

# A COMPARATIVE STUDY OF PERIPHERAL INHIBITION IN DECAPOD CRUSTACEANS

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(With Two Text-figures)

## INTRODUCTION

ALL the peripheral muscles of the decapod Crustacea receive, as far as is known, at least one inhibitory axon (Wiersma, 1941). A detailed study of the mechanism of this peripheral inhibition has been made for only a few muscles. Marmont & Wiersma (1938) examined especially the inhibition of the opener muscle of the crayfish, and Wiersma & Helfer (1941) the inhibition of the opener and closer muscles of the crab, *Cancer antennarius*. Although these studies were limited to so few muscles, significant differences in the nature of the inhibitory mechanisms of these preparations were found. The distribution of the inhibitory fibres has since been worked out for species of the *Astacura*, *Palimura*, and *Brachyura*, both for the anatomical relationships and the functional response to stimulation of the isolated inhibitory fibres (Wiersma, 1941). These recent additions to the knowledge of the distribution of inhibitory axons have made a more extensive comparative study of inhibitory mechanisms possible.

*Patterns of innervation.* In order to interpret the results of the present investigation it will be necessary to review briefly the distribution of efferent fibres for the muscles in the distal parts of the leg, as reported by Wiersma (1941). As can be seen in Fig. 1, innervation of these muscles in the three above-mentioned groups is identical with respect to the number and distribution of motor fibres. The inhibitory innervation consists always of three nerve fibres which show differences in distribution between the groups. These distribution patterns of the inhibitors may be briefly described as follows: In all three groups the *opener* muscle (abductor of dactylopodite) and the *main flexor* (of carpopodite)<sup>1</sup> are innervated by one inhibitory fibre (I). The second inhibitor (II) innervates the *stretcher* (extensor of propodite), and in *Panulirus* and the crabs runs only to this muscle. In *Cambarus*, however, the stretcher inhibitor also innervates the *closer* muscle (adductor of dactylopodite). The third inhibitor (III) shows great variation

<sup>1</sup> This has been established with certainty for *Panulirus* only, but is also very likely for *Cambarus* and the crabs.

in its distribution in the three groups. In *Cambarus* it innervates the *bender* (flexor of propodite) and the *extensor* (of carpopodite). In *Panulirus* it innervates the closer, the bender, the extensor, and the *accessory flexor* (of carpopodite).<sup>1</sup> In the crabs the third inhibitor is the 'common' inhibitor of Wiersma (1941), which innervates the opener, the closer, the stretcher, the bender, and the extensor, thus giving the opener and the stretcher a double inhibitory innervation consisting of the respective true inhibitors and the common inhibitor. In the paper mentioned, this way of innervation was studied only in *Cancer anthonyi*, but we found it to be exactly the same in the other species of crabs which have been used in this investigation, and in *Randallia ornata*. Since the latter species belongs to a different superfamily of the Brachyura, it is very likely that all true crabs show the same pattern.

In the following pages, in speaking of inhibition of the stretcher contraction of *Cambarus*, for instance, the term 'stretcher inhibitor-stretcher system' will be used, while in the tables roman numerals indicate the inhibitory fibres.

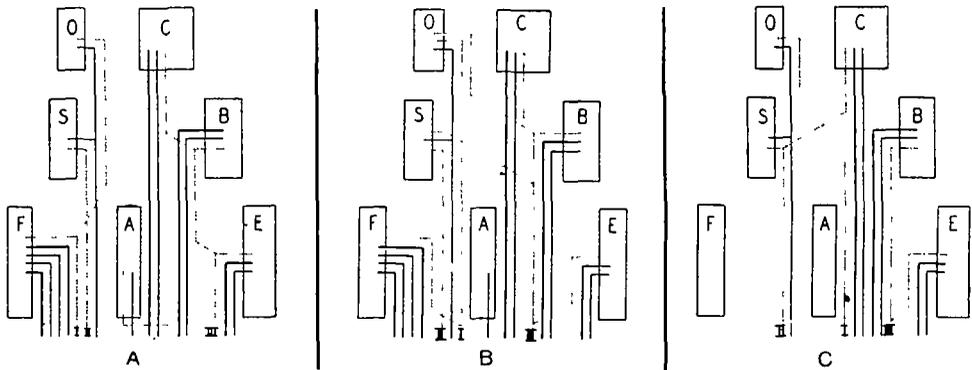


Fig. 1. Scheme of the innervation of the chelipeds and legs. A, *Panulirus*. B, crabs. C, *Cambarus*. Each line represents one axon. Full drawn lines= motor fibres, dotted lines= inhibitory fibres. Notice the similarity in number of the fibres in the different cases and the difference in distribution of the inhibitors. The muscles are represented by the letters: O= opener, C= closer, S= stretcher, B= bender, F= main flexor, A= accessory flexor, and E= extensor.

#### METHODS

The method of preparing single motor and inhibitory fibres which has been used throughout this investigation has been described in detail for the same preparations which were used here (Wiersma, 1941). In order to study the effectiveness of inhibition essentially the same method was used as that reported by Marmont & Wiersma (1938). In this method the lowest frequency of inhibition which will just suppress any shortening in the muscle when excitation and inhibition are started at the same time is determined for different frequencies of excitation. The necessary stimulating frequencies were obtained with two thyatron stimulators each of which had a range of frequencies more than ample to cover the physiological range. The stimulators were calibrated and were periodically checked as to frequency; in general only small and insignificant variations in the calibrations were found.

<sup>1</sup> This innervation of the accessory flexor was first demonstrated during the present investigation. In the crabs and the crayfish the innervation of this muscle has not yet been found.

The strength of stimulation was always kept low so as to obtain an impulse on each stimulus and yet avoid repetitive discharges. The completeness of the suppression of the contraction was determined by visual observation either with or without the aid of a binocular microscope, or by recording with an isotonic lever. Comparison of the values obtained by these methods showed no appreciable difference.

To study the effect of inhibition of the muscle action potentials, an apparatus was used giving series of two shocks at various frequencies. (In the present investigation a frequency of 45 per sec. was usually employed.) The interval between the two shocks could be varied from 0 to 8 msec. This apparatus has been described by Keighley and was used for the study of the potentials of the opener and closer of *Cancer anthonyi* (Wiersma & Helfer, 1941).

The animals which were used in the present investigation were, for the Astacura, *Cambarus clarkii*; for the Palinura, *Panulirus interruptus*; and for the Brachyura, *Cancer anthonyi*, *C. antennarius*, *Pachygrapsus crassipes*, and *Loxorhynchus grandis*.

## RESULTS

Determinations have been made for most of the inhibitory innervations as to (A) the effect of frequency of excitation on  $R_c$  ( $R_c = F_i/F_e$ : that is, the ratio between frequency of excitation,  $F_e$ , and frequency of inhibition,  $F_i$ , necessary just to suppress the contraction), (B) the  $R_c$  values of the different systems, and (C) the presence or absence of supplementary inhibition.

### A. THE EFFECT OF FREQUENCY OF EXCITATION ON $R_c$

Marmont & Wiersma (1938) have shown that in the opener of *Cambarus*  $R_c$  is remarkably constant for different frequencies of excitation. In the slow closer system, on the other hand, they found that only at low frequencies of excitation could an  $R_c$  value be determined, and that at slightly higher  $F_e$  inhibition was always incomplete no matter how high  $F_i$  was made. Most of the systems which have been investigated are less easily inhibited than the opener but more easily inhibited than the slow closer of the crayfish. It was found that the majority of the preparations in every system showed a remarkable constancy of the ratio at different frequencies within a certain range. Several examples of this constancy have been listed in Table 1. It should be noted in this table that in the preparation of the stretcher system of *Panulirus* the ratio remains constant despite a tenfold increase in  $F_e$ .

In systems in which  $R_c$  is high it is invariably found that at higher  $F_e$  the system cannot be completely inhibited with any  $F_i$ . Inhibition thus becomes ineffective above a certain frequency of excitation. These systems thus resemble the slow closer of the crayfish, but none other shows such an extreme case. In these systems it is easier, therefore, to find how this change in  $R_c$  is established. Of the two possibilities, (a) a gradual increase of  $R_c$  with an increase in  $F_e$ , or (b) a sudden change to a state in which inhibition is only partial; the latter is the one which is found. Sometimes a small range of  $F_e$  in which an increase in  $R_c$  seems to be present is

Table 1. *Constancy of R<sub>c</sub> with changes in frequency of excitation*

Animal	Inhibitor	Excitor	<i>F<sub>i</sub></i>	<i>F<sub>e</sub></i>	<i>R<sub>c</sub></i>
<i>Cancer anthonyi</i>	I and II	Stretcher-opener (both contractions)	12	20	0.60
			15	25	0.60
			18	30	0.60
			27	45	0.60
			37	60	0.62
			60	100	0.60
<i>Panulirus</i>	II	Stretcher	11	20	0.55
			16	30	0.53
			26	45	0.56
			35	60	0.58
			55	100	0.55
	105	200	0.53		
	III	Fast bender	36	30	1.20
			55	45	1.22
			70	60	1.17
119			100	1.19	
<i>Pachygrapsus</i>	III	Slow bender	15	45	0.33
			20	60	0.33
			35	100	0.35
			40	120	0.33

Table 2. *Effect of different frequencies of excitation on R<sub>c</sub> in cases in which inhibition becomes incomplete at high frequencies of excitation*

Animal	Inhibitor	Excitor	<i>F<sub>i</sub></i>	<i>F<sub>e</sub></i>	<i>R<sub>c</sub></i>
<i>Pachygrapsus</i>	III	Opener	55	45	1.22
			75	60	1.25
			—*	100	—
			75	60	1.25
<i>Panulirus</i>	III	Fast closer	60	45	1.33
			79	60	1.32
			—*	100	—
			80	60	1.33

\* *F<sub>i</sub>* 180 did not suppress *F<sub>e</sub>* 100, but completely suppressed *F<sub>e</sub>* 60.

Table 3. *Effect of different frequencies of excitation on R<sub>c</sub> in cases in which R<sub>c</sub> becomes lower at higher frequencies of excitation*

Animal	Inhibitor	Excitor	<i>F<sub>i</sub></i>	<i>F<sub>e</sub></i>	<i>R<sub>c</sub></i>
<i>Cancer antennarius</i>	I	Opener	17	30	0.57
			26	45	0.58
			35	60	0.58
			55	100	0.55
			62	120	0.51
			110	200	0.55
			60	300	0.20
			60	300	0.20
<i>Cancer antennarius</i>	II	Stretcher	16	30	0.53
			25	45	0.57
			37	60	0.61
			50	100	0.50
			60	120	0.50
			100	200	0.50
			80	300	0.26
			80	300	0.26

noticed, but this range is usually very limited in its extent. Thus, if  $Fe$  values are taken which are not too close together one will show the  $Rc$  which is constant, while the higher frequency cannot be inhibited (Table 2). The frequency of excitation at which inhibition in such systems becomes incomplete differs greatly. For example, the slow closer of *Cambarus* ceases to be completely inhibitable at a  $Fe$  of about 10 per sec.; for most of the fast closers the frequency is about 80–100; and in some preparations of slow systems the contractions fail to be completely suppressed at a  $Fe$  of about 200 per sec. That fatigue of inhibition can have an influence on these phenomena is shown by the fact that on repetition at one  $Fe$  a contraction may show at first the normal  $Rc$ , then show a state of only partial inhibitability, and, after rest, again show the normal ratio. At the higher  $Fe$ 's, however, even a completely fresh preparation will be only partially inhibitable. In these cases  $Rc$  cannot be applied to the incompletely inhibited states as it is by definition 'just complete' inhibition.

Some systems show an entirely different picture. In these, instead of being but partially inhibited at high  $Fe$ ,  $Rc$  drops suddenly to a much lower value. This occurs especially in systems in which  $Rc$  is normally quite low (see Table 3). It should be noted that in both cases the  $Fi$  which completely suppresses the contraction at  $Fe$  300 is even lower than the value which was necessary at  $Fe$  200.

Whereas the  $Rc$  of the large majority of all the preparations of the different systems was quite constant with frequency, there were a very few scattered preparations in which a definite increase in ratio took place and in some others a decrease. It is apparent that these phenomena are associated with some particular property of the individual preparation and are not of general occurrence. We have therefore omitted values of this sort from further consideration, and whenever such a preparation was encountered the values were considered not trustworthy and were discarded.

It can thus be concluded that frequency has no influence on  $Rc$ . This does not mean, however, that  $Rc$  is constant in every system, but that such inconstancies as occur are caused by factors other than the frequency of excitation. In the determination of the  $Rc$  of different systems these factors form a disturbing influence, as will be clear from the following paragraphs.

#### B. THE $Rc$ VALUES OF THE DIFFERENT SYSTEMS

In respect to constancy of  $Rc$  in one preparation of one system and of the same system in different preparations, the systems fall into three groups: (a) In a number very little variation is found in different determinations. (b) The second group is composed of those which show a rather widespread variation both within the same preparation and between different preparations. (c) A third group shows two distinct ratios each of which is usually constant.

*Group (a).* In systems with a constant ratio the variations of  $Rc$  hardly ever surpass the limits set by the accuracy of the method, namely, about  $\pm 8\%$ . Examples

of this group are the opener of *Cambarus* and *Panulirus*, the stretcher of *Panulirus*, and several other systems, including the slow bender of *Pachygrapsus*, which is the most easily inhibited of any of the systems studied.

*Group (b)*. Variable ratios are found most commonly in preparations which show a high ratio. It is likely that here the factors of facilitation and fatigue of the contraction and of the inhibition play a large part, which explains the difference in values often obtained in one preparation. It is possible, nevertheless, that there are here also real and rather large differences in the effectiveness of inhibition in different preparations. Though in this group the limits are much wider than in those in which the ratio is constant, it is quite possible to determine a mean value which in most cases does not differ from the extremes by more than  $\pm 15\%$ . Examples of this group are the closer inhibitor-fast closer systems of all the animals in which inhibition of this system is possible. In Table 5 the mean values alone are given for such variable systems and are marked with an asterisk.

Table 4. *Constancy of more than one ratio in the same system*

Animal	Inhibitor	Excitor	$F_i$	$F_e$	$R_c$	$F_i$	$F_e$	$R_c$
<i>Loxorhynchus</i>	I	Opener	13	30	0.43	17	30	0.57
			20	45	0.45	25	45	0.55
			27	60	0.45	34	60	0.57
			44	100	0.44	53	100	0.53
<i>Loxorhynchus</i>	II	Stretcher	12	30	0.40	14	20	0.70
			20	45	0.44	22	30	0.73
			27	60	0.45	33	45	0.73
			47	100	0.47	44	60	0.73
<i>Panulirus</i>	III	Slow extensor	26	45	0.58			
			35	60	0.58			
			63	100	0.63			
			11	25	0.44			
			13	30	0.43			
			22	45	0.49			
			26	60	0.44			
47	100	0.47						

*Group (c)*. The true opener inhibitors of the crabs are examples of systems which show two distinct values for  $R_c$ , each of which is remarkably constant. In this group one preparation may jump from one value to the other in two successive determinations, though most frequently the ratio in one preparation is constant at one value while that of another preparation (which may be of another leg of the same animal) is constant at the other. In Table 4 the values have been given for *Loxorhynchus* and for the slow extensor of *Panulirus*; in the two muscles of the crab the values are from different preparations, in the lobster the values were found in the same preparation.

The results of the determinations of  $R_c$  are given in Table 5 and Table 6. In Table 5 the ratios for the opener, closer, stretcher, and bender of all the animals are given, while Table 6 presents the results obtained from the extensor, flexor, and accessory flexor in addition to those from the four distal muscles of the lobster.

*Effectiveness of inhibition in the opener muscle*

The opener inhibitor of *Cambarus* has been previously reported to have a very effective inhibitory effect, giving an *Rc* of 0.41 (Marmont & Wiensma, 1938). In the present investigation in which more preparations have been used our mean value was also 0.41.

The opener inhibitor of *Panulirus* gives a ratio of 0.50, which indicates that exactly two excitatory impulses can be suppressed by a single inhibitory one.

The true opener inhibitor of the crabs shows, as has been pointed out, two distinct ratios. Both these ratios are about the same in the two species of *Cancer*, and are definitely higher than the corresponding ones in *Pachygrapsus* and *Loxorhynchus*, the lower ones in the *Cancers* being of the same order as the higher ones in the two other species.

The ratio for the common inhibitor in the crabs is always definitely higher than any of the ratios of the true opener inhibitor, and the values are in general much more variable. The ratios in the two species of *Cancer* for this inhibitor are, in contrast with the ones of the true opener inhibitor, lower than those of the other two genera.

Table 5. *Rc* values for the inhibitory systems of the four most distal muscles

Animal	Opener		Stretcher		Fast closer III	Slow closer III	Fast bender III	Slow bender III
	I	III	II	III				
<i>Panulirus</i>	0.50	—	0.5*	—	1.25*	0.80	1.25	0.75
<i>Cambarus</i>	0.41	—	0.41	—	∞	5*	1.25*	0.70
<i>clarkii</i>			0.65					
<i>Cancer</i>	0.60	1.0*	0.5	1.0*	1.0*	0.75*	1.00	0.75
<i>anthonyi</i>	0.75		0.6					0.55*
<i>Cancer</i>	0.53	0.93	0.53	1.0*	1.3*	0.65*	1.1*	0.50*
<i>antennarius</i>	0.75		0.75			0.95*		
<i>Loxorhynchus</i>	0.45	1.5*	0.45*	1.5*	1.5*	1.0*	1.4*	0.58
<i>grandis</i>	0.56		0.75					
<i>Pachygrapsus</i>	0.40	1.40*	0.45*	1.25	∞	0.45*	∞	0.33
<i>crassipes</i>	0.50		0.65			0.6		

\* Indicates that these systems show a rather wide variation.

*Effectiveness of inhibition in the stretcher muscle*

The ratio of the stretcher inhibitor system is in most cases very similar to that of the opener inhibitor system. In the majority of the preparations in all animals exactly the same ratio was found for the two systems when both inhibitors were stimulated simultaneously on the same electrodes.

Sometimes exceptions are encountered in certain preparations, although it is never found that a constant difference occurs in all preparations of a certain system. In some systems it is the opener, in others the stretcher, which is occasionally more difficult to inhibit.

In *Cambarus* the stretcher shows besides the same value as the opener a second value which is higher. In *Panulirus* the ratio is less constant than that of the opener, but does not show a definite second ratio.

The true stretcher inhibitor of the crabs shows two ratios which are not always exactly the same as those of the opener. Both lower and higher values are found, as can be seen from Table 5. In *Loxorhynchus* this is clearly demonstrated in Table 4, in which the lower values for the two muscles are about the same, but the higher values are different, that of the stretcher being noticeably higher.

The common inhibitor-stretcher systems of the different crabs give essentially the same ratios as those of the common inhibitor-opener systems of the same species.

#### *Effectiveness of inhibition in the closer muscle*

In all of the animals the slow closer contraction can be inhibited, and in most of them with relative ease. The most outstanding exception is the slow closer contraction of *Cambarus*, which, as was pointed out by Marmont & Wiersma (1938), can be inhibited only at low frequencies of excitation and at these only with difficulty. The very high  $R_c$  which they reported (6.2) has been confirmed by our observations, which gave ratios of the same order, approximately 5.0. The other slow closer systems show a range from about 1.0 in *Loxorhynchus* down to values as low as 0.4 in *Pachygrapsus*.

The fast closer contraction is not inhibitable in either *Cambarus* or *Pachygrapsus*. In both of these cases the muscle responds with a single twitch to a single impulse in the fast fibre, although this contraction in *Pachygrapsus* is relatively weak and in *Cambarus* is very strong. Not only was it impossible to inhibit the twitch contractions, but inhibition also was found to have no effect whatsoever on tetanic contractions. In the other animals where no visible contraction is obtained on a single impulse, there is definite inhibition although the ratios are either about 1.0 or well above it.

#### *Effectiveness of inhibition in the bender muscle*

As in the closers there are always two types of contraction in the bender. Again, the slow contraction is in every case easier to inhibit than the fast. In general the values for the fast contractions are about the same as those for the fast contractions of the closer muscle in the same species, but *Cambarus* presents a most interesting exception. In this animal both the slow and fast bender contractions are easily inhibited, the slow showing a ratio of about 0.7, the fast one of 1.2, values which check closely with those obtained by Marmont & Wiersma (1938), even though the latter determinations were made on a much smaller number of preparations. In *Pachygrapsus*, however, the fast bender is, as the closer, uninhibitable. In this animal, in contrast to *Cambarus*, a single impulse in the fibre for the fast bender gives a rather strong twitch contraction of the muscle. The fact that the slow bender contraction of *Pachygrapsus* shows a very low and constant value of 0.3, the lowest ratio obtained in any preparation, has already been mentioned. In general the slow bender contractions appear to show a definitely lower ratio than the corresponding slow closer contractions.

*The effectiveness of inhibition in the muscles of the meropodite of Panulirus*

As has been mentioned, in Table 6 are summarized the results from the muscles of the meropodite in the lobster. There are several points on which some comment should be made. The fast extensor contraction is here rather easily inhibited and yields the lowest value found for any fast contraction, namely, 0.80. It is interesting to note that this value for the *fast* extensor is much lower than that of the other fast systems innervated by the same inhibitor (closer and bender), and corresponds quite well with the values obtained for the similarly innervated *slow* bender and *slow* closer.

The four contractions of the main flexor show clearly that the 'slower' the system is the more easily it is inhibited. This enlarges the findings of van Harreveld & Wiersma in this muscle (1939). It may be noted that only the *slowest* flexor contraction is inhibited with about the same effectiveness as the opener which is inhibited by the same fibre. The fastest of the main flexor contractions is very difficult to inhibit, and the values tend to be quite inconstant, ranging from about 1.5 to 3.0.

Table 6. *Rc* values for the inhibitory systems of *Panulirus interruptus*

System	Opener I	Stretcher II	Closer III		Bender III	
			Fast	Slow	Fast	Slow
<i>Rc</i>	0.50	0.5*	1.25*	0.80	1.25	0.75

System	Extensor III		Accessory Flexor III	Flexor I			
	Fast	Slow		Fast	2nd fast	2nd slow	Slow
<i>Rc</i>	0.8	0.45 0.6	0.60	2.0*	0.80	0.70	0.52

\* Indicates that these systems show a rather wide variation.

## C. THE PRESENCE OR ABSENCE OF SUPPLEMENTARY INHIBITION

Marmont & Wiersma (1938) have shown that in the opener of *Cambarus* reduction of the muscle action potentials during inhibition is present only when the inhibitory impulses arrive within a restricted time limit before the excitatory ones. Inhibition in which the muscle action potentials are reduced has been called supplementary as against simple in which no reduction occurs. Wiersma & Helfer (1941) have shown that in certain inhibitor systems of *Cancer* no supplementary inhibition can be obtained no matter at what interval the excitatory and inhibitory impulses arrived at the muscle. These systems, therefore, show simple inhibition only. This was shown to be the case in the inhibition of the slow closer and of the opener by the common inhibitor. The true opener inhibitor of *Cancer* did, however, show supplementary inhibition which is in accord with the findings of Marmont & Wiersma (1938) on several other crabs. The present investigation concerns itself mainly with the determination of which systems are capable of giving supplementary inhibition and which ones are not. In contrast with the earlier papers the quantitative effects were treated more superficially.

In *Cambarus* it was found that besides the opener, the stretcher can be made to show reduction in muscle action potentials during inhibition. Marmont & Wiersma (1938) described the phenomenon for the opener of the crayfish thus: 'the action currents are reduced somewhat gradually at the onset of the supplemented inhibition but rise immediately (rebound) to their normal height when the inhibiting impulses are stopped.' In the present investigation the gradual onset and sudden rebound was also observed for the stretcher system (Fig. 2A). No sign of reduction of the action potentials was found in the inhibition of the bender and closer contractions in the crayfish. It is of particular interest to note that the inhibitor which produces the phenomenon in the stretcher fails to do so in the closer.

It was found possible to elicit supplementary inhibition in both the true opener inhibitor and the true stretcher inhibitor system in all the crabs (Fig. 2B). In all cases the supplementary inhibition shows the characteristics reported by Wiersma & Helfer (1941) for the opener system of *Cancer*, namely, the gradual depression at the onset of inhibition and the gradual growth upon release in contrast to the

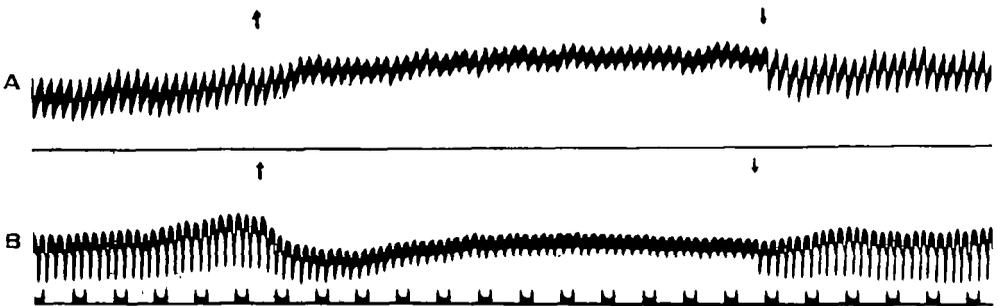


Fig. 2. Supplementary inhibition of action potentials of the stretcher muscle. A, *Cambarus*. Notice the immediate rebound to full height at the end of inhibition. B, *Cancer anthonyi*. At the end of inhibition the action potentials grow gradually to full height.  $\uparrow$  = onset of inhibition.  $\downarrow$  = release of inhibition. In both cases the inhibitory stimulus was given 2 msec. before the excitatory one. Frequency 45 per sec. Time 0.1 sec.

immediate rebound of the crayfish. In no case could supplementary inhibition be obtained with the common inhibitor. This enlarges the findings of Wiersma & Helfer (1941) with this inhibitor for the opener and the closer to include the other muscles which it innervates. Especial attention has been paid to the inhibition of the slow bender contraction of *Pachygrapsus* because it is the most easily inhibited contraction. Nevertheless, we have never obtained any signs of supplementary inhibition in this muscle. It should be pointed out in this regard that the slow closer and the slow bender contractions of *Pachygrapsus* do not lend themselves very well to this sort of investigation because of the small magnitude of the muscle-action potentials which they give (in sharp contrast to the very large magnitude of the fast-action potentials of these muscles). We have not paid especial attention to the presence or absence of the small depression of facilitation and the subsequent growth of the muscle-action potentials on release of simple inhibition which Wiersma & Helfer (1941) reported, but noted that it was present in several cases.

In *Panulirus* not one of the systems has ever shown any signs of supplementary inhibition. The four most distal muscles and the extensor have been carefully examined in this respect. As the complete absence of action-potential reduction in at least the opener and stretcher muscles was unexpected, a large number of preparations of the opener and of the stretcher have been made, but not one of these has given any indication of the phenomenon.

#### DISCUSSION

It is surprising that the ratio of the frequency of inhibitory impulses to the excitatory impulses for just complete inhibition is constant with changes in the frequencies of stimulation. Such constancy has been seen in nearly all of the preparations observed. The question arises how this constancy of  $R_c$  can be brought about. It is a well-established fact that in the muscles of decapod Crustacea the strength of a contraction depends on the excitatory frequency, and that with increase in frequency there is in these muscles not only an increase in contraction strength by algebraic summation but also by facilitation,<sup>1</sup> which results in successive nerve impulses becoming more and more effective. The effect increases the quicker the impulses follow each other. That this facilitation does not make contractions obtained on higher frequency stimulation relatively more difficult to inhibit than at low frequencies, which would result in an increase in  $R_c$ , can find its explanation only in a simultaneous increase in effectiveness of the inhibitory processes. It can be proven that this is indeed the case. If inhibition is started before excitation it is found that it can completely suppress a contraction caused by a frequency of excitation which it cannot suppress if both are started at the same time. Wiersma & van Harreveld (1934) found in *Eupagurus bernhardus* that if a frequency of inhibition is selected which cannot suppress but can only slightly reduce a test contraction when both are started at the same time, continued stimulation of the inhibitor suppresses successive test contractions more and more until fatigue of the inhibitory mechanism sets in and the contractions become again less completely suppressed. Under the same circumstances all inhibitory systems were observed to give similar results. The counterpart of this experiment, i.e. to test the influence of short inhibitions during continuous stimulation of the excitor, was performed with the following result: stimulating at frequencies near  $R_c$  values the effect of the inhibitions becomes gradually less and less, even during the time in which the contraction begins to diminish through fatigue. This shows that facilitation of the contraction makes it less inhibitable, even if the mechanical effect diminishes. It can thus be concluded that both excitation and inhibition undergo facilitation and that these facilitations balance each other at the  $R_c$  values.

Although the  $R_c$  value gives a measure of the relative rate of the facilitations of

<sup>1</sup> Facilitation of excitation has been shown to work at two places, for there is a facilitation of the action potential and of the contractile mechanism (Wiersma & van Harreveld, 1939). In the present discussion we have taken these two together, thus deliberately simplifying the picture. It seems possible that in the inhibitory process two similar facilitations are active, but at present it is not fruitful to discuss the theoretical consequences of such an arrangement.

excitation and inhibition of a system it is not possible to measure the absolute rates. However, the results obtained point to some conclusions. In a doubly motor innervated muscle inhibited by one fibre it is likely, for instance, that the two ratios for the two contractions can be taken as a measure of the relative strength of the excitatory facilitations. It thus becomes evident that in such cases as the fast closer contraction of *Cambarus* and the fast closer and fast bender contractions of *Pachygrapsus*, which are uninhibitable at any frequency, the ineffectiveness of inhibition seems to be directly associated with the excitatory process. In the case of the opener and stretcher, which usually show the same ratio, it is logical to conclude that the two facilitations, inhibitory and excitatory, of these muscles are the same. In the cases in which there is a difference in the ratios of these two muscles this will be most probably due to a difference in the inhibitory facilitation, since it seems unlikely that the excitatory facilitation of the same motor axon would change in one muscle and not in the other.

Since they share a single inhibitory fibre it might appear logical to consider the similarity of the  $R_c$  values for the fast extensor, the slow bender, and the slow closer systems of *Panulirus* as indicative of a similarity in the facilitation of the excitatory processes of these contractions. That such is not necessarily the case, however, is seen in *Cambarus*, in which the closer and stretcher are innervated by the same inhibitor, yet the stretcher contraction gives  $R_c$  values which are of the order of one-tenth those of the slow closer contraction. Any tenfold difference between the facilitation of these contractions is certainly not present.

It is clear that constant ratios depend on a great number of factors. If there is still facilitation of excitation or of inhibition from a preceding volley the ratio will shift to one side or the other. This will show at the same frequency on repetition. This factor can be excluded, however, by allowing sufficient time between stimulations and also by very short durations of the tests. Both have been utilized as much as practicable in the experiments. That the fast systems have, nevertheless, given rather inconstant ratios may well be due to the fact that these systems are generally rather inconstant. For instance, repetition of the same stimulation often results in contractions which are noticeably different, even though a prolonged rest period is given, which may well indicate variations in the facilitation of the excitation.

The appearance in certain preparations of two often well-defined ratios cannot be considered to be due to such an inconstancy. This type of variation is presumably due to a shift of one or the other of the facilitations to a different level which is the same in different preparations. This shift must be a very sudden one in those instances in which the second level was observed shortly after the first in the same preparation. The reasons for such a shift and for the constancy of the two levels are unknown.

The incomplete inhibitability of certain preparations at the higher frequencies of excitation which are completely inhibitable at lower frequencies is, however, presumably not due to any peculiarity of the facilitations. The most likely explanation is that the inhibitory mechanism is stimulated with too high a frequency,

and that certain of the impulses fail to have an effect. That this explanation is reasonable is shown by the fact that in preparations in which the ratio is favourable to the inhibitor, the excitor may fail first in a similar way. In such a case the ratio will be constant up to high values of excitation but will drop at still higher ones. It may be remarked that failure of excitation or inhibition does not influence the ratio of subsequent contractions at lower frequency levels, these being quite normal.

In crabs the opener and stretcher muscles are inhibited by two different fibres. The functional significance of this arrangement is unknown (see Wiersma, 1941), but it is of interest to note that the  $R_c$  values of the two inhibitors show a certain relation. In the two *Cancers*, where the true opener inhibitor is relatively ineffective, the common one is relatively effective, whereas in *Pachygrapsus* and *Loxorhynchus* a relatively effective true opener inhibitor is accompanied by a rather ineffective common inhibitor.

In previous papers it has been pointed out that the facilitation of the excitatory mechanism is most likely not due to spatial facilitation by the involvement of more and more muscle fibres but to a gradual increase of contraction strength in each muscle fibre. This means that the muscle fibre of the crustaceans does not contract in an all-or-none fashion. The results obtained with inhibition give additional support to this view. The absence of any effect on the muscle action potentials in most inhibitions shows that the inhibitory mechanism must be located after the process of this muscle action potential and since there is an innervation of each muscle fibre in many places the inhibition must be present at all these places. It is thus thought that inhibition like excitation is a gradual process in each muscle fibre, or better, at each nerve ending on each muscle fibre.

One of the most significant observations arising from the present investigation is the apparent lack of correlation between the ease with which an inhibitor is able to suppress the contraction of the muscle which it innervates and the presence of the phenomenon of supplementary inhibition. It would seem quite plausible that to obtain a maximum efficiency of the inhibitory process it would be necessary not only to block the transmission step between the muscle action potential and the contractile mechanism, but to suppress the earlier transmission step between the nerve action potential and the muscle action potential as well. This is, however, definitely not the means whereby certain of those systems showing a low  $R_c$  achieve their effectiveness, e.g. the opener system of *Panulirus* and the slow bender system of *Pachygrapsus*.

The presence of supplementary inhibition in both the opener and stretcher muscles of crayfish and crabs, muscles which have separate inhibitory axons, shows that the phenomenon is not limited to one inhibitor. At the same time the absence of the phenomenon in other muscles inhibited by these same inhibitors, for instance the closer of *Cambarus*, indicates clearly that the mechanism involved in supplementary inhibition is not to be found in the inhibitory innervation, but in the muscle itself. It is apparently an extra process whereby inhibition can be assured in those few systems in which the phenomenon can be demonstrated. *Panulirus* seems to be totally without this type of inhibition.

## SUMMARY

The effectiveness with which different contractions in a number of muscles can be inhibited was investigated. As a measure of this effectiveness the frequency of inhibition which can just inhibit a contraction with a given frequency of excitation was determined. It was found that in all systems the ratio ( $R_c$ ) of such inhibitory frequencies to that of the excitatory frequencies they can suppress was constant for a wide range of frequencies.

At high frequencies either the inhibition or the excitation may become less effective. This is explained by failure of the respective system to function normally at such a frequency.

The effectiveness of inhibition of different systems was determined. Some systems show a very constant  $R_c$  value; in a second group  $R_c$  varies within wider limits; and a third group shows two distinct  $R_c$ 's sometimes in the same preparation at different times.

$R_c$  values have been found to vary widely. For instance, in the bender inhibitor-slow bender system of *Pachygrapsus* three excitatory impulses are suppressed by one inhibitory impulse; in the closer inhibitor-slow closer system of *Cambarus* one excitatory impulse needs five inhibitory impulses to counteract its effect. The fast closer contraction of *Cambarus* and the fast closer and fast bender contraction of *Pachygrapsus* were found to be uninhibitable, i.e. no effect of inhibition whatsoever was noticed on any of these contractions. All three systems are distinguished by giving a mechanical response to a single stimulus in contrast with all the inhibitable systems which do not respond to single impulses.

Reduction of the action potentials during inhibition is obtainable in only a few systems, namely, the opener inhibitor-opener and the stretcher inhibitor-stretcher systems of *Cambarus* and the crabs. (In the crabs this applies only to the 'true' inhibitors.) In all other systems, including every system of *Panulirus*, no reduction of the muscle action potential is obtained.

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