THE TRIPLE INNERVATION OF CRAYFISH MUSCLE AND ITS FUNCTION IN CONTRACTION AND INHIBITION

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(With Seven Text-figures)

INTRODUCTION

The double innervation of the abductor muscle of the claw of *Astacus fluviatilis* was first described by Biedermann (1887). Each muscle fibre of the abductor is innervated by a branch of two different axons, which form a ramification on the surface of the muscle, and are the only nerve fibres innervating this muscle. The ramification is of such a nature that the two fibres always divide at the same place, a mode of branching later described by Mangold (1905) as diplotomic. Mangold confirmed the double innervation not only of the abductor muscle of *Astacus*, but found a similar supply in many other muscles of this animal. He enlarged Biedermann's findings to include the muscles of *Hydrophilus*, and those of many other insects (e.g. beetles, dragonflies and caterpillars). Hoffmann (1914) confirmed the double innervation of the abductor muscle, but found that in other muscles of the cheliped the innervation is not so simple. In general they receive more than two axons, the adductor of the dactylopodite three to five fibres, the flexor of the carpopodite four fibres. In these muscles the ramification is somewhat more irregular than in the abductor muscle, because the fibres do not divide always at the same point. In the finest branches, Hoffmann always found only two nerve fibres going to one muscle fibre, so that the innervation of these muscles would be essentially the same as that in the abductor muscle.

Biedermann (1887) used the double innervation of the abductor muscle to explain the peripheral inhibition, which he found in this muscle. One of the fibres to the muscle would be a fibre causing contraction; the other, when stimulated, would evoke an inhibition of this contraction. Biedermann showed this peripheral inhibition by stimulating the whole nerve bundle with electrodes inserted into the shell after cutting the tendon of the adductor muscle. With weak faradic stimuli, he obtained contraction of the abductor muscle, which he explained as a stimulation of the excitatory fibre only; on increasing the strength of the stimulus, the contraction disappeared, owing to simultaneous stimulation of the inhibitory fibre. Hoffmann

(1914) confirmed and improved Biedermann's experiments by cutting parts of the nerve. He described a peculiar course of the fibres innervating the abductor and the adductor of the dactylopodite and the extensor of the propodite in *Astacus fluviatilis*. The extensor has two nerve fibres that run in the meropodite in a definite bundle, which is separated from the rest of the nerve; this bundle is thin compared to the rest of the nerve trunk. The motor fibres for the adductor muscle are located in the main nerve trunk. The two axons going to the extensor of the carpopodite do not end in this muscle; one of them runs on to the abductor, the other to the adductor of the dactylopodite, joining there the other fibres which innervate the latter muscle. The nerve trunk of the meropodite which contains the three to five fibres for the adductor muscle, also includes one fibre going to the abductor. Thus the two fibres for the abductor muscle run in two different bundles in the meropodite. In marine crustaceans it has been possible to isolate these two main bundles and to obtain (i) contraction of the muscle by stimulating the thin nerve bundle, and (ii) inhibition of this contraction by simultaneous stimulation of the thick bundle. This has been done by Knowlton & Campbell (1930) in *Homarus americanus*; by Wiersma (1933) in several marine crustaceans, by recording the contractions of the abductor and the adductor muscle with different levers. Wiersma & van Harreveld (1934) used preparations of *Eupagurus bernhardus* in which the adductor muscle was put out of action by cutting its motor supply.

Though all workers on this subject have observed peripheral inhibition, several explanations, other than that postulating special fibres for the excitation and the inhibition, have been put forward. Fröhlich (1908) gives two different explanations. On the one hand, he attributes the inhibition to fatigue, assuming that the abductor muscle is easily tired by strong and frequent stimuli; on the other hand, he thinks that the double innervation may be the cause of the inhibition; the impulses reaching the muscle fibre from two different sources might extinguish each other. He thought it possible that the stimulation of either of the two nerve fibres innervating the abductor muscle might sometimes cause contraction, sometimes inhibition, according to the state of the muscle. This latter idea was afterwards taken up by Segaar (1929). Fraenkel-Conrat (1933) explains the peripheral inhibition as Wedensky inhibition due to current escape of the stimulating current.

In a previous paper (1936) we have shown that the slow and the twitch contractions of the adductor of the dactylopodite are evoked by the stimulation of two different axons. We could not find any other fibre that had a detectable effect on this muscle. If we identify the end ramification of these two axons with the double innervation as described by Hoffmann, there would remain no anatomical structure for the peripheral inhibition, which as we mentioned above, has been demonstrated by several authors. Explanations of the inhibition, which do not need the assumption of special inhibiting fibres (Fröhlich, Segaar, Fraenkel-Conrat), would then be the most probable. We studied the innervation of the muscles of the cheliped of *Cambarus clarkii* thoroughly, hoping to find anatomical structures that would make it possible to explain both the double motor innervation and the peripheral inhibition in these muscles. During the investigation we came to the conclusion that the

anatomical picture of the innervation of the crustacean muscles as given by Biedermann, Mangold and Hoffmann is, at least for some of the muscles of the cheliped of *Cambarus*, incomplete.

THE INNERVATION OF THE MUSCLES OF THE CHELIPED

Method. We used methylene blue to stain the nerve fibres, as did earlier investigators. We did not get very good results by injection of the dye as recommended by Mangold. Much better preparations were obtained by bathing the muscle in a dilute (1/20 to 1/30 per cent) solution of the dye[1] in a physiological salt solution (van Harreveld, 1936). The preparation of the muscle to be stained is very important. The surface of the muscle on which the ramification of the innervating fibres is situated must be prepared and freed from connective tissue without injuring or even touching the nerve fibres, since after even very slight injury they stain badly or not at all. The preparations were bathed for 20–30 min. in the methylene blue solution, rinsed, and kept for some time in moist air or in physiological solution, during which time the colour of the fibres becomes more intense. Good preparations were either fixed or photographed. For fixing, the best results were obtained by using a solution having the formula of Bethe (B. Romeis, 1928, p. 497). This solution fixes the blue colour of the thin branches excellently, but the result of the fixation of the main axons of the ramification is always poor. We therefore made photographs of the main fibres: this had to be done in a short space of time, as the staining with methylene blue lasts only for a limited time after the preparation has been completed. Here we encountered other difficulties, the stained preparations often showing fibrillar contractions, and the uneven surface of the muscles making focusing difficult.

The extensor muscle of the carpopodite. We shall begin with a description of the innervation of the extensor of the carpopodite, which is situated in the meropodite, because it shows a "complete" innervation. This muscle is pennated having its origin on one side of the shell of the meropodite; its antagonist, the flexor of the carpopodite, is fastened on the other side. Between these two muscles lies a thick nerve bundle, from which arises, near the joint between the ischio- and meropodite, a small nerve bundle going to the extensor of the carpopodite. This small bundle forms a ramification on that surface of the muscle which faces its antagonist. This bundle going to the extensor contains (besides a few fibres that do not have any connexion with the muscle and are presumably sensory) three fibres that form the ramification, of which the smallest branches disappear between the muscle fibres. The type of branching of these three fibres is similar to that described by Biedermann for the two fibres of the abductor muscle of the dactylopodite, since the three nerve fibres always branched at the same place and even the smallest branches, disappearing between the muscle fibres, still consisted of three nerve fibres (Figs. 1 and 2). Following the nomenclature of Mangold we called this type of branching

[1] Methylene blue for vital staining of the National Aniline and Chemical Company, Inc. (New York, N.Y.).

"triplotomic". The three fibres in the main bundle and in the first divisions are so thick that they can be prepared and handled separately, thus excluding the possi-

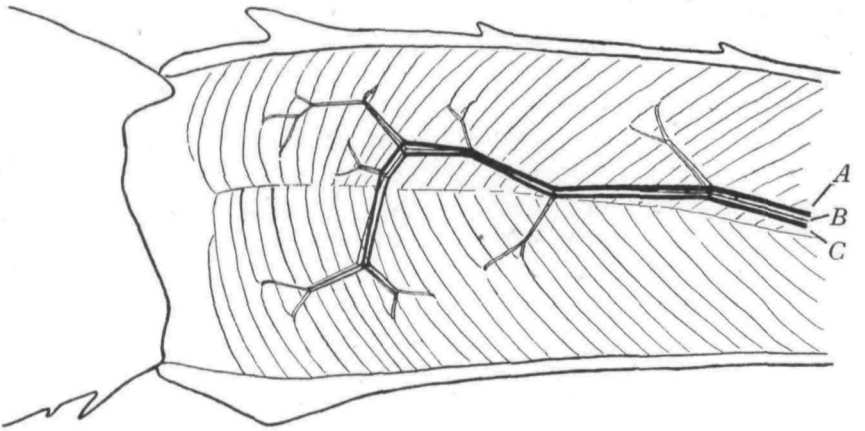

Fig. 1. Sketch of a triply innervated muscle (extensor of the carpopodite). Methylene blue staining. *A*, the thick axon; *B*, the thin fibre; *C*, the intermediate axon.

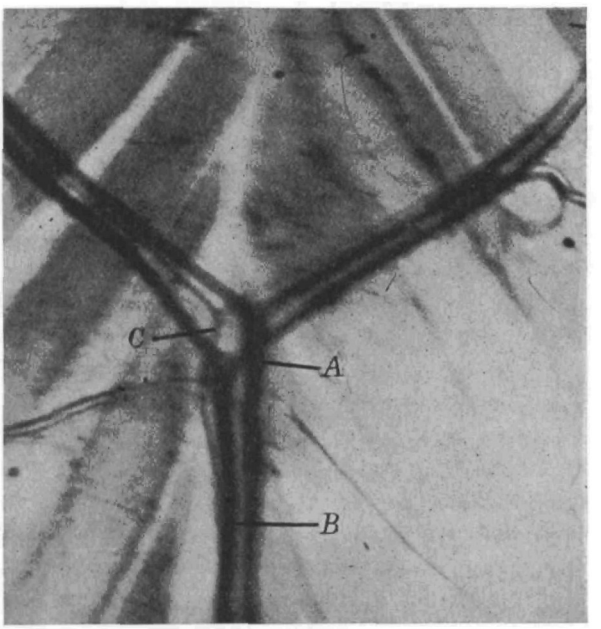

Fig. 2. Photomicrograph of triplotomic branching in the ramification of the extensor muscle of the carpopodite. Methylene blue staining (1/2500 for 20 min.). Magnification about 100 times. *A*, the thick fibre; *B*, the intermediate; *C*, the thin axon.

bility that the three fibres would be an artefact due to refraction. The same triplotomic branching is found on following the nerve to its finer divisions. In Table I is shown the relation between the sizes of the three fibres in a number of prepara-

tions. The absolute diameter in this table has no immediate significance as we measured the fibres in any part of the ramification where they all three showed up clearly. The average ratio of the diameters was found to be 1·4 : 1 : 0·45. The thinnest fibre, at least in the main branches, stains rather differently from the thicker ones, reaching its maximum colour, at a different stage of the staining process to the others. This, and the fact that most of the authors on this subject concentrated their attention on the abductor muscle of the claw (which as we shall see later really has a double innervation), may be the reason that the triple innervation has until now been overlooked. Preparations in which the triple innervation can be seen over a large distance are very rare, because the fibres were in most of the ramification situated in such a way that one or two were covered by the others. In most preparations we could find evidence of the innervation of the muscle by three fibres and of the triplotomic branching. It will be understood that this mode of innervation can be found only in the unregenerated cheliped, and, except when otherwise stated, we shall, in the following pages, speak only of the unregenerated claw. In regenerated claws there are usually more than three fibres which run and branch on the muscle surface in a quite irregular way.

Table I. *Diameters of fibres innervating the extensor muscle of the carpopodite*

	Diameter			Ratio		
	Thick fibre μ	Intermediate fibre μ	Thin fibre μ	Thick fibre	Intermediate fibre	Thin fibre
1	28	20	11	1·4	1	0·6
2	35	20	7	1·8	1	0·4
3	20	16	5	1·3	1	0·3
4	18	14	5	1·3	1	0·4
5	32	21	12	1·5	1	0·6
6	34	21	7	1·6	1	0·3
7	24	18	5	1·3	1	0·4
8	28	23	11	1·2	1	0·5
9	27	18	7	1·5	1	0·4
10	25	18	9	1·4	1	0·5
11	21	18	11	1·2	1	0·6
12	32	25	13	1·3	1	0·5
			Average	1·4	1	0·45

The innervation of the other muscles of the cheliped. We investigated the other muscles of the mero-, carpo- and propodite of the claw by the same methods as were used for the extensor muscle in the meropodite. Each of these podomeres contains two antagonistic muscles. The muscles are all pennated; the thick, central tendon being attached to the next distal podomere. The muscle fibres are at one end attached to the shell, at the other end to the central tendon. In the meropodite we found a small third muscle lying alongside the flexor muscle of the carpopodite; the muscular portion is situated in the proximal half of the meropodite and is inserted with a long tendon to the carpopodite. In most of the muscles of the cheliped, the innervating axons form a ramification on that surface of the muscle which faces its

antagonist. The adductor of the dactylopodite is, however, an exception, here the axons divide below the surface of the muscle, the first main division being usually the only one visible from the outside. Between the two antagonists, in each podomere, runs the nerve supply of the claw, consisting mainly of sensory fibres, but containing also the motor and inhibitory fibres for the more distal muscles.

The flexor muscle of the carpopodite and that of the propodite are innervated in exactly the same way as the extensor of the carpopodite, namely by three axons branching in a triplotomic manner on their surface.

Though we made a large number of preparations, we did not succeed in finding the triple innervation in the extensor muscle of the propodite and in the abductor of the dactylopodite. We are convinced that these muscles are innervated by only two nerve fibres as has been claimed by Biedermann, Mangold and Hoffmann, in *Astacus fluviatilis*. Hoffmann described for these two muscles and for the adductor of the dactylopodite the unusual innervation that we have mentioned above. By means of methylene blue preparations of *Cambarus* in which we prepared the whole innervation of these three muscles, we were able to confirm most of Hoffmann's results. The two fibres that we described in a previous paper, causing the twitch and the slow contraction of the adductor muscle of the dactylopodite, can be easily found in methylene blue preparations where they enter this muscle, and can be traced back to the nerve bundles in the meropodite. The two fibres for the extensor of the propodite could also be traced back into the meropodite, where they run in the thin bundle that is separated from the main nerve trunk. The two nerve fibres branch diplotomically on the surface of the extensor muscle, but do not end there. One of them can be followed distally till it reaches the abductor muscle of the dactylopodite ending there in the ramification on that muscle. During its course to the abductor it is joined by a fibre that in the mero- and carpopodite runs in the main trunk with the two fibres for the adductor muscle. Together they follow their course and form the diplotomic ramification on the surface of the abductor muscle. The second fibre of the two innervating the extensor of the propodite is rather variable in thickness distal to this muscle. Sometimes it is of relatively large diameter and can be traced joining the two motor fibres for the adductor muscle; the three fibres then show triplotomic branching at their first main division. In the majority of the preparations, however, this fibre, having innervated the extensor of the propodite, becomes very thin, and it is then difficult to prove that it joins the fibres for the adductor muscle. In Fig. 7 we give a scheme of the innervation of the six principal muscles of the cheliped as made out from our anatomical and physiological data. The figure shows the triple innervation of both the flexor and the extensor of the carpopodite, the flexor of the propodite and the adductor of the dactylopodite, as well as the double innervation of the extensor of the propodite and of the abductor of the dactylopodite. Most of the fibres innervating these muscles have been traced over long distances, some of them even over several joints, but never did we see any branching of them except the ramification on the muscles. This is of importance in relation to the explanation that Tonner (1933) has given for peripheral

tone since he assumes the existence of branches of the motor fibres going to sensory end-organs and thus making axon reflexes possible.

During the physiological experiments that we shall describe in the following pages, a number of isolated axons with known motor or inhibitory function were obtained; the diameters of these fibres were measured and the data collected in Table II. The diameters of these fibres vary from 25 to 90μ.

Table II.* *Diameter of motor fibres of the cheliped*

Preparation	Flexor of carpopodite μ	Extensor of carpopodite μ	Flexor of propodite μ	Extensor of propodite μ	Abductor of dactylopodite μ	Adductor of dactylopodite μ
I	*a* 60 *b* 30	—	—	—		*a* 60
II	*b* 24	—	—	*b* 40		*a* 72 *b* 51
III	—	—	—	*b* 36		*a* 51
IV	*a* 40 *b* 30	*a* 57 *b* 57	*a* 30 *b* 27	*b* 42		*a* 60 *b* 36
V	—	—	—	*b* 42 *c* 27	*c* 30	—
VI	—	—	*a* 37 *b* 28 *c* 28	—	*c* 30	*a* 48 *b* 36
VII	—	—	*a* 45 *b* 33	—	—	—
VIII	—	—	*a* 50 *b* 45 *c* 33	—	*c* 25	*a* 90 *b* 42

* In this table the fibres indicated with an "*a*" are fibres for the fast contraction, those indicated with a "*b*" for the slow contraction and those with a "*c*" are inhibitory fibres.

As can be seen from the scheme (Fig. 7), the four distal muscles of the cheliped are innervated by eight motor fibres, divided into two groups; one group contains two fibres, that run in the meropodite, in what we called the thin nerve bundle, and the other consisting of six fibres running in the meropodite in the main nerve trunk. We tried to find these fibres on the cross-section of the nerve bundles in the meropodite. It is not always possible to identify the thick fibres in the cross-section, but in a preparation such as that shown in Fig. 3 we can do this with reasonable certainty. Here we see a bundle of thick fibres containing six axons, and another bundle with two more thick fibres. All these fibres belong to the thickest of the whole nerve trunk. It is likely that the two fibres running separately from the other large axons are the fibres for the extensor muscle of the propodite. As mentioned above, one of the fibres for the abductor runs with the two motor fibres for the adductor of the dactylopodite, and for the physiological experiments it is known that the fibres for the adductor often run together with the axons for the flexor of the propodite; this makes it likely that the small bundle of six large fibres is formed by the two fibres for the adductor, the fibre for the abductor and the three fibres for the flexor of the

propodite. In the remaining part of the cross-section we see a number of other fibres with a fair diameter; where they run to and what is their function is quite uncertain, but it has been shown that their stimulation has no effect on the muscles of the cheliped.

THE FUNCTION OF THE NERVE FIBRES

Method. The method used to prepare single, functioning, nerve fibres is essentially the same which we have described for the two motor fibres of the adductor of the dactylopodite (van Harreveld & Wiersma, 1936). The motor fibres for the other muscles are also isolated by splitting the nerve into bundles and testing each bundle with faradic stimulation through micromanipulated electrodes. In order to locate inhibitory fibres it is necessary to use two sets of electrodes as the excitatory fibres

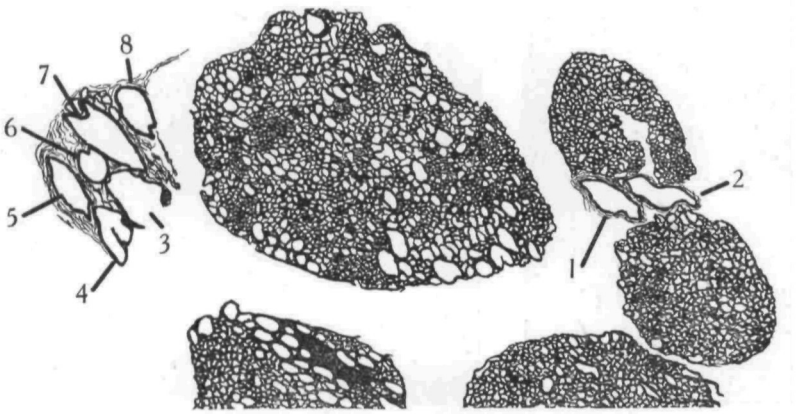

Fig. 3. Drawing of the cross-section of part of the nerve bundle in the meropodite of *Cambarus clarkii*. Fibres 1 and 2 are the axons running to the extensor muscle of the propodite. The bundle of six large fibres (3–8) contains the three fibres for the flexor of the propodite, the two fibres for the adductor of the dactylopodite and the fibre for the abductor of the dactylopodite. Fibre 3 lacks part of its neurilemma. In the part of the cross-section that is not in the picture we did not find any other fibre comparable in size with the ones just mentioned.

have to be stimulated at the same time. The difficulty of obtaining satisfactory preparations is, however, much greater than when only the motor fibres of the adductor have to be prepared, as it is necessary to work with a number of fibre bundles, and the size of the required fibres, especially of the inhibitory type, is often much smaller, as can be seen from Table II. The number of unsuccessful preparations has been rather large, as often one or more of the fibres looked for have been damaged during preparation. According to the preparation desired the cheliped has been mounted in different ways, and the shell opened from different sides.

The flexor muscle of the propodite. As described above, this muscle has a triple innervation, and was preferred above other muscles with a "complete" innervation as the nerve can be prepared over a long distance in the meropodite. In order to register the contraction with the muscle pulling in a vertical direction, the cheliped

is fixed with a clamp at the carpopodite and the meropodite is opened from the underside. The muscles in the meropodite are removed and the preparation of the nerve is started by isolating a bundle which contains both the motor axons for the adductor as well as those for the flexor of the propodite. The two adductor fibres are then prepared and discarded by cutting them as close to the carpopodite as possible. The next step, usually, is to locate the fairly thick axon for the fast contraction of the

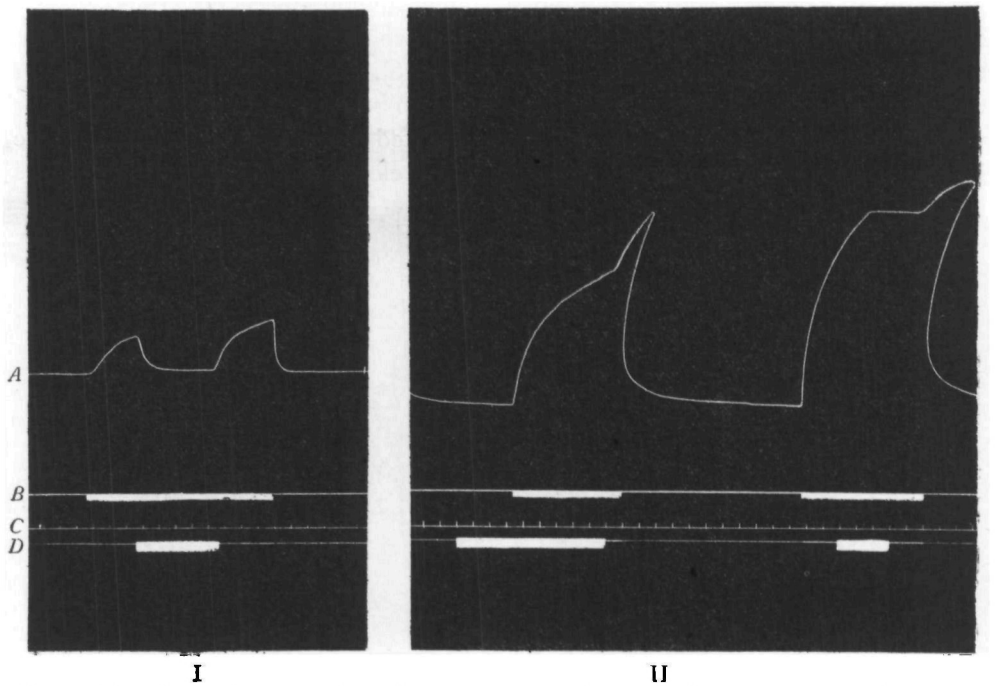

Fig. 4. Inhibition of the slow and the fast contraction of the flexor of the propodite, all three axons for this muscle isolated. I, slow contraction by stimulation of the isolated fibre of intermediate size (30μ); II, fast contraction by stimulation of the thickest fibre (36μ). Both curves are from the same preparation. The inhibition is obtained by stimulation of the thinnest fibre (24μ). *A*, the isotonic muscle contraction; *B*, the stimulation of the excitatory fibre, thus in I this is the fibre for the slow contraction, in II that for the fast contraction; *C*, the time in seconds; *D*, the stimulation of the inhibitory fibre. The coil distance was for the excitatory fibre 13·5 cm., for the inhibitory fibre 15·5 cm. Curve I shows the complete inhibition of the slow contraction during the stimulation of the inhibitory fibre. In II the same strength and frequency of the inhibitory stimulus gives only partial inhibition of the fast contraction. In the first curve of II the inhibition is started before the excitatory stimulation, in the second one the inhibitory stimulation is started after the beginning of the stimulation of the fibre for the fast contraction. Notice the difference in speed of the slow and the fast contraction, especially that the uninhibited onset of the fast contraction is very quick.

flexor of the propodite. If this preparation has been successful, the remaining bundle, when stimulated, does not, in general, elicit any visible contraction, because it contains an inhibitory fibre in addition to the fibre for the slow contraction. It is then necessary to isolate different single axons, keeping them all intact, until one is found that gives slow flexion. If this stage has successfully been reached, this axon is left on one pair of stimulating electrodes, and the other pair is used to find the inhibitory fibre. The procedure is then again the same as for the isolation of excitatory

fibres; first the whole bundle is stimulated and if inhibition of the slow contraction of the flexor muscle results, the bundle is divided in parts, each part tested separately until the single inhibitory fibre is found. As we were very careful to use only normal claws, we have never found more than three axons for the flexor, although after obtaining these three fibres we have tried to get inhibition from other bundles, but the result was always negative. We may conclude that the three axons, whose physiological properties are described above, are identical with those described in the anatomical section of this paper. The measurement of the fibres showed that the thickest axon is the one causing the fast contraction; the axon with the intermediate diameter evokes the slow contraction; and stimulation of the thinnest axon gives inhibition.

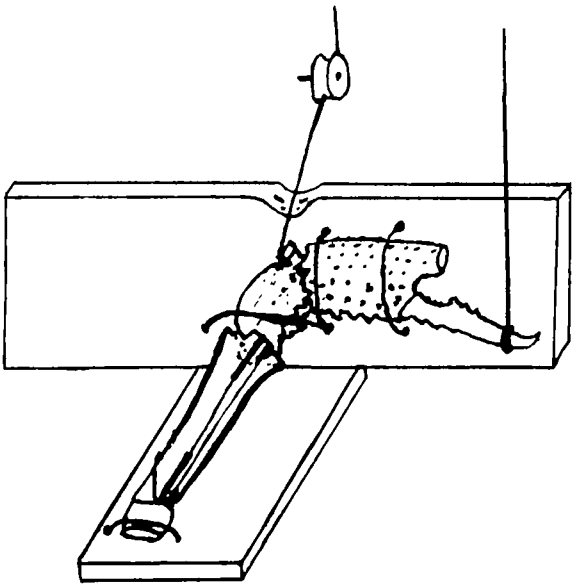

Fig. 5. This picture shows the mounting of the claw for the simultaneous registration of the extensor of the carpopodite and the abductor of the dactylopodite. For explanation see text.

We have made several records of this inhibition, stimulating alternatively the fibres for the slow and the fast contraction together with the inhibitory fibre. The record (Fig. 4) shows that both the slow and the fast contraction can be inhibited, but with the same frequency and strength of stimulation of the inhibitory fibre the effect on the slow contraction is much more marked. Although we do not intend here to go into further detail about the slow and the fast contractions, we must mention that the fast contraction of this muscle is not quite the same as the twitch of the adductor of the dactylopodite, as single induction shocks elicit no apparent mechanical response, whereas faradic stimulation of the same strength elicits a large contraction.

The abductor of the dactylopodite and the extensor of the propodite. To record the contraction of these two muscles at the same time, the cheliped is mounted on a wooden support as shown in Fig. 5. A small part of the shell of the propodite, to

which the tendon of the extensor is attached is cut loose and fastened to a string, the immovable tip of the claw is cut off and another string is fastened to the dactylopodite. In this way both muscles pull directly on their recording levers. In a number of preparations the small nerve bundle, containing the two fibres running to the extensor of the propodite was prepared. Stimulation of this bundle always gives abduction of the dactylopodite, but very often the extensor of the propodite stays completely at rest, though no other bundle will elicit a contraction in this muscle. On further preparation of the bundle the contraction appears, however, and is then an exact duplicate of the contraction of the abductor muscle, following it in all its movements. By further preparation it can be shown that contraction of both muscles is evoked by stimulation of one single axon; no other fibre has ever been found to cause a contraction in either of the two muscles. These muscles, unlike those with triple innervation, have only one motor axon. It is clear that the axon, described above, which innervates both muscles, is the motor axon, so that these two muscles may be considered as two parts of a single motor unit. In normal preparations the inhibitory fibre for the abductor muscle never runs in the same nerve bundle as the two fibres for the extensor of the propodite. In regenerated claws, however, it has often been located in the immediate neighbourhood of the excitatory fibre; but in these claws the situation of the bundles is abnormal. In these regenerated claws the motor fibre for the extensor of the propodite and the motor fibre for the abductor are sometimes two different axons.

If a preparation of the excitatory and of both the inhibitory fibres is required, the technique is somewhat different from that described above. The thin bundle containing the excitatory fibre is first loosened from the main nerve and placed on one pair of electrodes. The remaining nerve bundles are investigated with the other pair of electrodes. It was found that the inhibitory axon of the abductor muscle also runs in the same nerve bundle as the motor axons for the adductor and the three axons of the flexor of the propodite, but it is always a separate axon. Only after this axon has been prepared is the thin nerve bundle, containing the two fibres for the extensor of the propodite, attacked and these axons isolated.

Fig. 6 represents a record from such a complete preparation. The two inhibitory axons are stimulated in turn, and as will be seen, inhibition of only that part of the motor unit to which they run, is effected. This proves better than any other experiment performed, the reality of inhibitory fibres, as all other possibilities are here completely excluded. If both inhibitory fibres are put on one pair of electrodes, inhibition of both muscles occurs, of course.

The other muscles of the cheliped. The motor innervation of the adductor of the dactylopodite has been described in a previous paper. The two motor axons are easily found. Experiments which were performed to test inhibition of the adductor by stimulation of the inhibitory fibre of the extensor of the propodite have under special conditions yielded result (Wiersma & Marmont, unpublished).

The muscles in the meropodite are less suitable for physiological investigation than the ones described. The length of the nerve available for the isolation of single axons is very much shorter, as the ischiopodite itself is short. In some very large

specimens we have been able to isolate single motor axons; for both muscles a fast and a slow contraction evoking fibre have been found (see Table II). No satisfactory preparation of inhibitory fibres could be made as the distance is too short to prepare several fibres over a long enough length. In view of the complete agreement between the anatomical and physiological data in the other muscles, there seems no doubt that here too the triple innervation represents a fast, a slow and an inhibitory fibre.

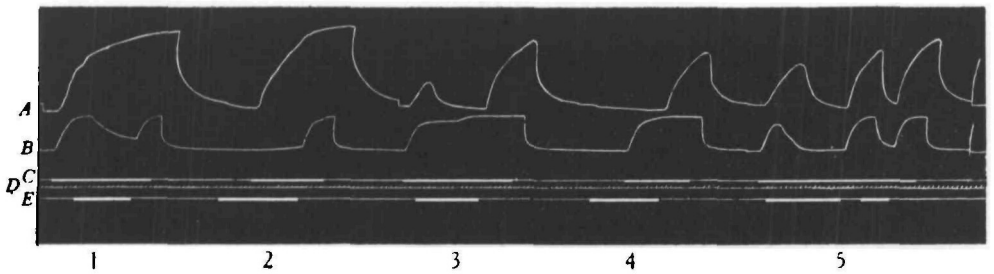

Fig. 6. Excitation and inhibition of the motor unit formed by the extensor muscle of the propodite and the abductor of the dactylopodite. The common excitatory and the two inhibitory fibres were isolated. *A*, the contraction of the abductor; *B*, that of the extensor; *C*, the stimulation signal of the common motor axon for the extensor and the abductor (coil distance 16 cm.); *D*, the time in seconds. *E*, the stimulating signal of the inhibiting stimulus; in 1 and 2 of the inhibitory fibre for the extensor; in 3 and 4 of the inhibitory fibre for the abductor; in 5 of both inhibitory fibres (coil distance 16 cm.). 1. Inhibition of the extensor by stimulation of its inhibitory fibre during a contraction of both muscles. The abductor shows no inhibition. 2. The inhibitory stimulation of the fibre for the extensor was started before the excitatory stimulus. The abductor contracts as quickly as in 1, but the extensor shows a very reduced contraction as long as both stimuli are on. On stopping the inhibition this muscle also contracts strongly. 3. Inhibition of the abductor by stimulation of its inhibitory fibre during a stimulation of the common excitatory fibre. The contraction of the abductor is completely inhibited. The slight depression in the contraction of the extensor is not significant as it does not coincide with the inhibitory stimulus. 4. The inhibitory stimulus of the fibre for the abductor is started before the excitatory stimulus. There is complete inhibition of the abductor as long as the inhibiting stimulus lasts; no effect on the extensor. 5. Both inhibitory fibres on one pair of electrodes. Both muscles contract and are inhibited simultaneously.

DISCUSSION OF THE SCHEME GIVEN IN FIG. 7

In Fig. 7 the exact number of axons innervating the six muscles of mero-, carpo- and propodite are sketched in. In normal chelipeds, no more and no fewer axons, having a detectable effect on these muscles, have been found. In regenerated claws, however, the number of axons innervating the muscles is much larger. To the left of the figure are seen the muscles receiving their motor fibres from the small bundle in the mero- and ischiopodite, viz. abductor of the dactylopodite, extensor of the propodite and flexor of the carpopodite. In addition to the motor fibres, the inhibitory fibre for the extensor of the propodite and the third (presumably inhibitory) fibre for the flexor of the carpopodite are shown in the same bundle. The right side of the figure shows the thick nerve bundle, which contains six motor fibres, namely two each for the adductor, the flexor of the propodite and the extensor of the carpopodite. The inhibitory fibres for the abductor, for the flexor of the propodite and the third, presumably inhibitory, fibre for the extensor of the carpopodite run in this thick bundle.

From the distribution of the motor fibres into two bundles, running separately in the ischiopodite, it is possible to explain, to a certain degree, the behaviour of the claw after it has been removed from the animal. After the initial effect of the cutting, the cheliped usually takes a certain position, in which it may remain for several minutes. It bends at the joint between the mero- and carpopodite, stretches at the joint between the carpo- and propodite and the dactylopodite is abducted. This shows that all the motor axons in the smaller bundle remain in the excitatory state, while those in the larger bundle have stopped discharging.

In accordance with the two kinds of contraction that the two excitatory fibres of the adductor evoke in this muscle, the motor fibres may be divided into two groups:

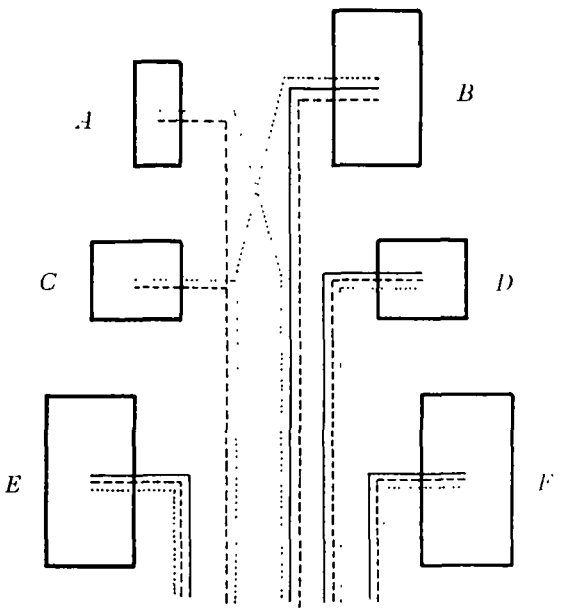

Fig. 7. Scheme of innervation of the six principal muscles of the cheliped. *A* and *B*, the abductor and the adductor of the dactylopodite; *C* and *D*, the extensor and the flexor of the propodite; *E* and *F*, the flexor and the extensor of the carpopodite. The full-drawn lines indicate the fibres for the fast contraction, the broken lines those for the slow contraction and the dotted lines the inhibitory fibres.

one, indicated by a full drawn line (see Fig. 7), causing a fast; the other indicated by a broken line, causing a slow contraction. It has been pointed out that the difference of the two kinds of contraction is not as marked in all the double motor innervated muscles as in the adductor of the dactylopodite. The main reason for distinguishing between axons evoking the fast and others evoking the slow contraction, as given in the scheme, is the shape of the mechanical contraction only, and this may require confirmation.

In reality the podomeres of the claw are twisted at the joints. In Fig. 7 the rotation has been straightened out by turning each joint in the same direction about 135°, so that the nerve bundles, though crossed in the animal, are parallel in the scheme.

SUMMARY

The innervation of the muscles of the cheliped of *Cambarus clarkii* has been investigated both anatomically and physiologically. It is found that at least three of the six principal muscles of the cheliped show a triple innervation. Each of these muscles in the unregenerated claw is innervated by three axons only. Such "completely" innervated muscles are the extensor and flexor of the carpopodite and the flexor of the propodite. The extensor of the propodite and the abductor of the dactylopodite have only a double innervation. Both these muscles together are innervated by three axons; one fibre gives branches to each of the muscles, a second to the abductor only, and a third to the extensor. An extension of the last fibre can, in a number of cases, be seen to join two fibres for the adductor of the dactylopodite, making the innervation of this muscle complete. In other preparations the third fibre for the adductor is very thin.

It is shown for the flexor of the carpopodite that each of the three fibres of this "completely" innervated muscle has a different function. Stimulation of the thickest fibre gives a fast contraction, stimulation of the second in size a slow contraction and stimulation of the third fibre, the smallest, causes inhibition. Both the fast and the slow contraction can be inhibited, but the slow contraction is more sensitive in this respect (see Fig. 4). It is shown that the axon which the abductor and the extensor of the propodite have in common is of the excitatory type. This makes these muscles two parts of one motor unit, each part having its own inhibitory fibre.

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