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THE ACTION OF QUININE AND QUINIDINE ON THE CONTRACTIONS OF STRIATED MUSCLE

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In 1939 Harvey studied the effect of quinine on skeletal muscle and found that it had an action both on the neuromuscular junction and directly on the muscle fibre. He concluded that the direct action of the drug was to cause a longer persistence of the contraction wave. Since then, Hill (1949) has introduced the concept of the active state of muscle, the process underlying the contraction, and it has become possible to account for the mechanical effects of several drugs on the contractions of skeletal muscle by an action on this process (Goffart & Ritchie, 1952; Hill & Macpherson, 1954; Ritchie, 1954*b*). In the present experiments the action of quinine on the muscle fibre was investigated to see how far the effects of this drug could be explained in terms of this concept.

It was found that quinine prolonged the time course of the active state of frog's muscle, and the same effect was obtained with its optical isomer, quinidine.

METHODS

Experiments were done on the tibialis anterior and soleus muscles of cats *in situ*, and on the isolated frog's sartorius muscle.

Experiments on cats. The cats were usually anaesthetized with chloralose (80 mg/kg), but in some experiments they were decerebrated or spinalized under preliminary ether anaesthesia. Twitch and tetanic contractions were elicited either directly, or indirectly via the tied sciatic nerve, by means of rectangular electrical shocks. The duration of the shocks for indirect stimulation was 30–100 μsec, if not otherwise stated, and their strength five times threshold value: for direct stimulation the duration was 1 msec and the strength such that the twitch contractions were equal to those evoked with maximal indirect stimulation. Tetanic contractions of $\frac{1}{2}$–4 sec duration were evoked, using frequencies of stimulation ranging from 16 to 500 shocks/sec. In the experiments with direct stimulation, the animals were fully curarized by an intravenous injection of tubocurarine chloride; the curarization was regularly checked by indirect stimulation of the muscle. For direct stimulation, a silver wire was wound round the tendon to form one electrode; the drill fixing the femur served as the other.

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The muscles were attached to the flat steel spring of a Brown-Schuster myograph. The tensions developed by the muscles were recorded either on a smoked drum by means of a long aluminium lever or, as in most experiments, on a cathode-ray oscillograph by connecting the tip of the spring to an R.C.A. 5734 mechano-electronic transducer valve.

Injections of 1% (w/v) quinine hydrochloride and quinidine sulphate dissolved in saline were made, if not otherwise stated, into the external iliac artery (Zaimis, 1953). In a few experiments on the tibialis anterior muscle the method of close arterial injection (Brown, 1938) was used.

Experiments on frogs. The isolated frog's sartorius muscle was suspended throughout the experiment in a bath containing 80 ml. Ringer's solution (NaCl 0·675%, KCl 0·015%, $CaCl_2$ 0·040%, w/v) buffered with sodium phosphate to pH 7·0. In most experiments tubocurarine chloride (1/50,000, w/v) was present. The muscle was directly stimulated at many points of its surface simultaneously by means of a multi-electrode assembly (Hill, 1949). The tension recorder was the R.C.A. transducer valve. Active state curves of the muscle were obtained using the quick release method described by Ritchie (1954*b*). The quinine and quinidine solutions, added to the bath, were dissolved in Ringer's solution and the final concentration of the drug in the bath was between 10^{-4} and 2×10^{-5}, w/v.

RESULTS

Experiments on the tibialis anterior and soleus muscles of the cat

The effect of quinine on single isometric twitches. When 5–15 mg quinine was injected into the external iliac artery, there was an increase in the peak tension developed by the tibialis anterior muscle in response to a single shock to the nerve. This effect started within 10 sec of the injection, was maximal in about 3 min, and lasted for over half an hour. These observations confirm those made by Harvey (1939) and are illustrated in Fig. 1, where a record of the single isometric twitch, made 6 min after the injection of 15 mg quinine, has been superimposed on a similar record made immediately beforehand. As shown in this figure, quinine caused an increase not only of the peak tension, but also of the contraction time, i.e. the interval between the stimulus and the peak of the contraction. With close arterial injections quinine produced these changes when given in doses as small as 1–2 mg. In the experiment of Fig. 1 the peak twitch tension increased by 36% and the contraction time by 16%. The increases in twitch tension varied greatly from experiment to experiment, between 25 and 140%, whereas the changes in the contraction time were always smaller and varied between 3 and 25%. Similar results were obtained with direct stimulation of muscle, where neuromuscular transmission was completely blocked by tubocurarine chloride.

Quinine caused similar but smaller changes in the response of the cat's soleus muscle. Thus, in a typical experiment, after 15 mg quinine was injected, the peak tension and contraction time of the soleus muscle were increased by 27 and 8% respectively, whereas the corresponding increases for the tibialis anterior muscle were 90 and 15%.

In some experiments the duration of the stimuli was increased from 30–100 μsec to more than 1 msec, with the result that the contractions in response to a single shock increased; this was caused presumably by repetitive

firing of the nerve. In these experiments quinine usually produced a slight, transient or prolonged, decrease of the peak 'twitch' tension (Fig. 2A, B) which was sometimes followed by an increase. With the shorter duration stimuli (30–100 μsec) this same pattern of events was seen in experiments where the injection of quinine had been preceded up to half an hour earlier by an arterial injection of eserine (Fig. 3A, B).

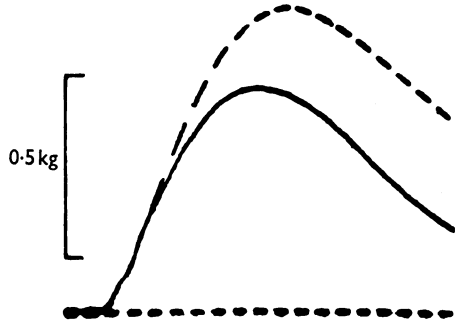

Fig. 1. Records of single, indirect twitches of cat's tibialis anterior muscle, before (solid line) and 6 min after (interrupted line) 15 mg quinine hydrochloride. Time bars, 3·3 msec.

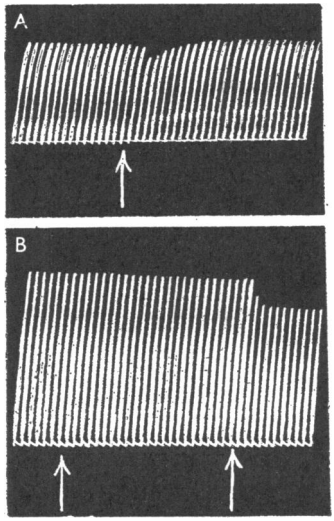

Fig. 2.

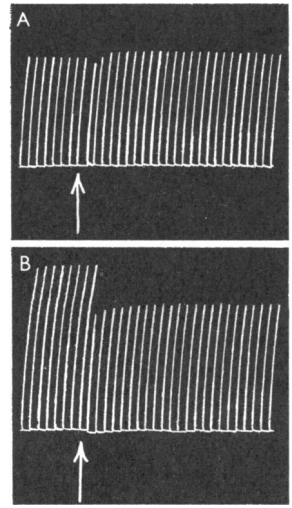

Fig. 3.

Fig. 2. Records of contraction of cat's tibialis anterior muscle in response to single shocks to the nerve of 1 msec duration every 15 sec. A: 10 mg quinine hydrochloride at arrow. B: 2 ml. saline at first arrow, 10 mg quinine hydrochloride at second arrow.

Fig. 3. Records of contraction of cat's tibialis anterior muscle in response to single shocks to the nerve of 100 μsec duration every 15 sec. A: 15 mg quinine hydrochloride at arrow, preceded 30 min earlier by 150 μg eserine sulphate. B: 15 mg quinine hydrochloride at arrow, preceded 10 min earlier by 150 μg eserine sulphate.

The effect of quinine on the twitch cannot be attributed to the fall in arterial blood pressure which occurred after the injection of quinine, because the fall was transient whereas the effects on the contraction lasted for up to half an hour. Furthermore, the close arterial injection of 1–2 mg of quinine produced only a very slight fall in blood pressure but had the same effect on the contractions of the tibialis anterior muscle. It is also unlikely that changes in the local blood supply of the muscle were responsible for the effects on the contraction, since they could be observed during arterial occlusion, which by itself produced no marked changes in the mechanical response of the muscle.

The effect of quinine on tetanic contractions. Quinine reduced the fusion frequency of both the tibialis anterior and soleus muscles, i.e. the smallest frequency of stimulation at which a fused mechanical response is obtained. In a typical experiment an arterial injection of 15 mg quinine reduced the fusion frequency of the tibialis anterior muscle from about 90 shocks/sec to about 60 shocks/sec.

The general effect of quinine on the tetanic tension depended on the frequency of stimulation. At frequencies lower than fusion frequency, quinine increased both the peak tension developed and the mean tension which the muscle was able to maintain. At frequencies higher than fusion frequency, quinine produced no change in the peak tetanic tension, although the ability of the muscle to maintain its tension during prolonged stimulation was greatly decreased. These findings are contrary to those of Harvey (1939), who found that even at a low frequency of stimulation the peak tetanic tension was decreased after quinine.

Typical results are shown in Fig. 4 for the peak tetanic tension of the tibialis anterior muscle at different stimulation frequencies and in Fig. 5 for the tension which the muscle was able to exert at the end of a tetanus of 4 sec duration. In the experiment of Fig. 4, tetani of about 4 sec duration were given to the motor nerve at 2 min intervals and at frequencies of stimulation ranging from 16 to 400 shocks/sec. The relation between peak tetanic tension and frequency of stimulation was obtained (solid circles). The ringed point in this curve corresponds to fusion frequency. At higher frequencies the peak tetanic tension remained constant: at lower frequencies it decreased with decreasing frequency in the manner described by Brown & Burns (1949). When the experiment was repeated two or three times at half hourly intervals, this relation was found to be unaltered. After 15 mg quinine the curve was shifted to the left (open circles). The peak tension developed by frequencies of stimulation greater than fusion frequency remained constant.

As shown in Fig. 4, the peak tension developed at the frequency of stimulation at which fusion was judged to occur was *slightly less* than the maximum tetanic tension: one would expect, however, that at fusion frequency the peak tension developed would be *equal* to the maximum tetanic tension. This

discrepancy can be explained by the fact that the value of fusion frequency determined experimentally depends on the sensitivity of the recording system (Ritchie, 1954*a*) and that in the present experiments this sensitivity was such that the whole of the tetanic contraction was recorded. Fusion frequency determined under these conditions is likely to be smaller than the true fusion frequency.

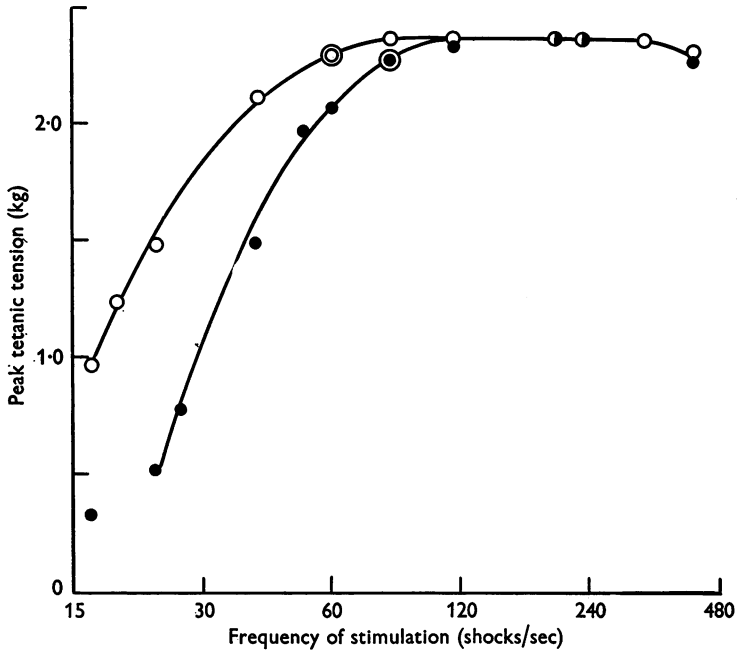

Fig. 4. Relation between the peak tetanic tension of the cat's tibialis anterior muscle and the frequency of indirect stimulation before (solid circles) and after (open circles) 15 mg quinine hydrochloride. The ringed point in each curve indicates the fusion frequency.

In Fig. 5 the tension which the muscle exerted at the end of a 4 sec tetanus at varying frequencies of stimulation and which is usually smaller than the peak tetanic tension is shown before and after quinine. Quinine shifts the whole of this curve to the left.

Experiments on the isolated frog's sartorius muscle

The effect of quinine on the active state of muscle. The effects of quinine on the twitch contractions of cat's muscle described above are similar to those described by Hill & Macpherson (1954) and by Ritchie (1954*b*) for the effect of nitrate on the frog's sartorius muscle. The effect of nitrate was ascribed by these authors to a prolongation of the time course of the active state of this muscle. We therefore examined the effect of quinine on the active state of

muscle. The isolated frog's sartorius muscle at 0° C was used and the active state was measured by the method described by Ritchie (1954*b*). When quinine was added to the Ringer's solution in which the muscle was suspended in final concentrations of between 10^{-4} and 2×10^{-5} (w/v), the duration of the active state increased. Fig. 6 illustrates a typical experiment where quinine produced a prolongation of the time course of the active state which amounted to over 30%.

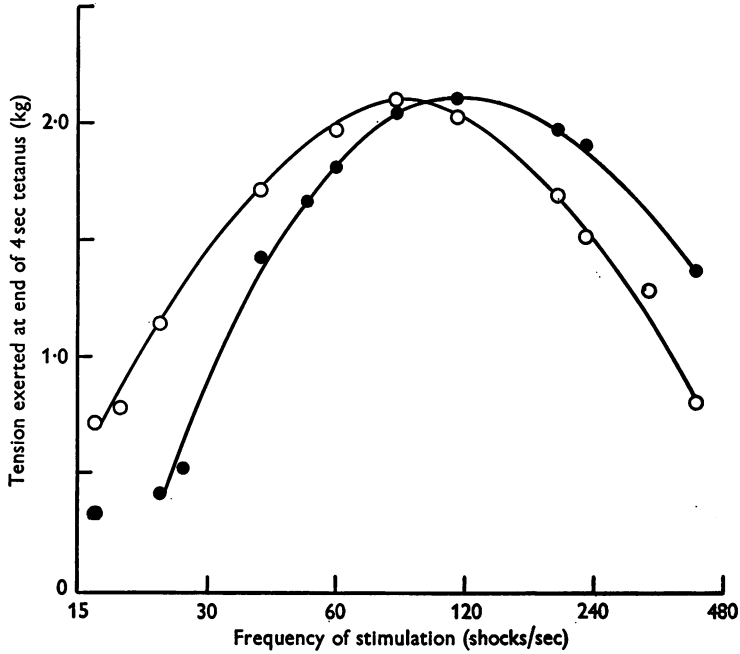

Fig. 5. Relation between the tension of the cat's tibialis anterior muscle at the end of a tetanus of 4 sec duration and the frequency of indirect stimulation before (solid circles) and after (open circles) 15 mg quinine hydrochloride. Same muscle as in Fig. 4.

In these experiments quinine increased the contraction time of the muscle but there was little or no increase in the peak tension developed in the single isometric twitch such as was found in cat's muscle. However, according to Ritchie & Wilkie (1955) this dissimilarity may well be associated with the fact that the twitch/tetanus ratio is high in frog's muscle at 0° C (more than 0·75), whereas it is small in cat's muscle (about 0·25). The twitch/tetanus ratio of the frog's muscle was therefore reduced experimentally by connecting an elasticity (a rubber band or an unstimulated muscle) between the preparation and the tension recorder. This reduced the twitch/tetanus ratio to about 0·2. Under these circumstances quinine increased not only the contraction time but also the twitch tension, as in cat's muscle. A similar result was obtained when the

twitch/tetanus ratio was reduced by raising the temperature of the muscle from 0° C to about 15° C.

The effect of curarine and eserine on the prolongation of the active state produced by quinine. In these experiments active state curves were determined every 15 min; each determination required 4–5 min. The results are best

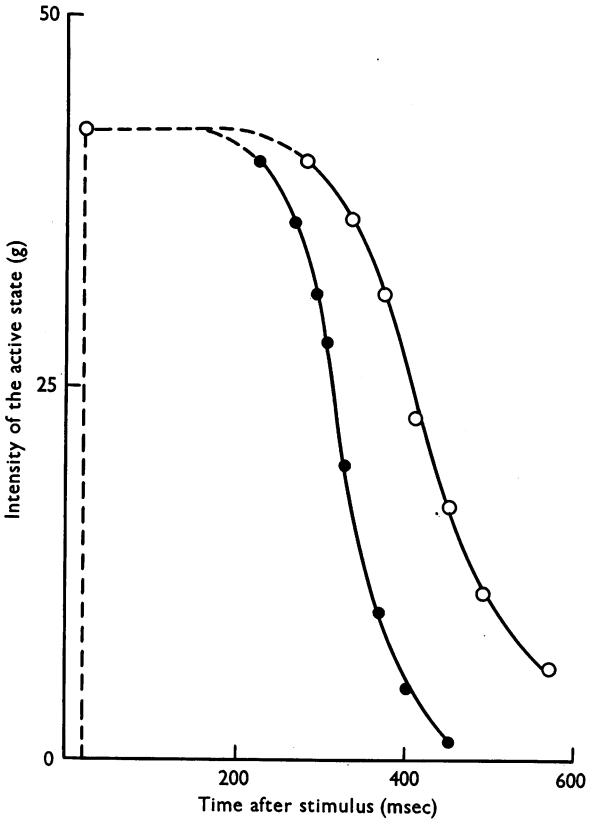

Fig. 6. Active state curves of frog's sartorius muscle at 0° C before (solid circles) and 45 min after (open circles) soaking in 10^{-4} (w/v) quinine hydrochloride.

illustrated by considering the time after the stimulus at which the intensity of the active state had fallen to a given tension, for example a third of the maximum tetanic tension. As shown in Fig. 7, this time was not altered either by tubocurarine chloride (2×10^{-5}, w/v) or by eserine sulphate (10^{-5}, w/v). Furthermore, neither tubocurarine nor eserine prevented the action of quinine in increasing this time. In Fig. 7 this effect of quinine is shown for the sartorius muscle of one leg after previous treatment with tubocurarine and eserine (Fig. 7A) and for the muscle of the other leg after treatment with tubocurarine alone (Fig. 7B). It can be seen that the effect of quinine was the

same in both muscles. Similar results were obtained in experiments where the muscle of the other leg was not previously treated either with tubocurarine or with eserine.

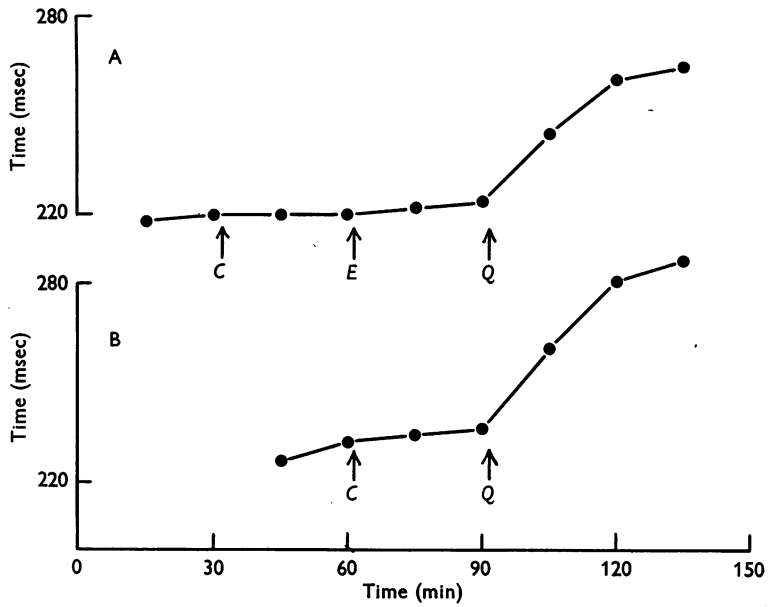

Fig. 7. The effect of tubocurarine, eserine and quinine on the duration of the active state of frog's muscle at 0° C. The ordinates are the times after the stimulus at which the intensity of the active state had fallen to one-third of its maximum intensity. Curve A: left sartorius. At *C*, *E* and *Q* tubocurarine chloride (final concentration 2×10^{-5}, w/v), eserine sulphate (final concentration 10^{-5}, w/v) and quinine hydrochloride (final concentration 10^{-4}, w/v) respectively were added to the bath. Curve B: right sartorius, same frog. At *C* and *Q* tubocurarine chloride and quinine hydrochloride (same concentrations as in curve A) were added to the bath.

The effects of quinidine on skeletal muscle

All the effects which have been described for quinine were also found with quinidine when given in the same doses. In the experiments with cat's muscles the twitch tension and contraction time were increased, fusion frequency was decreased, and the curves relating peak and maintained tetanic tensions to stimulation frequency were shifted to the left. Furthermore, the time course of the active state of frog's muscle was increased and this effect was unaffected by tubocurarine or eserine.

DISCUSSION

Changes produced in the duration of the active state of muscle by quinine and quinidine can account for many of the effects of these drugs on muscular contraction. If a muscle were composed entirely of contractile material, its isometric myogram and its active state curve would be identical. As Hill (1949)

has pointed out, the reason why they are not is because muscle contains an elastic component in series with the contractile material. During the development of muscle tension this component has to be stretched and the rate of this stretching is limited both by the force/velocity relation of the contractile material and by the stress/strain characteristic of the series elasticity. The consequence is that muscle tension rises gradually, and during a single twitch is curtailed after a time by the decay in the intensity of the active state, the driving force underlying the contraction. Quinine prolongs the time course of the active state in frog's muscle and so allows tension development in a single twitch to continue over a longer period of time before it is stopped by this decay. Peak twitch tension and contraction time are in consequence both increased. The finding, illustrated in Fig. 1, that quinine does not change the rate of tension development in the early stages of a twitch is explained on the grounds that during this time there is no decay in the intensity of the active state and the isometric myogram is determined only by the characteristics of the series elastic component and the force/velocity relation, neither of which presumably are affected by quinine. Goffart & Ritchie (1952) suggested that the effect of adrenaline on some mammalian skeletal muscles might be explained by a similar action of this drug on the active state; and it has recently been shown that the increased peak twitch tension and contraction time produced by the nitrate ion on frog's skeletal muscle is, in fact, caused by such a prolongation (Hill & Macpherson, 1954; Ritchie, 1954*b*).

The active state curve can at present be determined only for relatively slow muscles such as frog's muscle at 0° C, and not for the faster mammalian muscles. However, it is most likely that quinine has the same action on the duration of the active state of cat's muscles as that observed for frog's muscles, because the effects of the drug on the twitch contractions of frog's and cat's muscles are so similar. Many of the observed effects of quinine on tetanic contraction of cat's muscle can be well explained by an action on the duration of the active state. The decrease in fusion frequency after quinine can be accounted for because, as shown by Ritchie (1954*a*), this frequency is related to the duration of the plateau of the active state; an increase in this duration must cause a decrease in fusion frequency. Furthermore, the effect of quinine in increasing the tension developed at any particular frequency which produces an unfused tetanus is also accounted for by an increase in the duration of the active state. During an unfused tetanus, the peak tension developed by the muscle is less than the maximum tension which the muscle can exert, because the internal activity of the muscle decays between each shock. When the time course of the active state is prolonged, this decay becomes less. The consequence is that greater peak and mean tensions are developed. During a fused tetanus, however, the decay in the active state is not a factor involved in determining the shape of the isometric myogram. Therefore, quinine by

causing a prolongation of the time course of the active state does not affect the maximum tension in a fused tetanus.

The prolongation of the time course of the active state is not the only action of quinine on striated muscle. The finding that tetanic contractions are not well maintained cannot be explained in terms of an action on the active state of muscle. Harvey (1939) has demonstrated that quinine has additional actions on skeletal muscle. It increases its threshold to electrical stimulation, slows the propagation of the action potential along the fibres, and greatly increases its refractory period. This increase in refractory period can account, as was suggested by Harvey (1939), for the effect of quinine on the maintenance of tetanic tension.

There are some differences between Harvey's results and ours. In our experiments the decrease in the ability of the muscle treated with quinine to maintain its tension during a tetanus was apparent only when these contractions were evoked with frequencies of stimulation greater than 100–180 shocks/sec (Fig. 5): in his experiments this was found in tetani evoked with 50 shocks/sec. A second point of difference was that Harvey observed a decrease in maximum tetanic tension in muscles treated with quinine, whereas we did not. These differences in results are not due to the use of different doses or modes of injection of the drug, for in many of our experiments these were the same as Harvey's. However, there was one major difference between Harvey's technique and ours which may partly explain the differences in results. We usually used a rigid isometric recorder and observed that during a prolonged tetanus at high frequencies of stimulation the maximum tension was exerted only for a brief period at the beginning of the contraction. Harvey, on the other hand, usually used a lever writing on a smoked drum: the inertia of a system like this is such that the rapid initial development of tension is not faithfully recorded. In fact, when we used a recording system similar to Harvey's, we observed a decrease in the recorded maximum tetanic tension after quinine similar to that reported by Harvey. We cannot, however, explain why Harvey found a decrease in maintained tetanic tension at a much lower frequency than either Ravin (1940) or we did. He obtained a decrease at a frequency where we found, even with the smoked drum technique, an *increase* in maintained tetanic tension.

Harvey reported that after the potentiating effect on twitch tension of a dose of eserine had worn off, quinine no longer increased the twitch tension, and he remarked that this action of eserine was difficult to explain. After we had found that eserine had no effect either by itself, or on the action of quinine, on the active state of frog's muscle, we repeated his experiments and found that the modification of the effect of quinine on cat's muscle produced by eserine was very similar to that found in experiments where repetitive firing of the nerve occurred because of the long duration of the nerve shocks.

In experiments where there is repetitive firing, quinine tends to decrease twitch tension by increasing the refractory period and tends to increase it by prolonging the duration of the active state. The net result is that quinine can produce a decrease, a slight increase, or no effect at all on the twitch tension. The effect of eserine in reducing or preventing the action of quinine in indirectly stimulated muscles can be well explained if we assume that even some time after giving the eserine, when the potentiating effect of the drug had largely subsided and the contractions were again steady, there was still repetitive firing at the neuromuscular end-plate. This assumption is strengthened by the fact that in experiments where eserine prevented the potentiating action of quinine, as in that illustrated in Fig. 3A, the twitch contractions just before the quinine was given, although steady, were greater than they were before the eserine had been given: and by the finding of Brown, Burns & Feldberg (1948) that after an injection of another anticholinesterase, D.F.P., repetitive firing occurs over a long period of time and persists even when the twitch tension has fallen to below its original value. There is thus no need to postulate, as Harvey has done, a direct action of eserine on the muscle fibre; indeed, from our experiments on frog's muscle (Fig. 6A), there is no evidence for such an action.

The effect of quinine on peak twitch tension is not very pronounced in frog's muscle at 0° C, because in this muscle the twitch tension rises nearly to tetanic tension before it is curtailed by the decay in the active state. Ritchie & Wilkie (1955) have pointed out, however, that in this muscle the effect on twitch tension of a prolongation of the time course of the active state can be exaggerated when the muscle is made to pull against an external elasticity. This reduces the twitch/tetanus ratio, and for a large part of the time during which twitch tension is being developed the decay in the intensity of the active state is continuously modifying the response of the muscle. Quinine given under these circumstances has a large effect on twitch tension. Similar reasoning may account for the fact that the effect of quinine on the cat's tibialis muscle is larger than on the soleus muscle, because the tibialis is the faster muscle and has the smaller twitch/tetanus ratio.

The effects of quinine and its optical isomer, quinidine, on striated muscle are identical. We think that the main action of both these drugs on cat's and frog's muscle is to prolong the time course of the active state. The fact that the time of onset of the effect of quinine on the twitch of cat's muscle *in vivo* is different from that on isolated frog's muscle does not negate this conclusion. For the drug will reach the surface of the fibres of the cat's muscle in a relatively short time through the blood capillary network: in the isolated frog's muscle, however, the drug has to diffuse through the intercellular spaces, and this will take some time.

SUMMARY

1. Quinine increases the peak twitch tension and contraction time of the cat's soleus and tibialis anterior muscles and of the frog's sartorius muscle.

2. Quinine decreases the fusion frequency of the cat's soleus and tibialis anterior muscles.

3. Quinine has no effect on the maximum tetanic tension of the cat's soleus and tibialis anterior muscles but increases the peak tension developed during an unfused tetanus.

4. Quinine decreases the maintained tetanic tension of the cat's soleus and tibialis anterior muscles evoked by high frequencies of stimulation, but increases this tension when evoked by low frequencies of stimulation.

5. Quinine increases the duration of the active state of frog's muscle. This change accounts for the observed increase in twitch tension and contraction time of this muscle.

6. Tubocurarine and eserine have no effect on the active state of frog's muscle, nor do they affect the action of quinine on the active state.

7. Quinidine produces the same effects as quinine on muscular contraction.

8. It is suggested that prolongation of the duration of the active state is the cause of various of the effects of quinine and quinidine on the contraction of cat's muscle.

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