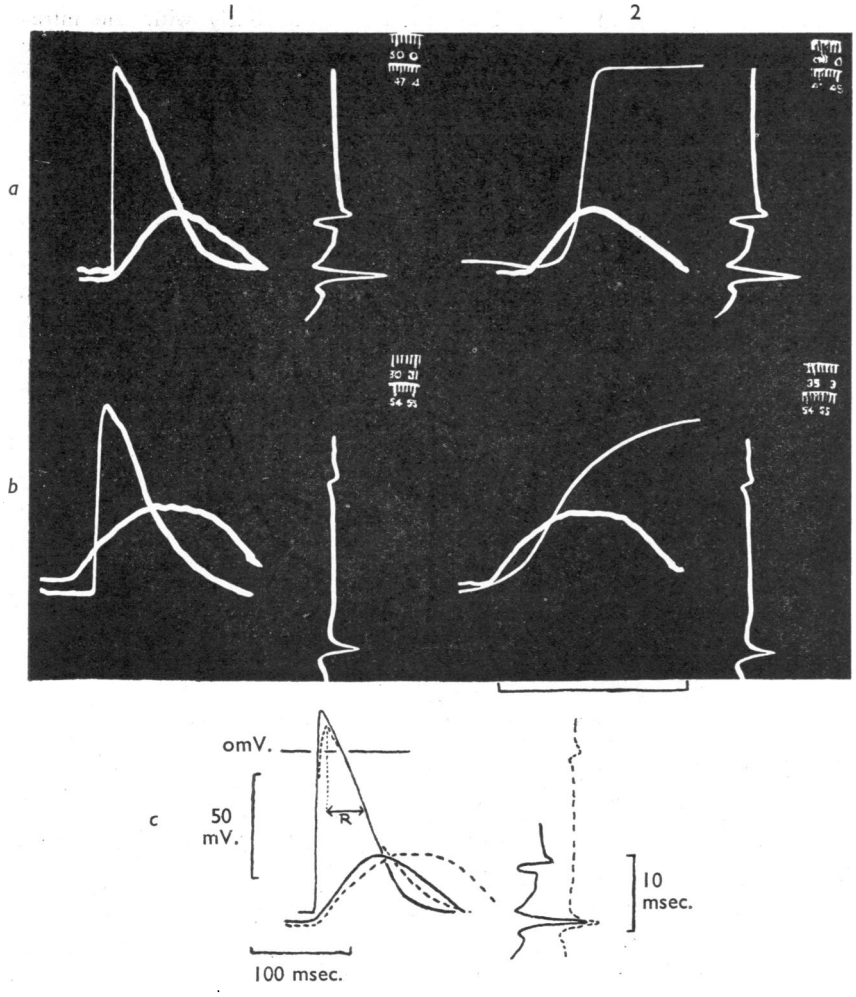

FIG. 1.—The effect of quinidine on contraction, conduction velocity and intracellular potential. *a*, Controls. Left-hand frame (1). The upper trace is the intracellular potential at a slow sweep speed. Immediately below it is the record of contraction at the same sweep speed. The vertical trace to right records external action potentials from left and right atria, from which conduction velocity can be calculated. The figures in the inset show the time at which the photograph was taken to within 0.1 sec. (see text). Right-hand frame (2). The trace recording intracellular potential has been speeded up about × 40, so that only the upstroke of the action potential is seen. *b*, Records from the same area after one hour of exposure to quinidine 9×10^{-6}. Conduction velocity and rate of rise greatly reduced. The time scale (5 msec.) for the fast sweep in *a*2 and *b*2 is given below *b*2. *c*, Tracings of *a*1 and *b*1 superimposed, with time and voltage scales. The height of the action potential was reduced by quinidine, but the duration was unchanged, except at the tail. The duration of 1/2 repolarization (R) was actually reduced.

had no effect upon any of the activities measured. In the presence of quinidine 6×10^{-5}, the atria became electrically inexcitable, and so attention has been directed to the effects of concentrations between these extremes. In order that the atria should follow the stimulus rather than their natural pacemaker, the frequency of stimulation had to be a little faster than that of the pacemaker. Stimuli of 5 msec. duration were employed. The stimulus strength was gradually increased until the atria began to follow, and was then doubled. In the presence of concentrations of quinidine 10^{-5} or less the atria were always still able to follow the stimulus at the control frequency, although the strength and duration of the stimulus had sometimes to be increased, by not more than 50%.

The effect of quinidine 9×10^{-6} is shown in Fig. 1. The top left-hand frame shows the intracellular potential at a slow sweep speed and immediately below it a record of the contraction at the same sweep speed. The external potentials have been displayed on the vertical trace to the right of this, and in the top right-hand corner the inset indicates that the exposure was taken at 47 min. (lower number) 0.3 sec. The top right-hand frame was taken a few seconds later, at 47 min. 6.1 sec., the speed of the oscilloscope sweep recording the intracellular potential having meanwhile been increased about 40 times. Thus only the upstroke of the intracellular potential can be seen on this trace. The two frames formed part of a series of control observations whose results have been summarized in the line relating to

experiment 15 in Table I. When the set of control observations was complete the atria were exposed to quinidine 9×10^{-6}, and after an hour a second series of observations was made, from which the lower two frames in Fig. 1 have been taken.

A major difficulty in making comparisons of this kind was the variation in the records of intracellular potentials taken from different sites. The rate of rise and the duration of the action potential were found to be more variable than the resting potential and the height of the action potential. The fibres were far too small to be seen individually, and it was, therefore, impossible to return to the same fibre for a second observation. Fortunately, however, fibres in the same bundle usually gave very similar records, although they might differ from those in a neighbouring bundle. A map of the bundles in the microscope field was therefore drawn and three or four sites were numbered. Several entries were made at each site and the records were compared with others taken subsequently from the same site during exposure to quinidine. The lower two frames in Fig. 1(*b*) were thus records taken 1 hr. 7.5 min. later from the same site as that from which the upper records were taken. Comparison of the two right-hand frames indicates that conduction velocity and the rate of rise of the action potential had greatly decreased in the presence of quinidine. Tracings of the records from the left-hand two frames have been superimposed in Fig. 1(*c*), and show that the height of the action potential had been decreased by quinidine. The resting potential (measured from a zero line on a succeeding frame, not shown) had actually increased by 3 mV., although there was no statistically significant change in resting potential calculated from all the observations in this experiment. The main change was a large reduction in the "overshoot." The duration of the action potential measured at half the action potential height was unchanged, although the tail of the repolarization phase was a little prolonged. Since, however, the phase of depolarization was greatly lengthened, the phase of repolarization alone, measured as the time from the peak of the action potential to half-repolarization (the distance R in Fig. 1), was actually shorter in the presence of quinidine. Thus quinidine, so far from slowing down the flow of ionic current responsible for repolarization, may actually speed it up as far as the half-repolarization point. Hoffman, Kao, and Suckling (1957) showed that a local response could occur in cardiac muscle when repolarization had reached only half the original resting potential. The absolute refractory period was approximately equal to the time taken to repolarize to half the resting value. An acceleration of the first half of the repolarization phase would thus explain why quinidine, in spite of slowing down the depolarization phase, does not prolong and may even shorten the absolute refractory period (Love, 1926).

Effects of Concentrations Above 16 mg./l.

When the concentration of quinidine was raised to 1.6×10^{-5}, the atria no longer followed a stimulus at the control frequency. In order to continue driving the atria, the frequency of stimulation had to be reduced, usually by only a few beats/min., but in the presence of the highest concentrations of quinidine, by as much as half the control rate. As soon as the rate was lowered the observations could no longer legitimately be compared with the control records. In normal atria merely reducing the frequency of stimulation prolonged the duration and increased the rate of rise of the action potential, and such effects were exaggerated in the presence of quinidine. No control observations at the reduced frequency necessitated by high quinidine concentrations could be obtained in normal atria, because the latter followed their natural pacemaker instead of the stimulus as soon as the frequency of stimulation was reduced. If the pacemaker region was cut away the value of the measurements was made uncertain by ignorance of the extent to which trauma might have interfered with normal activity.

The upstroke of the action potential may be divided into three phases: (1) a slow "foot," (2) a fast and almost linearly rising central portion, (3) a more slowly rising terminal phase. Although quinidine caused a slowing of the rate of rise of the central portion as well, it became clear that the terminal phase exhibited the greatest slowing, as is shown in Fig. 2. The effects of increasing concentrations of quinidine are shown in four experiments. In *a* the control observations have been given as well as those illustrating the effect of quinidine 5×10^{-6}. In *b*, *c*, and *d* records taken in the presence of quinidine only are shown. In *d*, the presence of quinidine 6×10^{-5}, the atria eventually became inexcitable, but the records indicate that conducted electrical responses continued to occur after the contractions had ceased to be measurable, since the intracellular electrode was in the right atrium but the stimulus was applied to the left atrium.

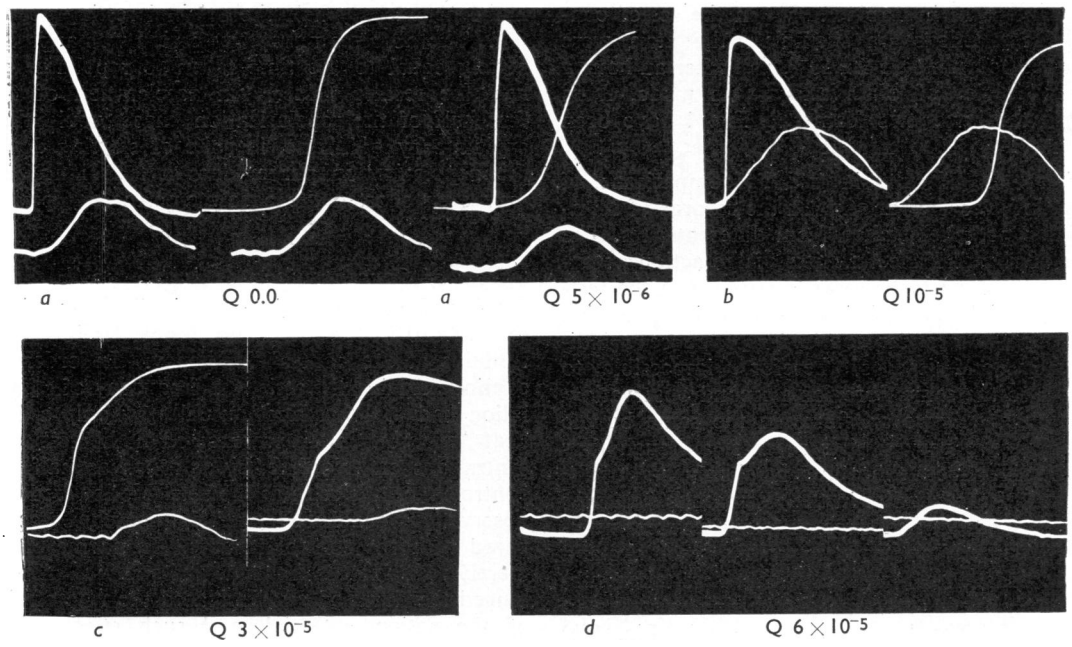

FIG. 2.—*a* to *d*. Effect of increasing concentrations of quinidine (Q) on contraction and intracellular potential. It is clear that the terminal part of the upstroke of the action potential was especially retarded. In *c* and *d* the traces were all at the slow sweep speed. In *d* contractions were no longer measurable although the action potentials were conducted.

When the atria began to fail in the presence of high concentrations of quinidine, they would sometimes do so by responding, say, to three stimuli, but failing to respond to the fourth. When the fifth stimulus arrived, after the interval of the missed beat, the atrium was found to have recovered sufficiently during its period of rest to respond with a larger contraction, and a faster rate of rise of the action potential. Conduction velocity was also increased. In Fig. 3*a*, an action potential at the beginning of failure in the presence of quinidine 3×10^{-5} is shown, with a third phase of depolarization so slow that a concavity had been formed in the upstroke. The next record depicts the responses to six successive stimuli photographed on the same frame. The first occurred just after a double interval, so that the contraction was large and there was no concavity in the upstroke of the action potential. In the responses to the next three stimuli the concavity or "step" became more and more pronounced, and the contractions progressively smaller. The atria failed to respond to the fifth stimulus altogether, but during the interval of the missed beat recovery again took place to such an extent that the sixth response resembled the first. The resting potential was unchanged throughout.

Effects of ACh in the Presence of Quinidine

Briscoe and Burn (1954) showed that when isolated rabbit atria had stopped in high concentrations of quinidine, they could be restarted with ACh. Marshall and Vaughan Williams (1956) found that atria stopped by cooling could also be restarted by ACh, and it was suggested that both phenomena might be explained by the effect of ACh in increasing the rate of entry of depolarizing ions (Marshall, 1957; Vaughan Williams, 1958). The present experiments provided the opportunity of observing directly the effect of ACh on the rate of depolarization in the presence of quinidine, and a measurement of this kind is shown in Fig. 3*b*. After control observations had been made, examples of which are illustrated, the atria were exposed to quinidine 2.5×10^{-6}, whereupon they failed to follow the stimulus at the original frequency. The frequency was then lowered (from 120 to 105 stimuli/min.) until the atria again followed every stimulus, but the reduction in frequency resulted in larger contractions and a greatly prolonged duration of the phase of repolarization. There was, nevertheless, still a marked concavity in the upstroke of the action potential. ACh was then added to the bath to give a final concentration of 10^{-6} (w/v), and the

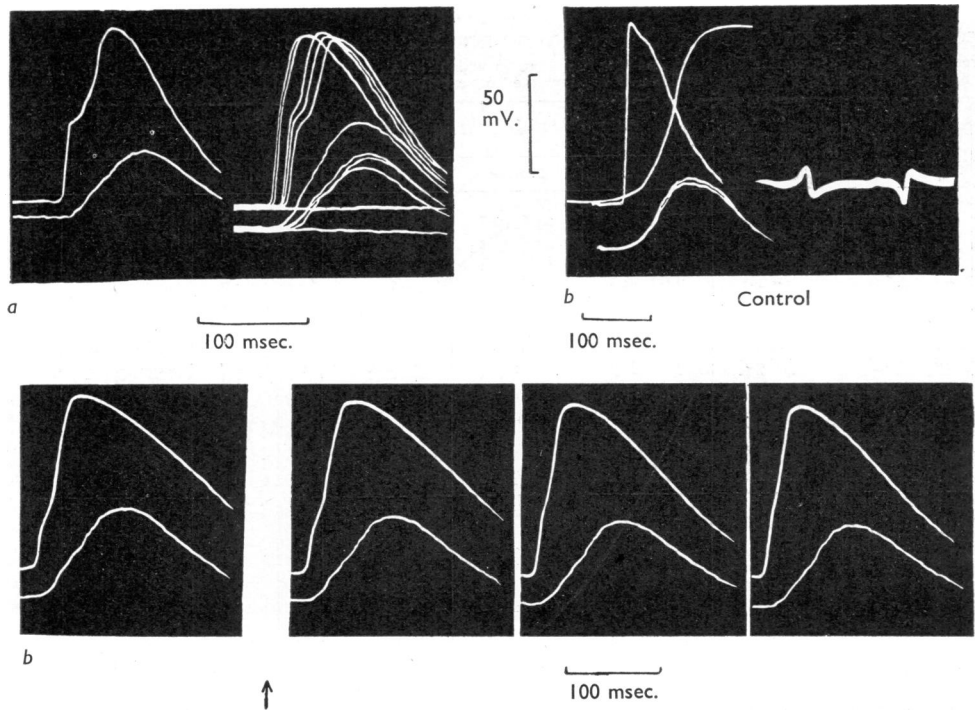

FIG. 3.—Effect of lowered frequency and ACh in increasing the rate of rise of the action potential. *a*. In the presence of quinidine 3×10^{-5}. Left, atria still responding to every stimulus. Right, responses to six successive stimuli. The first response shown followed a missed beat, so that the rate of rise was faster, without a "step," and the contraction was larger. The step appeared progressively in the upstroke of the action potential during the next three beats and contractions became smaller. The response failed altogether on the fifth stimulus, so that the response to the sixth, after the interval of the missed beat, resembled that to the first. *b*. Experiment to illustrate the effect of ACh 10^{-6}. At bottom left the frequency was reduced until responses occurred to every stimulus in the presence of quinidine 25 mg./l. There was nevertheless a "step" still apparent, which was almost abolished when ACh speeded up the rate of rise of the action potential. The lower records were all made in the presence of quinidine 2.5×10^{-5}. At the arrow, ACh 10^{-6} was added to the fluid bathing the preparation.

succeeding photographs show that it caused an increase in the rate of rise of the action potential, especially in the third phase, so that the concavity almost disappeared.

Analysis of Results

The effects of quinidine on contractions, on the intracellular resting and action potentials, and on the duration of the first half of the phase of repolarization in all the experiments have been summarized in Table I. Each estimate represents the mean of several sets of observations taken at different sites in each experiment, and the standard errors of the means have been indicated when there were four or more sets of observations. The horizontal broken line divides those experiments in which the atria still followed the stimulus at the control frequency from those in which the frequency had to be reduced It is evident that quinidine, except in one experiment, had no dramatic effect on the resting potential, but caused a highly significant ($P<.001$) reduction in the height of the overshoot. It has been concluded that the action of quinidine cannot be attributed to a change in resting potential. This contrasts with the effects of quinidine on Purkinje tissue (Weidmann, 1955b).

The duration of the first half of the phase of repolarization was either shortened or not significantly ($P>.05$) changed in the presence of quinidine 10 mg./l. or less. Above 16 mg./l. it was necessary to reduce the frequency of stimulation, and there is evidence (Hoffmann and Suckling, 1954) that this alone could lead to a prolongation of the duration of the action potential. It was not possible to say, therefore, to what extent the lengthening of the phase of depolarization in the presence of toxic concentrations of quinidine was due to the quinidine or to the reduction in frequency. It may be noted, however (Fig.

TABLE I

EFFECTS OF QUINIDINE ON CONTRACTION AND ON INTRACELLULAR RESTING AND ACTION POTENTIALS

The results given above the horizontal broken line are those in which the atria followed the stimulus at the control frequency, while in those below the frequency had to be reduced.

Expt. No.	Conc.	% Change in Contraction	Resting Potential (mV.)		Action Potential (mV.)		Time for Half Repolarization (msec.)	
			Before	During	Before	During	Before	During
2	10^{-6}	0	72 ±0·6	78 ±4	98 ±0·6	101 ±0·4	58 ±0·3	57·5±0·6
3	$2{\cdot}5\times10^{-6}$	−26	69 ±0·4	68·6±0·7	87 ±0·4	87·2±0·1	59	58
4	3×10^{-6}	−11	72 ±0·6	72·3±1·2	98 ±0·7	92·5±0·8	58 ±0·3	56·6±0·9
5	5×10^{-6}	−20	74·5±0·7	74·3±0·5	102 ±0·5	92 ±0·1	85 ±1·7	74 ±0·3
6	,,	−26	73·5±1·3	70 ±0·3	93 ±0·7	81·5±0·5	51 ±0·4	36 ±3·4
7	,,	−2	72·3±0·3	71·6±0·4	94·5±0·2	88·5±0·5	60·7	62
8	,,	0	73·2±0 2	72 ±0·2	96 ±0·1	90·2±0·2	53·2±3	51·8±0·4
9	,,	−2	73·2±0·2	74 ±0·4	99 ±0·2	93·5±0·1	58 ±0·6	57·3±0·2
10	6×10^{-6}	−7	71·5±0·5	72·5±1·3	84·5±0·2	85 ±0·7	41 ±0·4	42 ±0·1
11	7×10^{-6}	−27	74·2±0·7	77·4±1·2	99·1±0·9	95·5±1·0	61·7±3·8	61·5±2·7
12	8×10^{-6}	−1·5	73 ±0·3	76 ±0·5	95 ±0·3	93·5±0·7	62·5±0 6	69 ±0·4
13	,,	−4	71 ±1·9	70 ±0·9	99·5±2·1	90 ±1·1	70·5±1·2	87 ±2
14	,,	−3	77·8±1·4	73 ±1·6	98·5±0·8	90 ±0·7	57·7±1·4	56·8±2
15	9×10^{-6}	+6	80·5±1·3	82·5±2·2	102 ±2·5	94 ±1·7	64·3±6	53·1±3·8
16	10^{-5}	−25	76	77	97·5	93·5	39	44·5
17	,,	−21	72·3±0·3	71·3±1·2	94·5±0·2	87 ±0·7	61	70
18	,,	−24	73·2±0·2	69·5±0·4	96 ±0·1	88·7±0·2	53·2±3	58·5±0·3
19	,,	−32	73·2±0·2	71·3±0·6	99 ±0·2	92·6±0·2	58·6±0·5	67·5±0·9
20	$1{\cdot}6\times10^{-5}$	−17	71 ±1·6	70·5±1·3	99·5±2·0	86 ±0·7	71 ±0·9	92 ±3
21	,,	−17·5	77·6±1·4	68·5	98·5±0·8	81 ±0·9	58 ±1·2	92
22	$2{\cdot}5\times10^{-5}$	−52	72 ±0·8	72 ±0·6	93·5±1·2	86 →72	72	122
23	,,	−66	72 ±0·6	< 49	98 ±0·6	< 42	58 ±0·4	>61
24	$2{\cdot}6\times10^{-5}$	−29	71 ±1·7	72·5	99·5±2·1	80 ±0·9	70 ±1·6	>92
25	3×10^{-5}	−57	72·3±2·7	68·3±0·4	94·5±0·2	< 89	61 ±2	65·4±2·6
26	,,	−63	73·2±0·2	67·7±0·3	96 ±0·2	< 87	53 ±3	55·5±6
27	,,	−49	73·2±0·2	71	99 ±0·1	84 →73	58	>69
28	4×10^{-5}	−31	71 ±1·5	71·2	99·5±1·7	84 →61	70	146

6), that in three experiments in concentrations above 16 mg./l. the prolongation was not great in spite of the quinidine *and* the reduced frequency. It is apparent that a change in the first half of the phase of repolarization cannot be concerned in the mode of action of therapeutic levels of quinidine. The absence of change, or the actual shortening of this phase in some experiments, is of interest, however, since it provides an explanation for the failure of quinidine to prolong the absolute refractory period.

The variation between atria was wide, and the graphs shown in Figs. 4, 5 and 6 have been drawn in order that comparisons may be made between experiments without the complicating factor of different control values for each experiment. The means of the control values of all the observations of action potential (98 mV.), resting potential (73 mV.) and half-time of repolarization (56 msec.) have been drawn as horizontal lines in Figs. 4, 5 and 6 respectively. The changes produced by quinidine in each experiment have then been plotted as changes from the common mean control in proportion to the actual change observed from each individual control. In this way the trends of the changes produced by increasing concentrations of quinidine have been clearly brought out. The vertical lines separate observations taken at this control frequency from those taken at a reduced frequency. There was no evidence of a change in resting potential in the presence of quinidine 10^{-5} or less. At higher concentrations there was a slight downward trend, but it was not highly significant ($P<.03$). In contrast, there was a striking and significant fall in the height of the action potential ($P<.001$) which would have been larger at concentrations of quinidine greater than 10^{-5} had it not been necessary to reduce the frequency of stimulation at these high concentrations.

Conduction velocity was always reduced by quinidine, and the change was doubtless a direct consequence of the slower rate of rise of the action potential. The maximum rate of rise was measured from the tangent of the fast central portion of the upstroke. The mean rate of rise was calculated from a measurement of the horizontal distance between the points at which the rising line of the upstroke cut horizontal lines 2.5 mV. above the most negative and 2.5 mV. below the most positive values of the action potential (*vide* Vaughan Williams, 1958). The measurements of maximum and mean rates of rise of the action potential and of conduction velocity are given in Table II, and the changes in the maximum and mean rates of rise produced by quinidine, referred to the mean of all the controls, have been plotted in Fig. 7.

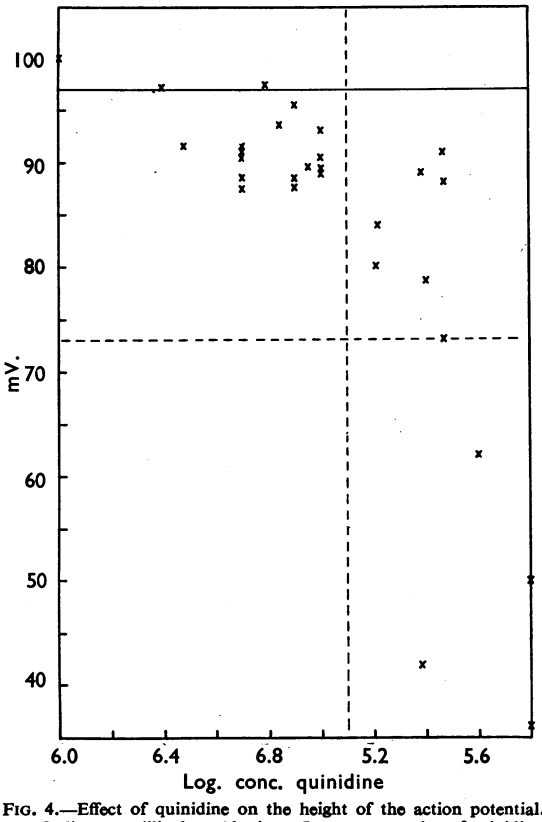

FIG. 4.—Effect of quinidine on the height of the action potential. Ordinate: millivolts. Abscissa: Log concentration of quinidine. The horizontal solid line represents the mean of all the control action potential heights. The horizontal broken line represents the mean of all the control resting potentials. The vertical broken line divides results taken from atria following the stimulus at the control frequency from those taken at a reduced frequency on the right.

The Effect of Quinidine on Contractions

Quinidine always produced a relaxation of the resting length of the atria. Provided the concentration was not greater than 10^{-5}, the effects on contraction were small (Table I). It is clear from Fig. 1 that in this experiment, although the contraction was no smaller in the presence of quinidine than in the control, its peak occurred later. The time from the beginning of the contraction to its peak was measured in all experiments, but although quinidine usually increased this duration by a few msec. the variation in the zero-peak time in control contractions was such that the change produced by quinidine was not statistically significant ($P>.05$).

Results During Recovery

In six experiments (Nos. 8, 11, 13, 15, 18 and 19) observations were made at intervals for more than an hour after the atria had been returned to the control solution from the quinidine solution. The changes produced by quinidine were slowly reversed, but only in experiment 8 (after quinidine 5×10^{-6}) was recovery complete at the end of one hour. A puzzling observation, made in all the experiments, was that during the early phase of recovery the duration of the action potential became *longer*. In the experiments 11, 13, 15, 18, and 19 the durations of the first half of repolarization became 71.4, 94, 80.4, 68 and 81 msec. respectively, which may be compared with the observations made before and during exposure to quinidine given in Table I. No explanation is offered for this phenomenon, but it is mentioned because the effect was regularly observed.

DISCUSSION

A puzzling feature of the action of quinidine has been that while there was no doubt that the maximum frequency at which atrial muscle could

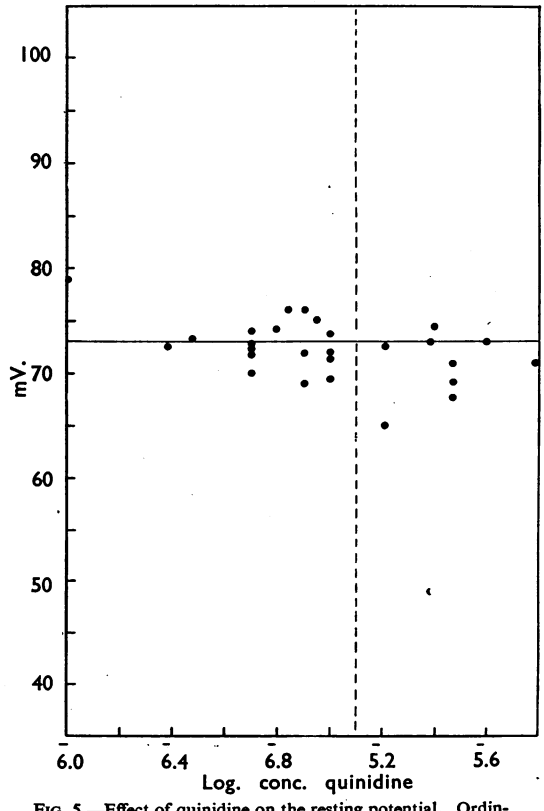

FIG. 5.—Effect of quinidine on the resting potential. Ordinate: millivolts. Abscissa: log concentration. The horizontal solid line represents the mean of all the control resting potentials. For explanation of vertical broken line, see legend to Fig. 4.

TABLE II

EFFECTS OF QUINIDINE ON CONDUCTION VELOCITY AND ON THE MEAN AND MAXIMUM RATES OF RISE OF THE INTRACELLULAR ACTION POTENTIAL

See Table I for explanation of dotted line.

Expt. No.	Conc.	Maximum Rate (V./sec.)		Mean Rate (V./sec.)		Conduction Velocity (M./sec.)	
		Before	During	Before	During	Before	During
2	10^{-6}	64 ±3	64 ±4	18·8	18·4	0·46	0·454
3	$2{\cdot}5\times10^{-6}$	47·4±1·3	22·1±0·3	14·2±0·3	7·5±0·2	0·634	0·300
4	3×10^{-6}	64 ±5	34·4±5·1	19·6	12·4	0·464	0·420
5	5×10^{-6}	107 ±1·2	59 ±4	20·2	12·6±0·4	0·308	0·185
6	,,	48 ±4	22 ±2	16·5±0·2	10 ±0·5	0·306	0·245
7	,,	56 ±3	20·3±0·8	25 ±0·4	12 ±0·2	0·368	0·327
8	,,	52·3±1·4	32·8±1·2	31 ±0·3	16·3±0·9	0·391	0·333
9	,,	45·6±3·2	37·5±1·8	28·1±0·8	15·3±0·3	0·388	0·340
10	6×10^{-6}	75 ±11	57 ±4·5	17·6±0·5	15·1±0·2	0·662	0·565
11	7×10^{-6}	90·5±5	58 ±6·5	29 ±2·1	12·2±2·2	0·332	0·235
12	8×10^{-6}	78·3±1·6	60·2±2·4	30 ±1·2	19·9±0·7	0·444	0·388
13	,,	83 ±2·1	41·5	34·2±1·3	12·9	0·441	0·375
14	,,	64 ±5	46 ±7	20·8±0·9	19·3±0·3	0·410	0·386
15	9×10^{-6}	120 ±9·2	36 ±4·8	54 ±12	16 ±1·4	0·720	0·270
16	10^{-5}	65·5	48			0·710	0·530
17	,,	56 ±3·2	16·6±3	25 ±0·36	7·7±2·1	0·368	0·246
18	,,	52·3±1·4	28·8	31 ±0·3	10·5±1·1	0·391	0·232
19	,,	45·9±3·7	25·7±4·8	28·1±0·8	9·7±0 6	0·386	0·246
20	$1{\cdot}6\times10^{-5}$	83 ±2·9	21 ±1·7	34 ±2	7·8±1·2	0·440	0·298
21	,,	64 ±5·1	33 ±3	21 ±1·2	9·1±0·4	0·420	0·247
22	$2{\cdot}5\times10^{-5}$	55	3·4	17·2	2·1	0·470	0·360
23	,,	64 ±4	< 7·3	19·6	4·2	0·460	0·280
24	$2{\cdot}6\times10^{-5}$	83 ±3	< 14·9	34 ±1·6	3·9±0·6	0·440	0·242
25	3×10^{-5}	56 ±3	< 7·15	25·2±0·4	< 2·3	0·368	0·240
26	,,	52·3±1·4	< 9·6	31 ±0·3	< 3·1	0·390	0·210
27	,,	45·6±3·2	< 7·6	28·2±0·7	< 2·6	0·380	0·222
28	4×10^{-5}	83 ±2·1	< 7·4	34·6±1·1	< 2·8	0·440	0·204

respond to a stimulus was reduced, that is, the "effective" refractory period was lengthened, there was also evidence that the duration of the action potential, measured with external electrodes, was not prolonged by quinidine (Wedd *et al.*, 1942), and that the absolute refractory period, defined as the minimum interval at which a *local* non-propagated response could be elicited, was unchanged or shortened (Love, 1926). In the present experiments an attempt has been made to resolve this paradox by an analysis of intracellular action potentials recorded from single fibres of isolated rabbit atria in the presence of a wide range of concentrations of quinidine.

The Importance of Concentration

Simultaneous measurements have been made of contraction, conduction velocity and intracellular potential in isolated rabbit auricles in the presence of a wide range of concentrations of quinidine sulphate. A major difficulty in interpreting the results has been to decide what concentrations in the isolated organ bath correspond to therapeutic and toxic levels of the drug in man. Sokolow and Edgar (1950) measured the concentrations of quinidine in the blood of thirty patients in whom auricular fibrillation had been successfully terminated. The average serum concentration was 5.9 mg./l., and 75% of the results fell between 4 and 9 mg./l. In some patients the concentrations reached were sufficient to evoke toxic manifestations. Nausea was experienced by several patients whose serum levels were 2, 5, 6.3, 7.0 and 10 mg./l. Tinnitus occurred in patients at 2.2, 4.3, 4.6, 5.4, and 11 mg./l.; diarrhoea at 4.2 and 6.3 mg./l.

In the present experiments an independent estimate of toxicity could be made from changes in the contractions which were simultaneously recorded. At concentrations of 10 mg./l. or less the contractions were not reduced by more than a quarter, and the atria were always able to follow the stimulus at the control frequency which was a little faster than the original frequency of their natural pacemaker. At concentrations of 16 mg./l. and above the reductions in the force of contraction became much larger, and the atria would no longer follow the stimulus, so that the frequency of stimulation had to be reduced.

At concentrations of quinidine of 10 mg./l. and less the results were quite clear cut. Quinidine produced no significant change in resting potential, nor was there any lengthening of the time required for repolarization to half the height of the action potential. There was, however, a prolongation of the tail of the repolarization phase. In all the experiments, at concentrations between 1.0 and 10 mg./l. inclusive, there was a great slowing in the rate of rise of the action potential,

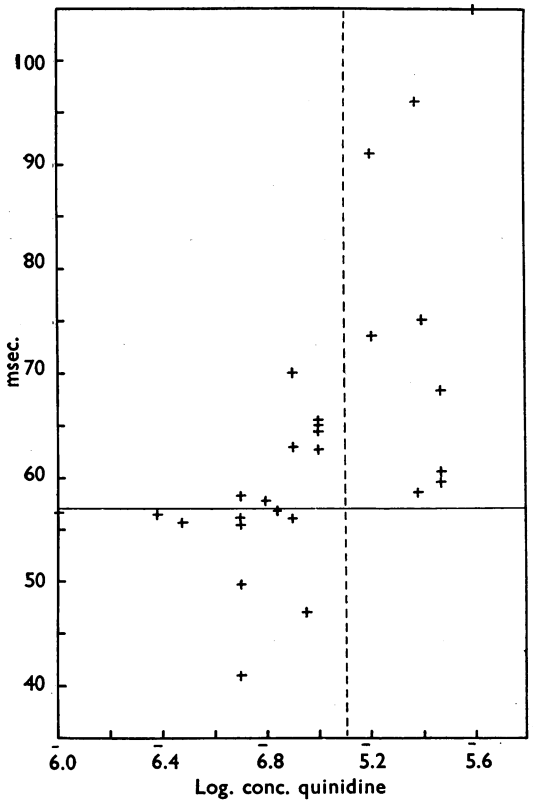

FIG. 6.—Effect of quinidine on the half-time for repolarization. Ordinate: milliseconds. Abscissa: log concentration. At concentrations of below 8 mg./l. the duration was unchanged or reduced. The increase in duration at higher concentrations was partly, or perhaps mainly, due to a reduction in the frequency of stimulation. The horizontal line represents the mean of all the controls. For explanation of vertical broken line, see legend to Fig. 4.

and the height of the overshoot was reduced. There was always also a slowing of conduction velocity.

Refractory Period

The results explain why quinidine, while making no difference to the absolute refractory period, prolongs the effective refractory period. There has long been evidence that the absolute refractory period of cardiac muscle is approximately synchronous with the duration of the action potential (Adrian, 1921 ; Blair, Wedd, and Young, 1941). It has been established (Weidmann, 1955a ; Hoffman *et al.*, 1957) that a second propagated response can take place in a cardiac fibre when repolarization has proceeded to 2/3 of the full resting potential, that is to about −62 mV. in a Purkinje fibre whose resting potential is −96 mV.

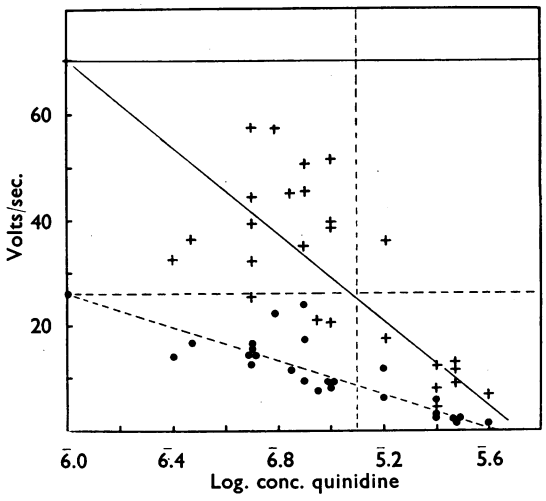

FIG. 7.—Effect of quinidine on the rate of rise of the action potential. Ordinate: volts/sec. Abscissa: log concentration. Crosses and solid line, maximum rate of rise. Circles and dotted line, mean rate of rise calculated from points 2.5 mV. above and 2.5 mV. below the most negative and positive values respectively of the action potential. Horizontal lines, means of all controls.

It is clear, therefore that the terminal phase of repolarization from −62 to −96 mV. could have no influence upon the effective refractory period, since a propagated response can already be elicited when repolarization has reached −62 mV. Hoffman *et al.* (1957) also showed that a local non-propagated response could be produced a little earlier, when repolarization had proceeded to only −45 mV., or half the resting potential, thus accounting for an absolute refractory period which was a little shorter than the effective refractory period.

For propagation to occur a certain minimum rate of change of the membrane potential must occur to overtake accommodation. Very slowly rising currents fail to excite. Weidmann (1955a) showed that the rate of rise of the action potential was a function of the level of the membrane potential at which the action potential "took off." If a stimulus was introduced when the membrane was repolarized to −65 mV., the action potential took off with a rate of rise much faster than if the stimulus arrived when repolarization had proceeded to only −55 mV. Hoffman *et al.* (1957) confirmed the findings of Weidmann (1955a), and showed that the critical rate of rise for propagation occurred when the response took off at about −50 mV. At −45 mV. a local response was evoked, but the rate of rise was insufficient for propagation.

In the presence of quinidine the rate of rise was greatly reduced. The membrane had therefore to repolarize further before the critical take-off point was reached at which the rate of rise was fast enough for propagation. The duration of the terminal phase of repolarization, normally unimportant, thus became significant in determining the effective refractory period. The absolute refractory period was unchanged, because the duration of the half-time for repolarization was not prolonged by quinidine.

Toxic Concentrations

At concentrations of 16 mg./l. and more it was necessary to reduce the frequency of stimulation, which would have increased the conduction velocity and the rate of rise of the action potential in normal atria. But conduction velocity and the rate of rise were further reduced by high quinidine concentrations in spite of the lowered frequency. In several experiments the duration of the action potential was greatly prolonged by quinidine concentrations of 16 mg./l. and more, but this may have been due to the reduced frequency. West (1955) found that the duration of the action potential was prolonged by quinidine: but since his preparations were beating spontaneously, the prolongation could have been due to the slowing of the rate of the pacemaker by quinidine. If, then, it may be assumed that concentrations of 10 mg./l. or less correspond to therapeutic levels of the drug, the mode of action of quinidine cannot be due to a change in the half-time for repolarization. The absence of slowing, and, in some experiments, the acceleration of the first half of the phase of repolarization is of interest, however, in providing an explanation for the observation that the absolute refractory period is unchanged or shortened by quinidine.

Phase of Depolarization

There are at least three ways in which a reduction in the rate of rise of the action potential could be produced. (1) A fall in the concentration differences inside and outside the fibre of the ions which carry the depolarizing current. The overshoot was reduced by moderate concentrations of quinidine, and might be abolished by 25 mg./l. or more. If the depolarizing ion is sodium, this would imply interference with the sodium pump. (2) A fall in the resting potential. This is evidently an unimportant factor in the mode of action of quinidine. It is also improbable that the intracellular concentration of potassium is altered by quinidine, though the total flux might be reduced. (3) An interference with the reaction which precedes or accompanies the flow of depolarizing current. This reaction has a high Q_{10}. The rate of rise is greatly reduced by a lowering of temperature from 36° to 26°, whereas the resting and action potentials are unaffected (Vaughan Williams, 1958). It is certainly a possibility that quinidine interferes directly with this reaction of depolarization.

There is as yet no evidence whether sodium is the ion which carries the current during depolarization in the rabbit auricle. In the cat auricle Burgen and Terroux (1953), and in Purkinje fibres Draper and Weidmann (1951), found that reduction of external sodium reduced the overshoot, but in these experiments sodium chloride was replaced by sucrose and saccharose respectively; so that there was a deficiency in the total ionic strength. In the guinea-pig ventricle, when external sodium was replaced by choline chloride, the overshoot was little affected (Corabœuf and Otsuka, 1956). There is evidence that in some tissues ions other than sodium can enter the cell during depolarization (Fatt and Katz, 1953; Hodgkin and Keynes, 1957; Fatt and Ginsborg, 1958).

At high concentrations of quinidine, the upstroke of the action potential developed a "step." A fast phase of depolarization (Fig. 3) was cut off early, yet depolarization proceeded at a slower rate to an overshoot. It is possible that the depolarization was cut short in the fibre in which the electrode was situated, but continued in distant fibres so that a further depolarization was observed, conducted back to the electrode electrotonically. Against this explanation is the observation that the "stepped" rising phase was observed at all sites penetrated in the presence of high quinidine concentrations, in several different auricles. Another possibility which might be considered is that the early fast phase of the step represents the operation of the sodium-carrier, and that the second slower phase is produced by current carried by other ions. Whatever the explanation for the "step," the early fast phase was accelerated and augmented by ACh, implying a direct action by ACh on the phase of depolarization (Vaughan Williams, 1958). In the experiments of Corabœuf and Otsuka (1956), although the final overshoot voltage reached in low sodium solutions was not reduced, the rate of rise was much slower. The sodium-carrier therefore might normally be the fastest but is not necessarily the only mechanism for transporting depolarizing current.

The problem could be solved by a method with sufficient resolution to measure the flux of ions during the different phases of the action potential,

but measurements of this kind have so far been found possible only in the slowly acting ventricle of the turtle (Wilde, O'Brien, and Bay, 1955). Measurements of average fluxes of ions can give only limited information, because the concentration differences responsible for the resting and action potential heights can remain unchanged over a very wide range of absolute fluxes, as shown by their resistance to temperature change (Burgen and Terroux, 1953; Trautwein, 1953; Trautwein, Gottstein and Federschmidt, 1953; Vaughan Williams, 1958).

In conclusion, it is apparent that the present work has some relevance to the hypotheses of Holland (1957) and Armitage (1957) concerning the rôle of potassium in the mode of action of quinidine in preventing fibrillation. These authors found that lowering the external potassium reduced the depressant effect of quinidine on isolated rabbit atria. It was concluded that quinidine acted by reducing potassium outflux, and so prolonging the duration of the action potential. Holland (1957), however, found that quinidine, even in as high a concentration as 50 mg./l., had by itself only a slight effect on potassium outflux, though its antagonism to ACh could be demonstrated. Burn, Gunning, and Walker (1956), Armitage (1957), and Armitage, Burn, and Gunning (1957) likewise took the view that the effect of changes in external potassium concentration acted mainly by altering the duration of the action potential. It was argued that by raising the external potassium the concentration difference assumed to be responsible for potassium outflux during repolarization was reduced, so that the phase of repolarization was prolonged. Against this must be set the evidence of Weidmann (1955a, 1956) and Carmeliet and Lacquet (1956) that raising the external potassium does not lengthen, but shortens, the cardiac action potential. On the other hand, if it is accepted that a reasonably high level of excitability with an adequate rate of rise of action potential is a prerequisite for the onset of fibrillation, the effects of potassium could be interpreted in another way. Raising the external potassium, by lowering the resting potential, would automatically reduce the rate of rise, and make fibrillation less probable. ACh, by increasing the rate of rise, would make fibrillation more probable. Quinidine reduces the rate of rise, and this action would be opposed by low external potassium, because the latter, by increasing the resting potential, would automatically accelerate the rate of rise.

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