MICROANATOMY OF THE ABDOMINAL STRETCH RECEPTORS OF THE CRAYFISH (ASTACUS FLUVIATILIS L.)

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Since Alexandrowicz (1951) first described muscle receptor organs in the abdomen of *Homarus vulgaris* and *Palinurus vulgaris*, several authors have studied the physiological and pharmacological properties of such organs, using mainly various species of crayfish as their objects of study. These investigations were based mainly upon the histological evidence presented by Alexandrowicz, concerning the microanatomy of the muscle receptor organs of lobsters (*Homarus* and *Palinurus*, belonging to the families of Homaridae and Palinuridae).

A study of the microanatomy of the muscle receptor organs (stretch receptors) of a representative species of crayfish (family of Astacidae) seemed to us interesting and necessary. We have found important differences between the microanatomy of the stretch receptors of *Astacus* and that described by Alexandrowicz (1951) for *Homarus* and *Palinurus*, differences which are of special interest with respect to the recent analysis of the stretch receptor physiology by Kuffler and Eyzaguirre. In addition some general features of the receptor neurons have been noted which have not been described previously.

Method

The abdominal stretch receptors were dissected from large specimens of *Astacus fluviatilis* L. Males were used exclusively and the receptors have been taken from the second and third abdominal segments. The method of dissection has been principally that described by Wiersma, Furshpan, and Florey (1953). The receptor muscles and the supplying nerve were mounted in their original orientation on a small glass frame (4 × 4 mm.) by means of a fine nylon thread (10μ in diameter). When lifted out of any solution the frame carried a film of fluid thus protecting the extremely delicate structure of the receptors from damage when it had to pass through the surface into another solution.

Methylene blue has been used in but a few preparations; most of the stretch receptor organs were stained according to the sodium hydroxyde silver method of

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Schulze (Romeis, 1948, pp. 417). Several variations of this method were tried. The following was found to be most satisfactory: 5 per cent formaldehyde (12.5 per cent formalin) for 24 hours (fixation). Washing in distilled water; 12 to 24 hours. 0.02 N NaOH for 24 hours. Distilled water (changed several times), 16 hours. 10 per cent $AgNO_3$ for 24 hours (if less concentrated silver nitrate is used, the impregnation is often better but the connective tissue also becomes impregnated, which is not the case if more concentrated $AgNO_3$ is used). For reduction a solution of 2.5 per cent hydroquinone and 5 per cent formalin in distilled water was used in dilutions 1:50 and 1:80. The reduction has to be observed under the microscope and must be interrupted as soon as the axis cylinders become black and the cell bodies brown. It is best to have several samples of distilled water ready and to place the preparation quickly into one after the other as soon as appropriate reduction is achieved.

The preparations were mounted (without the frame) in Canada balsam.

The sensory neurons and the efferent fibers show different staining properties and in many cases the sensory apparatus was extremely well impregnated while the impregnation of the efferent innervation was only poor (and *vice versa*).

RESULTS

Topography of the Muscle Receptor Organs

The two muscles of the abdominal muscle receptor organs are located dorsal to the most medial fibers of the musculus superficialis medianus (Schmidt, 1915). This is in contrast to the lobsters in which the receptor muscles (RM) are lateral to the same muscle (Alexandrowicz, 1951). The medial location of the receptor muscles has also been noted in *Cambarus clarkii* Girard by Wiersma *et al.* (1953). As in *Cambarus* the two receptor muscles are not encapsulated in a common sheath of connective tissue as is the case in lobsters, except in the region of the two sensory cells (Fig. 1). There is one nerve trunk running toward the RM's which contains the sensory and efferent fibers. It fans out before reaching the RM's. It is covered by a thin sheath of connective tissue. The nerve trunk sends out branches to the superficial muscles and to the few fibers which constitute a small and short muscle bundle dorsomedial to the medial superficial muscle. We have never observed any other nervous supply of the RM's.

The Stretch Receptor Muscles

The muscular portion of the organ consists of two bundles of muscle fibers. Following Alexandrowicz's nomenclature we shall call the medial bundle RM 2 and the lateral bundle RM 1. As in *Homarus* and *Palinurus* (Alexandrowicz, 1951) the fibers composing RM 2 are thinner and show a finer cross-striation than the fibers of RM 1. But even in RM 1 the fibers are much thinner than those of the superficial muscles.

In contrast to the lobsters the receptor muscles do not have a non-contractile, tendinous, intercalated region in the area innervated by the sensory

dendrites. The fibers can be followed right through that area and do not exhibit any change in their cross-striation. This has been studied with the polarization microscope in the living organs but can also be seen in the silver-stained preparations. In the sensory area the muscle fibers diverge so that the diameter of the bundle increases considerably around the location of the sensory cells (see Fig. 2). The fibers of RM 1 receive efferent nerve endings also in the region innervated by the sensory dendrites and we have to assume that this region of RM 1 is just as contractile as the rest of this

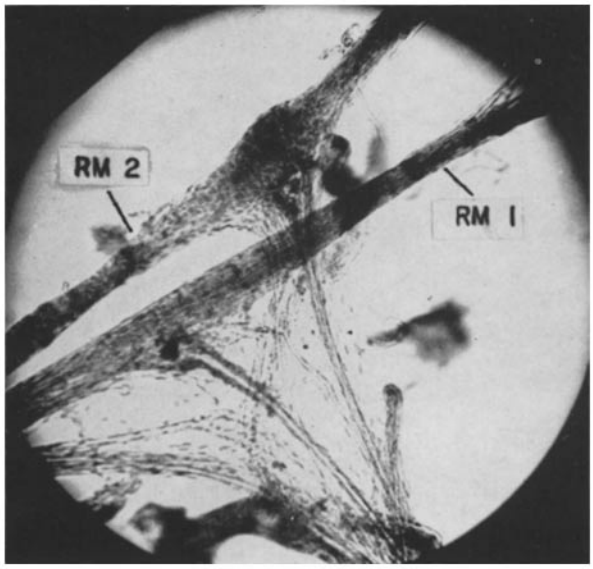

FIG. 1. Photomicrograph of a living, unstained stretch receptor organ of *Astacus*. Receptor muscles (RM) and sensory neurons are shown in their original orientation. × 120.

muscle bundle. RM 2 shows a particular enlargement at the site of insertion of the sensory dendrites. This enlargement is mainly due to one stout dendrite and its massive branching in that part of the muscle, but an accumulation of connective tissue around the sensory innervated area also contributes its part

The Sensory Neurons

For simplification we shall designate the sensory neuron (SN) which belongs to the muscle RM 1 as SN 1, and the sensory neuron of RM 2 as SN 2. These neurons show marked differences in their morphology, although they have many other features in common. For example both have their cell

bodies close to the muscle and the axons are not clearly differentiated from the cell bodies but rather represent a prolongation of the cell body which gradually diminishes in diameter. In our animals these sensory axons had the largest diameter of all the axons of the stretch receptor innervation, at least up to a distance of 5 mm. from the receptor muscles (Figs. 1, 7, and 8). In a distance of 3 to 5 mm. this diameter amounted to 8 to 10 μ in animals of 10 to 12 cm. body length.

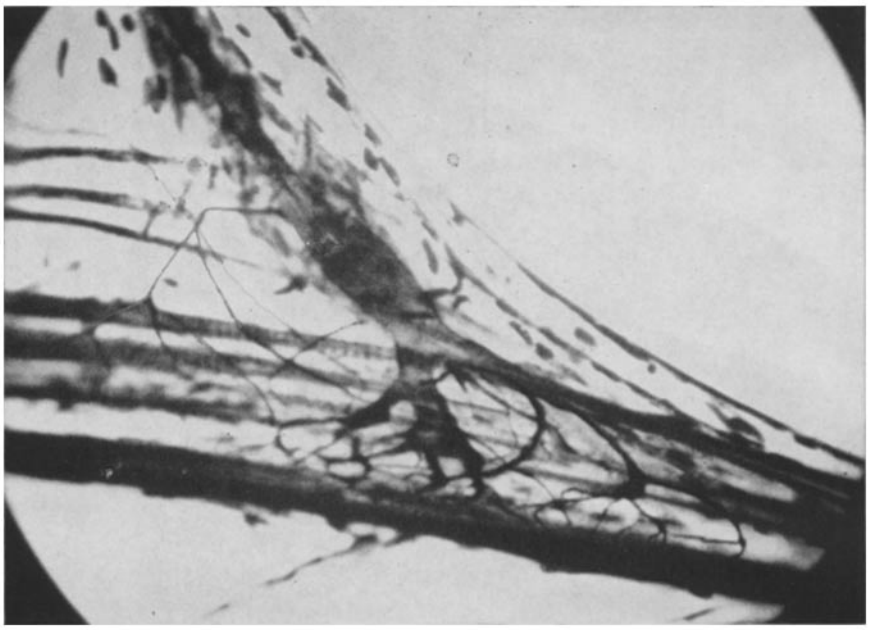

FIG. 2. Photomicrograph of the sensory innervated area of RM 1 and the sensory cell with its dendrites and axon. Note the divergence of the muscle fibers and their cross-striation. The dark spots around cell body and axon are nuclei of connective tissue cells. Silver impregnation. × 200.

The dendrites of the sensory neurons enter the respective muscle bundles. The shape, orientation, and mode of branching of the two types of sensory neurons (SN 1 and SN 2) showed a remarkable constancy in all animals investigated.

The dendritic system of SN 1 consists of three major parts: (1) a long dendrite, extending rostrally rather parallel to the RM 1; (2) a stout and rather short dendrite whose main direction is perpendicular to the extension of the muscle fibers; and (3) a system of one to four very thin dendrites which leave the cell body on the opposite side of the first long dendrite. Fig. 3 shows the

outlines of eight cells of the type SN 1 and a scheme which summarizes their morphology as far as it can be followed in the unstained, living preparation.

Silver staining reveals the further course of the dendrites: they all send their final endings to the different muscle fibers. It is interesting to note how branches from one dendritic system run towards the main area of branching of another dendritic system. Fig. 4 gives a typical example. The three den-

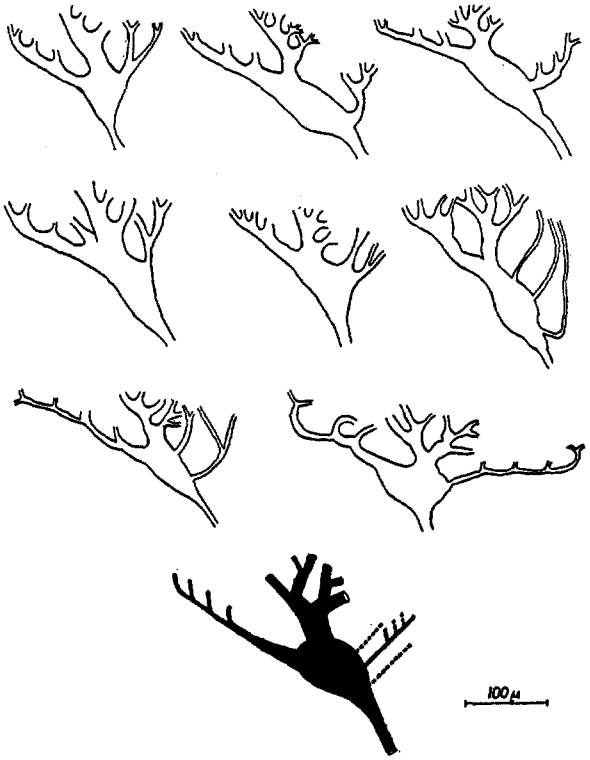

FIG. 3. Outlines of several cells of type SN 1, as observed in unstained, living preparations. The black figure represents a scheme of the dendritic systems (see text).

dritic systems of one SN 1 cover a surprisingly large area and extend over 500μ and more of the length of the receptor muscle.

When the dendritic branches reach their designated muscle fiber they bifurcate in a characteristic T-shape, the ends running in opposite directions along the muscle fiber. If further bifurcations occur, the new branch leaves perpendicular to the original ending which continues its course, and at the shortest distance bifurcates again in T-fashion at the same muscle fiber. Fig. 5 represents a scheme of the dendrites and nerve endings of SN 1 and

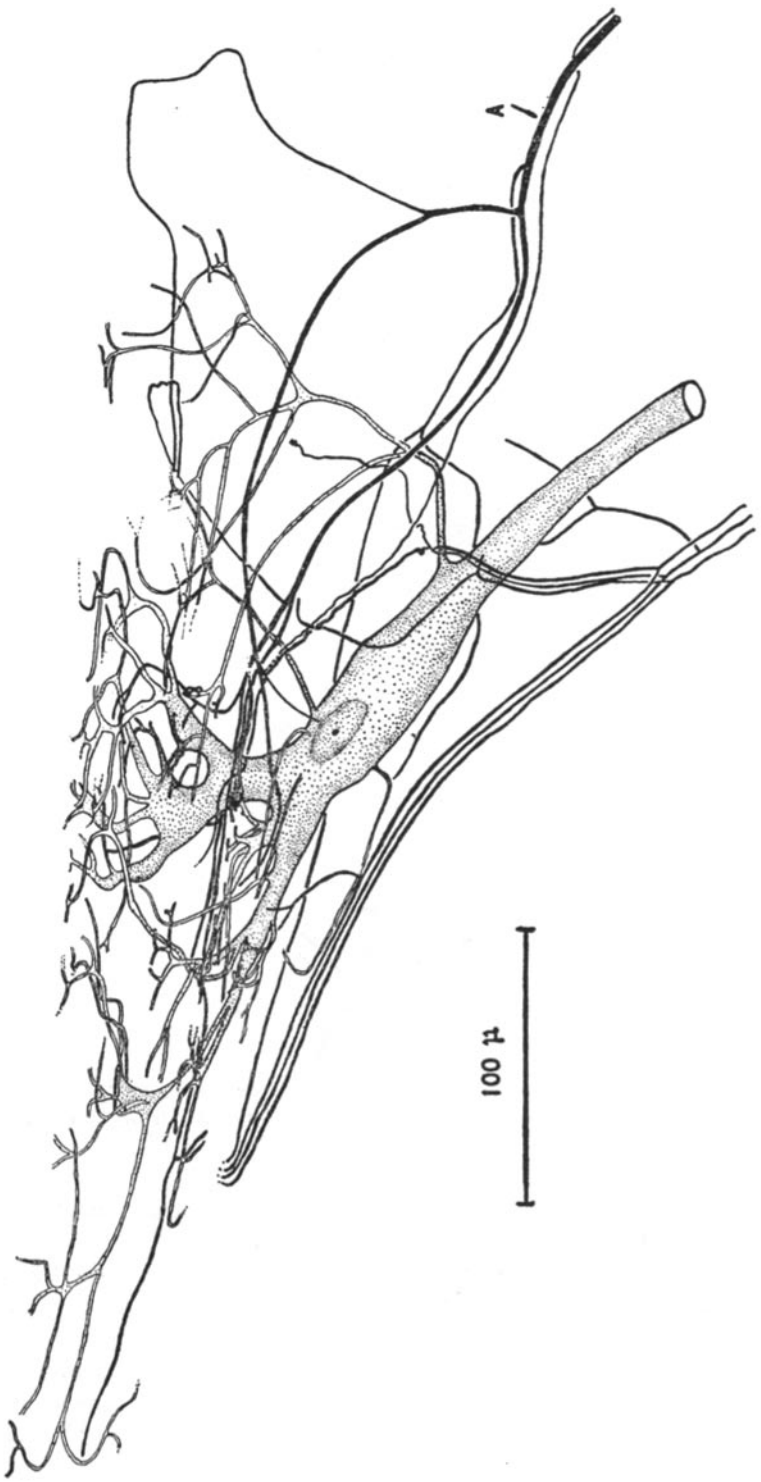

FIG. 4. Drawing of a silver-stained SN 1 (same as in Fig. 2). *A* = the "accessory" fiber.

their relation to the muscle fibers of RM 1. The photomicrograph in Fig. 6 shows that these nerve endings are not straight fibers but curled. It is likely that they straighten out as the muscle fibers are stretched. According to the microscopical picture the contact between nerve endings and muscle fibers is a very loose one.

The morphology of SN 2 and its dendrites and endings differs greatly from that of SN 1 in that the cell body is more stretched in the direction of the axon and the dendrites leave the cell body all in about the same direction. There are three to 4 dendrites, one of them being rather stout. The branching heads of these dendrites meet in a common area and comprise a bulk of nerve

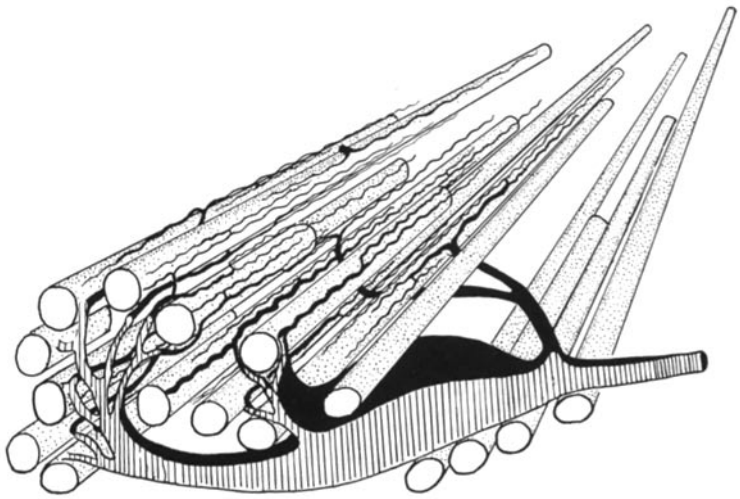

FIG. 5. Schematic drawing of SN 1 and the relation of its endings to the muscle fibers.

endings which seem to be rather short and which go in all directions. No correlation could be found between the orientation of these nerve endings and that of the muscle fibers. Fig. 7 gives an example of a cell of SN 2.

The Efferent Innervation

In describing the efferent stretch receptor innervation of *Homarus*, Alexandrowicz distinguishes (*a*) two main motor fibers, each innervating one receptor muscle, (*b*) one thick accessory fiber which innervates the area which is occupied by the endings of SN 1 and SN 2. This fiber also innervates both receptor muscles, giving off branches all over the length of these muscles. (*c*) One thin accessory fiber which innervates the areas of endings of SN 1 and SN 2 and which probably also takes part in the innervation of the muscle

fibers. (*d*) A number of small fibers whose origin and function could not be clearly established, and which mainly innervate RM 1.

The efferent innervation of the abdominal stretch receptors of *Astacus* differs markedly from that of *Homarus*. Figs. 8 and 9 give examples of total preparations.

(*a*) There is only one fiber which can be compared with the two "main motor fibers" of *Homarus*. This fiber is of large caliber (7 to 10 μ) and runs as far as could be observed (5 mm.) without branching towards the cell body of

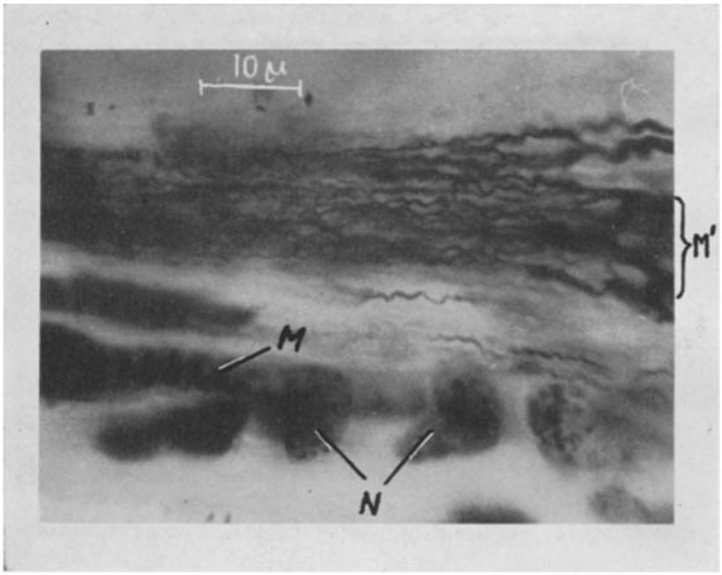

FIG. 6. Photomicrograph of endings of SN 1 which cover a muscle fiber (*M'*) which is only faintly stained. *M* = another muscle fiber, *N* = nuclei of connective tissue. Silver impregnation. × 2500.

SN 2, in the neighborhood of which it bifurcates and sends one branch each in opposite directions along RM 2. These branches give off endings which run towards the various muscle fibers. In contrast to *Homarus* RM 1 is not innervated by a fiber of large diameter.

(*b*) There is one fiber which could be compared with the thick accessory fiber of *Homarus*. It is also of large caliber (5 to 10 μ in diameter) and runs along the nerve trunk without branching until it comes near the sensory cell bodies. Here it bifurcates twice, sending two branches to each nerve cell. Fig. 10 shows in how many different ways this is achieved. The final endings of this fiber go around the dendrites in loops and finally intermingle with the sensory nerve endings (Figs. 4, 7, 8, and 11). The exact relationship of the two types of endings could, however, not be established since their size reaches

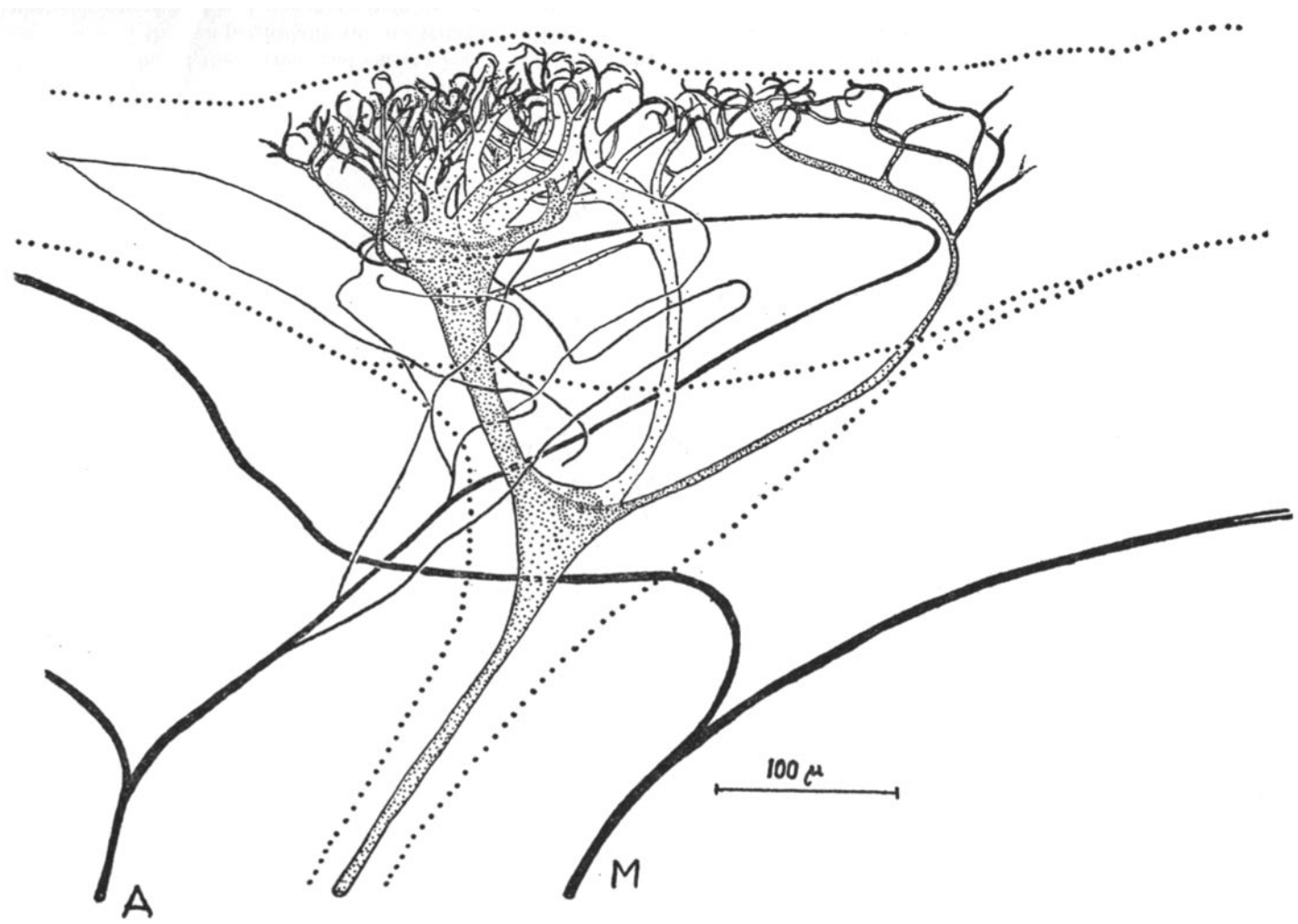

FIG. 7. Drawing of a silver-stained preparation of SN 2. *M* = motor axon, *A* = accessory fiber.

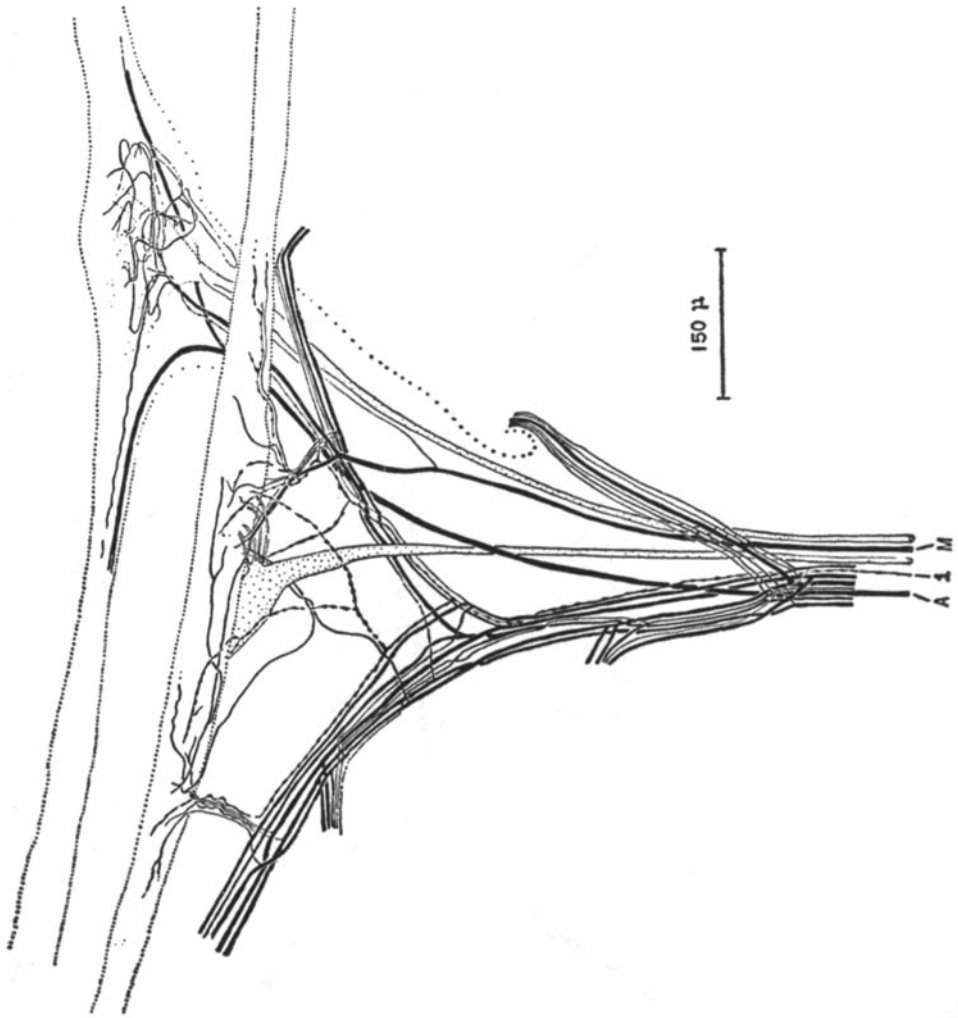

FIG. 8. Drawing of the afferent and efferent innervation of one stretch receptor organ (from a silver-stained preparation). In this case one fiber shows a particularly varicose structure; it is marked *i* in this diagram. *M* = motor fiber, *A* = accessory fiber. The photomicrograph of Fig. 1 was taken from the same organ.

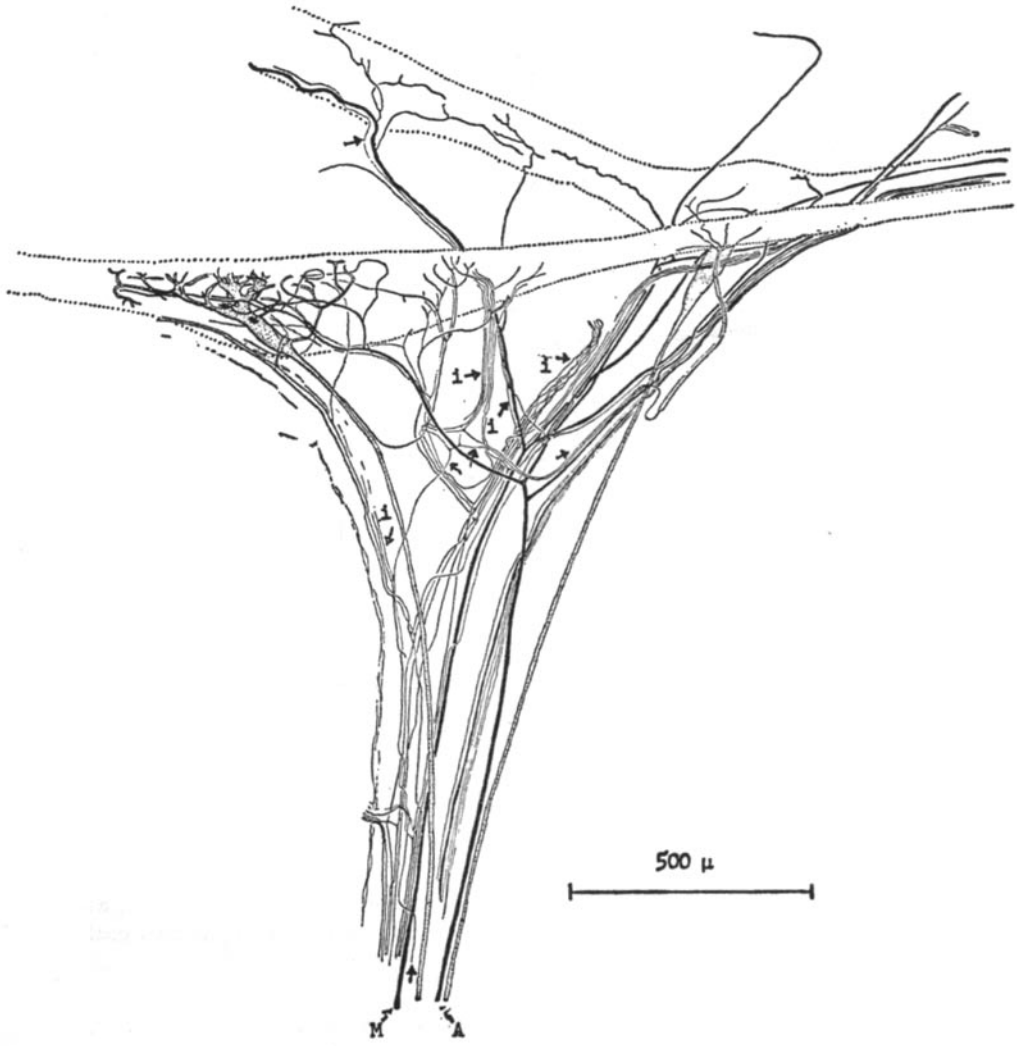

FIG. 9. Drawing of the afferent and efferent innervation of one stretch receptor organ of *Astacus* (from a silver-impregnated preparation, same as in Figs. 2 and 4). i = thin fiber which supplies RM 1 and RM 2. M = motor fiber to RM 2, A = accessory fiber.

the limit of visibility with the light microscope. This nerve fiber behaves like the thick accessory fiber only with respect to the sensory neurons, but it does not take part in the innervation of the receptor muscles outside their sensory area.

(*c*) In none of our preparations could we find an indication of the existence of a fiber that would correspond to the thin accessory fiber of *Homarus*.

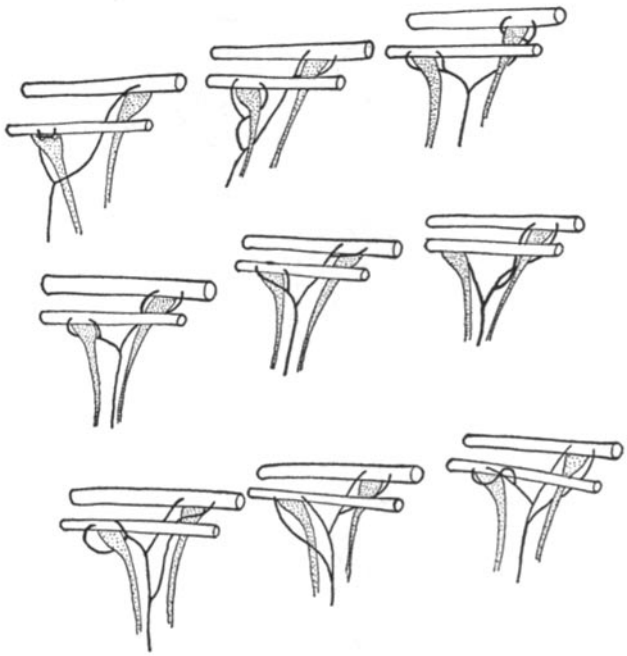

FIG. 10. Schematic drawing of the mode of branching of the accessory fiber, as observed in different preparations. Note in how many different ways the final goal is reached.

(*d*) RM 1 receives a multiple innervation by several thin efferent fibers. These fibers branch several times before reaching the muscle, sending off bundles of five to six fibers to the superficial muscles. Those branches which finally reach RM 1 have a diameter from 1 to 3 μ. Their endings cover the entire length of RM 1, including the sensory innervated area. One of these fibers (i in Figs. 8 and 9) also innervates RM 2, as could be observed in a few preparations.

DISCUSSION

There seems to be no doubt that RM 1 and RM 2 receive a different innervation, and we have reason to believe that at least the motor innervation

of each muscle is independent from that of the other. It is known that the muscles of decapode crustaceans are innervated by at least two efferent axons, one being the motor axon, the other an inhibitory fiber. Since there is no in-

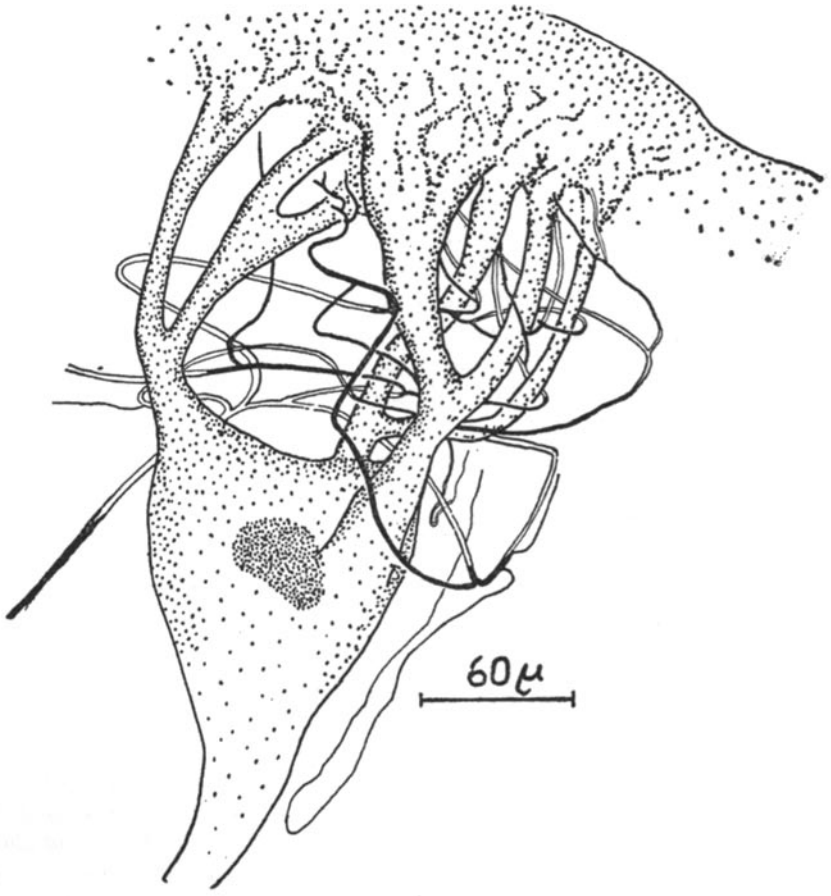

FIG. 11. Semischematic drawing of the relationship between the branches of the accessory fiber and the dendrites and endings of SN 2 (from a silver-impregnated preparation).

dication that RM 2 receives more than two efferent fibers, one of them must be an inhibitory axon. The inhibitory fibers are usually thinner than the motor fibers (Van Harreveld and Wiersma, 1939) and one might justly assume that the thin fiber which innervates RM 2 is an inhibitory fiber, while the thick efferent fiber is its motor axon (M in Fig. 8). Since the thin fiber also innervates RM 1 both muscles might be inhibited by the same fiber.

While the motor fiber of RM 2 does not seem to participate in the innervation of the superficial muscles, there is no doubt that the same fibers which innervate RM 1 also supply the superficial muscles. There is much evidence that all the efferent fibers which innervate the same muscle or group of muscles, bifurcate at the same place (for references see Wiersma, 1941). We have observed the same principle in the efferent innervation of the superficial muscles and RM 1. We found, however, in several instances certain deviations. One fiber of a bundle may, for instance, divide too early. In this case its two branches will run alongside in the same bundle to the place where the next general bifurcation takes place. If the bundle consists originally of five fibers, there will now be six. At the next point of general bifurcation this fiber does, however, not divide with the others, so that the bundle divides into two bundles of five branches. We can therefore generally assume that the number of branches found in any nerve bundle supplying the superficial muscles or RM 1 represents the true number of fibers which innervate these muscles. Since most of the bundles consist of five branches we believe that RM 1 receives a quintuple efferent innervation. The different motor innervation of RM 1 and RM 2 is of particular interest in view of recent experiments of Kuffler (1954) which show that electrical stimulation of the efferent supply of the receptor muscles causes a fast, or twitch contraction in RM 2 and a slow, or tonic contraction in RM 1.

It is also of interest to compare the spacial distribution of the efferent nerve endings in the two receptor muscles: In RM 1 the endings of the various fibers are rather evenly distributed over the whole length of the muscle, while in RM 2 the region around the sensory area is free of motor endings. This would mean that during motor stimulation the sensory area of RM 1 is shortened while that of RM 2 is stretched.

If we now consider the sensory neurons, the most conspicuous difference between SN 1 and SN 2 is the mode in which the dendrites divide. One is tempted to believe that the different structure of the final nerve endings of these two sensory cells is responsible for their different behavior. As we know from the work of Wiersma *et al.* (1953) and Kuffler (1954), SN 1 shows a slow adaptation and fires at a constant rate if a constant amount of stretch is maintained, while SN 2 fires only if a considerable amount of stretch is reached and if the rate of stretch is big enough. From the behavior of SN 1 it can be deduced that the extension of the nerve endings is directly correlated with the final impulse frequency (see also Florey, 1955). These nerve endings are in parallel with the muscle fibers so that any stretch of the muscle fibers is directly conveyed to the nerve endings (see Fig. 5). The endings of SN 2, however, are pointing in all directions and it would take a considerable amount of stretch to orient them more or less parallel to the muscle fibers. Only then could any additional extension of the muscle bring about a stretching of the nerve endings and thus fire the neuron.

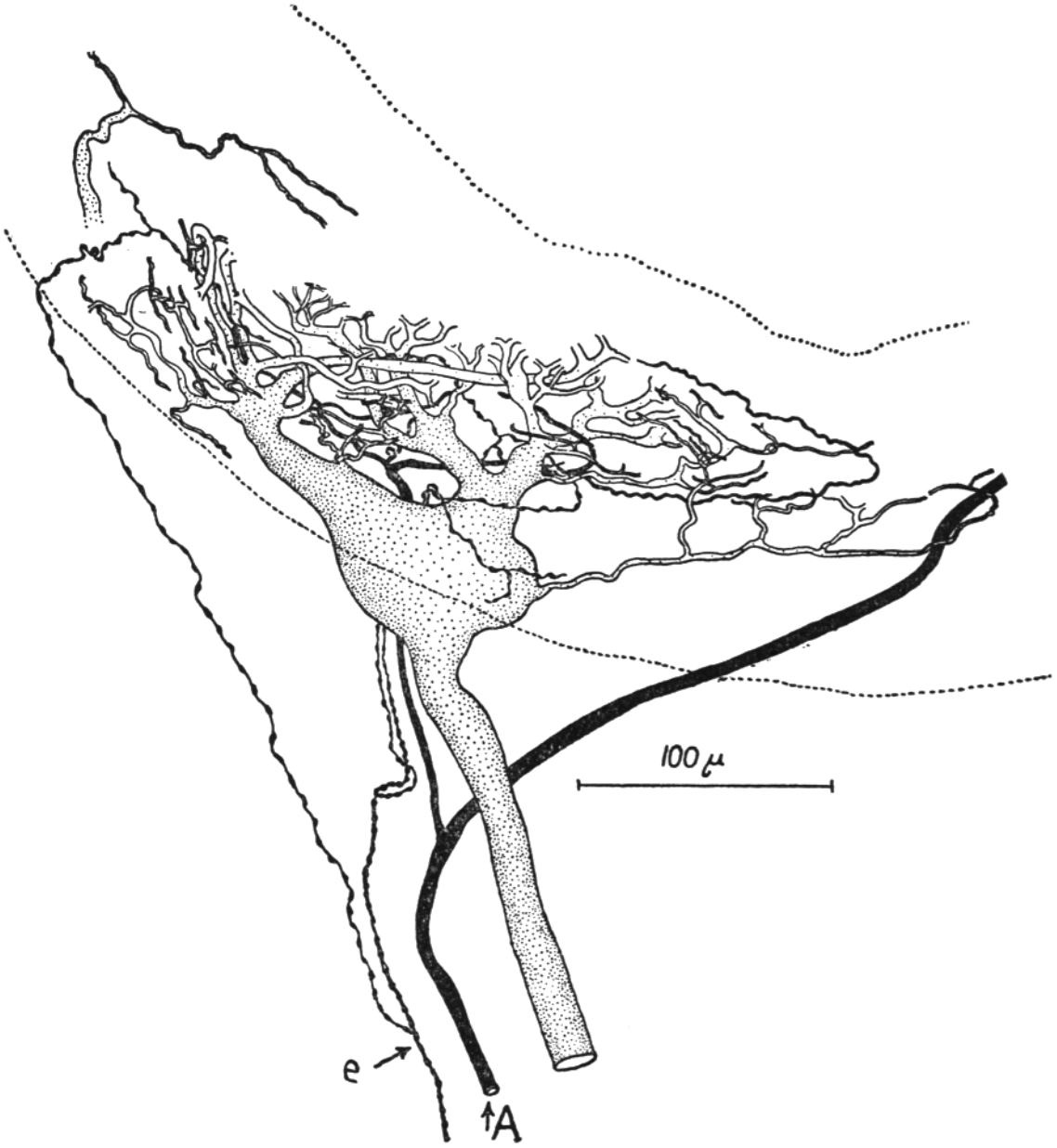

FIG. 12. Drawing of one SN 1 which seems to receive endings of another fiber besides those of the accessory fiber (from a silver-impregnated preparation). *A* = accessory fiber, *e* = branch of one of the thin fibers supplying RM 1.

Beside the motor fiber which supplies RM 2 there is another fiber which does not participate in the innervation of the superficial muscles: this is the fiber which corresponds to the "thick accessory fiber" (Alexandrowicz, 1951) of *Homarus* and *Palinurus*. As we have shown, the endings of this fiber do not take part in the innervation of muscle but enter into an intimate relationship with the sensory endings. They are efferent fibers and are most likely concerned with the regulation of the sensory discharge. For the lobster, where there is a thick and a thin accessory fiber, Alexandrowicz has suggested that one of them is excitatory, one inhibitory. Since there is only one such fiber in the crayfish it could be either excitatory or inhibitory. The histological evidence alone is not sufficient to permit a final decision. Kuffler and Eyzaguirre (1955) have, however, shown by electrophysiological methods that in the crayfish both sensory neurons receive an inhibitory neuron. It is therefore justifiable to call the fiber which in the crayfish corresponds to one of the two accessory fibers of the lobster, an inhibitory fiber.

We have considered the possibility that at least SN 1 receives an excitatory fiber which would replace the thin accessory fiber of the lobster. In only one preparation have we observed that endings of one of the thin fibers supplying RM 1 also have endings at the cell body and dendrites of SN 1. Fig. 12 shows the situation. Since it is possible that the stained nerve endings represent only part of the system it is uncertain whether the picture presents the true relationship of these endings and the sensory neuron or whether the actual endings do not turn from the sensory structures to the muscle fibers. Physiological evidence is needed to clarify the situation.

There is no doubt that the efferent innervation of the abdominal stretch receptors of the crayfish *Astacus* differs greatly from that of the lobsters *Homarus* and *Palinurus*. Unfortunately we have no silvered preparations of stretch receptors of the American crayfish *Cambarus*. This makes it somewhat difficult to generalize our findings for all the Astacidae. We have, however, studied a number of freshly dissected, and formalin-fixed stretch receptors of *Cambarus virilis* Hagen. Anatomical localization, shape of the sensory cells, and pattern of cross-striation of RM 1 and RM 2 are similar to those of *Astacus*. There is also no tendinous, intercalated region in the sensory innervated area of the receptor muscles.

SUMMARY

Microanatomical studies on the abdominal stretch receptor organs of the crayfish *Astacus fluviatilis* L. have been carried out in order to establish a basis for the physiological work that has been, and is being carried out on stretch receptors of various species of crayfish.

Important differences have been found between these organs and those previously described by Alexandrowicz for the lobsters *Homarus vulgaris* and *Palinurus vulgaris*.

With the aid of silver-impregnated preparations the relationship of sensory endings and muscle fibers has been shown as well as the pattern of the efferent innervation. The physiological significance of the histological findings has been discussed.

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