

## ON THE EXCITATION OF CRUSTACEAN MUSCLE

## IV. INHIBITION

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(With Five Text-figures)

IN the second paper of this series (Pantin, 1936) the responses of the muscles of various limbs to stimuli of different frequency and intensity in *Carcinus maenas* were described. Particular attention was paid to the flexor of the dactylopodite of the walking legs. If, while the frequency is kept constant, the intensity of the stimulus is gradually raised, the response of the muscle at first remains unaltered. But when a certain critical intensity is reached, the strength of the contraction diminishes. This is true of all the muscles investigated. Even in the crusher-claw adductor a sufficient increase in intensity results in a failure of the response. This failure is due to the excitation of the inhibitory nerve, the threshold of which, in normal preparations, is invariably higher than that of the motor nerve. The existence of inhibitory nerve fibres in crustacean limbs rests upon excellent evidence. The most convincing proofs are those in which the inhibitory nerve supply to the muscle has been stimulated separately from the motor supply to the muscle. These have been given in experiments reviewed and confirmed by Hoffmann (1914) and Knowlton and Campbell (1929).

In the following experiments the same methods were employed as those described in an earlier paper (Pantin, 1936). Silver-silver-chloride electrodes were pushed into the intact meropodite. Stimuli consisted of condenser discharges controlled by a neon lamp. All contractions were recorded isometrically.

## THE NATURE OF INHIBITION

Fig. 1 shows the responses of the cutter-claw adductor to short batteries of stimuli. The responses of this muscle are similar to that of all the others studied except the adductor of the crusher claw. In the figure the effects of increasing the intensity from the threshold upwards is seen for different frequencies. At each frequency the sequence is the same. When the threshold for excitation is passed there is a short range over which the rate of contraction of the muscle is independent of the intensity of the stimulus. Beyond this intensity, the rate of contraction decreases rather suddenly. With further increase of intensity the rate of contraction

remains unchanged. The extent of inhibition is thus independent of intensity once its threshold is passed.

We have already seen in an earlier paper that for simple stimulation of the motor nerve, the rate of contraction increases progressively with the frequency. This relation can be seen in Fig. 1. The figure also shows that exactly the same relation holds during inhibition. When the stimulus is sufficiently strong to cause inhibition, the rate of contraction still increases progressively with the frequency, though at any particular frequency the rate of contraction under the influence of inhibition is less than the normal rate.

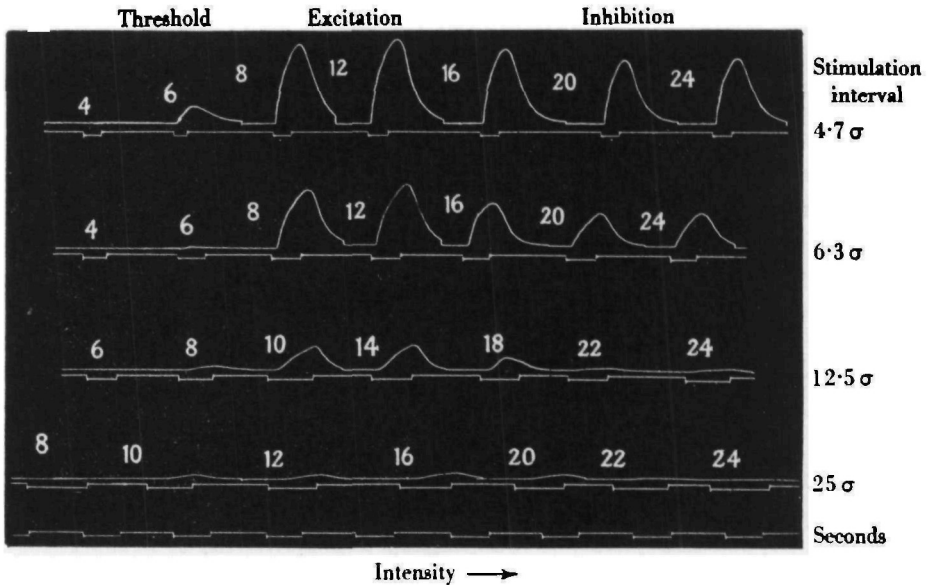


Fig. 1. Responses of adductor of cutter claw to stimuli of increasing intensity at increasing intervals between shocks. Note: at threshold not every shock is effective.

Since, in spite of inhibition, the rate of contraction still increases with the frequency, at a sufficiently high frequency (200–300 per sec.) almost maximal contractions can be attained. Under these circumstances inhibition has barely any influence on the contraction rate (cf. Fig. 1). This has the important consequence that the effectiveness of inhibition rapidly diminishes as the frequency of excitation increases.

The fact that inhibition is scarcely effective at high frequencies and only approaches completion when the interval between stimuli is large has important consequences. In the first place, it completely precludes the possibility that this inhibition is connected with the Wedensky phenomenon. This hypothesis was put forward by Fröhlich (1908) and more recently by Fraenkel-Conrat (1933). The progressive decrease in the effectiveness of inhibition with increasing frequency is precisely the reverse of the condition required for Wedensky inhibition. In the latter, conduction to the muscle is impaired because the impulses are sent down the

nerve at such high frequency that each falls in the relative refractory phase of its predecessor. In *Carcinus* limbs this is only achieved at frequencies of about 300 per sec. But the responses of the muscles to stimuli of this frequency show little or no trace of true inhibition. True inhibition has therefore nothing to do with Wedensky phenomena.

In *Carcinus*, at extremely high frequencies, there does appear to be a decline in the rate of contraction, though contraction does not fail altogether. This effect may be connected with the Wedensky phenomenon, but it is not connected with true inhibition which we have just discussed. The reduction of response at such

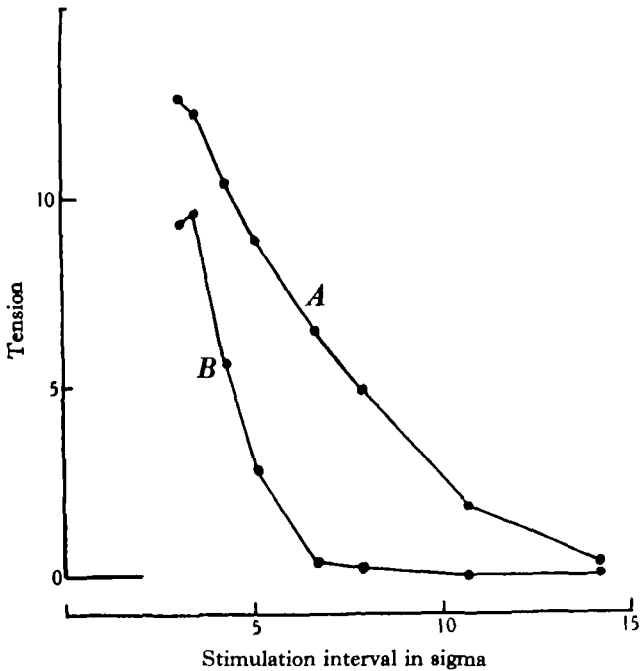


Fig. 2. Tension developed after stimulation for 0.25 sec. Abscissa = time interval between shocks. A, motor excitation. B, motor excitation + simultaneous inhibition.

very high frequencies is evident as soon as the threshold for excitation of the motor nerve is passed, even though the intensity is below the threshold of true inhibition. Such treatment rapidly impairs the neuromuscular junction. For this reason the effect is probably an artefact of no functional significance.

When the motor nerve is excited by a series of stimuli the rate of contraction is controlled by neuromuscular facilitation (Pantin, 1934, 1936). Each successive shock facilitates the transmission of subsequent impulses to more and more of the muscle fibres. This power of facilitation increases as the interval between each pair of shocks becomes shorter, so that the higher the frequency the more rapid the rate of contraction. In Fig. 2 the rate of contraction is shown at different frequencies of stimulation. The muscle employed was the flexor of the dactylopodite of the walking

leg. The rate of contraction is measured as the tension developed after 0.25 sec. of stimulation. The ordinate is the stimulation interval corresponding to the frequency. Curve *A* shows the usual increase in rate of contraction with frequency for simple motor excitation at intensities just above the threshold. Curve *B* is obtained in the same way but for intensities just above the threshold of inhibition. It will be seen that the rate of contraction increases with frequency in both cases, but when inhibition is present the rate of contraction at all frequencies is depressed. The rate of contraction is controlled by neuromuscular facilitation. We thus arrive at the conclusion that inhibition is essentially a depression of neuromuscular facilitation.

#### INHIBITION AND HUMORAL ACTION

There is a significant parallel between this inhibition and the mode of action of certain drugs on the neuromuscular junction. This aspect of inhibition is en-

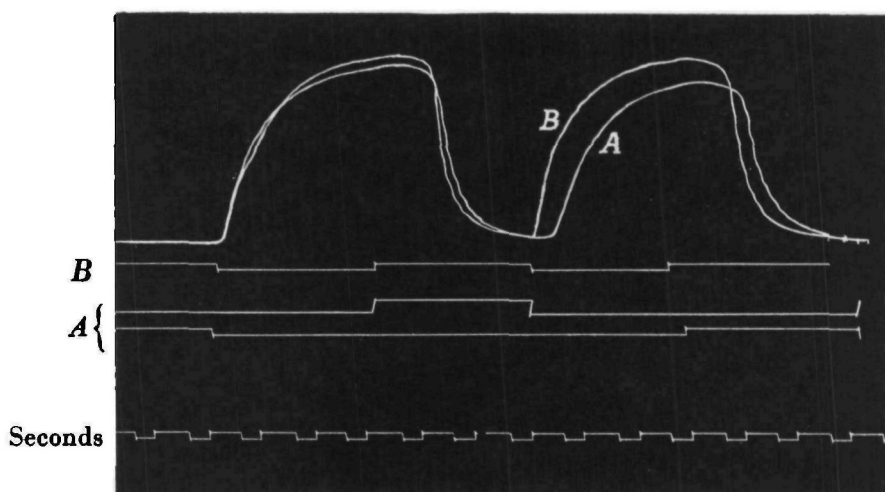


Fig. 3. Curve *A*, inhibition. Curve *B*, temporary absence of stimulation. *A*, lower signal, excitation at 80 per sec. *A*, upper signal superimposed inhibition.

hanced by certain of its properties. It was shown in an earlier paper (Pantin, 1934) that the rate of development of tension in response to a low-frequency stimulus is much slower than the redevelopment of tension following a quick release during a tetanus. This is because the initial development of tension depends on the progressive facilitation of excitation to more and more muscle fibres, while in the quick release all the fibres are already in action. Fig. 3, however, shows that the redevelopment of tension after inhibition is as slow as, or even slower than, the initial development. This means that in a muscle under complete inhibition neuromuscular facilitation has been temporarily abolished, and when inhibition ceases the tension is restored by progressive refacilitation as in the resting muscle.

Biedermann made the important observation that the effects of inhibition sometimes persisted after the inhibitory stimulus had ceased. Further evidence

can be obtained concerning this by comparing carefully the fall and redevelopment of tension produced by complete inhibition with the effects of cessation or commencement of simple excitation of the muscle. In Fig. 3 is shown a prolonged tetanus in response to stimulation at a frequency of about 80 per sec. During the course of this a brief period of inhibition is given. Superimposed upon this tracing is a record of the normal relaxation of the muscle. In this case excitation has been cut off at a moment corresponding to the onset of inhibition in the first tracing. Excitation is subsequently recommenced at a moment corresponding to the cessation of inhibition in the first tracing. In the first tracing there is thus a sequence of excitation, inhibited excitation, excitation; while in the superimposed record there is excitation, no excitation, excitation. The figure shows that the onset of inhibition is such a rapid process that the fall in tension produced by it is identical with that of normal relaxation. On the other hand, the redevelopment of tension after inhibition, though resembling the normal contraction in form, differs from this in an important respect. After inhibition has ceased there is a definite period of delay before redevelopment of tension commences. In normal legs in good condition this delay amounts to about  $\frac{1}{4}$ – $\frac{1}{2}$  sec. In fatigue it may be prolonged to more than a second.

The effect of inhibition presents an analogy to the action of curare on the neuromuscular junction in vertebrate skeletal muscle, and resembles also in many ways the action of various toxic and anaesthetic substances. If the crab leg is perfused with a suitable artificial medium, the responsiveness of the muscle to stimulation of the nerve remains for a considerable period (Pantin, 1934). The addition to the perfusing solution of small amounts of shed crab's blood or muscle extract which have been left exposed to the air rapidly causes failure of the response of the muscle. Experiments show that this failure is, as in the case of inhibition, essentially a depression of neuromuscular facilitation. Increase in the concentration of certain ions such as Mg acts in a similar way. The concentration of Mg in sea water is sufficiently great, compared with concentration of the blood, to exercise a decided depression of neuromuscular conduction. Prolonged perfusion with sea water may even abolish the response of the muscle to stimulation of the nerve.

There is evidence that this is a junctional effect, for under the influence even of high Mg concentration the muscle can still be caused to respond to direct stimulation. The muscle is therefore still active. It is not, on the other hand, the nerve itself which is paralysed, for the work of numerous authors (*e.g.* Furusawa, 1929) shows that the condition of the nerve may be actually improved by bathing in sea water. The region affected must be the neuromuscular junction.

The analogy of inhibition to such chemical actions and the persistence of inhibition after stimulation of the inhibitor nerve has ceased, naturally suggests that inhibition itself has a humoral origin. The work of Dale (1934) and Parker (1932) has shown that humoral action is a widely distributed phenomenon. Attempts were therefore made to see whether inhibition was accompanied by the production of a diffusible substance which could be passed on to independently stimulated muscles.

Two legs from the opposite sides of a crab were taken, and in both the extensor

muscle of the dactylopodite and its overlying chitin were carefully removed. The two legs were then bound together so that the flexor muscles of the two limbs lay over one another. Electrodes were placed in the upper part of each limb and one of the legs was slowly perfused with an artificial solution previously described (Pantin, 1934). The thresholds for excitation and inhibition were found in each muscle. The nerve of the unperfused limb was then excited at a low frequency to give a normal slow contraction for a long period. The muscle was fatigued before the experiment because this fatigue exaggerates the inhibitory effect.

While a low-frequency tetanus was being maintained in this muscle, the other limb was stimulated above the inhibitory threshold, and responded in the usual way by a contraction of subnormal intensity. If an inhibitory substance were formed during this process, the perfusion might be expected to carry it to some extent over the surface of the tetanised muscle and thereby produce inhibition. Out of some twenty successful experiments four gave evidence of inhibition of the second muscle following excitation of the inhibitor nerve of the first. The inhibition was delayed for a period of some 15 sec. after the onset of stimulation of the inhibitory nerve in the first perfusing leg, and disappeared about  $\frac{1}{2}$  min. after cessation of the inhibitory stimulus. Since the large proportion of experiments did not show any obvious effect, the experiments are not conclusive.

#### RECIPROCAL INHIBITION

It is well known that excitation of the nerve in the amputated crustacean limbs may cause reciprocal action of its muscles. In general, the opener or extensor muscle is activated at lower intensities, while at higher intensities this muscle relaxes and the adductor or flexor begins to respond. According to Biedermann (1887) this reciprocal action is due to the relation of excitation and inhibition in the two muscles. He stated that a weak stimulus caused excitation of the opener (= extensor) of the claw, and inhibition of the adductor (= flexor). A strong stimulus inhibited the extensor and excited the adductor. The existence of reciprocal control of the muscles is easily verified, but the present experiments do not wholly agree with Biedermann's interpretation of its mechanism. According to him, the threshold for excitation of the adductor is actually higher than the threshold for inhibition. This condition is the reverse of that shown in Fig. 1. In the present experiments, all the muscles without exception behave as Biedermann found the opener (= extensor) muscle to do, that is, the threshold for excitation was always below the threshold for inhibition.

Before analysing Biedermann's interpretation we will consider what is actually observed to take place in reciprocal action of the muscles. In these experiments, both legs and claws were used. Fig. 4 shows simultaneously recorded responses of the extensor and flexor of the dactylopodite of the walking leg of *Carcinus*. It was found that these could be obtained with the least operational damage to the limb by carefully splitting the dactylopodite from its tip to its base so that each muscle pulled one half independently of the other. The tensions developed in each half

of the split dactylopodite were then registered with weak isometric levers. In Fig. 4 the responses are shown to increasing intensities of stimulus at a frequency of about 200 per sec. applied over the nerve in the meropodite. In the responses of both muscles the first thing to appear is excitation, while at a higher intensity this is converted into inhibition. The only difference is that in the case of the extensor, inhibition is very much more powerful than in the case of the flexor. We have already seen that at frequencies of 200 per sec. inhibition exerts very little effect in the flexor, and the small fall in tension at the inhibitory threshold in Fig. 4 agrees with this. At this same high frequency, on the other hand, inhibition of the extensor is complete.

With fresh legs in good condition, the relations of the various responses are invariably obtained in the order shown. There is first excitation of the extensor, then inhibition of the extensor and excitation of the flexor, and finally inhibition of the flexor. The exact reciprocal nature of the action of the two muscles depends upon the respective thresholds of excitation and inhibition in them. In normal

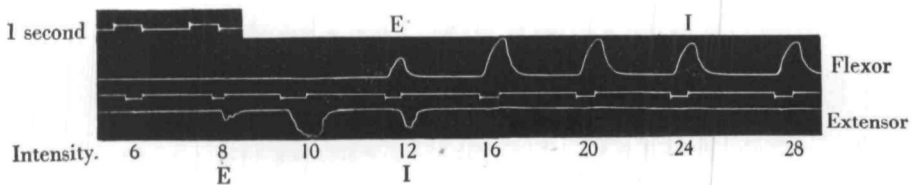


Fig. 4. Reciprocal action in muscles of dactylopodite of leg with increasing intensity of stimulus. Frequency 208 per sec. *E*, threshold of excitation. *I*, threshold of inhibition.

preparations, the threshold for excitation of the flexor and inhibition of the extensor are almost identical. They are, however, never quite the same, and all the thresholds may shift independently, particularly during gross fatigue and other adverse conditions. These shifts may take place to such an extent that excitation of both muscles may take place together or the threshold of inhibition may become so low as almost to abolish excitation. It appears, therefore, that the nice balance of reciprocal responses found in the normal limb is due to the special values of the thresholds of excitability for the excitor and inhibitor nerves supplying each muscle.

The independent relationships of excitation and inhibition in the two muscles can be followed in the intensity-duration curves. Fig. 5 shows a sequence of such curves obtained with *Carcinus* leg using repetitive neon-lamp stimuli of different durations and frequency 20 per sec. The method has been fully described in a previous paper (Pantin, 1936). During fatigue, or under the influence of adverse perfusion media, these four intensity-duration curves can be caused to shift independently so that it would appear that we are dealing with four separate excitable systems, whose arbitrary thresholds in the normal limb are found to be in a certain order so that progressive increase of intensity brings about co-ordinated muscular action.

It is remarkable that in four separate nerves, cut off from all central connection when the limb is amputated, the thresholds should bear so constant a relation to another, when stimulated by arbitrarily placed electrodes.

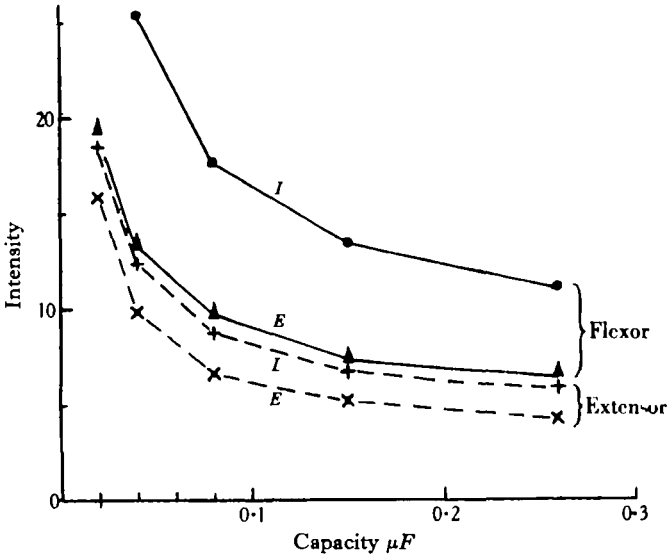


Fig. 5. Muscles of dactylopodite of leg. Relation of threshold for excitation (*E*) and inhibition (*I*) for neon lamp condenser discharges of increasing capacity.

#### DISCUSSION

Biedermann interprets the reciprocal action of the muscles in the crustacean limb by saying that a weak stimulus causes excitation of the extensor muscle and inhibition of the flexor, while a stronger stimulus causes excitation of the flexor and inhibition of the extensor. According to the present experiments, the phenomena are to be interpreted rather differently. A certain weak stimulus causes excitation of the extensor. It does not, however, cause inhibition of the flexor, it simply does not excite it at all. A stimulus of greater intensity will cause inhibition of the extensor and excitation of the flexor, as Biedermann found; but if the intensity of stimulus is raised still more, this is followed by true inhibition of the flexor.

The present experiments differ from those of Biedermann and many earlier workers in two important respects. First, induction shocks were not generally employed as a stimulus; secondly all contractions were recorded isometrically. This second distinction is of importance for where slow contractions are concerned and where inhibitory action depresses the tension which can be developed, highly misleading results can be obtained with isotonic recording levers. With these, significant changes in tension can be entirely overlooked.

Compared with brief condenser discharges such as were used for stimulation in the present experiments, ordinary physiological induction coils supply stimuli of a much more complex character. The fact that make shocks have a different intensity from the break shocks means that there is necessarily an increase in the effective



frequency of excitation as the intensity of the stimulus is raised, and with the strong induction shocks that have frequently been employed on crustacean nerve there is a decided liability to multiple excitation. These factors together cause a very great increase in effective frequency of excitation as the intensity of the stimulus is raised. This increase in effective frequency with intensity greatly complicates the response of crustacean muscle because the extent to which inhibition can be effective depends, as we have seen, on the frequency. At frequencies of stimulation ordinarily obtained with a physiological induction coil (say 100 per sec.) inhibition is complete if the inhibitory nerve is excited. But if the effective rate of excitation is raised to 2-300 stimuli per sec., inhibition is scarcely effective at all.

The responses to the stimuli of an induction coil as ordinarily used follow a complex sequence as the intensity is increased. At ordinary frequencies of stimulation a relatively slow contraction is produced in the muscle as soon as the threshold intensity is passed. A further increase in intensity reaches the threshold for inhibition and the contractions are abolished. But a still further increase in intensity causes strong and rapid contractions to appear, owing to the fact that the effective rate of excitation is now so great that inhibition is ineffective. As suggested in a previous paper (Pantin, 1934) a similar explanation applies to the responses obtained with alternating current of increasing intensity. A similar sequence of slow contraction, inhibition, and rapid contraction, is obtained here also.

Biedermann in his experiments found in the adductor muscle that inhibition was caused at a lower threshold than excitation. He demonstrated this inhibition by its power to suppress tonic contraction in the muscle. Such tonic contraction is probably due to discharge of impulses from the injured nerve (Barnes, 1934). In this tonic condition the slow contraction obtained by stimuli of ordinary frequencies at the true threshold intensity can produce scarcely any additional response. Under these conditions the first effect to be noted is the inhibition of the tonic contraction when the threshold of the inhibitor nerve is reached. A further increase in intensity, as we have seen, raises the effective frequency of excitation so that contractions now appear despite inhibition. There is thus the false appearance of inhibition preceding the threshold for excitation. With condenser discharges of short duration and limbs which do not show marked injury tonic discharges, the complex effects found with induction coils do not occur, and the simple sequence of thresholds, first of excitation, then of inhibition, is found, as in Fig. 1, for every muscle.

In conclusion, it is of interest to consider the functional significance of inhibition itself. Figs. 1 and 3 show the remarkable fact that inhibition is in some ways less effective than mere absence of stimulation. It is only complete at low frequencies of excitation, and is subject to certain delays. This is remarkable, for on *a priori* grounds it would seem that the animal should be able to effect a perfect reciprocal control of its antagonistic muscles without the aid of any inhibitory fibres at all. A consideration of this problem suggests a solution of considerable general interest. Examination of the living animal shows that the muscles of its limbs are normally in a condition of slight tone. The observations of Barnes on the nervous impulses associated with tonic contractions of the muscles allow one to interpret this con-

dition of tone as being due to a background of low-frequency excitation continuously present. The existence of such a low-frequency background of nervous impulses greatly modifies the manner in which the neuromuscular system will normally act. Blaschko, Cattell and Kahn (1931) showed how the interjection of extra stimuli during low-frequency excitation of the nerve in Crustacea could give rise to large and lasting increases of tension in the muscle. This highly important effect we have shown in a previous paper is characteristic of other muscles as well as those studied by these authors. In the presence of a low-frequency background of excitation, therefore, all that is required for the animal to contract its muscles is the momentary interjection of a few extra impulses at a higher frequency. But once this state of contraction is attained, it continues as long as the low-frequency stimulation endures. If in fact the animal maintains a low-frequency background of tonic impulses, there is only one way in which it can bring such tetanic contractions to an end, that is, by inhibition. Fig. 3 shows how effectively this can be done in spite of concurrent excitation of the motor nerve.

We are thus brought to the possibility that the normal mode of control of tension in crustacean muscle is not by the continuous discharge of impulses during the maintenance of a tetanus as in vertebrate skeletal muscle, but that the contraction is initiated by a brief train of impulses of higher frequency and is terminated by inhibition. The state of tension is thus not so much continuously maintained as put into action by a first "word of command", and abolished again by a second.

#### SUMMARY

1. The nature of inhibition in the muscles of the limbs of *Carcinus maenas* has been studied. In all the muscles there is a definite threshold for inhibition which is normally higher than the threshold for excitation. Under the influence of inhibition the rate of contraction of the muscle is depressed. This depression is greatest at low frequencies of excitation. It is scarcely appreciable at higher frequencies. This absolutely precludes the possibility that inhibition is connected with the Wedensky phenomenon.

2. It is shown that inhibition is essentially due to a depression of neuromuscular facilitation, so that compared with normal excitation a higher frequency of stimulation is required to cause a response by summation.

3. Certain evidence suggests that inhibition is of a humoral nature. The effects of inhibition last longer than the duration of excitation of the inhibitory nerve, and there is slight evidence that an inhibitory substance may be transmitted by artificial perfusion so as to cause depression of facilitation in a second muscle.

4. The relation of excitation and inhibition in the reciprocal action of the extensor and flexor muscles of the dactylopodite of the limb is examined. In both muscles excitation occurs at a lower intensity than inhibition. The reciprocal responses obtainable in the amputated limb are due to the respective thresholds of the excitor and inhibitor nerves of each muscle. A weak stimulus excites the extensor, but has no action on the flexor. A strong stimulus inhibits the extensor

and excites the flexor, these thresholds being close together. A still stronger stimulus inhibits the flexor. This interpretation differs from that originally given by Biedermann, and the reasons for this are discussed.

5. The functional significance of inhibition is discussed. It is pointed out that as a means of bringing contraction to an end inhibition is less efficient than mere absence of excitation. But it is also pointed out that in the resting limb there is probably a background of low-frequency excitation continually present. By itself this suffices only to maintain the muscle in tone. Under these conditions, however, the muscle can be made to contract by a brief series of rapid stimuli (Pantin, 1936). Such a contraction once produced is thereafter maintained and can only be brought to an end by inhibition. This mechanism is contrasted with neuromuscular action in the skeletal muscle of the vertebrates.

Part of this work was done while holding the Cambridge University Table and the Bidder Fund at the Stazione Zoologica, Naples. I wish to express my sincere thanks to Dr R. Dohrn and his staff for their hospitality and for the great facilities they gave me. I also wish to thank my wife for valuable help during this work. The cost of some of the apparatus was defrayed by a grant from the Government Grant Committee of the Royal Society.

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