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COUPLING OF MEMBRANE POTENTIAL TO CONTRACTION IN CRUSTACEAN MUSCLES

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The mechanism, whereby the changes in the surface membrane of the muscle fibre which are reflected in the electrical potential changes taking place on excitation bring about contraction, remains virtually unexplored in any neuromuscular system (Hill, 1950; Hoyle, 1957; Gelfan, 1958). The only theories ever proposed for striated muscles have tried, like the disputed (Sten-Knudsen, 1954) window-field theory (Bay, Goodall & Szent-Gyorgyi, 1953; Csapo & Suzuki, 1957), to link contraction directly to the electrical field which is set up by the propagated action potential. Such a theory, even if it were tenable elsewhere, could not in any case be applied in the Crustacea. Contraction in most crustacean muscles occurs when there are only very small junctional potentials (j.p.s) and the depolarization of the membrane occurs almost synchronously over the whole surface of the muscle fibre owing to the distributed nature of the nerve terminals (Fatt & Katz, 1953*a*; Hoyle & Wiersma, 1958*a*). There is thus no appreciable potential difference between different parts of the fibre during the excitation. Only the potential difference between the inside and the outside of the membrane is changed and the extent of this may be very small.

One possibility which has been very tentatively suggested (Fatt & Katz, 1953*b*) is that contraction in crustacean muscle starts at a particular level of membrane potential. Thereafter, presumably, greater depolarization causes more and more contraction. The mean level of depolarization produced by the summed j.p.s would then be the principal factor determining contraction. This theory is in line with the currently accepted hypothesis of excitation in smooth muscle fibres and in the slow motor system of the frog. In the guinea-pig taenia coli the tension has been shown to be inversely related to the membrane potential (Bülbring, 1955). In the slow skeletal muscle system of the frog the tension is related to the depolarization plateau produced during repetitive excitation (Kuffler & Vaughan Williams, 1953).

The nature of the physico-chemical phenomena which in these muscles might couple the membrane potential to contraction cannot, in the present state of our knowledge, even be hinted at. It is known that the link can be partially or completely uncoupled by various chemical treatments and it is hoped that some of these may shed light on the mechanism. In the taenia coli the tension can also be related directly to the height and duration of recurring small spike potentials. But following treatment with DNP this correlation is abolished (Bülbring & Lüllman, 1957). The membrane is excited so that it becomes depolarized, and both spike frequency and duration increase simultaneously. But in spite of the increased membrane activity the muscle relaxes, uncoupling of the two events has been achieved by the drug.

In ordinary frog muscle, also, the spike mechanism can be dissociated from contraction by the simple expedient of raising the osmotic pressure of the bathing fluid $2\frac{1}{2}$ times with sodium chloride solutions. The contraction is reduced and eventually abolished, although the action potential as recorded with an internal electrode is actually increased somewhat in height (Hodgkin & Horowicz, 1957). The link in this muscle used to be regarded as an all-or-nothing one by many authors, but its lability has now also been disclosed in experiments in which the chloride of the Ringer's fluid is replaced by nitrate, bromide or iodide. The twitch tension is increased in that order (Hill & Macpherson, 1954), apparently owing to an increased duration of action of the coupling mechanism. The phenomena are immediately reversible when normal Ringer's fluid is substituted, which indicates that they occur at the fibre surface where, presumably, the coupling mechanism is situated.

In view of the nature of the excitatory and inhibitory events occurring in crustacean muscles, these should provide excellent material for the investigation of the coupling mechanism. There are several different excitatory processes (Hoyle & Wiersma, 1958*a*) and most of these can be partially or completely uncoupled by natural events such as inhibitory transmitter action (Hoyle & Wiersma, 1958*b*).

The purpose of the present investigations was to study the relationship between the membrane potential and tension in crustacean muscles, with a view to determining the extent to which the two might be coupled and to attempt to shed some light on the nature of the coupling mechanism.

METHODS

The preparations investigated were the same as those described in the previous papers (Hoyle & Wiersma, 1958*a*, *b*). Of these certain ones were given particular attention. These will be described in turn.

RESULTS

Opener of Cambarus

In all muscle fibres examined, stimulation of the excitor nerve fibre at a frequency adequate to evoke a contraction leads to a reduction in the level of the membrane potential. The extent of this reduction varies from fibre to fibre, as does the height of individual j.p.s. The reduction is approximately correlated with the height of the j.p.s but there is a considerable degree of scatter. Thus in fibres showing j.p.s of similar height (6 mV at 50/sec) the membranes were depolarized by as little as 4 mV (maintained) in some fibres to as much as 20 mV (maintained) in others.

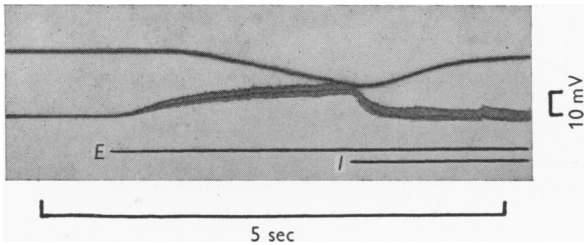

Fig. 1. Membrane potential changes associated with excitation and inhibition in the stretcher of *Panulirus*. Upper trace, tension (downward deflexion); lower trace, intracellular record of membrane potential changes, direct-coupled amplification. Frequency 35/sec approx. The upper horizontal line marks the duration of the excitatory stimulation, the lower one that of inhibitory stimulation. Note that the membrane becomes slightly more polarized during inhibition with excitation than during rest.

The extent of the depolarization in individual fibres was related to the frequency of stimulation of the motor axon in a manner similar to that found in the *Panulirus* closer (Hoyle & Wiersma, 1958*a*, Fig. 2). Repetitive inhibitory stimulation given alone commonly polarized the membrane by 0·1–10 mV. In many fibres, however, and sometimes in every fibre of a given muscle, it depolarized the membrane by 0·1–4 mV.

We will consider first only those fibres in which the inhibitory axon causes either no potential or a polarizing one. In these fibres, if inhibition is given during excitation, it effects a considerable reduction in the maintained level of depolarization produced by the excitatory stimulation. The membrane potential may even be polarized beyond the resting level. An example of a similar phenomenon from another muscle during simple (β) inhibition is shown in Fig. 1. The extent of the polarization is a function of the frequency of inhibitory stimulation as also is the tension developed.

Some values obtained during an experiment on the opener are illustrated in Fig. 2. The motor axon was stimulated at a frequency of 45/sec and the full tension allowed to develop. Then the inhibitory axon was stimulated, at

several frequencies. At each frequency the maintained level of membrane potential was measured and also the tension, whilst the excitatory frequency was kept constant. Complete mechanical inhibition occurred at an inhibitory stimulation frequency of 30/sec. At higher frequencies up to 2 mV more polarization could be effected. This shows that higher frequencies of inhibition can cause an appreciably larger effect, as is also evident from the fact that they can inhibit higher frequencies of excitation completely.

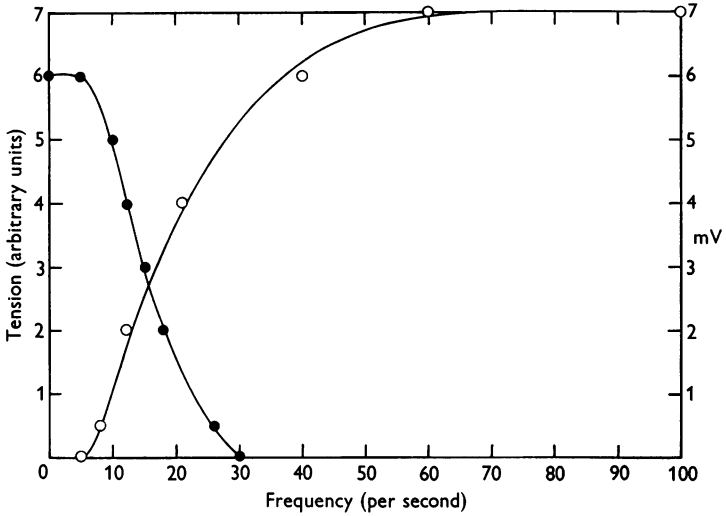

Fig. 2. Tension, ●, of whole muscle, and membrane polarization, ○, measured in a single representative muscle fibre as maintained polarization above potential level reached during excitation alone. Different frequencies of inhibitory stimulation were given, whilst the excitor was stimulated at 45/sec; opener of *Cambarus*.

Isolated results from a few fibres unfortunately do not necessarily represent the general activity in the muscle. It would be essential in this kind of experiment to record from a large number of muscle fibres taken at random in order to obtain a mean value which would be statistically significant, and this has not been attempted in our investigation. However, if we may provisionally regard the fibre of Fig. 2 as being a reasonably typical example, which we think it was for muscles in this state, some features of the relationship between tension and membrane potential can be described. These are as follows: (1) There is a threshold level of depolarization at which contraction starts; when inhibitory action restores the membrane potential to this same level the contraction becomes completely inhibited (not shown in Fig. 2). (2) There is a minimum frequency of inhibitory stimulation at which the membrane polarization becomes evident; this was at only 5 stimuli/sec in the experiment quoted. (3) The tension starts to fall at the least frequency at which the inhibitory stimulation starts to reveal a maintained polarization. (4) Every

degree of tension can be obtained by varying the frequency of stimulation of the inhibitor axon. (5) There is an inverse relationship, which is approximately linear, between the tension developed and the maintained membrane potential change, from frequencies of 10–80 stimuli/sec.

These findings are clearly compatible with the hypothesis which links tension to membrane potential, but they do not constitute evidence that there is a causal relationship. It would be necessary to suppose that a shift of membrane potential of only a fraction of a millivolt is an adequate stimulus for contraction, at the threshold. In a bi-stable system a very small change is adequate to effect the transition from one stable mode to the other, or to release a trigger and initiate new events. But we are clearly not dealing here with either a bi-stable system or a trigger action. The energy change after the 'threshold' is exceeded is a graded, not an abrupt process. For, as previously stated, in this muscle it is not possible to believe that only a few muscle fibres would be involved in weak contractions. We must, therefore, consider the possibility that the electrical events are merely associated with some other event which is the real determinant of coupling.

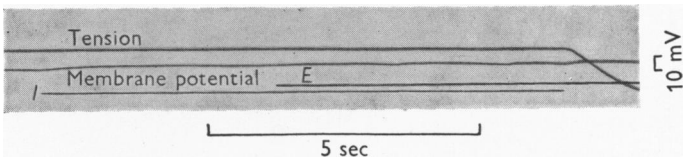

Fig. 3. Relationship between tension (upper trace) and membrane potential (lower trace) in a *Cambarus* opener preparation in which inhibitory stimulation caused depolarization; upper horizontal line, excitation; lower, inhibition. Notice that during simultaneous stimulation of both axons the junctional potentials gradually change in height; this was due to slight differences in frequencies of the excitatory and inhibitory stimulations.

Returning to the membrane changes, in those muscle fibres which show a depolarization during inhibitory stimulation, the excitatory and inhibitory depolarizations may initially sum. But as the depolarization continues beyond a certain level, which is not very critical, the inhibitory action becomes polarizing. Cutting off inhibition during simultaneous stimulation of the excitor and inhibitor when the membrane potential has reached this level may then cause slight further depolarization (Fig. 3), restoring it again causes a slight polarization. Thus the existence of depolarizing inhibitory potentials does not provide convincing evidence against coupling of the membrane potential and contraction.

Results similar to those obtained in the opener of *Cambarus* have been observed also in some of the other muscles examined, notably the openers and stretchers of *Cancer antennarius* and *C. anthonyi*. The stretcher of *Panulirus interruptus* gave similar results too, with the exception that depolarizing inhibitory potentials were never found. Typical results from some of these

experiments are shown in Fig. 4. It will be seen that in each case the tension is inversely related to the membrane potential. Inhibitory stimulation given alone causes a polarization in all these instances. When excitatory stimulation is given at the same time the membrane potential remains at the higher level or falls slightly. Immediately the inhibitory stimulation is turned off the potential falls quickly but by a small amount. In the *Panulirus* stretcher muscle stimulated at 65/sec the potential drops by 10 mV. In the *Cancer* stretcher, however, the corresponding value is only 1 mV. At lower frequencies of stimulation, which just cause a contraction, these values were all reduced by about two thirds.

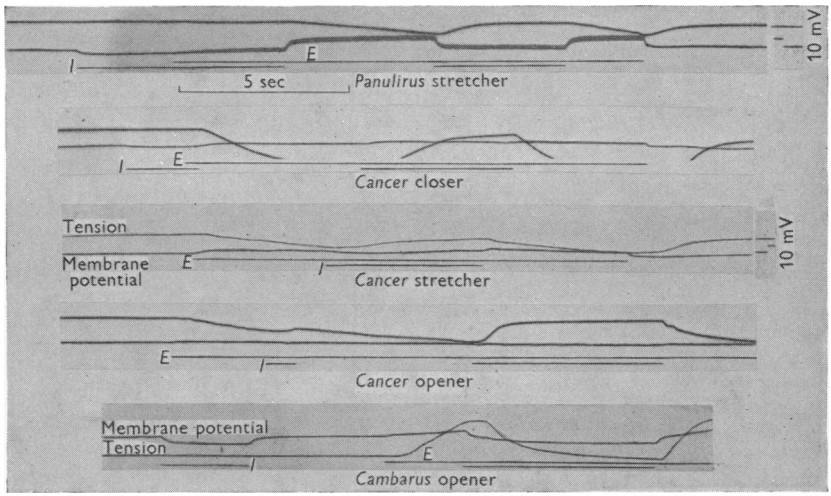

Fig. 4. Relationship between tension (upper traces) and membrane potential (lower traces) during excitatory and inhibitory nerve stimulation in various crustacean preparations. The upper horizontal line marks in each record the duration of the excitatory, and the lower line of the inhibitory stimulations. For *Cambarus* opener membrane potential is recorded in the upper, tension in the lower trace. Increasing tension is a downwards deflexion except in the *Cambarus* opener, where it is upwards.

Double motor innervation and inhibition

The depolarization brought about by the slow and fast motor axons of the 'closer' muscles of the walking legs of *Cancer* and *Panulirus* have been examined and correlated with tension. The depolarizations in the crabs were very small. The observations for *Panulirus* have been presented in an earlier paper (Hoyle & Wiersma, 1958*a*). At the lower frequencies of stimulation the slow axon effects a greater extent of maintained depolarization relative to the magnitude of the j.p.s than the fast. At higher frequencies there is no significant difference in the maintained depolarizations produced by the two

fibres. Tensions are correspondingly similar. The principal difference between the 'slow' and 'fast' systems lies in the greater speed of onset of the latter's contraction.

Inhibitory stimulation of the same frequency as the excitatory one is equally effective in counteracting the two membrane depolarizations and inhibits both contractions fully. Results from the *Cancer* closer are illustrated in Fig. 5. These are records of the largest potential changes which could be found in the preparation. Approximately the same amount of final tension was produced by each system, but tension was developed more quickly by stimulation of the fast axon. The tension fell away equally quickly for the two systems when inhibition was turned on. The rate of relaxation during inhibition was exactly that of normal relaxation following cessation of excitation alone. The development of the maintained membrane potential change during stimulation of the fast axon is detectably faster than that during slow. The differences are, however, extremely slight.

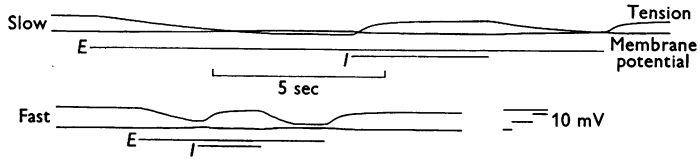

Fig. 5. Relationship between tension (upper traces) and membrane potential (lower traces) during inhibitory and excitatory stimulation of both 'slow' and 'fast' nerve fibres of the closer of *Cancer antennarius*. Intracellular records from the same muscle fibre.

That there is equality between the rate of natural relaxation and that effected by inhibitory stimulation at a frequency which gives complete mechanical inhibition has been reported previously for a number of systems (van Harreveld & Wiersma, 1939). This is a significant observation as it suggests that inhibitory action uncouples the excitatory process in an abrupt fashion as soon as inhibition is turned on. The restoration of the membrane potential in *Cancer* takes place slowly, although it is very fast in *Panulirus* and *Cambarus* (Fig. 1). It seems doubtful whether in *Cancer*, at least, the membrane potential change as such is the factor which determines the tension.

Potential and tension changes during simple (β) and supplemented (α) inhibition

The tension has been correlated with maintained membrane potential in cases of simple and supplemented inhibition in the openers of *Cambarus* and *Cancer*. Supplemented inhibition does not occur in *Panulirus*. Some of the results have been described already (Hoyle & Wiersma, 1958*b*).

Since, during supplemented inhibition the j.p.s are attenuated, the polarization effect should be greater than in simple inhibition. This is the case; polarization is effected at a greater rate and may also achieve a higher level during

attenuation (Hoyle & Wiersma 1958*b*, Fig. 6). On the hypothesis that tension development is coupled to the membrane potential, supplemented inhibition should cause a faster relaxation than simple inhibition. This effect was not found. This, therefore, also suggests that inhibitory action may not be the result of the potential change. The potential change is not coincident with the tension change but precedes it by many milliseconds. Thus it is not a secondary accompaniment of the actual contraction or relaxation of the muscle substance.

The paradox state

One of the most intriguing problems in the physiology of crustacean neuromuscular transmission has remained unexplored since it was raised as the result of experiments by Wiersma & van Harreveld (1938) on the doubly-motor-innervated closers of *Blepharipoda* and *Randallia*. They found that stimulation of the fast axon at a low frequency (about 12/sec) evoked large electrical responses but no contraction. By contrast, stimulation of the slow axon at the same frequency evoked a contraction, but the electrical responses were so small as to be hardly detectable. The phenomenon was discovered in experiments utilizing condenser-coupled amplifiers and outside leads, so it was possible that the slow axon was evoking the contractions by depolarization without the presence of rapid junctional potentials. Alternatively, only a few muscle fibres might be active, though giving powerful contractions from the slow system, as may be the case in *Pachygrapsus* closer muscle (Hoyle & Wiersma, 1958*a*). These possibilities could only be resolved with the aid of intracellular recording, which has been used in the present experiments.

Unfortunately, only one large specimen of *Randallia*, which is by far the best species for exposing the muscle surface without damage, has been available. In this specimen it was found that the excitatory j.p.s occurring to both slow- and fast-axon stimulation were remarkably uniform in size in all muscle fibres tested, whether from the proximal, middle or distal part or at the surface of the muscle or from a deep layer. Both the size of the j.p.s and the amount of depolarization were greater for the fast axon than for the slow (Fig. 6). This was true for all fibres examined.

At the start of the experiments there was a contraction in response to fast-axon stimulation at 10/sec and this was larger than the response to the slow axon at the same frequency. But after about half an hour the muscle, in both legs studied, went into the paradox state. Then contraction ceased in response to stimulation of the fast axon at 10–20/sec, although the j.p.s and also the maintained depolarization remained unchanged. The contractions in response to stimulation of the slow axon remained good over the same frequency range.

We have found the phenomenon also in *Blepharipoda*, although the electrical responses were very small and showed some degree of overlap between 'fast' and 'slow' responses in different fibres. It also occurs regularly in the closer of

Cambarus, after the twitch on a single impulse in the fast axon has declined with ageing of the preparation, and we obtained it occasionally in the 'closer' of *Panulirus*. Most other doubly-motor-innervated muscles, however, do not show it. Thus, we have not found it in the extensor of *Panulirus*, which in other ways resembles the closers of *Randallia* and *Blepharipoda* (Hoyle & Wiersma, 1958*a*). For here the fast system gives slow, smooth and powerful

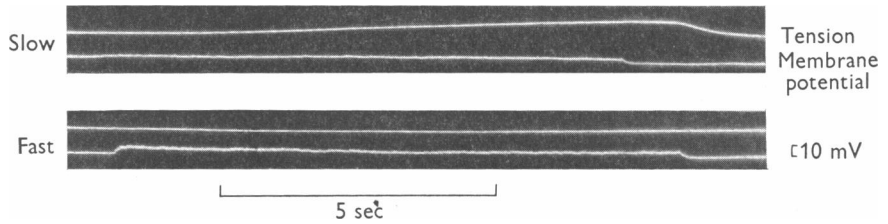

Fig. 6. Relationship between tension (upper traces, increasing tension upwards) and membrane potential in closer of *Randallia*. Intracellular direct-coupled records from same muscle fibre during stimulation of 'fast' and 'slow' axons at 15/sec.

contractions at frequencies of excitation even lower than 10 /sec. At this frequency the largest j.p.s are some 4 mV in height and there is no maintained depolarization (Fig. 7).

When the closer of the *Cambarus* pincher is in the paradox state, the fast junctional potentials (f.j.p.s) are quite large, of the order of 10 mV (Fig. 8). At frequencies at which the paradox is evident there is no appreciable maintained depolarization. The slow junctional potentials (s.j.p.s) are then barely measurable. In most muscle fibres they do not cause any discernible maintained depolarization, but there are a few in which an appreciable one develops. Whether the slow contractions at paradox frequencies can be ascribed in this muscle to those fibres which do show these maintained depolarizations, as in the example illustrated (Fig. 8), is problematical.

The remarkable differences in the mechanical responses of the fast and slow systems in the paradox state and the different associated electrical effects make it improbable that they are simply due to quantitative differences in the release of, and reaction to, a common transmitter substance. Instead, it appears that different chemical transmitters must be liberated at the terminals of the two axons. This adds considerable weight to the arguments advanced in the first paper (Hoyle & Wiersma, 1958*a*) for different transmitter substances in all cases of double motor innervation.

Our findings confirm the existence of the paradox state as a physiological phenomenon in three crustaceans. The intracellular leads show that the findings with external recordings by Wiersma & van Harreveld (1938) are paralleled by similar observations from each individual muscle fibre, with

respect to the relative height of the j.p.s, and that they are not due to different reactions of a mixed population of muscle fibres.

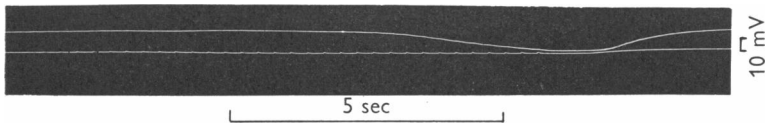

Fig. 7. Tension (upper trace) and membrane potential (lower trace) from extensor muscle of *Panulirus* during stimulation of the 'fast' nerve fibre. Note long period of slow growth of electrical responses, slow summing mechanical responses. The 'slow' nerve fibre did not evoke a mechanical response at this frequency (3·5/sec).

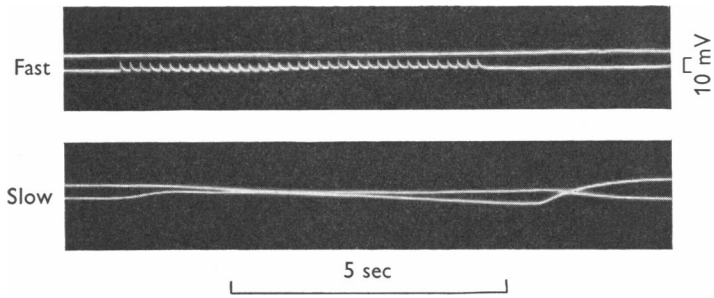

Fig. 8. Closer of *Cambarus* in paradox state. Leads from an exceptional muscle fibre during stimulation at 6/sec. Upper trace, tension; lower trace, membrane potential. Note the maintained depolarization in this fibre during the slow response.

DISCUSSION

The observations described above, particularly those on the paradox, render untenable any hypothesis which couples the tension of muscular contraction directly to the membrane potential. Although it was found that in most of those crustacean preparations which do not have frequent spike and twitch responses there is a correlation between the extent of total contraction and the maintained depolarization level during excitation at higher frequencies, there is often no such correlation at the lowest frequencies which cause contraction.

As an alternative we suggest that the clues to the various problems of excitation lie in the coupling mechanism and the manner of its activation during nervous action. The transmitter substances of most 'slow' and 'opener-stretcher' systems and some 'fast' systems can evidently initiate contraction without at the same time evoking membrane potential changes larger than 1 mV. These substances could be exerting this effect in any of three different ways. In view of the diffuse nature of the innervation they could be themselves crossing the membrane and act on the coupling mechanism directly. Or they could be causing increased permeability of the membrane to a minor ('key') ion selectively so that its movement (inwards or outwards, as the case may be)

affects the coupling mechanism. The movement of calcium ions, for instance, might work as a link in this way. Thirdly, they may initiate the release in the membrane of a substance which in turn diffuses inwards and excites the coupling mechanism. In the first and third possibilities the permeability changes could be incidental accompaniments of the primary action.

The 'fast' transmitter substance produces in the paradox states f.j.p.s which clearly do not affect the coupling mechanism until they reach a critical height. If permeability increase to a 'key' ion is the fundamental process we could interpret their failure to initiate contraction on the basis that the permeability of this 'key' ion is not sufficiently affected by the electrical events of the j.p. In contrast, the 'slow' transmitter action would from the beginning change the permeability to this 'key' ion.

The large spike, when it occurs, must give a large excitatory 'boost' to the coupling mechanism, which may, for example, be brought about by increasing the permeability to the 'key' ion very markedly, if this is the initiating process, or by releasing the substance which effects coupling if that is the pathway. The ionic movements during spiking are different from those occurring during pure j.p.s, particularly in regard to the repolarizing phase. These ionic movements would include movements of the 'key' ion.

There may be muscle fibres in Crustacea which do not contract in response to the changes produced by pure j.p.s alone, but only to secondary responses of the kind which become spikes when large enough. But in other cases both slow and fast j.p.s are able to evoke contraction. The 'slow' system, however, is almost invariably more efficient in this respect, which may denote that the s.j.p.s either give a proportionally greater release of 'coupling-substance' or are more efficient toward changes of the 'key' ion permeability.

The phenomenon of inhibition can be regarded as an uncoupling of the excitatory process by means of an inhibitory transmitter substance. With coincident timing of the arrival of excitatory and inhibitory nerve impulses, this may be associated in some systems with attenuation of the excitatory potentials, but this latter process, earlier called supplemented inhibition, we do not consider itself an inhibitory mechanism. The electrical manifestations of excitatory transmission do not, in any case, as has been argued above, always give an indication of the true excitation. The action of the inhibitory transmitter substance appears to be a direct one, probably on the coupling process. Thus, for example, it may block the transfer of the 'key' ion or prevent its action, or it may prevent the release of a 'coupling substance'. In some such mechanism may be hidden the reason why 'fast' contractions are invariably more difficult to inhibit than 'slow' ones of the same magnitude.

These investigations have shown that the excitatory and inhibitory mechanisms encountered in the eight species of crustaceans studied are of a considerable variety. They do not fall into any simple scheme of classification,

for even in the one outlined above some phenomena, like the effectiveness of spikes in causing contraction and the inability to inhibit them, as well as the generation of secondary processes as a result of the j.p.s, are not accounted for without further hypotheses. Generalizations based on the results of observations on one muscle, or even on several muscles of one species, would be misleading in most instances.

The number of peripheral motor axons is fixed and the total number of nerve cells in the central nervous system is small in Crustacea (Wiersma, 1957). Hence, the plasticity of the neuromuscular junction affords an important site for adaptive changes. Alterations in habitat or in way of life may find an original reflex giving too weak or too strong a contraction for the new need, but by changes in the junctional transmission the adaptive correction can be obtained. It is therefore not difficult to visualize how the many variations of the transmission processes, present in the decapod crustaceans, have come about.

SUMMARY

1. The mechanism is unknown whereby the surface membrane changes of muscle fibres occurring during excitation bring about contraction. It seems that crustaceans, in which there are two or more excitatory mechanisms and also one or two inhibitory ones operating on the same muscle fibre, offer a specially favourable situation for the study of this problem.

2. In many instances the total tension of a muscle can be related to the membrane potential of typical component muscle fibres over a wide range during nervous excitation and inhibition at various frequencies.

3. This relationship breaks down, however, in muscles which exhibit the so-called 'paradox' state (i.e. large electrical and little or no mechanical response to stimulation at low frequency of one motor axon, and the converse response to the second axon).

4. It is suggested that the electrical changes are related to membrane changes or movements of 'minor' (perhaps Ca^{2+}) ions which are more significant in initiating contraction than the electrical potential change itself.

5. It is suggested that the inhibitory transmitter substances act directly on these more significant events when they cause mechanical inhibition; the potential change which may occur is incidental.

6. The great variety of neuromuscular mechanisms encountered in crustaceans is the result of the paucity of motor nerve cells. Elaboration of peripheral mechanisms has formed a means by which in part the evolution of motor function in crustaceans has been achieved.

REFERENCES

BAY, Z., GOODALL, M. C. & SZENT-GYORGYI, A. (1953). The transmission of excitation from the membrane to actomyosin. *Bull. math. Biophys.* **15**, 1–13.

BÜLBRING, E. (1955). Correlation between membrane potential, spike discharges and tension in smooth muscle. *J. Physiol.* **128**, 200–221.

BÜLBRING, E. & LÜLLMAN, H. (1957). The effect of metabolic inhibitors on the electrical and mechanical activity of the smooth muscle of the guinea-pig's taenia coli. *J. Physiol.* **136**, 310–323.

CSAPO, A. & SUZUKI, T. (1957). A preliminary note on excitation-contraction coupling. *Proc. nat. Acad. Sci., Wash.*, **43**, 278–281.

FATT, P. & KATZ, B. (1953*a*). Distributed 'end-plate potentials' of crustacean muscle fibres. *J. exp. Biol.* **29**, 433–439.

FATT, P. & KATZ, B. (1953*b*). The effect of inhibitory nerve impulses on a crustacean muscle fibre. *J. Physiol.* **121**, 374–389.

GELFAN, S. (1958). Muscle. *Ann. Rev. Physiol.* **20**, 67–96.

HILL, A. V. (1950). A discussion on muscular contraction and relaxation: their physical and chemical basis. *Proc. Roy. Soc.* B, **137**, 40–87.

HILL, A. V. & MACPHERSON, L. (1954). The effect of nitrate, iodide and bromide on the duration of the active state in skeletal muscle. *Proc. Roy. Soc.* B, **138**, 325–338.

HODGKIN, A. L. & HOROWICZ, P. (1957). The differential action of hypertonic solutions on the twitch and action potential of a muscle fibre. *J. Physiol.* **136**, 17.

HOYLE, G. (1957). *Comparative Physiology of the Nervous Control of Muscular Contraction.* Cambridge University Press.

HOYLE, G. & WIERSMA, C. A. G. (1958*a*). Excitation at neuromuscular junctions in Crustacea. *J. Physiol.* **143**, 403–425.

HOYLE, G. & WIERSMA, C. A. G. (1958*b*). Inhibition at neuromuscular junctions in Crustacea. *J. Physiol.* **143**, 426–440.

KUFFLER, S. W. & VAUGHAN WILLIAMS, E. M. (1953). Properties of the 'slow' skeletal muscle fibres of the frog. *J. Physiol.* **121**, 318–340.

STEN-KNUDSEN, O. (1954). The ineffectiveness of the 'window-field' in the initiation of muscle contraction. *J. Physiol.* **125**, 396–404.

VAN HARREVELD, A. & WIERSMA, C. A. G. (1939). The function of the quintuple innervation of a crustacean muscle. *J. exp. Biol.* **16**, 121–133.

WIERSMA, C. A. G. (1957). On the number of nerve cells in a crustacean central nervous system. *Acta physiol. pharmacol. néerl.* **6**, 135–142.

WIERSMA, C. A. G. & VAN HARREVELD, A. (1938). The influence of the frequency of stimulation on the slow and the fast contraction in crustacean muscle. *Physiol. Zool.* **11**, 75–81.