STUDIES ON THE ACTIVATION OF CRUSTACEAN MECHANORECEPTORS. THE MOVEMENT RECEPTORS OF THE CRAB PACHYGRAPSUS CRASSIPES RANDALL AND THE MOVEMENT AND STRETCH RECEPTORS OF THE CRAYFISH PROCAMBARUS CLARKII (GIRARD)

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ABSTRACT

The activation of crab movement receptors and of crayfish movement and stretch receptors was studied. It was found that the adequate stimulus to movement receptors is a change of the length of the elastic strand into which they send their distal processes. One group of movement receptors responds to shortening of the strand; and another group to its lengthening. A hypothesis is presented that accounts for the differential sensitivity of these two groups of cells, and the difference between movement and position receptors in the same sense organ. It was found that the movement receptors may be activated by nicotine as well as by mechanical means; and that nicotine activation differs in a significant manner from mechanical activation. The unusual spiking pattern of movement receptors was examined using intracellular and extracellular recording. The mode of activation by nicotine and the intracellular recording data are combined to form a partial explanation of the movement receptors' discharge pattern. Caffeine was found to block the mechanical activation of movement receptors and it is concluded that this is due to a direct effect on the mechanotransducer membrane. Several drugs which affect the crayfish stretch receptor were found to have no effect on movement receptors and conversely nicotine and caffeine were found to have no effect on stretch receptors. Stretch receptors were subjected to the same mechanical stimuli used on the movement receptors and the responses of the two types of end organs compared. The similarities and differences of movement and stretch receptors are discussed.

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I. INTRODUCTION

The problem of how stimulus energy is converted by sensory receptors into neural signals has been of interest for many years. Since the work of Adrian and coworkers (1, 2, 3, 4) on the responses of muscle spindles and cutaneous touch and pressure receptors, a large amount of information has accumulated relating to this problem. The earlier work was limited to recording the spike responses of the afferent nerves and comparing these responses to the parameters of the stimuli. Using this approach certain salient features of the receptors became known, most of which were recognized by Adrian and Zotterman (3, 4). They found, by recording from the afferents of single receptor units, that a muscle spindle discharges at increasing frequency as the tension on the muscle is increased; that the discharge is smoothly rhythmic except at very low frequencies; and that, when the muscle was stretched to a particular tension and maintained at that tension for a prolonged period, the discharge frequency of the receptor rose abruptly at the onset of the stretch but then fell to a lower value. This last, the phenomenon of adaptation, was also observed by these authors in the touch receptors and in the pressure receptors, identified as Pacinian corpuscles, of the toe pad of the cat. The adaptation of the spindle, touch receptors and pressure receptors differed in that the frequency of discharge of the spindle decreased to a plateau value if the tension was maintained, but did not drop to zero; whereas the discharge of the touch receptors subsided completely after a very short time and that of the pressure re-

ceptors, although not stopping completely, became irregular. It was observed that abrupt unloading of the muscle spindle caused the afferent to go silent for a period of 0.75-1.3 seconds after which spikes again appeared, the frequency rising to that characteristic of a resting spindle -- about 6/sec. Primarily on the basis of this last, Adrian and Zotterman reasoned that adaptation was not due to an increase in the refractory period of the nerve but to a decrease in the sensitivity of the end organ to the stimulus. A muscle spindle is thus capable of presenting two kinds of information in its discharge: it will indicate the speed of onset of the stimulus, since the maximum frequency of the initial part of its discharge is determined by this factor; and it will indicate the magnitude and duration of the stimulus during the later portion of its discharge after adaptation has occurred. The pressure receptors indicate the intensity of the stimulus by their frequency of discharge at the onset of the stimulus, but adapt in such a brief time that little information is given by them concerning the duration of the stimulus. Although no detailed study was made of the touch receptors' characteristics, they adapt so rapidly that it is unlikely that they signal more than the fact that stimulation took place. It was not shown that they can indicate duration or intensity of stimulation.

Recording the spike discharges in the afferent fibers of a receptor and comparing the form of these discharges with the stimuli did not yield much information regarding the nature of the processes within the end organs. The questions of why adaptation took place and why the spike frequency was in some way a function of the intensity of the stimulus

could not be answered from such a "black box" approach. That the answers to these questions have in part been revealed is the result of a fortunate set of circumstances that have made three types of mechanoreceptors particularly useful in this connection. All three types are so constructed and situated that it is possible under the proper conditions to record electrical changes from very near the end organ of the receptor. These three receptors are the vertebrate muscle spindle and Pacinian corpuscle, and the crustacean stretch receptor. In all three a potential has been observed, called the receptor potential, that is antecedent to and apparently responsible for the triggering of the spike discharge. The three types of receptor will be treated separately below.

The receptor potential of the muscle spindle was first recorded by Katz (5), using an electrode placed on the afferent nerve very close to its exit from the muscle mass. He found that when the muscle was stretched the spike discharge set up was accompanied by a slow shift of the baseline potential. Before the first spike of a discharge the potential slowly shifted, then the first spike appeared followed by repolarization, after which the slow shift once more manifested itself followed by another spike and so on. The receptor potential had to pass a threshold value to initiate a spike, and the frequency of spiking was proportional to the magnitude of the receptor potential. Application of procaine to the preparation abolished the spikes but did not greatly affect the slow potential. In the absence of spikes there was no repolarization until the stretch was released. The receptor potential, in the absence of spikes, showed adaptation: it rose rapidly when the muscle was

stretched and then fell slowly to a steady value proportional to the level of steady stretch of the muscle. When the muscle was abruptly unloaded the receptor potential fell to a value less than that which it had before the application of stretch and then rose to its resting level. Thus in all ways that could be observed, the amplitude of the receptor potential behaved just as did the frequency of the spike discharge. Katz reasoned that the receptor potential was the immediate consequence of the stretch of the spindle endings and that it acted as a cathodal stimulus to the more central portion of the sensory axon; the spike frequency would thus be set by the magnitude of this potential. Further, adaptation was a property of the receptor potential and not of the part of the nerve where the spikes arose.

The crustacean stretch receptors behave in a manner similar to the muscle spindle. These receptor organs were discovered by Alexandrowicz (6) in the lobster, Homarus vulgaris. Every organ consists of a pair of muscle bundles of unequal size, each with an associated nerve cell. There are two of these organs at each abdominal joint, one on either side of the middorsal line, and they span the joints in such a manner that Alexandrowicz suggested they might be receptors for the position of one segment on another. The dendrites of the sensory cells, which are usually few in number and of large diameter, penetrate the middle region of the muscle bundle with which the cell is associated and the axon, emerging from the opposite pole of the cell, runs to the ventral nerve cord. The microanatomy of stretch receptors, especially their dendrites, was investigated by Florey and Florey (7), in the

crayfish, Astacus fluviatilis, using methylene blue or silver staining. They found that the structures of the terminal portions of the dendrites of the two cells were different: the dendrites of SN1, the sensory neuron of the thin muscle bundle, branched extensively and were quite long showing a preferred orientation parallel to the fibers of the muscle; whereas the dendrites of SN2 were shorter, less branched, and exhibited no obvious orientation with respect to the muscle.

The two components of the receptor organ differ in their physiological characteristics as well as structurally. The first physiological findings regarding these receptors were those of Wiersma et al. (8, 9); using the crayfish, Cambarus clarkii. The stretch receptors in this animal differ but slightly from those in the lobster and they were found to indeed be sensitive to flexion of the abdomen and to direct stretch. The thinner muscle and its sensory cell, collectively called RM1 by these authors, responded to stretch in a manner almost identical to the muscle spindles investigated by Katz. Adaptation in this receptor is incomplete; the spike discharge occasioned by stretching dropped in frequency if a maintained stimulus was applied, and reached a steady level characteristic of the degree of stimulation. Under conditions of maintained stretch an RM1 might continue to discharge at a fairly constant, regular rate for several hours. The frequency of the adapted discharge was a function of the degree of stretch and the frequency before adaptation appeared to be dependent on the rate at which the receptor was stretched. It was shown that the muscular portion of the receptor is not necessary for a response; but that stimulation of the motor nerve to the

muscle causes a contraction that can elicit a discharge. Wiersma and coworkers also found that the stretch receptors are stimulated by acetylcholine (ACh) in concentrations as low as 10^{-8} gm/ml in stretched preparations. This drug causes a silent RM to discharge and will accelerate the discharge of an already active organ. High concentrations of ACh do not block the response of the organ. The effect of ACh is potentiated by eserine in low concentration but blocked by this same drug applied at high concentrations. By clamping the organs very close to the nerve cell, and by exposing fully relaxed organs to ACh, it is possible to demonstrate that the drug still exerts its effect; showing that the stimulation of the RM is a direct effect on the nerve cell and is not mediated through a contraction of the muscular portion of the receptor.

The fact that the RM's in both lobster and crayfish are separate from the rest of the dorsal musculature, except for some connective tissue, was taken full advantage of by Eyzaguirre and Kuffler (10, 11, 12, 13). The size of the sensory cells makes it possible to introduce into their interiors microcapillary electrodes for the measurement of the potential difference between the interior and exterior of the cells. Such measurement, performed on RM1, revealed a receptor potential such as that found with extracellular recording in the vertebrate muscle spindles. Graded stretch of the receptor gives rise to a proportionately graded decrease of the resting potential of the cell; and, when the potential has decreased to a certain value, spikes are set up which invade, and may be recorded in, the soma. Each spike

repolarizes the soma to the resting level following which the potential again drops, producing another spike. As the degree of stretch is increased, the rate of rise of the receptor potential also increases, triggering the spikes at briefer intervals. Until rather high levels of stretch are reached the threshold potential for spike initiation does not change and each spike is followed by repolarization to the resting level; but under conditions of strong stretch the repolarization phase of each spike no longer reaches the resting potential, the average internal potential declines, and the threshold voltage for spiking shifts toward a lower voltage. Sudden release of stretch was observed to produce an "off" effect, a slight hyperpolarization of the soma, which was especially prominent if the preceeding stretch was a strong one. Eyzaguirre and Kuffler proposed that stretching the dendritic endings of the nerve cells causes a proportional depolarization, which they name "generator potential," in these terminals that is conducted electrotonically to the soma region and there initiates the spike. The invasion of the soma by the spike then wipes out the depolarization there; but it is once again reestablished by the persistent potential change in the dendrites, which are not reached by the spike. They offer no explanation for the mechanism of adaptation; but do state that the "off" hyperpolarization is a manifestation of this process, unmasked by the abrupt cessation of the excitatory process.

The larger of the two components of the stretch receptor organ, RM2, acts in a somewhat different manner from RM1 (9). If the abdomen of the crayfish is flexed to an extent which produces a steady

discharge from RM1, RM2 gives a burst of impulses of rapidly declining frequency and quickly becomes completely silent. In isolation it is found that RM2 responds to stretch of medium amplitude in the aforementioned manner; but that if the stretch is increased to still higher levels then it reacts similarly to RM1. In the intracellular recordings of Eyzaguirre and Kuffler (10,12) it was shown that the receptor potential of RM2 increases steeply at the onset of stretch, its rate of rise and total amplitude being dependent on the rate of stretch and the amplitude of stretch, and then decreases rather rapidly if the stretch is maintained constant. If the constant level of stretch is low the receptor potential decays below the spike threshold and only if the steady stretch is quite large does the receptor potential remain above the spike threshold after adaptation is complete. It is clear that such behavior is merely a modification of that already described for the vertebrate muscle spindle and the crayfish RM1.

The Floreys proposed (7) that the explanation for this difference between RM1 and RM2 lay in the anatomy of the dendrites. They reasoned that if the adequate stimulus to the dendritic endings were longitudinal stretch then the endings of SN1 would be oriented in such a way as to be properly stretched by a very small extension of the muscle; whereas the endings of SN2, since they point in all directions, would first have to be pulled out parallel to the axis of the muscle and then stretched. In this manner would the difference in threshold of the two receptors be explained. In order to account for the rapid adaptation of the RM2 they invoked an unstable coupling between the endings

of the SN2 and the muscle fibers such that when the muscle came to rest at a new length the SN2 terminals would slide back to their original orientation and would no longer be stimulated. It appears that a more likely explanation for the differences between RM1 and RM2 is presented by Krnjević and van Gelder (14) who investigated the relation of the tension in the muscle bundles to the firing frequency. Using stretch receptors of Astacus fluviatilis they made direct measurements of the tension in the muscle bundle using a strain gauge system, and compared the tension so measured with the instantaneous firing frequency of the receptors. They found that as the length of the muscle bundle is increased in small steps the tension rises in a stepwise manner; overshooting at each step and then decaying to plateau values between increases in length. The authors state that the adapted firing frequency is perfectly correlated with the plateau tension and that the unadapted frequency at each step correlated closely with the overshoot of the tension. There was little difference between the slow (RM1) and fast (RM2) receptors except that RM2 required slightly higher tension than RM1 to attain the same firing rate, and RM2 did not maintain a given tension well--apparently the muscle extended. There was a slight undershoot in the measured tension on sudden relaxation which alone could account for the electrical "off" effect noted by Eyzguirre and Kuffler. Krnjević and van Gelder state that since the changes in measured tension comport so well with the accompanying changes in output frequency it appears that the differences in response between slow and fast receptors may be attributed almost entirely to the

mechanical characteristics of the muscle bands. A further consequence of these findings is that the amplitude of the receptor potential in the RM's must be directly proportional to the tension of the band since the spiking frequency is proportional to the tension and to the amplitude of the generator potential. The mechanoreceptive portions of the dendrites are linear transducers of tension up to the limit imposed by the potential difference across the membrane; and the generator potential faithfully records changes of tension as well as the level of steady tension. It is not unreasonable to suppose that the vertebrate spindle endings share this property.

Although Adrian and Umrath (2) had found in their experiments that the pressure receptive Pacinian corpuscles of the cat gave a maintained irregular discharge under constant compression, later work on this receptor proved them to have been in error, probably due to the mechanism they used for stimulation. Alvarez-Buylla and Ramirez de Arellano (15) measured the receptor potential of the Pacinian corpuscle using an electromechanical system for stimulation. In essence the stimulation consisted of compression of the corpuscle by the tip of a small rod. The rod was moved by an electromagnetic coil system which embodied sufficient stability to hold the stimulus quite constant at various intensities. They found that the receptor potential was graded with the intensity of the stimulus and that it adapted very rapidly to maintained compression. Furthermore, a similar receptor potential appeared at the termination of a maintained compression, the polarity of which was the same as that at the onset.

The only difference between on and off response lay in the amplitude of the potentials; the on potential was larger than the off potential for a given size of stimulus. This property of the Pacinian corpuscle was confirmed by Gray and Sato (16) who also found that there was apparently a threshold for the receptor potential. Unless the stimulus intensity was high enough no receptor potential was detectable with external leads, but since they were not recording with intracellular electrodes it is possible that responses to very low intensity stimuli were undetectable in the noise of their records. More important than this apparent threshold for the amplitude of the stimulus was the observation that the appearance of the receptor potential depended on the rate at which the corpuscle was compressed, if the stimulus rose to its final intensity too slowly there was no observable potential. In a series of experiments Loewenstein and coworkers (17,18,19) demonstrated that the properties of the Pacinian corpuscle set forth above were characteristic of the terminal, unmyelinated portion of the afferent fiber where it lay inside the capsular structure of the corpuscle. By stripping off as much as 99% of the capsule they were able to demonstrate that the receptor potential, measured at the point where the afferent leaves the capsule, is the result of a generator potential set up by compression in the unmyelinated segment; and that any part of the unmyelinated segment can evolve such a potential. The rapid adaptation is also a characteristic of this unmyelinated segment: the time course of the generator potential set up by a compression is exactly the same whether the compression be immediately released or

maintained for several seconds. With this decapsulated preparation it was shown that a given spot of the ending became refractory after evolving a generator potential and that stimulation of two spots at once produced summation of the generator potentials recorded when either spot was stimulated alone. Loewenstein (20) proposes that the transducer membrane of the unmyelinated ending is a patchwork of receptor spots, each of which acts in an all-or-none manner when stimulated. He attributes the graded nature of the receptor potential to spatial summation, more of the spots being involved as the stimulus is increased because the mechanical disturbance spreads over a greater area. That each spot exhibits all-or-none behavior would explain the rapid adaptation of the receptor to a maintained stimulus and the necessity for the stimulus to reach its full value at a greater than minimal rate: the spots first involved by a slowly increasing stimulus would be refractory by the time additional spots are stimulated, so spatial summation would fail. In this work no threshold for stimulus amplitude could be observed!

Despite the differences between muscle spindle and stretch receptor on the one hand and the Pacinian corpuscle on the other with respect to threshold, refractivity and adaptation, there remains a basic similarity in the manner in which mechanical activity is converted to impulse signals in all three (21, 22). Mechanical stimulation elicits from the terminal endings of the sensory neuron a potential change which is graded in amplitude, the amplitude being proportional to the intensity of the stimulus. Regardless of whether adaptation is slow or fast some

part of the generator potential follows, in its amplitude, the intensity of the stimulus; and in turn acts as a stimulus to the nerve fiber, setting up spikes that travel to the CNS. The frequency of the spike discharge in the nerve is proportional to the amplitude of the generator potential and thus the duration of the discharge will indicate the duration of the generator potential and the instantaneous frequency of the spikes will mirror its instantaneous amplitude. In the receptors discussed above the frequency of spiking may range up to several hundreds of impulses per second and thus a single receptor unit can convey information about a wide range of stimulus intensities. This wide response range and high maximum frequency is characteristic of most receptor types that have been investigated (3, 4, 5, 9, 23, 24, 25, 26, 27).

The present work arises from the finding by Wiersma and Boettiger (28) and Wiersma (29) that certain receptors in the leg joint of various Decapod crustacea respond to stimulation in a manner which one is hard put to fit into the scheme of mechanoelectric conversion set forth above. The existence of these receptors was first found by Barnes (30, 31) who recorded the impulses set up in the leg nerves of a number of Decapods by movement of the joints. He found that spikes were present in the nerves only when the joints were in motion and that no signals appeared if the joint was maintained in a given position. He considered that the cessation of the discharge at the end of movement was a rather remarkable example of adaptation and commented on the rapid recovery from this adaptation: the discharge restarted as soon as the movement was resumed. Barnes dubbed these sensory units

"movement receptors."

In 1954 Burke (32) discovered in Carcinus maenas the sense organ responsible for this movement response by tracing a bundle of large fibers from the meropodite. The organ consists of an elastic strand which spans the joint between the propodite and the dactylopodite, running from the dorsal border of the closer muscle apodeme to a point on the shell of the dactyl. Located at the proximal end of the strand is a cluster of large nerve cells which send their axons into the main leg nerve along with the axons of numerous smaller cells situated more distally on the band. Similar "innervated strands" have been described by Alexandrowicz (33) and Alexandrowicz and Whitear (34) in the more proximal leg joints of various Decapods; and Wiersma (29) has found them at carpopodite-propodite and meropodite-carpopodite joints. According to Burke (32) the cells of the PD (propodite-dactylopodite) organ are bipolar and lie in a mass of connective tissue loosely bound to the elastic strand. Emerging from the distal pole of each cell a long (~ 500 μ) process runs distally and seems to end in the fibers of the strand. The ultrastructure of the distal processes of cells in the PD organ of Carcinus has been studied by Whitear (35) with the electron microscope, and has been found to resemble that found in cells of chordotonal organs. The distal processes of two cells approach each other and enter a cylindrical scolopale which is secreted by a non-nervous cell (or cells). The two distal processes lie in close apposition, one on either side of the tubular scolopale. Whitear was unable to find cells of any other structural type in the PD

organ; the only variation on this plan of organization appeared in the CP2 organ of Carcinus (36) where she observed many scolopales which contained one normal looking distal process and one that seemed to be degenerate.

The work on the physiological properties of these organs, begun by Barnes, was continued by Burke (32). Recording apparently from the whole PD nerve, he observed discharges to vibrations of 5 - 1000 cps, to passive and active movement and to maintained position at either side. With the organ isolated from the leg and held in clamps, he was able to show that discharges occurred both when the band was stretched and when it was relaxed; and that under these conditions it was still quite sensitive to vibration. On the basis of these data he suggested that the mechanism of stimulation of the movement receptors, as he too called them, was a transient deformation of the cell membrane produced by some kind of viscous drag. He did not make any speculations regarding the mechanism of activation of the position receptors in the organ. Unfortunately Burke did no single unit recording and so missed a number of very interesting features of these organs.

This deficiency was remedied by Wiersma and Boettiger (28) and Wiersma (29) who made single unit recordings from the PD, CP and MC organs of Carcinus maenas and a number of other Decapods. In the PD organ of Carcinus, Wiersma and Boettiger found four distinct types of receptors: units sensitive to movement of the joint in the closing direction, units sensitive to movement in the opening direction, units which responded with discharges proportional to the position of

the joint towards fully closed and others to the position of the joint toward fully opened. The position units reacted much as do spindle organs and stretch receptors; when the joint reached a certain position on the appropriate side of the arc they began to discharge slowly and irregularly and at positions closer and closer to the proper side they responded with higher and higher frequencies. The discharge of the position receptors was maintained for long periods, some showing slight adaptation and others no adaptation at all. Those that exhibited no adaptation are probably the most nearly perfect position receptors known (37): the frequency of their discharge is always a function of the position; and the speed with which any position is approached does not affect their output.

The manner in which the mechanical stresses produced by movement are coupled to the excitable membrane of the movement receptors so as to produce unidirectional responses is not at all clear. It was noted by Wiersma and Boettiger (28) that a number of thin elastic fibers cross the angle between the main strand of the PD organ and the closer tendon, and that since the angle between strand and tendon decreases when the strand is stretched by closing, and increases when the strand is relaxed by opening, the tensions of main strand and thin fibers are in opposite phase. Wiersma (29) has observed that the CP organs of Carcinus do not, particularly in older animals, usually respond equally well to both directions of movement. There are two organs at the CP joint and one usually responds best to flexion; the other to extension. Wiersma calls attention to the fact that both these organs

are being relaxed during the movements to which they are preferentially sensitive.

The movement units displayed a wide range of thresholds; the most sensitive responding to very slow movements of the order of one degree per second, with others responding only to more rapid movements. The most sensitive movement units are almost entirely unaffected by the position of the joint and respond identically near the fully opened and fully closed positions; whereas the less sensitive units may respond better toward one side or the other, usually toward the end of the movement in the direction to which they respond. The range of speed to which an individual unit responds with an increase in frequency is rather restricted: when the speed of movement exceeds the threshold of a unit it begins to discharge irregularly at a low frequency and as the movement is made more rapid the discharge becomes more regular and may rise in frequency to about 40 - 50/sec. Further increasing the speed of the movement, by as much as tenfold, does not produce a smooth rise of the frequency of discharge but may cause the appearance of double or triple spikes, the doublets or triplets recurring at about 40 - 50 per sec. The incidence of doublet or triplet firing is not, so far as is known, a smooth function of the movement speed; leading one to conclude that a single movement receptor is a poor indicator of absolute speed of movement. Wiersma and Boettiger referred to the maximum frequency attainable by a movement receptor unit as the "saturation frequency"; and Wiersma (37) has stated the opinion that this frequency is determined by an intrinsic timing process within the cell. He has

reached this conclusion as the result of examining two characteristics of the movement receptors: the way in which the discharge frequency increases between threshold and saturation, and the timing of the multiple firings at saturation. In the records from Carcinus movement receptors there is an occasional missing spike in an otherwise regular train at saturation. The interval left by the failure of a single spike is nearly twice the interval characteristic of the saturation frequency. At lower discharge frequencies the intervals between spikes appear to be integral multiples of the saturation interval. Wiersma feels that such behavior can only be explained by a cyclic change of excitability of the cell that makes firing more probable at certain times than at others. That the doublets and triplets recur at the same interval as single spikes, and that the frequency within a doublet or triplet is quite high support such a belief. Since the frequency in the multiple discharge is high, perhaps 150/sec., the refractory period of the cells cannot be so long as to account for the low saturation frequency.

A timing mechanism such as Wiersma proposes would be a radical departure from the common sort of spike generating mechanism already known to exist in other types of sensory receptors. The form of the generator potential in movement receptors may be quite unusual, and the relation of the generator potential amplitude to stimulus intensity may also be atypical. Alternatively, the unique character of the discharge of movement receptors may have its foundation in the coupling between generator potential and spike initiation.

The purpose of the present work is to investigate both the mechanical aspect of the stimulation of movement receptors in an attempt to account for unidirectional sensitivity, and the mechanism of spike generation within single cells as a test of Wiersma's hypothesis concerning impulse timing. For the sake of comparison a series of experiments was performed on crayfish stretch receptors subjected to the same kind of stimulation used on the movement receptors.

II. METHODS

Experiments were performed on the shore crab, Pachygrapsus crassipes (Randall), and the crayfish, Procambarus clarkii (Girard); both obtained from commercial dealers. Until used the crabs were kept in glass aquaria through which artificial sea water at approximately 15 degrees C. was constantly circulated. The water was filtered through fiberglass and charcoal on each circuit through the pumping system. The artificial sea water was made up according to the formula* given by Tyler (38), the salts being dissolved in distilled, deionized water. For this purpose the metal chelating agents in Tyler's formula were omitted. Crabs survived in this environment for as long as two months with little overt sign of deterioration, although it appeared that the response of the sense organs were not as good after a week as they had been when the animals were newly caught. For this reason new crabs were procured at two week intervals when possible. The crayfish were kept in concrete tanks in running tap water. Here too some degeneration of the sense organs seemed to take place, but the experiments reported on these animals were all performed on fresh batches.

The setup for stimulation and recording is shown diagrammatically in fig. 1. In order to achieve controlled stimulation of the mechanoreceptors a special system was devised and assembled based on an electromechanical transducer built to specification by the Stephens

*The artificial sea water is constituted as follows: NaCl-590 g. ; KCl-16.5 g. ; $MgCl_2$-308 g. ; $CaCl_2$-28.4 g. ; Na_2SO_4-93.6 g. ; $NaHCO_3$-5.1 g. ; H_2O q. s. p. 25 L.

Tru-Sonic Corp. This device employs the field magnet assembly of a high compliance 12-inch loudspeaker. A bifilar coil is wound on a light aluminum tube and mounted in the field of the magnet in such a way that the tube moves back and forth as the proper currents are sent through the windings. Attached to one end of the tube, and coaxial with it, is a three-inch long bar of aluminum; through the end of which extends an insect pin at right angles to the bar. With the transducer in place for stimulation of a PD organ this pin extends downward perpendicularly into a tiny hole in the shell of the dactyl of the crab leg; and as the bar is moved back and forth the dactyl is flexed and extended. When the transducer is used for stimulation of an isolated sense organ, either PD organ or crayfish stretch receptor, the aluminum bar is replaced with a spring loaded clamp. One end of the isolated organ is held in this clamp, the other end in a second clamp which is mounted on a micrometer drive. The peak to peak intensity and frequency of the stimulus are controlled through the transducer, and the average tension with the micrometer drive.

The current to drive the transducer is supplied by a push-pull transistorised amplifier driven by a Hewlett-Packard model 202A Low Frequency Function Generator which delivers sinusoidal, triangular or rectangular waveforms at very low distortion. The response of the entire stimulating system extends from DC up to several tens of cycles per second with the output excursion of the transducer reaching two centimeters peak to peak. The two centimeter excursion is more than sufficient to move the dactyl of the crab leg through its whole arc, thus

Figure 1

Block diagram of apparatus. 1, waveform generator; 2, power amplifier; 3, transducer with arm and pin attached; 4, input probe of cathode follower; 5, cathode follower preamplifier; 6, main scope; 7, monitor scope; 8, audio system. The insert depicts the way the transducer is set up for stimulation of isolated organs. 3, transducer with spring clamp attached; 9, second spring clamp on micrometer drive mount.

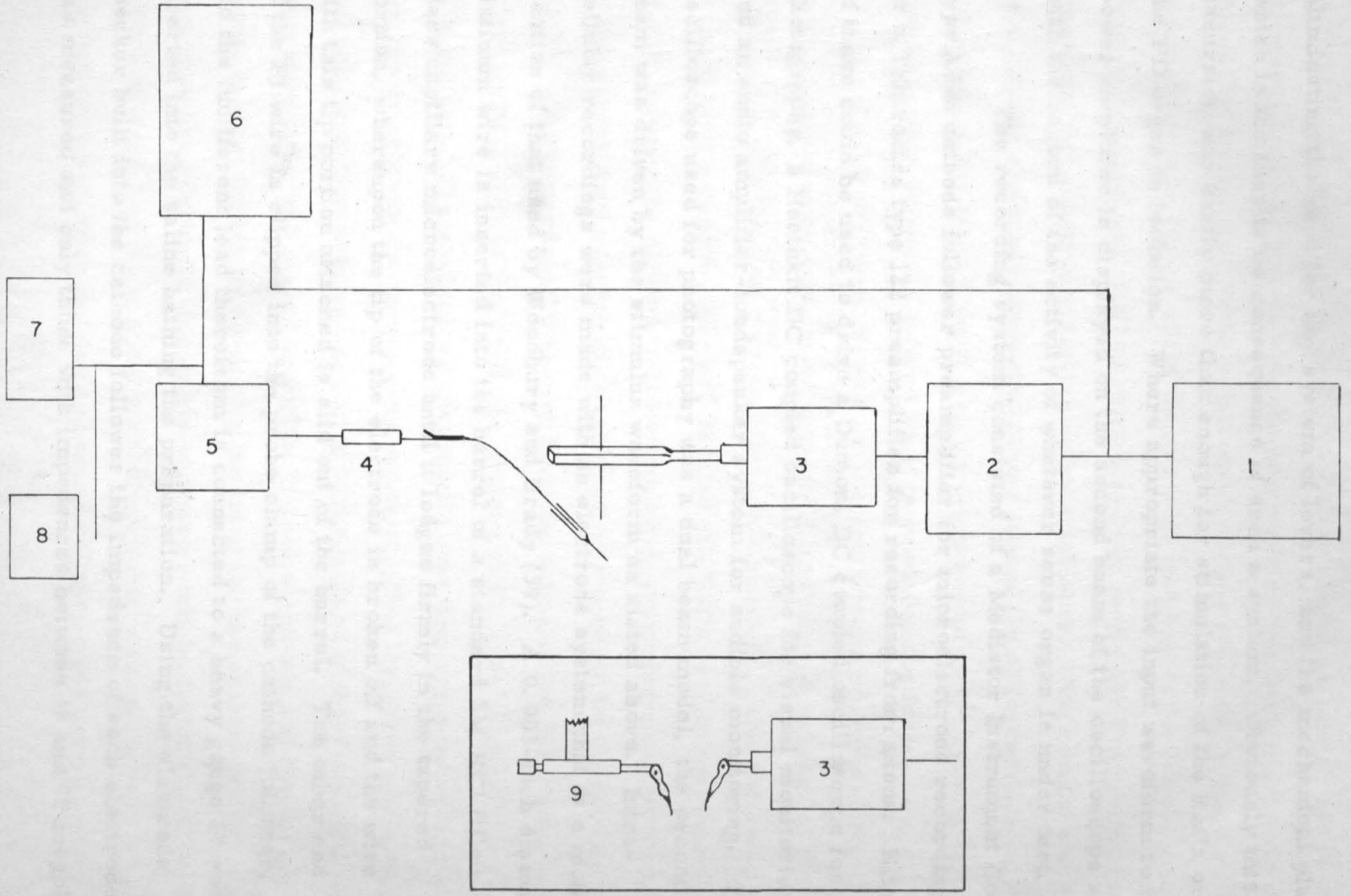


FIGURE 1.

eliminating the need for any system of levers, and the mechanical play which is the inevitable consequence of such a system. Obviously this excursion was vastly more than enough for stimulation of the RM's or the PD organ in isolation. Where appropriate the input waveform to the power amplifier is displayed on the second beam of the oscilloscope along with the record of the activity of whatever sense organ is under test.

The recording system consisted of a Medistor Instrument Corp. type A32b cathode follower preamplifier for microelectrode recording, or a Tektronics type 122 preamplifier for recording from axons. Either of these could be used to drive a Dumont DC coupled oscilloscope for photography, a Heathkit DC coupled oscilloscope for visual monitoring and an audio amplifier-loudspeaker system for audible monitoring. The oscilloscope used for photography was a dual beam model, the second beam was driven by the stimulus waveform as stated above. Intracellular recordings were made with an electrode system that is a modification of that used by Woodbury and Brady (39). A 0.003-inch diameter platinum wire is inserted into the barrel of a standard 3M KCl filled glass capillary microelectrode until it lodges firmly in the tapered portion, whereupon the tip of the electrode is broken off and the wire with this tip portion attached is slid out of the barrel. The other end of the Pt wire is clipped into the probe clamp of the cathode follower, and the indifferent lead therefrom is connected to a heavy gauge Pt wire inserted into the saline bathing the preparation. Using the electrode checker built into the cathode follower the impedence of each electrode was measured and only those with impedences between 15 and 75 megohms

were used. The probe was held in a Zeiss micromanipulator which allowed maneuvering the electrode into position and lowering the tip onto the cell to be impaled. The small mass of the tip portion of the electrode and the high compliance of the thin Pt wire resulted in the lowest attainable drag on a moving cell. When recording from axons the Tektronics preamplifier was substituted for the cathode follower. The axon(s) was lifted on a Pt hook electrode connected to one side of the differential input of the preamp, the other side of which was connected to the bathing saline, also through a Pt wire. A third Pt wire grounded the bath.

To record from the axon of a stretch receptor was relatively easy. The abdomen was cut off a crayfish and the ventral part of the shell along with the flexor musculature was removed. Careful removal of the medial extensor muscles revealed the RM's, which could then be placed between the clamps of the stimulating apparatus along with a two- to three-centimeter length of the afferent nerve which was picked up on the Pt hook electrode. To record from the axons of the PD organ, one of the walking legs of the crab was pinched at its base causing it to be autotomized. The shell of the meropodite was then removed on one side and the muscles in the meropodite removed. The leg nerve was cleaned and split with fine needles according to the method of Wiersma (40). Each subbundle was picked up on the Pt hook electrode and the dactyl moved back and forth by hand. By listening for the characteristic response of the PD bundle this group of fibers could be isolated and then split further if desired to yield recordings containing one or only a very few active units. When the desired degree of purity of response was

achieved, the resulting nerve filament was cut at its proximal end and lifted out of the water to give monophasic recording. The recordings from the PD organ of the crayfish chelipid were made in exactly the same manner.

Intracellular recording from the RM's was not a great deal more difficult than recording from their axons. The only difference in the procedure was that once the RM was mounted in the clamps a microelectrode was pushed into the cell body under direct microscopic control in transmitted light. Since the muscle bundle of the RM was prone to slip out of the way of the electrode tip, a small glass hook, maneuvered by a second micromanipulator, was used to support the RM until penetration was achieved, after which the hook was moved out of the way. To reach the PD organ for intracellular recording, the distal half of the shell of the propodite was cut away on the ventral side. The intact bridge of shell on the dorsal side served to support the articulation of the dactyl. The exposed fibers of the closer muscle were then removed with great care revealing the PD organ lying just ventral to the nerve emerging from the dactyl. Figure 2 is a diagrammatic view of the preparation as it looks at this stage of the dissection. The PD bundle was then separated from the nerve trunk and the portion of this trunk in the dissection field was removed. The leg was then pinned down in a wax bottomed dish and the microelectrodes inserted in transmitted light. Here too the great mobility of the elastic strand necessitated the use of the glass hook. Light was supplied through a lucite tube built into the wooden block on which the preparation dish rested, and a slab of lucite

Figure 2

Diagrammatic drawing of the PD organ in situ: 1, pin connecting transducer to dactyl; 2, dactyl; 3, elastic strand; 4, nerve trunk from dactyl; 5, cell mass of PD organ; 6, opener muscle; 7, propodite; 8, closer tendon exposed by removal of distal fibers of closer muscle; 9, region where thin fibers fan out from elastic strand; 10, closer muscle.

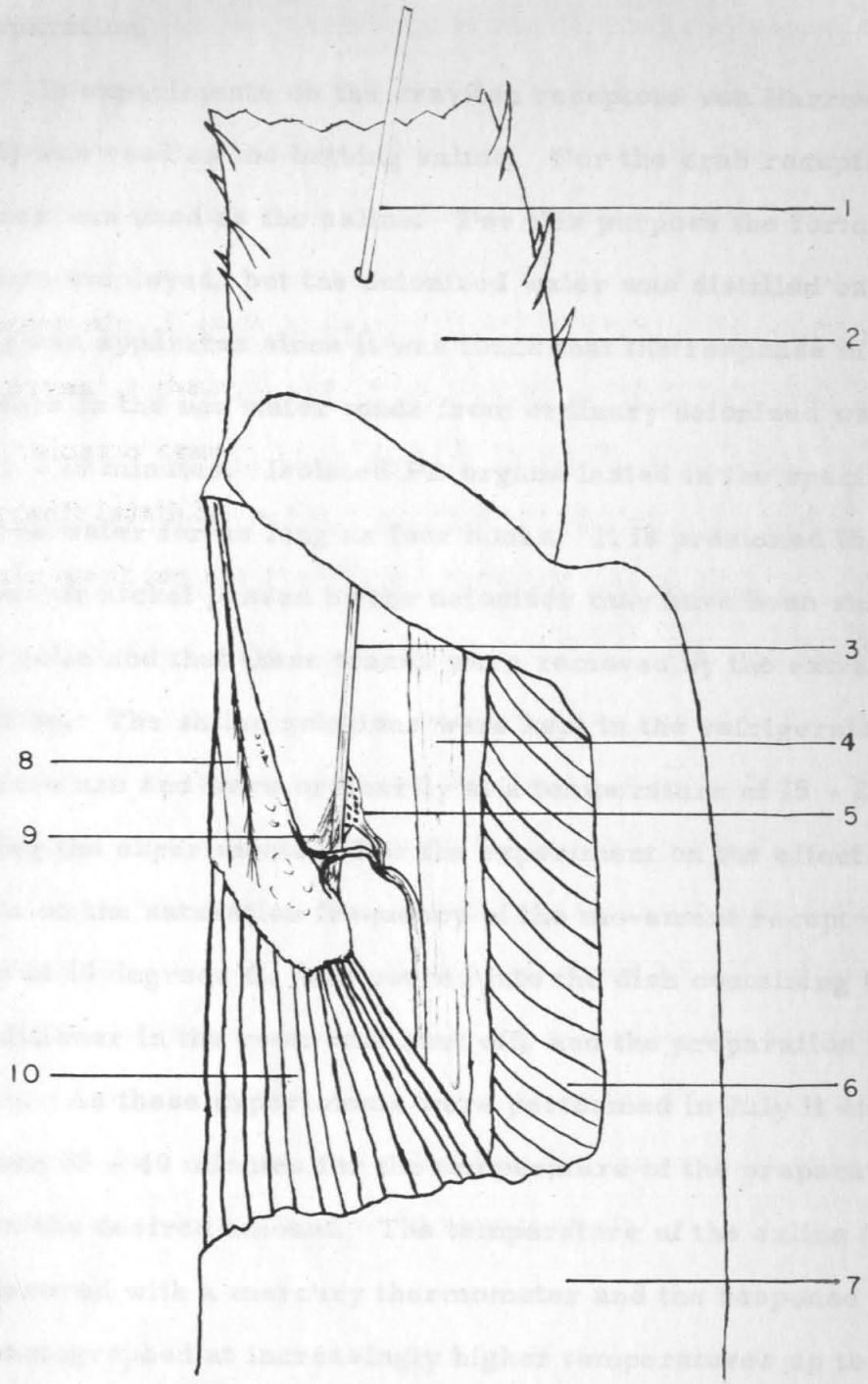


FIGURE 2.

embedded in the wax in the bottom of the dish permitted the light to reach the preparation.

In experiments on the crayfish receptors van Harreveld's solution (41) was used as the bathing saline. For the crab receptors artificial sea water was used as the saline. For this purpose the formula cited above was employed, but the deionized water was distilled once more in an all-glass apparatus since it was found that the response of the organs disappears in the sea water made from ordinary deionized water, usually within 5 - 10 minutes. Isolated PD organs lasted in the specially prepared sea water for as long as four hours. It is presumed that traces of copper or nickel passed by the deionizer may have been sufficient to kill the cells and that these traces were removed by the extra glass distillation. The saline solutions were kept in the refrigerator until just before use and were ordinarily at a temperature of 15 - 20 degrees C. during the experiments. For the experiment on the effect of temperature on the saturation frequency of the movement receptors, saline solution at 10 degrees C. was poured into the dish containing the leg, the air conditioner in the room was shut off, and the preparation allowed to warm up. As these experiments were performed in July it did not take more than 35 - 40 minutes for the temperature of the preparation to increase the desired amount. The temperature of the saline in the dish was measured with a mercury thermometer and the response of the organ photographed at increasingly higher temperatures up to 25 degrees C., at which temperature the response of the organ usually ceased irreversibly.

For the experiments on the pharmacological properties of the receptors the drugs were made up in the desired concentrations in the appropriate saline solution and kept in the refrigerator at all times when not actually in use. All concentrations, except for those of nicotine, are expressed in grams per milliliter; nicotine in the liquid alkaloid form was used and its concentration is in milliliters alkaloid per milliliter saline. Nicotine solutions were made up fresh at about weekly intervals since the drug decomposes even at lowered temperatures.

Drug solutions were applied with a medicine dropper drawn out to a fine tip. The tip was lowered into the bathing saline and the bulb squeezed as gently as possible. Even with extreme care a discharge was often provoked from the movement receptors by the application of the drugs. As a control, saline solution was applied in the same manner as were the drugs and an identical discharge was obtained. Apparently the discharge caused at the moment of the application of a drug was due to the agitation of the fluid. Such discharges were disregarded in the interpretation of the records. No attempt was made to control or determine the final concentration of any drug at the receptor; the drug solutions were added to an undetermined volume of saline at various distances from the receptor organs. It is felt that for the present purpose a knowledge of the final concentrations is unnecessary.

III. RESULTS

Controls

Since the stimulus parameters are indicated in the following records by the input voltage to the power amplifier of the stimulating apparatus, it was felt necessary to compare this input with the resulting excursion of the transducer. For this purpose a light aluminum vane was affixed to the bar on the transducer, which was arranged so that the vane interrupted a beam of light falling on a photocell. Movement of the vane changed the fraction of the surface of the photocell that was illuminated and the output voltage of the photocell was thus closely proportional to the position of the vane. Figure 3(a) is a record of the excursion of the transducer measured in this manner: the upper beam displays the input voltage to the power amplifier and the lower beam displays the output voltage of the photocell. At the highest rates of stimulation used in these experiments this correspondence may not be so good as shown due to the inertia of the moving parts of the system; and in fact when the system is fed a rectangular waveform a damped oscillation follows each switch of position. However, the output circuit of the amplifier provides a high degree of negative feedback and the damping factor of the amplifier is quite high, so it is felt that the inaccuracies that might be introduced by such distortion are insignificant in the interpretation of the data.

Although Wiersma and Boettiger (28) have stated that opening the propodite of Carcinus has little effect but for increasing the sensi-

Figure 3

(a) Input to power amplifier compared with output excursion of transducer. Input-upper trace; output-lower trace. (b) Activity in whole PD nerve before dissection of propodite. (c) As above but after the shell of the propodite has been opened and the hypodermis removed. (d) As in (b) and (c) but after the distal portion of the closer muscle has been removed.

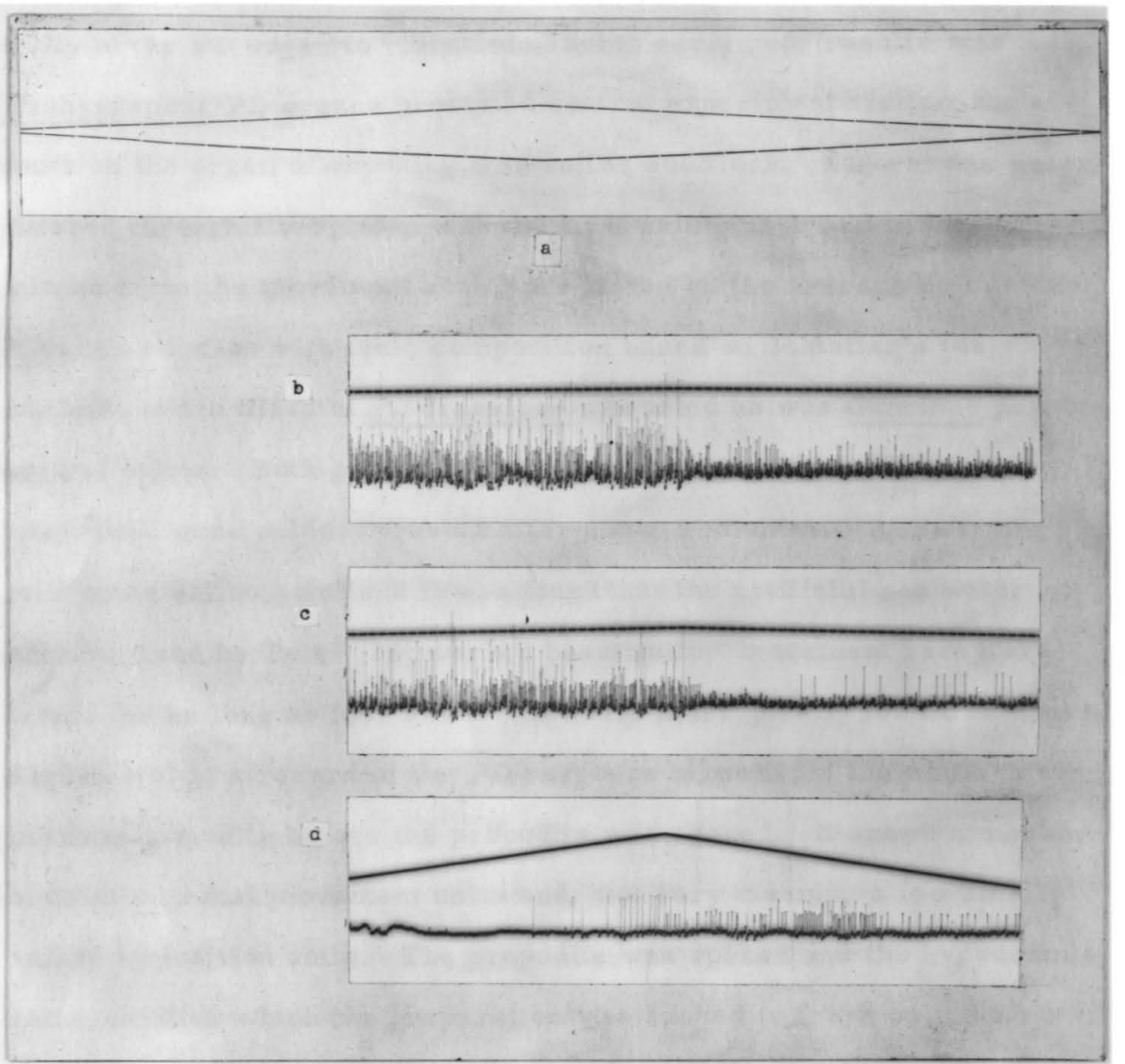


FIGURE 3.

tivity of the PD organ to vibrations, some early poor results with Pachygrapsus PD organs prompted control experiments to test the effects on the organ of exposing it to saline solutions. Natural sea water, filtered through fiberglass, was the first saline used and in it the response from the movement receptors lasted on the average 20 minutes. A saline solution with ionic composition based on Schlatter's (42) analysis of the blood of P. crassipes was tried as was Carcinus physiological saline. Both gave even worse results than natural sea water; responses were seldom present after about 5 minutes. After trying still other saline solutions it was found that the artificial sea water solution used by Tyler (38) worked beautifully: movement receptors lasted for as long as four and a half hours and typically for three hours. Figure 3(b) is a record of the PD response taken from the whole nerve in the meropodite before the propodite was opened. It shows a number of unidirectional movement units and, not very clearly, a few small spikes of position units. The propodite was opened and the hypodermis removed after which the preparation was soaked in fresh saline for 15 minutes, at the end of which time record 3(c) was made. Although the number of responding units is smaller it is clear that movement units are still responding in their typical manner. The entire distal mass of the closer muscle was then removed and record 3(d) was made. Here it can be seen that still fewer units are responding and that the more sensitive units seem to have been lost; note that the stimulus speed is higher than in record (c). The response was recorded monophasically in all three records with the proximal end of the nerve cut and floating

free. The flexion of the nerve trunk which was inevitable during the two steps of the dissection must undoubtedly have injured some of the nerve fibers and it is also likely that the spatial relationship of the electrode to the responding fibers changed from record to record. The preferential loss of the high sensitivity units may be explained by the observation, made many times, that these units are the most sensitive to mechanical damage: indeed if the dactyl is moved very rapidly, recording from a high sensitivity unit usually reveals a sudden high frequency discharge followed by silence. It must be stressed that the units that continue to respond do so in an entirely normal manner and that once the dissection is complete those units still responding will continue to do so in an unaltered fashion for several hours. Furthermore these records were made early in the series of experiments and as the experimenter's dissection technique improved with time the number of sensitive units which would respond after complete opening of the propodite and removal of the closer muscle became progressively greater. From the results of these control experiments it would appear that neither the dissection nor the bathing solution materially affected the response of those units that were left after these procedures.

Mechanical factors in the stimulation of movement receptors

Although Wiersma (29) has stated that shortening of the elastic strand is almost certainly the stimulus for most movement receptor cells in the CP organs of Carcinus, it was decided to recheck this conclusion with the PD of Pachygrapsus. The responses to passive opening

and closing were first recorded and then the strand shortened and lengthened by hand with fine forceps. The response to lengthening contained impulses identical to those elicited by closing, and similarly for shortening and opening. Yet the response of the PD organ may also be affected by other factors. The influence of the elastic fibrils crossing the angle between strand and closer tendon was investigated in two ways: the first was to cut these fibrils in situ and record the response of the organ; the second was to remove the organ from the leg and stretch and relax it directly under controlled conditions. Cutting the fanning fibrils, as I will call the thin elastic fibrils, produced no change in the response of units in the PD, either to opening or to closing. To achieve controlled stimulation of the organ in isolation, the ends of the strand were placed in the clamps of the stimulating apparatus as described in Methods. The response of the organ was picked up from a length of the PD bundle that was removed with the organ and the length of the organ adjusted with the micrometer mounted clamp until no position responses could be detected. According to Wiersma and Boettiger (28), this lack of position unit discharges indicates that the strand is near its resting length. Despite the great mechanical stresses that must have been placed on the receptor by this procedure, responses were found when the strand was shortened and lengthened. An example is shown in fig. 4(a) of the response of such a preparation. Examination of the record shows that with constant velocity stretch and relaxation of the elastic strand the movement receptors responded in a wholly normal manner. There are at least four units responding to extension of the

Figure 4

(a) Bidirectional response of an isolated PD organ. (b) Response of a single opening unit in the isolated organ.

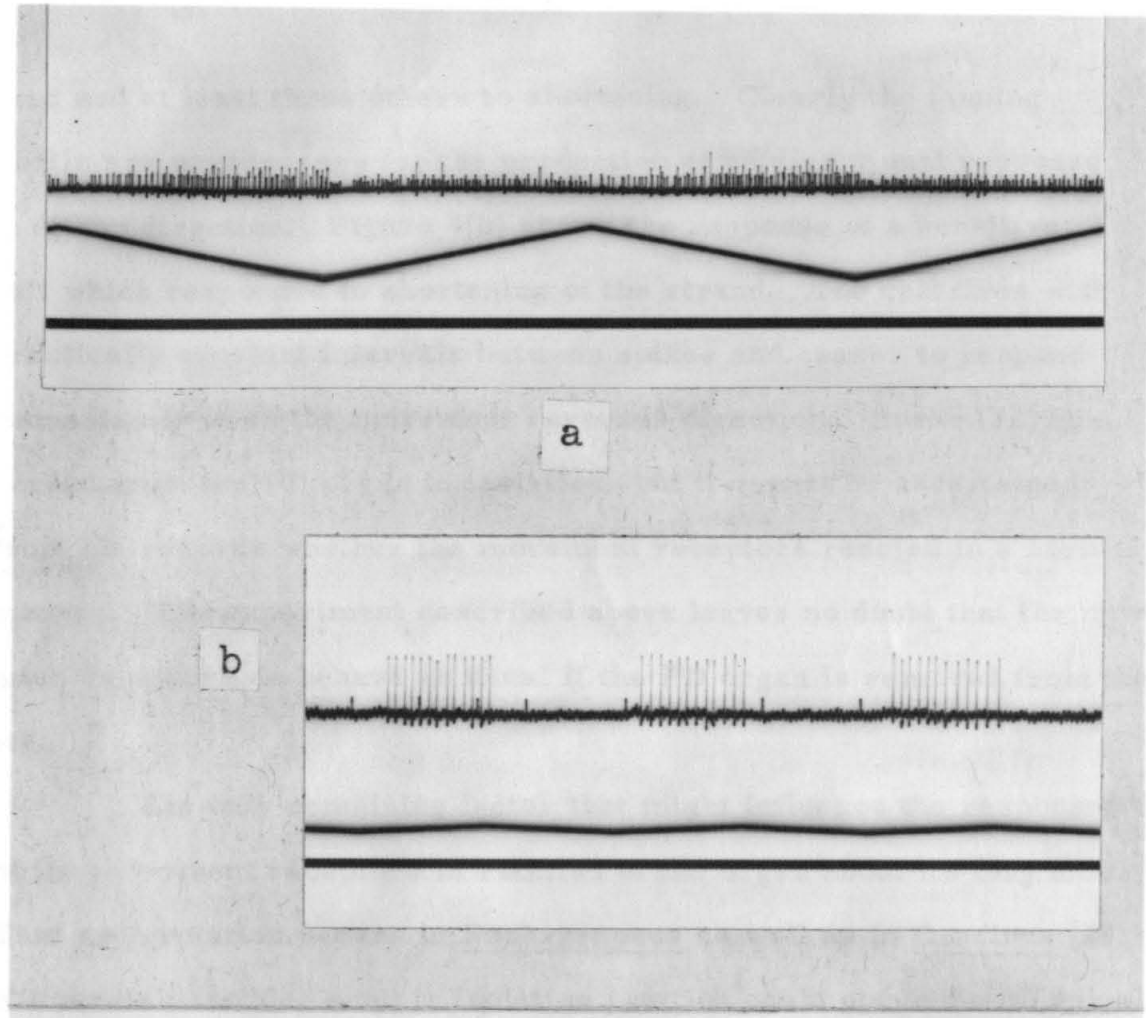


FIGURE 4.

band and at least three others to shortening. Clearly the fanning fibrils are unnecessary for the production of unidirectional response in either direction. Figure 4(b) shows the response of a sensitive unit which responded to shortening of the strand. The cell fires with practically constant intervals between spikes and ceases to respond immediately when the movement reverses direction. Burke (32) recorded from the PD organ in isolation, but it cannot be ascertained from his records whether the movement receptors reacted in a normal manner. The experiment described above leaves no doubt that the movement receptors do behave as usual if the PD organ is removed from the leg.

The only remaining factor that might influence the response of the movement receptors is rotation of the organ about its long axis. That such rotation occurs in Pachygrapsus as well as in Carcinus (28, 37) seems possible, even in isolation rotation could occur due to spiral orientation of the fibers of the strand, and experiments were performed to test the effect of such rotation on the responses of the movement receptors. An apparatus was constructed with a spring clamp mounted in a teflon bearing in such a manner that the clamp could be rotated about its own long axis. The distal end of the elastic strand was clamped while in its normal orientation and cut distal to the point of clamping. The end of the propodite, along with the dactyl, was then removed and the preparation carefully adjusted so that the axis of the clamp was parallel to that of the elastic strand and the length of the strand changed as little as possible (fig. 5). The rotating clamp apparatus was mounted on a

Figure 5

Diagrammatic view of the setup for rotation of the elastic strand. The propodite (1) has its distal end cut off to expose the distal stump of the closer tendon (2). The elastic strand (3) is held in a spring clamp (4) which is mounted on shaft (5). The shaft has attached to its opposite end a pulley (8) and runs in a teflon bearing (7) supported rigidly through a rod (6). A rubber band (9) runs to a second pulley, not shown, which is rotated by hand.

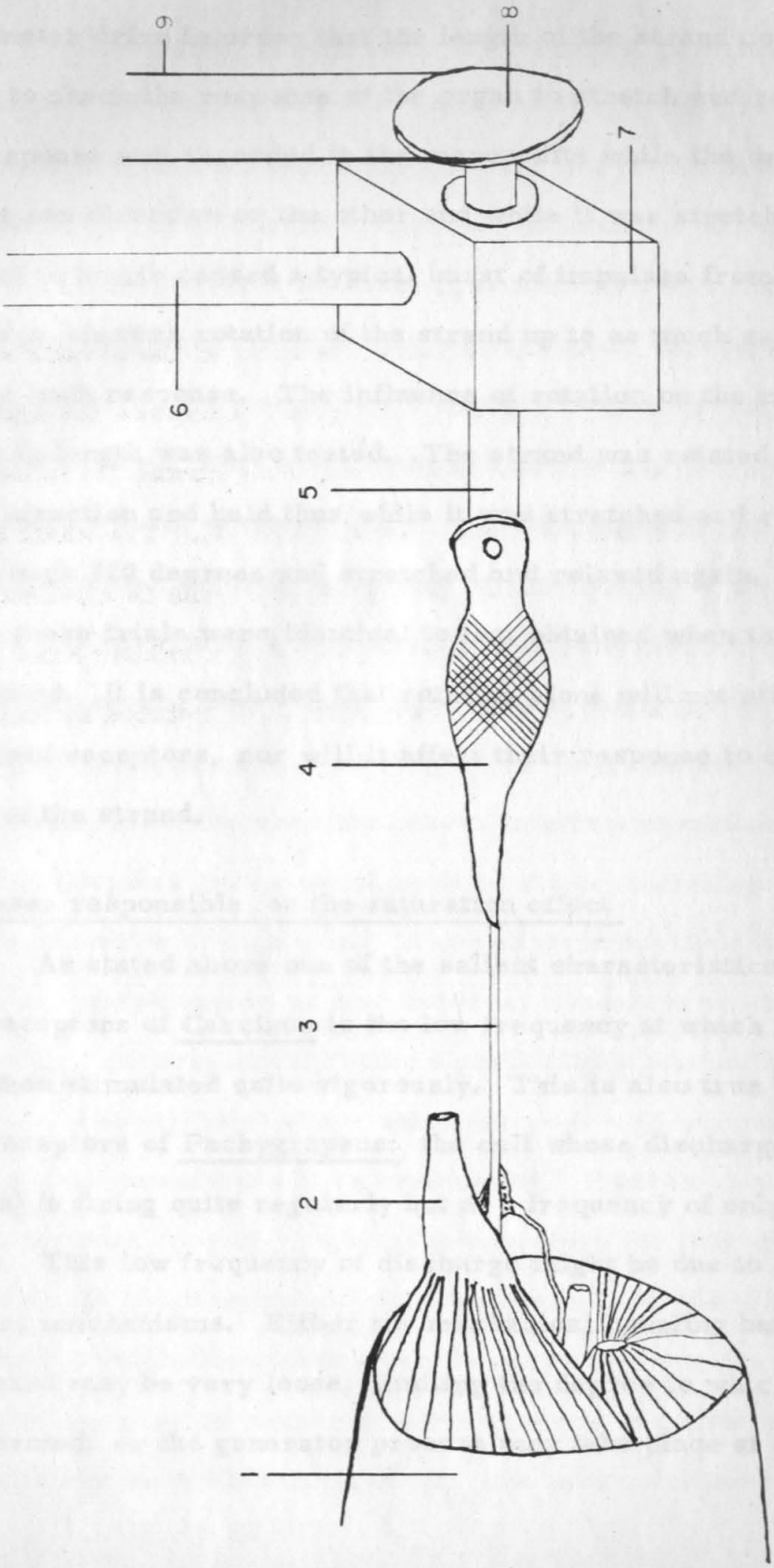


FIGURE 5.

micrometer drive in order that the length of the strand could be changed at will to check the response of the organ to stretch and relaxation. The response was recorded in the meropodite while the organ was rotated in one direction or the other and while it was stretched and relaxed. Changes in length caused a typical burst of impulses from the movement receptors whereas rotation of the strand up to as much as two full turns gave no such response. The influence of rotation on the response to change in length was also tested. The strand was rotated 360 degrees in one direction and held thus while it was stretched and relaxed, then turned back 720 degrees and stretched and relaxed again. The responses in both these trials were identical to that obtained when the strand was not rotated. It is concluded that rotation alone will not stimulate the movement receptors, nor will it affect their response to changes of the length of the strand.

Processes responsible for the saturation effect

As stated above one of the salient characteristics of the movement receptors of Carcinus is the low frequency at which they discharge even when stimulated quite vigorously. This is also true of the movement receptors of Pachygrapsus: the cell whose discharge is shown in fig. 4(b) is firing quite regularly but at a frequency of only a little over 40/sec. This low frequency of discharge might be due to one of three different mechanisms. Either the mechanical coupling between cells and strand may be very loose, limiting the degree to which a cell can be deformed; or the generator process may take place at such a distance

from the spike initiating locus that even when the generator potential is maximal the output frequency is limited by the depolarization spread to the spiking point; or finally, some process in the cell membrane may limit the frequency: Wiersma's hypothesis outlined above would be in this last category. Since the first alternative depends mainly on physical processes the change in saturation frequency with temperature might be expected to be quite small were this alternative the correct one. Either of the other two possible mechanisms ought to show a marked change with change of temperature; a change which might be reflected in the saturation frequency. To test this possibility a series of experiments were performed in which saturation frequency was determined at various temperatures. Such experiments suffer from the limits imposed by the temperature sensitivity of the preparation. At 25 degrees C. the preparation irreversibly ceases to give a response, and at 12 - 14 degrees the spikes get so small as to be almost indistinguishable from the baseline noise at high gain. In one experiment the saturation frequency was photographed at four different temperatures from 10 - 24 degrees C., the preparation being a particularly fortunate one. This was the only experiment where saturation was well maintained at all temperatures so only it will be considered. Figure 6 is a graph of the mean saturation frequency against temperature for this experiment. The points for the first three temperatures, 10, 14 and 19 degrees C., lie along a straight line, then a break occurs between 19 and 24 degrees. The actual location of the break in the curve is of course unknown and the dotted line so indicates. The Q_{10} has been calculated from the

Figure 6

Plot of the saturation frequency of a movement receptor as a function of temperature. Frequency on the ordinate; temperature on the abscissa.

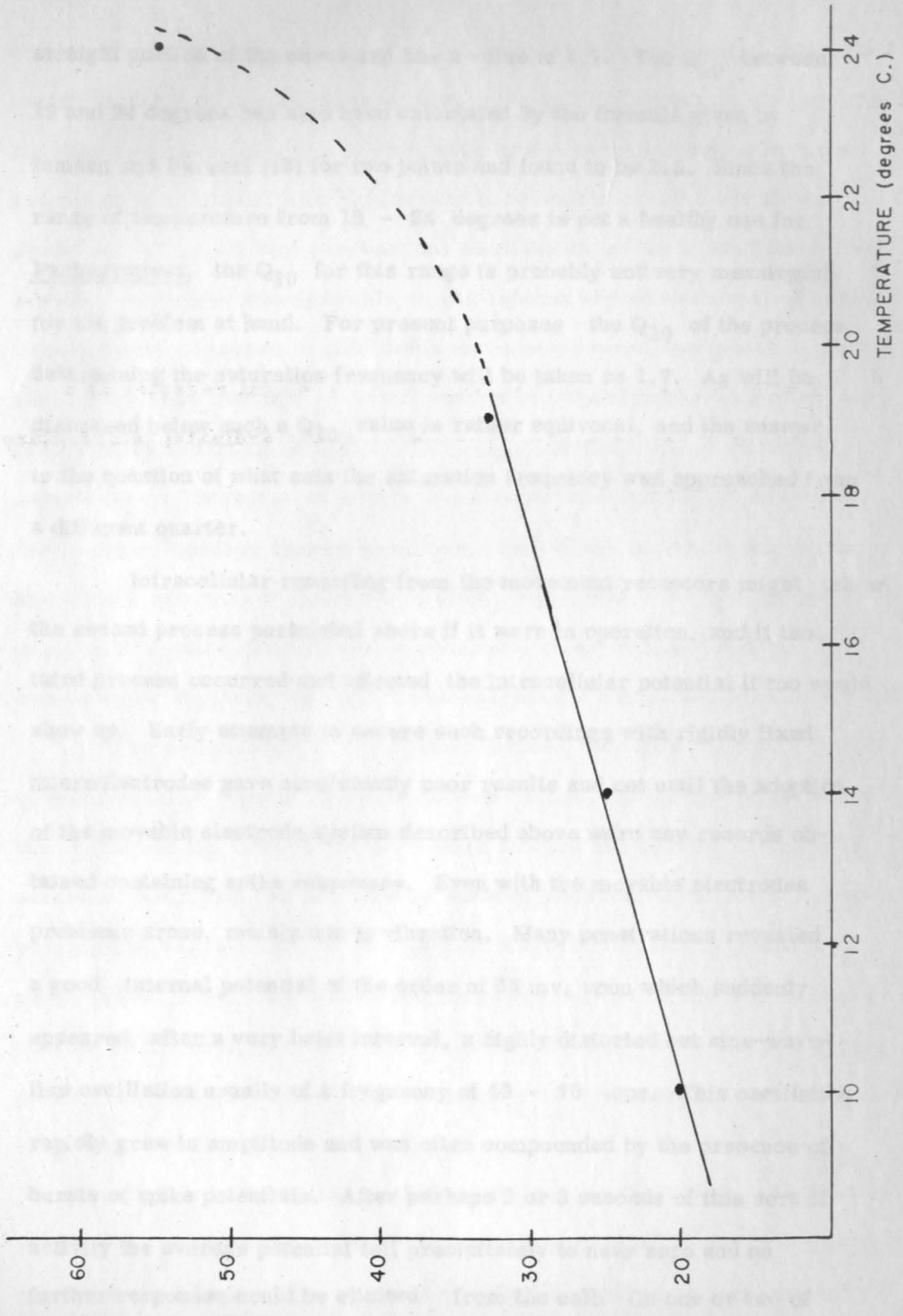

FREQUENCY
60
50
40
30
20
10
12
14
16
18
20
22
24
TEMPERATURE (degrees C.)

FIGURE 6.

straight portion of the curve and has a value of 1.7. The Q_{10} between 19 and 24 degrees has also been calculated by the formula given by Inmann and Perruzi (43) for two points and found to be 2.3. Since the range of temperature from 19 - 24 degrees is not a healthy one for Pachygrapsus, the Q_{10} for this range is probably not very meaningful for the problem at hand. For present purposes the Q_{10} of the process determining the saturation frequency will be taken as 1.7. As will be discussed below such a Q_{10} value is rather equivocal, and the answer to the question of what sets the saturation frequency was approached from a different quarter.

Intracellular recording from the movement receptors might show the second process postulated above if it were in operation, and if the third process occurred and affected the intracellular potential it too would show up. Early attempts to secure such recordings with rigidly fixed microelectrodes gave consistently poor results and not until the adoption of the movable electrode system described above were any records obtained containing spike responses. Even with the movable electrodes problems arose, mainly due to vibration. Many penetrations revealed a good internal potential of the order of 65 mv. upon which suddenly appeared, after a very brief interval, a highly distorted but sine-wave-like oscillation usually of a frequency of 40 - 70 cps. This oscillation rapidly grew in amplitude and was often compounded by the presence of bursts of spike potentials. After perhaps 2 or 3 seconds of this sort of activity the average potential fell precipitately to near zero and no further responses could be elicited from the cell. On one or two of

these occasions observation of the electrode revealed that it was vibrating in the horizontal plane at high rate and amplitude. It is thought that the two compliances, of thin wire and elastic strand, along with the mass of the electrode tip formed a resonant system which was probably triggered into mechanical oscillation by the vibration of the building (which is considerable in the laboratory where most of the experiments reported in this work were performed and which is in the right frequency range). A short series of experiments was attempted in the early morning hours, 2 - 5 A. M., but the results were not satisfactory. Moving the entire experimental setup to the basement floor of the building finally eliminated this disturbance to the extent necessary for intracellular recording. Apparently the movement receptors are quite sensitive to mechanical intervention, and this is compounded by their position on an extremely compliant elastic strand.

The compliance of the strand further increased the difficulties of intracellular recording since the cell mass, under the pressure of the electrode tip, would simply rotate around the axis of the elastic strand allowing the tip to slide by without penetration of a cell. Supporting the organ from below with the glass hook usually permitted penetration, after which the hook was lowered out of the way. In the course of lowering the hook the electrode usually came out of the impaled cell, and only about one in twenty tries resulted in a stable penetration.

Figure 7 illustrates an intracellular record which is typical of most of the results obtained. The resting potential of the cell was approximately 67 mv. The response is obviously that of a movement

Figure 7

Intracellular recording from opening unit in the PD organ. (a), (b) and (c), the same unit at three different speeds of movement. The spikes are small and have probably been blocked at a site distal to the recording electrode. Calibration: 10 mv., 200 msec.

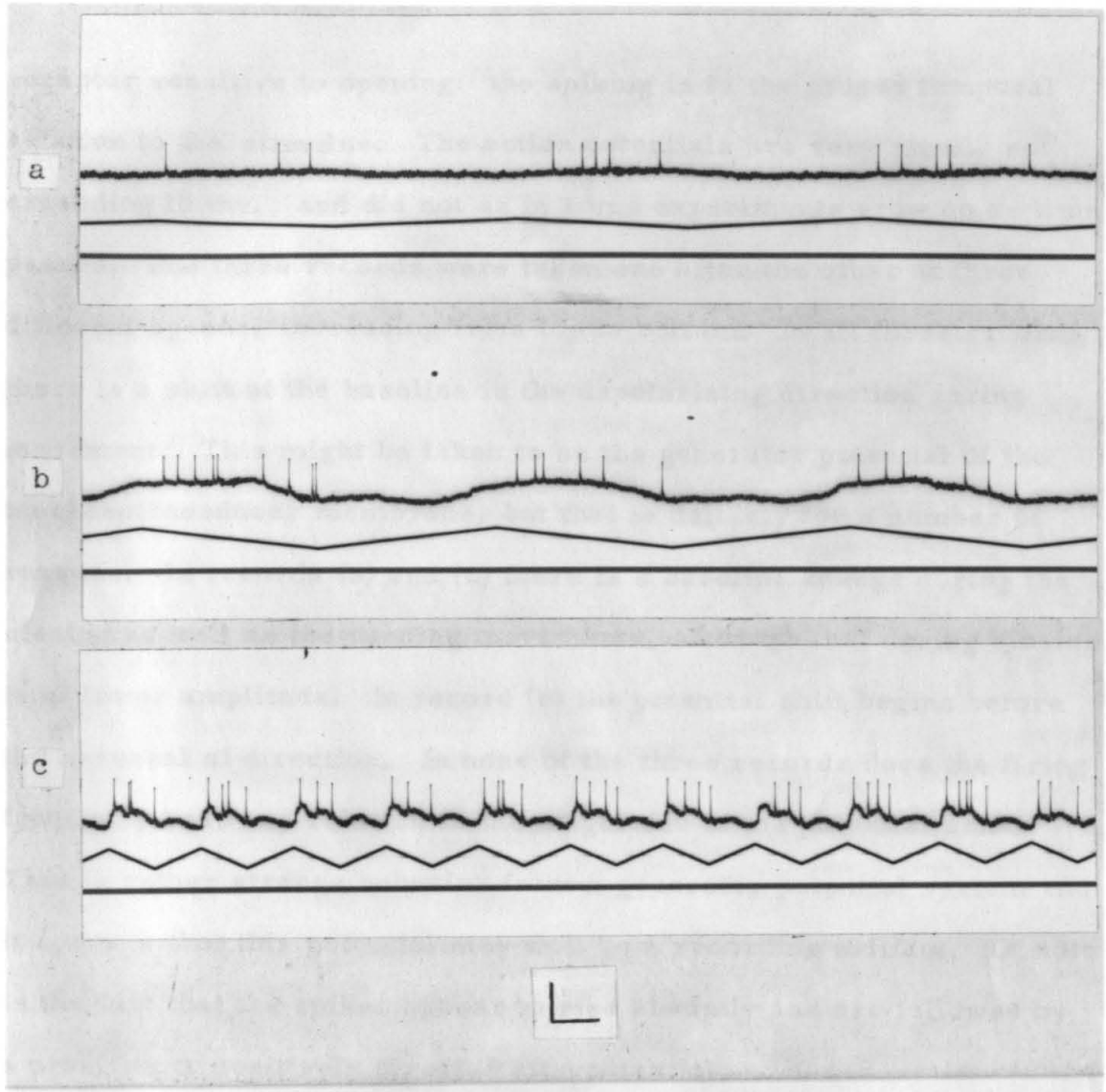


FIGURE 7.

receptor sensitive to opening: the spiking is in the proper temporal relation to the stimulus. The action potentials are very small, not exceeding 10 mv., and did not as in some experiments grow up as time passed. The three records were taken one after the other at three different speeds, increasing from top to bottom. In all three records there is a shift of the baseline in the depolarizing direction during movement. This might be taken to be the generator potential of the mechanotransducer membrane, but that is unlikely for a number of reasons. In records (b) and (c) there is a baseline change during the closing as well as the opening movements, although that during closing is of lower amplitude. In record (b) the potential shift begins before the reversal of direction. In none of the three records does the firing frequency bear any relation to the magnitude of the potential shift. This is rather strange behavior from a generator potential system and it appears that this potential may well be a recording artifact. Of note is the fact that the spikes appear to rise abruptly and are followed by a prominant, positively directed afterpotential.

These features are more clearly seen in fig. 8, taken from another opening-sensitive cell at higher film speed. This is the best intracellular record obtained from a movement receptor: the spikes are full grown, overshooting the zero level, and the electrode contributes very little noise. The very low amplitude noise that does show up on the base line and riding on the potential waves averages 0.5 mv. or less and varies in frequency, usually being above 100/sec. The shape of this low level activity is roughly sinusoidal, or if not sinusoidal

Figure 8

Intracellular recording from a sensitive opening unit of the PD organ. (a) and (b), responses to movements at two different speeds. (c), response to movement at a speed that was subthreshold for spike production. (d), response to closing. The dots near the tops of the records (a) and (b) indicate the positions of spikes which show only very faintly in reproduction. Calibration: 20 mv., 100 msec.

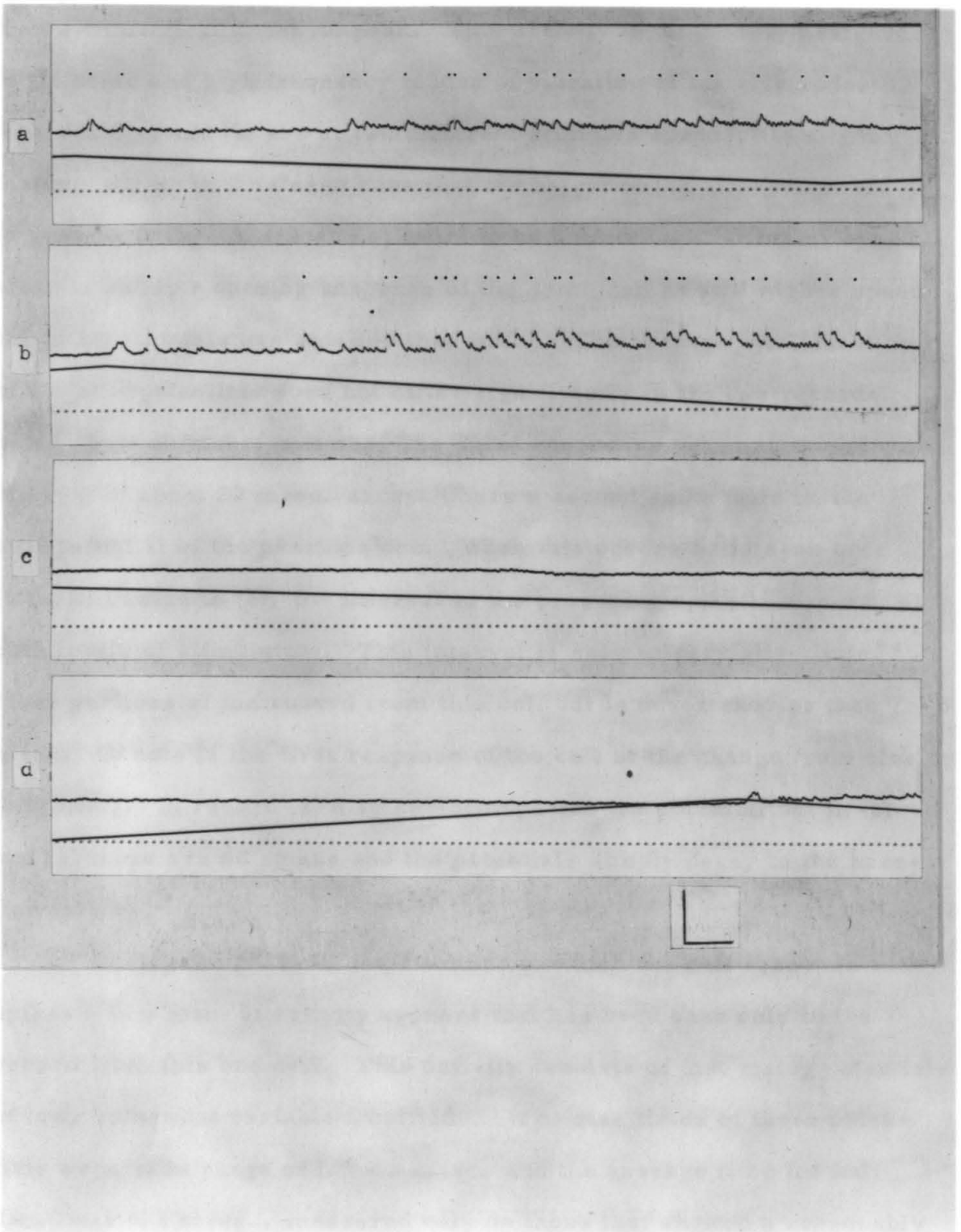


FIGURE 8.

then symmetrical about its peak. This activity is tentatively assigned to tip noise and high frequency modes of vibration of the electrode. Records 8(a) and (b) are at two different stimulus speeds, (b) slightly faster. Although it is seen here that the height of the afterpotentials is greater in (b), this does not seem to be a function of stimulus speed since in another opening response of the same cell at still higher speed the afterpotentials are smaller than in (b). The average total duration of the afterpotentials does not differ significantly in the two records, being about 20 msec. in both. The spike responses occur at a minimum interval of about 20 msec. except where a second spike falls on the afterpotential of the previous one. When this occurs as is seen once in (a) and twice in (b), the interval to the preceding spike is 8 msec. at both levels of stimulation. This interval is seen to vary slightly in other portions of the record from this cell but is never shorter than 7 - 8 msec. Of note is the first response of the cell at the change from closing to opening. In record (a) a spike accompanies the potential but in (b) and (d) there are no spikes and the potentials simply decay to the base-line level.

During the phase of the stimulus to which the cell responds with spikes a new form of activity appears that has been seen only in the record from this one cell. This activity consists of fast rising potentials of low, somewhat variable amplitude. The rise times of these potentials were in the range of 1.5 - 2 msec. and the average time for half decay was 4.2 msec., measured only on those that showed a reasonably smooth decay uncomplicated by noise. The amplitudes of these small

potential changes in (a) covered the range from 1 - 2 mv., the average height being 1.5 mv. Their amplitude is seen to be virtually unchanged in record (b) and the rate of their recurrence does not change markedly. In 8(c), taken at a subthreshold speed of movement, there are none of the 1 - 2 mv. potentials and they are similarly absent in (d) which shows a closing movement at the same speed as the opening in (b). In (d) the small potentials resume when the movement reverses to opening. The minimum interval between the potentials has a mean value of 8.8 msec. and is not observed to be less than 7 msec. Rarely there is what looks like summation of two potentials. In such cases the peak of the second may be at a slightly lower potential than the first, but this is difficult to ascertain with accuracy. In such cases of possible summation it is clear that the peak of the second potential is still at least 7 msec. later than the first. The only basis for calling this summation is that the second of the two potentials rises out of the falling phase of the first. As may be clearly seen in fig. 8 these potentials can occur during the spike afterpotential and preserve their identity perfectly. On the after potentials of several spikes a small potential falls at the top of the afterpotential. The minimum interval between the foot of the spike and the foot of the small potential again averages 8 msec. and does not go below 7 msec. Since a close examination of the records in fig. 8 discloses that the spikes usually take off from a step of approximately 0.8 - 1.5 mv., these small potentials will be called prepotentials; fuller reasons for this will be presented below in the discussion.

As in the records of fig. 7, no consistent slow shift of the base-

line potential was observed during the responses shown in fig. 8. In two instances measured the baseline potential became 0.5 and 1.1 mv. respectively more positive during opening than during closing, but in a third case became 0.5 mv. more negative during opening. Again as in fig. 7 no correlation of baseline potential shift with frequency could be found. The cell from which the records of figure 8 were taken was clearly in view and the electrode was seen to be in the large expansion of the soma.

Since the results of intracellular recording did not seem to provide a ready explanation for the low saturation frequency of the movement receptors another approach to the problem was sought. Since the third postulated alternative mechanism, essentially that proposed by Wiersma (37), implies that the rhythm exhibited by a cell may be invariant, a series of experiments was undertaken to determine if the saturation rhythm of a movement receptor can be influenced by antidromic stimulation. If the saturation frequency is determined by mechanical coupling or a limited generator potential amplitude at the spike initiating locus, or if a cyclic excitability change takes place in a region of the cell accessible to antidromic spikes, then an antidromically invading spike should be able to "reset" the rhythm of the movement receptor's discharge. Antidromic stimulation was delivered to the PD bundle in the meropodite with a pair of Pt hook electrodes driven by a Grass SD-5 stimulator. Several attempts were made to record the result of this stimulation with intracellular electrodes but they all failed, either because the impaled cell could not be identified

as a movement receptor or because antidromic impulses did not invade a cell that was so identified.

To circumvent this difficulty a different method was employed. The stimulating electrodes were applied to the cut proximal end of the PD bundle and a pair of recording leads were used to pick up an uninterrupted filament of the bundle at a point distal to that of stimulation. The recording leads were connected one to either side of the differential input of the preamplifier. By using such a connection it was hoped that the direction of travel of an impulse could be ascertained and antidromic spikes differentiated from orthodromic ones. The response of a single sensitive movement unit was recorded while it was driven at saturation speed by movement of the dactyl and the stimulator triggered at random times during the response of the cell. At low intensities of antidromic stimulation no impulses were observed traveling towards the organ from the stimulating electrodes, and at high intensities of antidromic stimulation the stimulus artifact was so great that it sometimes obscured all spikes occurring up to 50 msec. after the delivery of the shock. Yet by measuring the interval between the spike just before the shock and that just after the shock and comparing this interval with the average saturation interval of the cell it might be possible to determine whether the rhythm had been altered. In the time available for these experiments on the effect of antidromic stimulation, only one record was obtained from a sensitive cell that exhibited a high degree of rhythmicity in its response to movement. The data obtained from this cell do not permit even a tentative conclusion

regarding the effect of antidromic stimulation on its firing pattern.

Pharmacological properties of the movement receptors

One of the greatest difficulties encountered in the attempts to make intracellular recordings from the movement receptors is that of identifying the cell which is impaled; it may be a movement receptor, position receptor or even possibly a non-neural element. The only reliable criterion for such an identification is the observation of a spiking response properly correlated with movement. The early attempts to elicit such a response were complete failures and the possibility was recognized that spikes might not invade the portion of the receptor cell penetrated by the electrode. Thus various drugs were tested in an effort to find one that would stimulate the movement receptors and show whether an intracellular electrode could pick up the spiking response. This intent was not realized for a reason which will be discussed below, but since spiking responses to movement were finally recorded it became superfluous. Of greater interest in its own right was the manner in which certain drugs affected the PD receptor cells and for this reason a study was undertaken to compare the effects of a limited series of drugs on crab and crayfish PD receptors and crayfish stretch receptors. The results of this study are presented in Table 1 and the actions of each drug are described separately below.

Nicotine. This alkaloid has previously been found to effect Arthropod nervous systems. Davenport (46) showed that nicotine in low concentration increases the frequency and amplitude of the heart beat

TABLE I

THE EFFECTS OF VARIOUS DRUGS ON CRAB AND CRAYFISH PD RECEPTORS AND CRAYFISH STRETCH RECEPTORS

Drug	Crab Pd	Crayfish RM	Crayfish PD
ACh	None	Stimulates sense cell (8, 9).	None
Eserine	None	Potentiates effect of ACh (9).	None
Prostigmine	None	----	----
Atropine	None	Sets up discharge and blocks action of ACh (9).	----
Nicotine	Stimulates movement and position receptors.	No effect on sense cell. Causes slight contraction of muscle.	Stimulates movement and position receptors.
Nornicotine	Stimulates movement receptors.	----	----
Nicotinamide	Stimulates movement receptors.	----	----
GABA	None	Blocks activation by stretch or ACh (26, 44, 45).	None
Caffeine	Blocks mechanical activation but does not block nicotine stimulation.	None	No effect on PD sense cells. Causes contraction of closer muscle.

of Cancer magister and in high concentration causes systolic arrest, both presumably due to an action on the cardiac ganglion which drives the heart muscle. Several other investigators report that nicotine affects synaptic transmission in Arthropod CNS, first facilitating and then blocking. Roeder and Roeder (47) found this effect in the cockroach cord; Wiersma and Schallek (48, 49, 50) in a preparation utilizing the synapse between giant fiber and third root motor fibers of the crayfish cord; and Turner et al. (51) in the same synapse of Callianassa. Finally Ellis et al. (52) reported a general stimulatory effect followed by depression when nicotine was injected into a whole crayfish.

When applied to the PD organ of Pachygrapsus nicotine elicits a massive discharge. Single unit recording showed that the frequency attained by a single cell was quite high, higher than the saturation frequency of the same cell when driven by movement. For this reason the action of nicotine on the crab movement receptors was scrutinized in some detail. In fig. 9(a) may be seen two closing units of low sensitivity to movement. The shortest interspike interval exhibited by the unit with the smaller spike is 8 msec., the average interval is 14.8 msec. corresponding to a frequency of 67/sec. Such high frequencies are often observed in the rather insensitive units and this one is chosen as an example with an unusually high frequency during mechanical stimulation. Figure 9(b) shows the effect of nicotine applied in a concentration of 10^{-3} ml/ml. The larger response had been lost by the time of application of the drug and only the unit with the smaller spike responded. The average interval at the height of the discharge is

Figure 9

Action of nicotine on movement receptors. (a), response of two closing units to movement. (b), response of the smaller of the two units in (a) to nicotine, 10^{-3}. (c), response of another closing unit to movement. (d), response of the unit in (c) after administration of nicotine, 10^{-6}. (e), same unit as in (c) and (d) after the nicotine has been washed out.

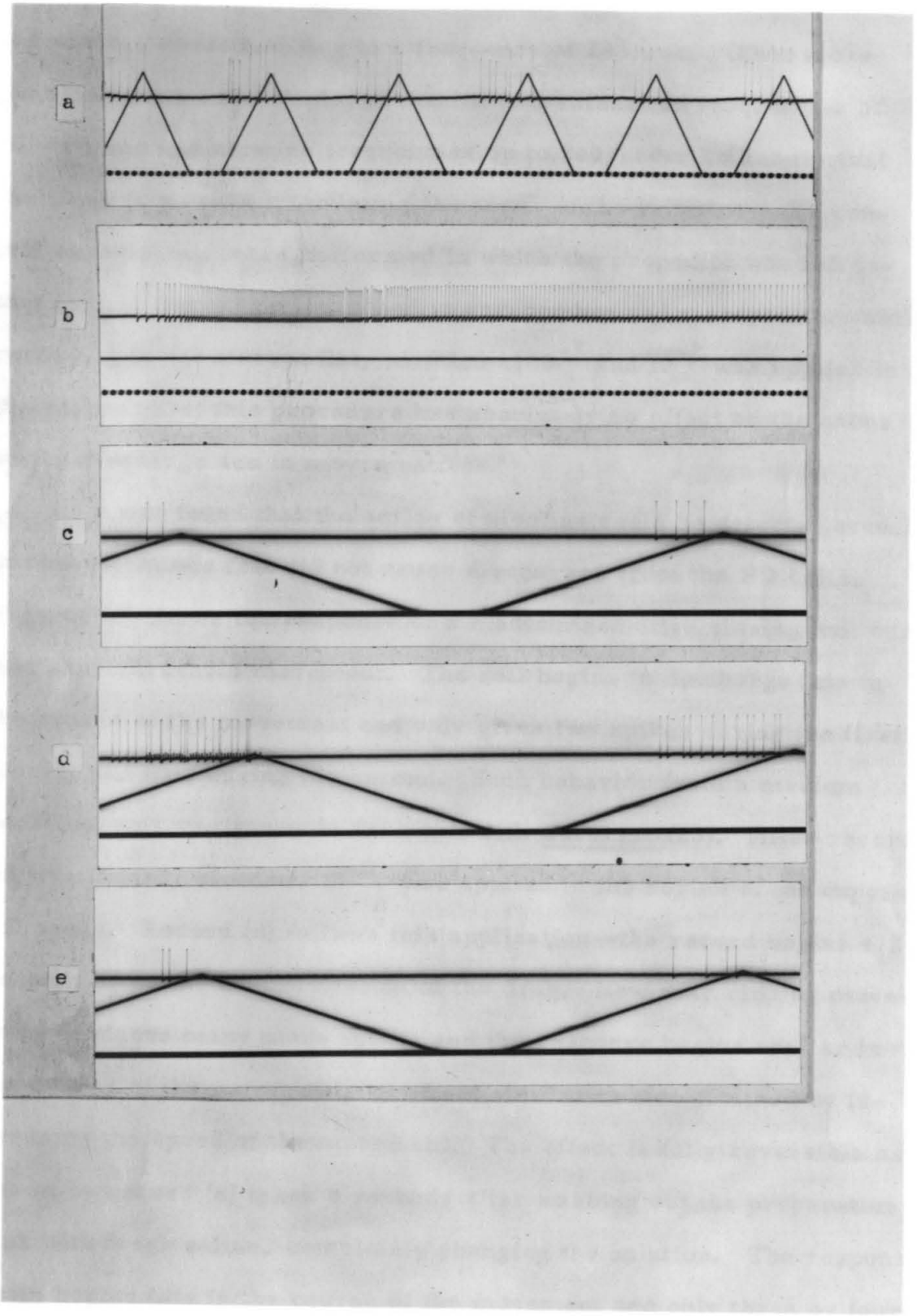


FIGURE 9.

3.5 msec., corresponding to a frequency of 285/sec. Other more sensitive units have displayed mechanical saturation frequencies of 40 - 55/sec and nicotine frequencies up to 240/sec. To assure that the nicotine was not stimulating the axons of the receptor cells control experiments were performed in which the propodite was left intact and the nerve trunk exposed in carpopodite and meropodite. While recording in the meropodite, nicotine at 10^{-3} and 10^{-2} was applied in the carpopodite; this procedure has absolutely no effect on the axons or the discharge due to movement.

It was found that the action of nicotine could be detected even in concentrations that did not cause discharges from the PD cells. Figure 9(c) shows the response of a medium sensitive closing unit during just suprathreshold movement. The cell begins to discharge late in the course of the movement and only gives two spikes during the first closing and four during the second. Such behavior from a medium sensitive unit was shown in the work with Carcinus (28). After record (c) was filmed, nicotine, 10^{-6}, was applied in the region of the exposed PD organ. Record (d) follows this application--the record begins 4.2 seconds after the administration of the drug. Now each closing movement produces many more spikes and the response begins earlier in the course of the movement, an effect similar to that obtained by increasing the speed of the movement. The effect is fully reversible as shown by record (e) taken 6 seconds after washing out the preparation dish with fresh saline, completely changing the solution. The response again begins late in the course of the movement and only three or four

spikes are generated. Careful examination of these three records reveals the presence of an opening position unit giving very small spikes that do not show up well in reproduction. In (c) it fires in perfectly typical fashion, silent at the closed position and discharging at about 80/sec. at the fullest opening used in the experiment. In record (d) it is firing throughout the entire record at about 70/sec., and by the time record (e) was made the frequency had dropped to 20/sec. in the closed position but rose to 86/sec. in the opened position. Thus it is shown that nicotine affects both movement and position receptors of the PD organ, and potentiation by nicotine of the effect of mechanical stimulation seems similar in both types of receptor. Nicotine could not be shown to block mechanical activation of movement receptors even at very high concentrations, nor did the effect of nicotine disappear so long as the drug was present in the solution bathing the PD organ. In one experiment where washing out of the preparation was delayed, a movement unit continued to fire at elevated frequency for 3 minutes. It did not seem necessary to find out for how long such a discharge would persist if permitted.

In most of the experiments where a cell was impaled with an intracellular electrode and identified as a movement receptor an attempt was made to obtain a record of the effect of nicotine. Every attempt failed; the resting potential of the cell simply disappeared abruptly with the application of the drug and it is assumed that the agitation of the bathing fluid caused the electrode to pull out of the cell.

Nornicotine and Nicotinamide. These two drugs are close structural relatives of nicotine and only differ slightly from it. When applied to the crab PD organ they showed exactly the same effect as nicotine in provoking a massive discharge and single unit recordings revealed that they too caused discharges of quite high frequency. Their action was not studied in subthreshold doses but it may be reasonably expected that in this they would also act just like nicotine.

Acetylcholine (ACh). Acetylcholine acts as a synaptic transmitter substance in vertebrates, activating certain specialized postsynaptic membranes (53). It is also active in Arthropods; it excites the hearts of many crustacea (46, 54), facilitates autotomy in Petrolisthes, facilitates synapatic transmission in the cockroach (47), and most significant in connection with this study stimulates the abdominal and thoracic stretch receptors of crayfish (8, 9, 26). In its action on vertebrate systems and also on crustacean hearts and the cockroach ganglion ACh effects the membranes of postsynapic cells only in the regions of the synaptic connections. The abdominal stretch receptors of the crayfish are not known to have any synapses on them that transmit cholinergically and the thoracic stretch receptors are entirely devoid of synapses, indicating that ACh can affect membranes that are not ordinarily activated by it. Since the effects of ACh and nicotine are practically indistinguishable in certain parts of the vertebrate nervous system, ACh was tested on the crab PD organs in view of their sensitivity to nicotine. ACh had absolutely no effect on either the movement or position receptors of the PD organ of Pachygrapsus.

Eserine and Prostigmine. Both these drugs are inhibitors of the enzyme cholinesterase which hydrolizes ACh. In vertebrates these agents potentiate the effects of ACh by protecting it from the enzyme and prolonging its action (53). Wiersma et al. (9) found that eserine potentiated the effect of ACh on the crayfish stretch receptor and Wiersma and Schallek (48, 49, 50, 56) showed that it affected transmission at the lateral synapses even though ACh had no effect there. Eserine and prostigmine were tested on the PD of the crab, first to see if they could unmask an effect of ACh and second to ascertain whether either would have an effect by itself. Neither drug affected the PD organ of the crab in any manner.

Atropine. This is another drug involved in the ACh system in vertebrates; it combines with the ACh receptor sites and makes them unavailable to ACh (53). Although atropine seems to have no effect on the CNS of the crayfish (57) it does increase the spontaneous activity of the ventral cord of Callianassa (51) and also blocks the effect of ACh on the crayfish stretch receptor while setting up a low frequency discharge (9). Atropine applied to the PD of Pachygrapsus had no effect.

Gamma aminobutyric acid (Gaba). Gaba has been identified as one of the components of an extract of mammalian brain that inhibits the discharge of crayfish stretch receptors (58, 59), and will show this inhibitory action when applied in its pure form. The abdominal stretch receptors of the crayfish are supplied with inhibitory nerves and Edwards and Kuffler (45) have shown that Gaba mimics the effect of this nerve;

however, Wiersma and Pilgrim (26) have demonstrated that Gaba also inhibits the thoracic stretch receptors (N cells) of the crayfish which are devoid of synaptic terminations. It was thus possible that Gaba would inhibit the PD cells which similarly lack synapses. Gaba in a wide range of concentrations had no effect on the PD cells of Pachygrapsus.

Caffeine. This alkaloid has a general stimulatory effect on the mammalian CNS, kidney and cardiac muscle (53), but was reported to have no effect on neuromuscular transmission in the crayfish (52). When applied to the crab PD organ caffeine was found to cause a decrease in the frequency of a response to movement and in sufficiently high concentration to abolish this response altogether. Washing of the preparation brings about the reappearance of the movement response within a few seconds. If nicotine is applied during the time that mechanical activation of the movement receptor is blocked by caffeine, a seemingly normal burst of impulses is recorded.

The responses of stretch receptors to movement

Since little is known of the response of other mechanoreceptors to the kind of stimulation employed on the movement receptors, and since the number of clear intracellular recordings from cells of the PD organ is so small, it was decided to investigate the response of the crayfish stretch receptors to constant velocity stimuli. Intracellular recording from the stretch receptors would also serve as a check on the internal recording technique. Stretch receptors were excised from

the animals and mounted in the stimulating apparatus as described above. The receptor was first stimulated with low amplitude rectangular movements until one spike was elicited at each small stretch. The transducer of course moved back to its original position between stimuli. In experiments where RM1 and RM2 were stimulated together and their responses recorded simultaneously from the afferent nerve, larger step stimuli were applied to be sure that the spikes of the two cells could be distinguished; this on the basis of their differing adaptation rates to maintained stretch. In all intracellular recording experiments only one of the receptors was used, the other was discarded.

The results obtained by stimulating both receptors together, stretching and relaxing them at constant speed, are shown in fig. 10(a). The response is from the afferent nerve and previous steady stimuli had shown the small spike to belong to RM2, the larger one to RM1. The three records were made with the same amplitude of stretch; only the rate of stretch and relaxation were increased from (a) to (a_2). The records show that both receptors are far from perfect indicators of stretch during the two phases of stimulation. The first spike of the train in each full cycle occurs at a lesser degree of stretch than the last spike of the train during the relaxation. In RM1 the first spike occurs at 27, 30 and 28 per cent of full extension for each of the three speeds, and the last spike at 45, 54 and 54 per cent of full extension during the relaxation. The corresponding figures for RM2 are 63, 64 and 67 per cent during stretch and 98, 96 and 93 per cent during relaxation. In both receptors the frequency of discharge increases smoothly

Figure 10

Responses of the RM's to constant velocity stimuli. (a), RM1 and RM2 stimulated and recorded together. (a_1) and (a_2), the same at two higher speeds, the smaller spikes are from RM2, the larger ones from RM1. (b), intracellular response of RM2 to stimuli just bracketing the spike threshold. (c), the same as (b) but from RM1. (d), intracellular response of RM1 to higher intensity and speed of stretch. Calibration: 20 mv., 200 msec.

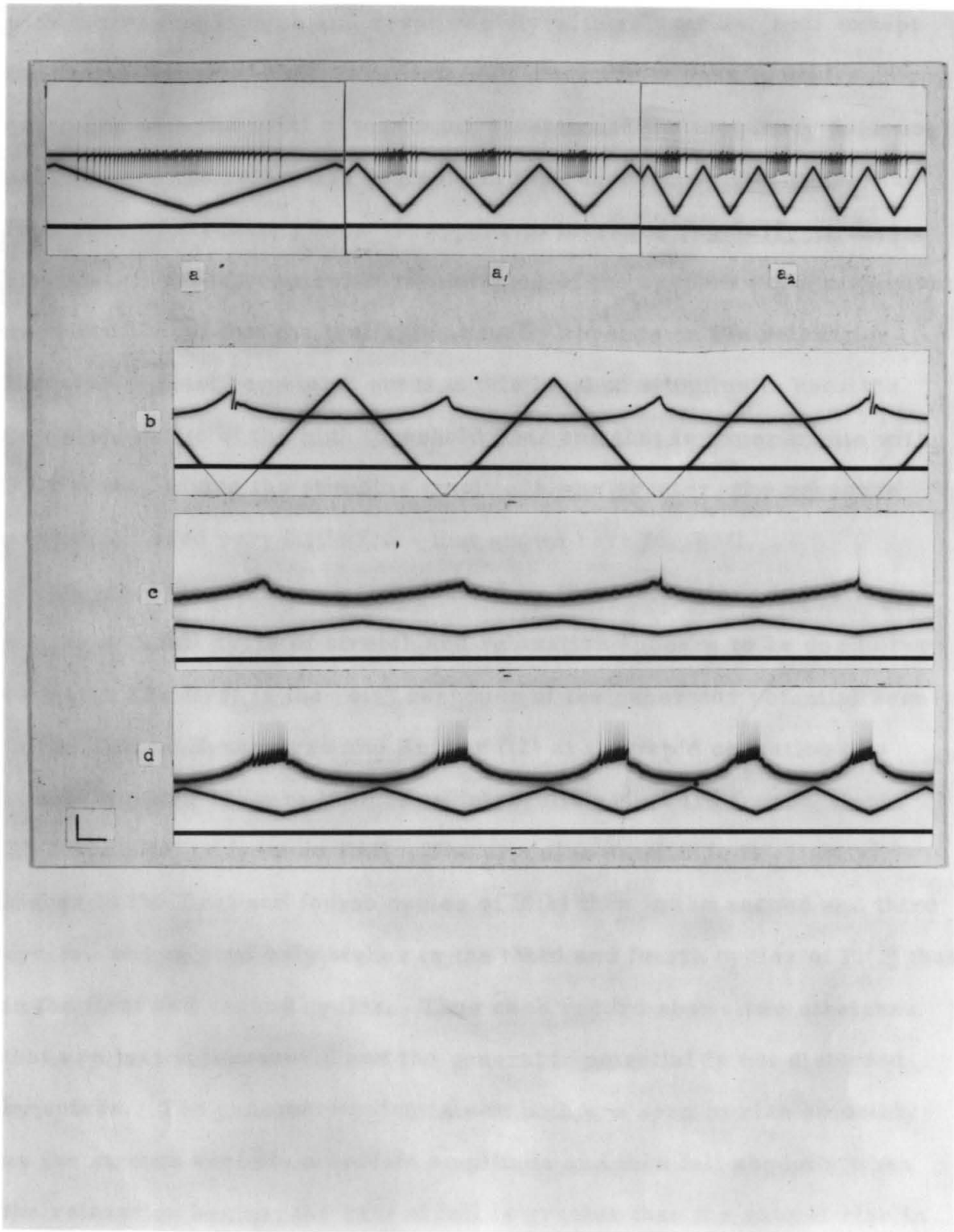


FIGURE 10.

with increasing stretch and drops rapidly with relaxation, and, except for the response of RM2 in record (a_2), the point of maximum frequency coincides with the point of maximum stretch and the frequency does not decline until the relaxation begins. In the response of RM2 in (a_2) the frequency of discharge does not appear to increase regularly as the stretch increases, somewhat reminiscent of the response of a movement receptor except that the last spike usually appears on the relaxation. However it must be pointed out that this level of stimulus is near the threshold range of the high threshold RM2 and that in experiments with RM2 alone, where the stimulus amplitude was greater, the response pattern differed very little from that shown here for RM1.

The unequal response exhibited by these receptors to the two phases of a full cycle of stretch and relaxation appears to be due to two causes. The first is the "off" response of the generator potential seen in the RM1 by Eyzaguirre and Kuffler (12) at the rapid cessation of a steady stretch. Figure 10(b) is an intracellular record from a single RM2 and 10(c) is from an RM1. The stimulus amplitude is slightly higher in the first and fourth cycles of 10(b) than in the second and third cycles, and is similarly higher in the third and fourth cycles of 10(c) than in the first and second cycles. Thus each record shows two stretches that are just subthreshold and the generator potential is not distorted by spikes. The generator potentials in both are seen to rise smoothly as the stretch exceeds a certain amplitude and then fall abruptly when the relaxation begins; the rate of fall is greater than the rate of rise in both receptors. Since the low slopes at the beginning and end of the

generator potentials make it difficult to place precise limits on the response and compare the time course of rise and fall qualitatively, it must suffice to state that the greater parts of the rising and falling phases look very much alike; only the parts right about full stretch differ greatly. Indeed once the rapid part of the falling phase is over, the remainder of the decay of the potential appears to be nearly a mirror image of the early part of the rise. Where the stimulation is just suprathreshold, spikes are elicited which hasten the rapid fall of the baseline potential. No experiments were performed in which higher levels of stretch were employed and an agent used to block the spikes so no statement can be made regarding the true shape of the generator potentials under conditions of suprathreshold stimulation. However the asymmetry of the spike discharge at such higher stimulus intensities may be due in part to a rapid decrease in the generator potential.

Such an asymmetry of the generator potential is not the only admissible cause for the unequal responses. Part (d) of fig. 10 shows the intracellularly recorded response of an RM1 to high amplitude stretch. The speed of stretch increases from left to right, the last two cycles being at the same speed. Here there is some asymmetry of the generator potential though not so obvious as in the foregoing records. In this series of responses the second mechanism that accounts for the fall of spike frequency may be seen. In each cycle of stretching the firing level of the spikes increases as the burst proceeds. This indicates that the threshold of the spike generating membrane is

rising, the average increase being 5.2 mv., but showing no systematic relationship to speed of stretch; and although the rate of the rise of the prepotentials of the spikes increases, the frequency does not go up linearly due to this compensatory rise of threshold. Indeed in the second and fifth cycles of stretch the generator potential just after the last spike may even be higher than just before the first spike yet it fails to evoke a fully regenerative response. Recordings from RM2 under similar stimulus conditions fail to show a clear-cut rise of the spike firing level, yet the more abrupt fall of the generator potential in RM2, if it behaves at higher stimulus levels as it does near threshold, may more than compensate for the lack of increase in the threshold for spiking. No records were made of the effect of overstretching an RM with this type of stimulus, but it seems safe to assume that overdepolarization would silence the spiking mechanism until the generator potential had dropped from its peak, whereupon spiking should resume only to cease again when the generator potential had fallen below the spike threshold.

Pharmacological properties of crayfish PD and stretch receptors

As pointed out above, the effects on the crayfish RM's of ACh, eserine, atropine and Gaba are already known, and these are listed in Table 1. Florey (59) had reported that nicotine 10^{-6} - 10^{-4} slightly accelerates the discharge of an active RM, and in a concentration of 10^{-3} stops the discharge. It was decided to recheck these data and additionally to test caffeine on the RM's; thus a comparison could be

made of the effects on both crab PD cells and RM's of at least six agents. It was found that nicotine in a wide range of concentrations (10^{-6} - 10^{-2}) has no effect on RM1 or RM2 if the receptor muscle is entirely slack. Only when the muscle is extended to a just subthreshold length does 10^{-3} nicotine elicit a response; and only when the receptor is already firing do lower concentrations of nicotine accelerate the discharge slightly. When the receptor muscle was slacked off to the point where it was thrown into a shallow curve and nicotine, 10^{-3}, applied during visual observation, it was seen that the curvature decreased--the muscle contracted. It must be concluded that the effect of nicotine on RM's is due to the isometric contraction of the receptor muscle. Caffeine had no discernable effect on either RM1 or RM2.

Examination of the first two columns of Table 1 immediately reveals a striking difference in the pharmacological properties of the crab PD receptors and the crayfish RM's. This difference might be due to the different species used or to a difference in the two types of receptors. In order to distinguish between these two possibilities, five of the drugs were tested on the movement and position receptors of the PD organ of the crayfish cheliped. These five were chosen as being representative of the different classes of agents used and are: ACh, eserine, nicotine, Gaba and caffeine. ACh and eserine, alone or in combination, were without effect, as was Gaba.

Nicotine stimulated both the movement and position receptors. The stimulation was weak with 10^{-5} but clear-cut with 10^{-4} and 10^{-3}. In these experiments the electromechanical system was not used;

movements were accomplished by hand. The only results accepted were those in which only one or two units responded to both mechanical and drug stimulation. Obviously the synergism between movement and nicotine could not be tested in this manner, but the graded effect of nicotine allows one to conclude that it is very likely this synergism exists in the crayfish PD receptors as well as in those of the crab.

Caffeine was tested on the crayfish PD units in concentrations ranging from 10^{-5} - 10^{-3}, and had no effect on the reactions of the receptor cells. On the first two or three occasions a discharge did occur when the caffeine was applied to the opened propodite, but this discharge was accompanied by an obvious contraction of the closer muscle. Ellis et al. (52) reported that caffeine had no effect on neuromuscular transmission or upon contraction in the crayfish closer muscle; yet the contraction due to caffeine was repeated four times. To pursue this result further is beyond the scope of this investigation. In order to eliminate the effect of this contraction the entire claw was pinned down as securely as possible and caffeine then applied. Under these conditions only a very slight movement of the dactyl occurred which was accompanied by two or three impulses in a very sensitive closing unit. Just afterward, the pins were removed from the dactyl and the response of the receptors checked. They responded quite normally.

DISCUSSION

The basis for unidirectional response

Although some hair type mechanoreceptors are known in the arthropods that will respond preferentially to bending of the hair in one direction (24, 60, 61), and a number of receptors have been reported to respond to movement (23, 27, 62, 63, 64, 65), in only one group of animals is a receptor known that responds wholly to movement of a joint in one direction only. That group is the Decapod Crustacea and here the cells responsible for this response are always found to lie in elastic organs spanning the joints they serve. Pringle (66) has found that responses to joint movement in the legs of Limulus polyphemus originate in elastic organs spanning the joints; but has not reported single unit analysis that would show whether the receptor cells are unidirectionally sensitive. Although bidirectional response to movement might be explained as being due to transient pressure changes on a deformation sensitive cell (23), such a mechanism does not suffice for the Decapod movement receptors. Nor can preferential stretching of a fast adapting type of stretch receptor be made to fit the data: apparently this is what Wiersma and Boettiger (28) had in mind when they suggested that the opening units of the crab PD might be associated with the fanning fibrils of the PD while the closing units might be associated with the main strand. The present work has shown that the fanning fibrils are entirely dispensable in the response of the PD organ, something that Wiersma and Boettiger subsequently inferred from the results of experiments on the crab CP organs. The

rotation experiments reported above dispose of another mechanism whereby tension changes might be transmitted to two groups of cells differentially. That is, if one group had its distal processes running around the strand in one sense, and another group in the other, then by differential rotation rates in different layers of the strand the two groups might undergo increases of tension in opposite directions of rotation. In fact rotation of the strand up to 720 degrees in either direction failed to stimulate the movement receptors. It must be pointed out that if rotation were the adequate stimulus for the movement receptors, they ought to be exceedingly sensitive thereto since the strand has never been observed, in situ, to rotate more than a few degrees.

It is therefore necessary to seek an entirely different mechanism than those of stretch or pressure. The experiments reported above make it entirely clear that the adequate stimulus for the movement receptors is the change of length of the strand; closing units respond to lengthening and opening units to shortening. Of course the tension in the strand must change concomitantly with the length; but it is as yet impossible to tell whether the movement receptors are responding to this change in tension or to their displacement with respect to other parts of the organ. That such unequal displacement of different components of the PD organ does occur in Pachygrapsus as it does in Carcinus (28, fig. 4) has been confirmed by direct visual observation. Two cells that could be easily identified were watched during opening and closing and were seen to change position relative to each other at the opened and closed positions. That two cells will change relative position is not absolute proof that

other elements move differentially; yet it is indicative that such may be possible. For present purposes it will be explicitly assumed that such differential movement of the parts of the organ does take place; and this in combination with the ultrastructural information from Whitear (35, 36) (see appendix) permits a hypothesis to be formed. If the scolopales of the cells of the organ enter the elastic strand at a high angle, and if the base of a scolopale is translated by the change in length of the band a distance different from that through which the apex of the scolopale is moved, then the scolopale must be bent. Providing that both distal processes in the scolopale lie in the plane of the strand then this bending will cause one of the distal processes to lie further out on the radius of the curve so formed. The distal processes will then be deformed unequally: the one on the outside of the curve will be lengthened and the other will be shortened. Stretching of the distal process may well be the truly adequate stimulus for the movement receptors as it is felt to be for stretch receptors. As hinted above, the fact that most of the scolopales in CP2 seem to contain only one normal distal process and the further finding that CP2 responds more strongly to one direction of movement than the other would strongly favor Whitear's assumption that one of the distal processes belongs to an opening unit and the other to a closing unit. Unfortunately for Whitear's suggestion of electrical interaction in the scolopale, the activity of one of the cells would most likely be a cathodal stimulus to the other and would stimulate not inhibit; besides which there could be no such interaction in a scolopale of a CP organ. The

hypothesis presented in this paper does not require any interaction of the cells in order to achieve a unidirectional response; this is taken care of entirely by the structural situation. In order to account for the prompt cessation of discharge at the close of a movement, it is only necessary to assume that the coupling between the scolopale of a movement receptor and the strand is of a viscous nature. When a movement ceased the apex of the scolopale might slip back slightly and reduce the curvature of the scolopale. The entire difference between PD movement and position receptors could be due to how tightly the scolopale was coupled to the fibers of the strand. Intermediate degrees of coupling could then be responsible for the response patterns of some units which partake of the character of both movement and position receptors (29). Even the differing thresholds of the movement receptors can be explained in the context of this hypothesis. If two cells move through different distances in the course of a movement then one must be subjected to more force than the other. Those cells that move farthest with respect to the rest of the PD organ should thus have the lowest thresholds to movement speed.

There are of course other conceivable mechanisms for the activation of the movement receptor, for instance crosswise shearing of the distal process. This does not contain an inherent explanation for unidirectional sensitivity, which alone makes it unattractive. Furthermore, the presence of the relatively massive scolopidial structure surrounding the distal process would be expected to greatly reduce the effect of shear forces. The very topic of the mechanism of stimu-

lation of arthropod mechanoreceptors is assiduously avoided by most authors, except that Cohen (24) has computed the threshold deformation in a statocyst cell distal process to be 0.5μ. The fact that no one has determined the orientation of the distal process with respect to the plane of maximal sensitivity in those mechanosensory hairs with a preferred direction of stimulation is to be regretted. It is obvious that the entire hypothesis presented above stands or falls on the orientation of the scolopidia and distal processes to the elastic strand of the PD organ. This has not been investigated so far, an unfortunate omission in Whitear's excellent work. It is hoped that this subject may be examined in the near future.

The saturation frequency and rhythmicity

In the section on results there were presented three mechanisms one of which might underlie the low saturation frequency of the movement receptors. They were: 1, limitation of the amplitude of the generator potential at the spiking locus due to decrement over a long distance; 2, the presence in the cell membrane of a rhythmic change in excitability which makes spikes much more probable at certain times than at others; and 3, limitation of the stress placed on the cell due to mechanical factors. I shall deal with each possibility separately in the order they are given.

Limitation due to generator potential decrement

According to Burke (32) the distal processes of the cells of the PD organ are generally about 200 micra in length. There is no reason

to suppose that the mechanotransducer membrane is anywhere but at the tip of the process, and some writers have implicitly assumed that the excitation generated at the mechanotransducer membrane would have to reach the region of the soma in order to trigger the production of spikes (21). This assumption is probably based on the finding by Case et al. (67) and Kuffler (68) that the spikes of the crayfish abdominal stretch receptors are set up in the axon of the sensory neuron and then progress both centrally to the CNS and distally into the soma. This assumption is surprising in view of the structure of certain proprioceptive organs in the basal joints of many decapods that have been described by Alexandrowicz and Whitear (34). These organs are innervated by the distal processes of cells whose somas are in the ventral cord and these distal processes must carry spikes; the sensory nerves of vertebrates are distal processes in the sense that the term is used here and they can carry spike impulses. It is remotely possible that Alexandrowicz and Whitear could have missed seeing the cell bodies of the organs they describe, but this is extremely unlikely.

Certainly the spikes of the movement receptors are not initiated in the cell soma; in the intracellular records from the soma (fig. 7, fig. 8) they are not preceded by a slow depolarization that one would expect to be present if the recording electrode were close to the point where the spike was set up. Thus the spike of the movement receptor must be initiated in the distal process of the cell and propagate through the soma. In view of this fact, one may conclude that the spike initiating

point is reasonably close to the mechanotransducer membrane; that the generator potential, if present in these cells, would not necessarily be expected to be limited by decremental conduction to a low value at the spike initiating locus; and that the first possible explanation for the low saturation frequency may be discarded.

Occurrence of a rhythmic excitability change

A mechanism in this category is the one proposed by Wiersma (37) to explain both the low saturation frequency and the apparent drop-out pattern of the discharge of the movement receptors of Carcinus; he has gone so far as to propose that the excitability rhythm might be manifested as an oscillation of the membrane potential of the cell. This oscillation could be either sinusoidal or relaxation-oscillation in form and would change the excitability of the cell with time since at its positive going peaks it would bring the spike initiating locus closer to threshold than at its negative going troughs. That such oscillatory potentials do occur in nerve cells has been abundantly documented; both spontaneous (21, 69, 70, 71) and triggered (72, 73, 74, 75) varieties have been observed. Both classes of oscillations have been found to be graded in amplitude and to be of remarkably constant frequency; either would serve quite well to account for the behavior of movement receptor cells. Most of the authors reporting such occurrences assume that the depolarization and repolarization phases of these oscillations are essentially the same as those proposed for the spike potential by Hodgkin and Huxley (76), but acting in a low grade manner rather than explosively.

It is either stated or implied that the only difference between oscillations and spikes is the inability, in the former, of the Na permeability change to become fully regenerative. Cole and Baker (77) have found an "inductive" component in the impedance of the squid axon membrane and Cole (78) has gone so far as to compute the natural undamped oscillation frequency of the squid membrane. He achieved values in line with the frequencies observed in this system. Cole does not speculate on the possible identity of the "inductive" component with one of the known membrane processes, but it is likely the regenerative Na conductance mechanism.

The most exacting examination of the intracellular records from movement receptors does not disclose an oscillation of the membrane potential, either during stimulation or when the cells are at rest. Since it has been assumed that the electrode in the soma is at a considerable distance from the spike initiating locus this is not conclusive evidence against the existence of an oscillation but a careful examination of the data which exist negates to some extent the most compelling argument which would lead one to believe an oscillation necessary. This argument is based on the supposed dropout pattern of discharge and the occurrence of doublet firing. The argument, by a process of exclusion, claims that only an oscillatory mechanism could be responsible for single impulses or high frequency groups occurring always at intervals that are integral multiples of some basic short interval. The movement receptor discharge in Pachygrapsus, though quite regular, did not seem to be as regular as might be expected so a count was made in a

good record of all the intervals of various length and the results plotted in the form of a bar graph. According to Wiersma's hypothesis a series of peaks should appear in such a plot corresponding to the basic saturation interval and its multiples. Except for doublets and triplets none of the intervals ought to be shorter than the fundamental saturation interval and very few intervals should have values falling between the peaks. The actual graph for Pachygrapsus is presented in fig. 11. There is a major peak at 20 msec., corresponding to a frequency of 50/sec., and a double peak at 39 and 41 msec. If the basic interval is taken to be about 10 msec., a frequency of 100/sec., one would expect the peaks near 20, 30, 40 and 50 msec. But the hypothesis is greatly strained by the broad peak at 13 - 16 msec. and the large one at 24 - 25 msec.; possibly the movement receptors of Pachygrapsus are less rhythmic than those of Carcinus and are poor tools for the exploration of this problem. To check this possibility the data of Wiersma and Boettiger (28, fig. 3) were subjected to the same analysis as those from Pachygrapsus; the result is presented in fig. 12. Here there are four graphs, since each part of their fig. 3 was derived from a different cell, and the comparison of intervals is only valid for a single cell. It is not easy to make exact measurements on printed figures, the contrast under the microscope is horrible, yet the internal consistency of the measurements makes it likely that the results so obtained are reliable for the present purpose. The data are presented in the same manner as in fig. 11, except that the length of the intervals must be given in arbitrary units. The average values of the shortest intervals have been computed and the arrows, inserted in a, b and c, indicate the integral multiples of

Figure 11

The distribution of interval lengths in the discharge of a single movement unit from P. crassipes. Interval length is shown on the abscissa; the number of intervals of each length is indicated on the ordinate.

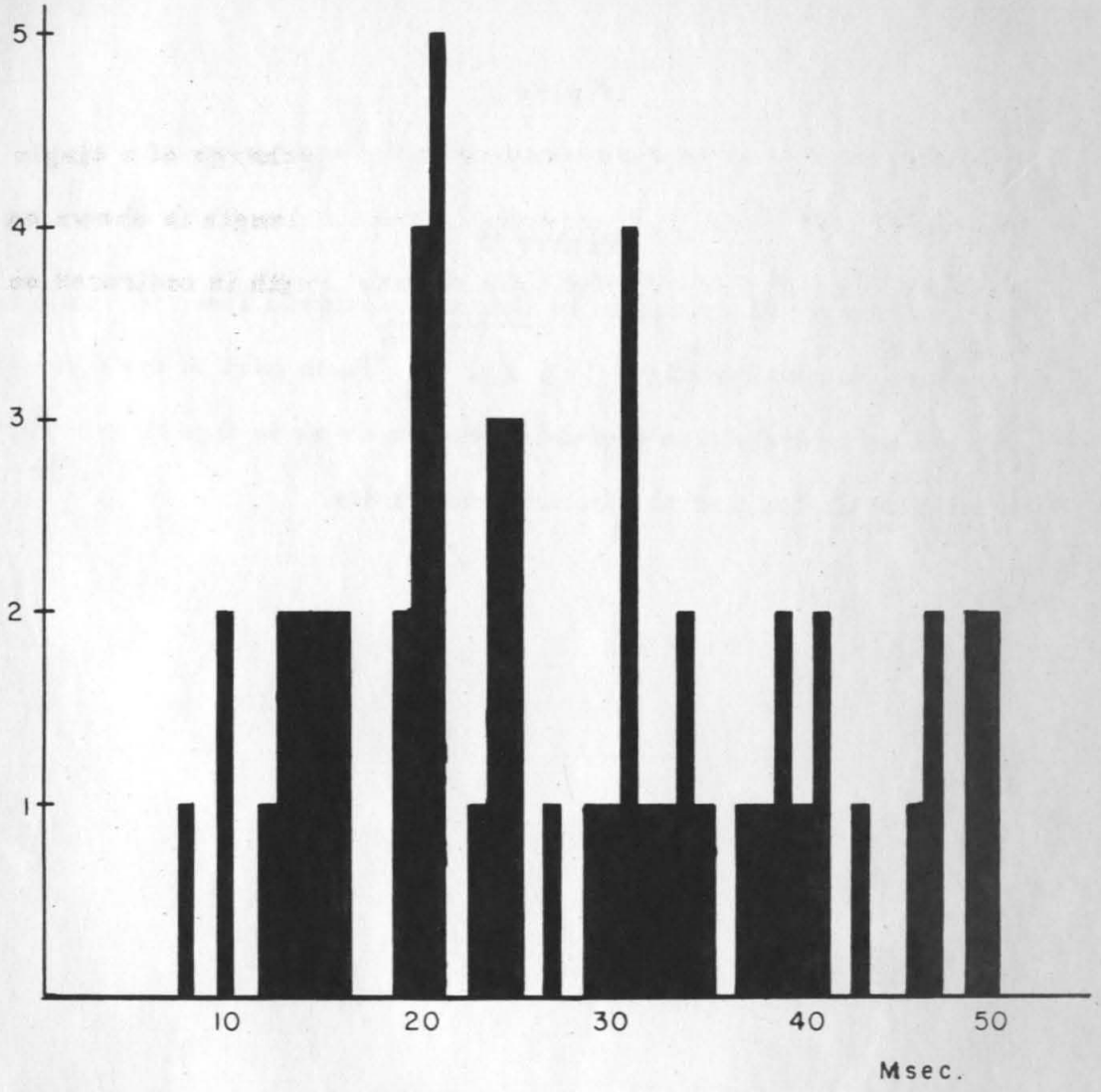


FIGURE 11.

Figure 12

Interval length distribution in Carcinus derived from the results of Wiersma and Boettiger (80, fig. 3). Each part of the figure is plotted separately; the method is the same as in fig. 11 except that the interval lengths are in arbitrary units.

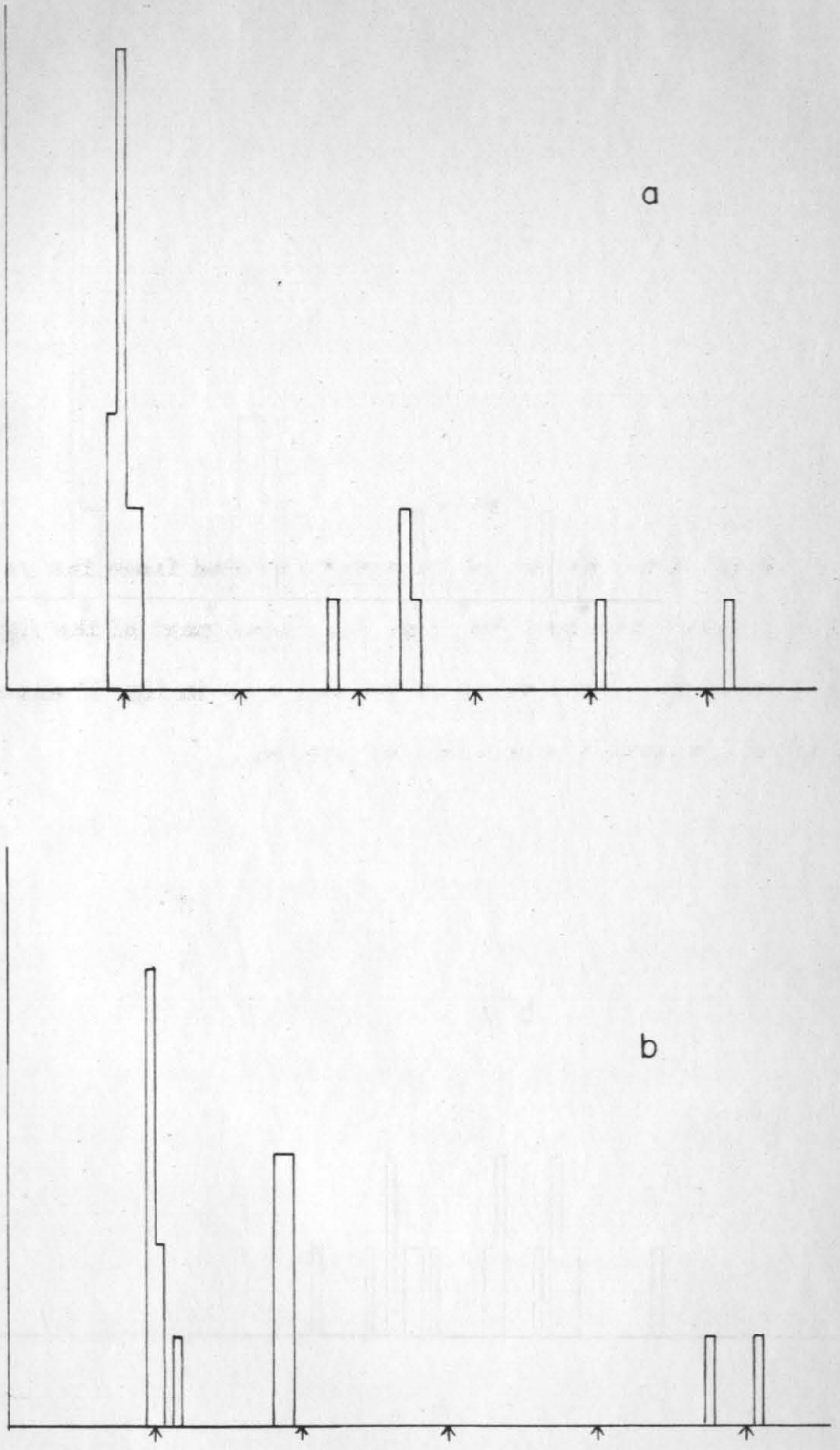
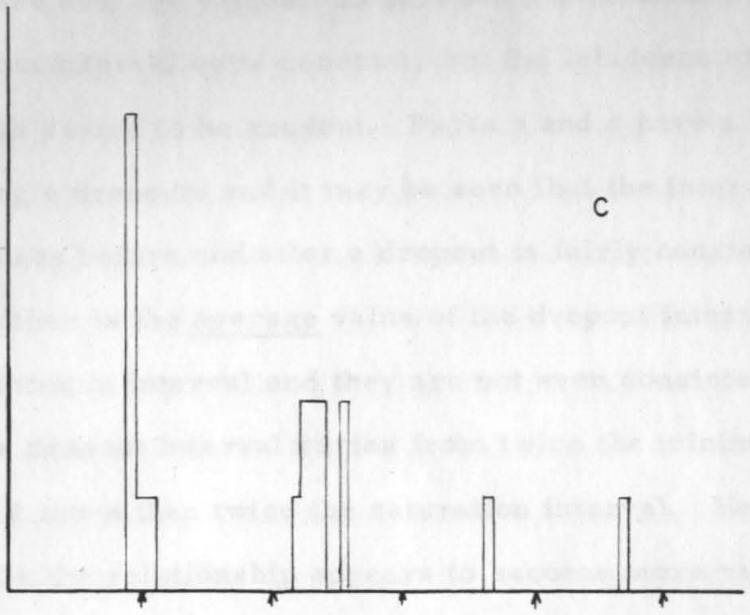


FIGURE 12.



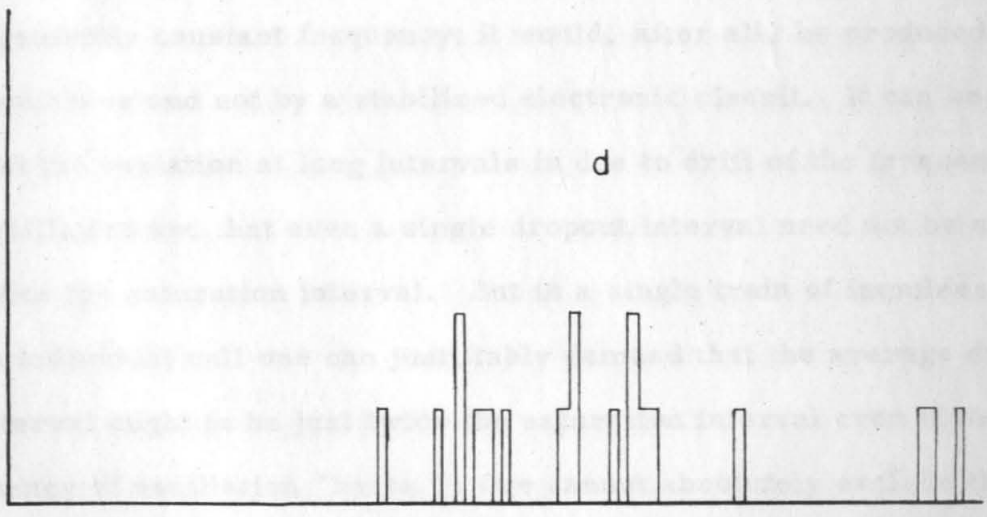


FIGURE 12 (cont.)

these average values. In part a the saturation was best and the minimum interval quite constant, but the incidence of spikes at longer intervals seems to be random. Parts b and c have a larger proportion of single dropouts and it may be seen that the interval between the two spikes before and after a dropout is fairly constant in both; yet in neither is the average value of the dropout interval exactly twice the minimum interval and they are not even consistent in the way in which the dropout interval varies from twice the minimum; in b it is less and in c more than twice the saturation interval. Here too at longer intervals the relationship appears to become more random. This is most apparent in part d where all the intervals are longer than saturation, and it is impossible to define any grouping of the interval durations or to derive a possible value for a minimum interval. One must admit that an oscillation such as Wiersma proposes would be unlikely to possess a perfectly constant frequency; it would, after all, be produced by a cell membrane and not by a stabilized electronic circuit. It can be claimed that the variation at long intervals is due to drift of the frequency of oscillation and that even a single dropout interval need not be exactly twice the saturation interval. But in a single train of impulses from an individual cell one can justifiably demand that the average dropout interval ought to be just twice the saturation interval even if the frequency of oscillation "hunts." One cannot absolutely exclude the possibility of oscillation in the excitability of movement receptors; but the data do not demand the existence of such a mechanism nor do they exclude others that could not account for near perfect rhythmicity.

In particular they do not exclude a mechanism such as that observed by Burkhardt (79, 80) in the crayfish stretch receptor. At low temperature, dropouts are recorded from the RM1; and intracellular recording reveals that at each dropout in the spike pattern a low amplitude oscillation with faster rise than decay takes the place of the missing spike and repolarizes the cell preparatory to the next full spike. This is probably the local potential of Eyzaguirre and Kuffler (12,13), a species of abortive spike that produces a refractory period. Such abortive spikes, variously called prepotentials, pseudo-pointes or local potentials, decay more rapidly than would be predicted for passive discharge of the membrane capacitance and are usually of all-or-none nature (81, 82, 83, 84). They are thought to be the immediately precedent step to the initiation of the spike, appearing alone only when the full spike fails to develop (20). The occurrence of such potentials as an antecedent step in the production of the movement receptor spikes might explain the low saturation frequency if the prepotentials had a long refractory period, for instance 10 - 15 msec. Then were nicotine to activate the spiking membrane directly, without the intervention of the prepotentials, the drug could produce a much higher frequency than mechanical stimulation. Or, alternatively, nicotine might drive the cell prior to the prepotential step but with such vigor as to override the prepotential mechanism and swamp its frequency limiting characteristic. The record of nicotine stimulation in fig. 9(b) holds possibilities in this respect. The train starts with eight spikes that are no closer than the closest spikes of this unit on mechanical stimulation in 9(a). Then there is a

rather quick transition to a higher frequency which rises smoothly higher still. As the frequency then begins to drop there occur four apparent dropouts and then another rather abrupt shift to a lower frequency at which the cell continues to fire for several seconds before the frequency starts to decrease again, this time smoothly. Such behavior might be due to the nicotine stimulation first activating and then swamping the prepotential frequency setter; and finally dropping to the point where the prepotentials once again took over in determining the maximum frequency.

The only potential changes of the required nature that are seen in the intracellular recording from Pachygrapsus movement receptors are the 1 - 2 mv. potentials that have been called prepotentials due to their seeming participation in the rising phase of the spikes. The maximum frequency of occurrences of these potentials is, however, in the range of 125/sec. in the one cell where they have been observed; and since this cell had a low threshold to movement it is not likely that its saturation frequency would be more than about 50/sec. This cannot be stated with certainty since an attempt to achieve saturation at the end of this experiment resulted in the loss of the penetration. Yet the probability that the saturation frequency would be quite low is so strong that it does seem impossible that 120/sec. potentials could represent the saturation frequency mechanism.

Limitation of drive due to mechanical factors

The third possible explanation for the low saturation frequency

postulates a poor coupling between the elastic strand and the sensory neurons such that the amount of stress which may be transmitted to a distal process is limited. The mechanism of coupling between strand and distal process postulated above (pp. 67, 68) contains an inherent limit on the drive to which a cell may be submitted; if the scolopale is bent because its base and apex move unequal distances, then the angle of bending cannot, as I see it, exceed 90 degrees. This sets an upper limit to the degree that a distal process can be bent and therefore to the output frequency of a cell. Unfortunately, since Whitear (35) claims that the structure of both position and movement receptors looks the same, this cannot explain the low frequency of saturation of the movement receptors. It is in the differences between movement and position receptors that an explanation must be sought; and a difference has already been postulated that will fit our need. It has been proposed above that the essential structural difference between movement and position receptors that explains their different sensitivities is how they are coupled to the fibers of the elastic strand; and that in order for the movement receptor response to cease at the end of movement their coupling must be rather loose or viscous. This would allow the scolopale of a movement receptor to straighten out when movement stopped, but would also imply that a movement receptor ought not to be as accessible to deformation as a position receptor. In the course of a movement the same slippage that releases the scolopale at the end of movement might already be occurring and dissipating the mechanical force applied to the scolopale. Thus the same mechanism that makes a movement receptor

respond only to changes of position may also be responsible for its low output frequency. This same mechanism may well be the basis for the irregular discharge at subsaturation levels. If the postulated slippage takes place, it would very likely be a jerky process; stimulation level at the distal process would not be a smooth function of speed, and the frequency would mirror this.

How then explain the doublet firing and the almost doubled saturation interval at a dropout? For the latter I do not see an explanation at present. It may be due to an abortive spike but none have been observed. Lippold et al. (84) have observed dropouts in the spontaneous discharge of a mammalian spindle cooled below 32 degrees C. Their recording is made at the exit of the afferent nerve from the muscle mass, close enough to the spindle to pick up the receptor potential; yet they see no sign of an oscillation or partial spike. They cannot explain the dropout phenomenon either. The occurrence of doublet and triplet firing is another matter. Eyzaguirre and Kuffler (13) have observed high frequency grouped firing in stretch receptors in response to a single antidromic impulse. They propose that this is due to electrically excitable activity in the proximal portions of the dendrites; this activity lasts longer than it takes for the spike initiating locus to recover its excitability and so triggers another spike. Asynchronous invasion of the several dendrites of the stretch receptor is assumed to be responsible for the long duration of some of these grouped discharges. I would propose that a similar mechanism is responsible for doublets and triplets in the movement receptors, and that the positively directed after-

potentials following the spikes are the electrical signs of this activity.

In the records of Wiersma and Boettiger (28) the second spike of a doublet follows the first at a very short interval and similarly in fig. 8 of the present work. In fact, in both parts (a) and (b) of fig. 8 the second spike of a doublet appears right at the peak of the positive afterpotential produced by the first spike of the doublet. That these positive afterpotentials are actually produced by the spikes must be so; nothing of their shape and time course is seen in the absence of spikes. This positive afterpotential may reach the spike initiating locus, which has probably recovered within the 8 msec. between spikes of the doublet, and set off a second spike if it adds to another drive produced by mechanical input. I think it safe to assume that the spike initiating locus fully recovers in 8 msec. since the refractoriness produced by a spike does not long outlast the spike. As the afterpotential decays the probability that any more spikes will be set up must decrease. This would lead to the prediction that whereas doublets are rare events, triplets are even more rare; and this is indeed the case, triplets are almost never observed in the movement receptors of Pachygrapsus. I think the fact that the afterpotential lasts for 20 msec., corresponding to a frequency of 50/sec., is purely fortuitous. If the afterpotential produced a refractoriness that set the saturation frequency it could not simultaneously produce doublet firing.

It was hoped that the series of experiments on the temperature dependence of the saturation frequency would yield evidence that might be used to distinguish whether saturation is due to passive or active

processes. By passive processes I mean those involving only mechanical factors such as coupling to the strand or bending of the scolopale; while under active processes I would include all those that occur in the cell membrane and are based on chemical reactions. A low Q_{10} could be taken to indicate the former, a high Q_{10} the latter. The actual value arrived at for the Q_{10} of the saturation frequency is 1.7, neither high nor low. Since it was arrived at using data from a single cell it may be completely unreliable, but I think its implications must still be considered. Most biologists seem to feel that a Q_{10} value close to one indicates a physical process whereas a value over two indicates a chemical process; the range in between is a sort of no man's land. The value of 1.7 may indicate either of two things: that a mechanical process is taking place but that its parameters are determined largely by chemical changes such as rearrangements of large protein molecules; or that the saturation process is determined by two active chemical reactions that have opposing effects. Inmann and Peruzzi (43) have calculated the Q_{10} of the Na permeability change in the Pacinian corpuscle to be 2.58; they are almost undoubtedly dealing with a single chemical reaction. One cannot conclude the same for the saturation process since its Q_{10} is not even in the same range. It is my opinion that while the Q_{10} of the saturation frequency is not decisive in indicating the nature of the underlying mechanism, neither does it exclude any of those considered.

Initiation of spikes in movement receptors

A theory may now be formulated concerning the sequence of

events in the initiation of spikes in the movement receptors, the entire sequence being localized in the distal process at a distance of as much as 200 μ from the recording site. The mechanical stimulus may or may not elicit a generator potential in the form of a slow shift in the membrane potential of the mechanosensitive membrane (5,12) that is graded in amplitude and proportional to the stress placed on the cell. Such slow shifts as have been observed in the intracellular records are not consistently in phase with the movements and do not show any correlation with the discharge frequency. A generator potential may be produced but go undetected due to the distance from the recording electrode to its site of production. The next step in the sequence, which is undoubtedly present, would be the production of the prepotentials. These might be triggered by the generator potential if present, or might be set off directly by the mechanical deformation. The production of brief local responses by maintained stimuli has been clearly shown to occur in the Pacinian corpuscle (15,16). Not every prepotential initiates a spike, but this occurs in other systems (83) and is due presumably to the operation of a second factor. Very likely the prepotentials are necessary for the production of spikes, subthreshold movements produce neither. The significance of the high frequency of the prepotentials observed in fig. 8 is unclear; but this high frequency does militate against the prepotentials as the saturation mechanism. Perhaps the spike initiating locus, although closer to the transducer membrane than the cell body, is still so far away that the intervention of a local regenerative process is necessary to assure the production of spikes.

I have alluded above to a second process besides the prepotentials that determines whether spikes will be generated. This is obviously the process that sets the saturation frequency and its nature cannot positively be determined from the data at hand. The results of the experiments on the effect of nicotine do not help here. Nicotine acts on the movement receptors in a seemingly graded manner; very low doses only add to the stimulation brought about by movement while higher ones can elicit discharges in the complete absence of movement. Data have been presented which indicate that nicotine activates the membrane which produces the prepotentials; it certainly doesn't exert its effect through the mechanoreceptive membrane for when this membrane is blocked by caffeine nicotine still works. Assuming the presence in these cells of a generator potential one can envisage that nicotine partially depolarizes the prepotential producing membrane so that a smaller generator potential can trigger it better. More nicotine will itself trigger the prepotentials and still more nicotine would depolarize the prepotential producing membrane to the point where it could not be repolarized at all; the stimulation to the spike producing locus would then be of high amplitude and prolonged duration.

Comparison between movement receptors and crayfish stretch receptors

In the behavior of the crayfish stretch receptors subjected to constant velocity stretching and relaxation may be found a single attribute to be applied to the movement receptors, if with caution. The "off" effect at the end of stretch may well occur in the movement receptors as well as in the stretch receptors. Its course in fig. 10(b), taken from

an RM2, is particularly exaggerated, probably by the form of the stimulus. That it is of greater magnitude in the fast adapting RM2 is particularly significant since such an "off" effect in the generator potential of the movement receptor would assure the prompt cessation of its discharge at the end of a movement. The fact that the output frequency of an RM2 does not change a great deal as the speed of stretching is increased indicates that the rate at which the adaptation process develops is tied to the rate of stimulus increase, something that has been assumed for the movement receptors. It is not likely that the change in spike threshold would be of great moment in the movement receptors since this change does not become effective until rather high discharge frequencies are attained (12).

I think it would be misleading to apply directly to the movement receptors the present theories that account for fast adaptation in the RM2, especially since it has already been shown that fast adaptation in RM2 is only a special case of the adaptation shown by all known stretch receptors. Just as misleading, in my view, are three reports that have described responses reminiscent of those of movement receptors, but recorded from known position receptors. In all three, sinusoidal stimulation was applied to position receptors and it was found that the point of maximum discharge frequency seemed to depend on the rate of stretching; yet all three experiments are subject to the same objections. The first is to the form of the stimulus. Since the rate of stretch changes progressively it is a chancy affair to state what is the exact correspondence between the receptors' discharge and the

rate of change of their length. Only constant velocity stretching at discreetly different rates disposes of this problem. More serious from the point of view of interpretation is the failure in all three reports to take into account the phenomenon of overstretch (9,12). This consists of a slowing and then complete stoppage of the discharge of a crayfish stretch receptor as the stretch on it is increased beyond a certain limit; and is due to the intense depolarization set up at the point of spike initiation by the generator potential. Under such conditions of intense depolarization the sodium conductance mechanism is inactivated and spikes cannot be evolved. All three of the reports state that as the rate of stimulation is increased the maximum frequency of discharge shifts to the phase of increasing stretch. Krnjevic and van Gelder (14) report that the tension in a stretch receptor does not rise linearly with length, but overshoots with a subsequent drop to a steady level characteristic of the new length. The degree of overshoot increases as the speed of the stretch increases. Thus in the experiments of Lowenstein and Finlayson (85) on insect stretch receptors and those of Lippold et al. (25) on muscle spindles, as the frequency of a sinusoidal stimulus is increased the overshoot of tension on each stretching phase would likewise increase with the result that at high frequencies the tension on the receptor might get up into the overstretch region even though a slower stretch to the same length would not result in overstretch. It is interesting that Lippold et al. insist that the shift cannot be due to adaptation because the receptors often go silent before the peak of the stretch is reached. Even the experiments of Pringle and Wilson (87)

though performed on the leg spines of Periplaneta americana, are subject to this objection since their results show that the "phase advance" produced by increasing frequency is similarly produced by an increase in stimulus amplitude with no change in stimulus frequency.

These considerations indicate that there is a fundamental difference between the PD receptors and most types of stretch receptors. Certainly the microanatomy of the ultimate dendritic extensions of the PD cells as revealed by Whitear is vastly different from that of the crayfish stretch receptors as found by Florey and Florey. It is possible, as stated above, that the ultimate nature of the adequate stimulus to both may be stretch of the mechanotransducer membrane; but until a study appears of the relation of distal process to strand in the PD organ this is only a guess. The difference between PD receptors and muscle receptor organs is merely emphasized by their pharmacological properties; they respond to entirely different sets of drugs. Most interesting are their properties with respect to the drugs which in vertebrates affect cholinergic cells: ACh, eserine, atropine and nicotine. A whole class of effects of ACh in mammals is labelled nicotinic because the effects of the two drugs are virtually indistinguishable; e.g., the stimulation of postganglionic autonomic units or of skeletal muscle (53). It is a widely held theory that ACh combines with specific "receptor" molecules in the membranes of the cells it acts upon and that nicotine mimics this effect because its structure is similar enough to that of ACh to allow it to combine with the same receptor molecules. Yet there are actions of ACh in mammals, the so-called muscarinic effects, that

are not duplicated by nicotine; presumably the "receptor" molecules are of a configuration that permits ACh to combine with them but not nicotine. It seems that the PD receptors must possess one sort of receptors that combine with nicotine but not ACh, and conversely for the RM's. Whether the sensitivity to cholinergic drugs in either class of receptors is of physiological significance is entirely unknown.

The action of caffeine on the crab movement receptors is very interesting. Gaba has been shown to inhibit the crayfish stretch receptors by "clamping" the membrane potential (45), preventing depolarization of the cell to the spike firing level. That a burst of spikes may be elicited from a movement receptor during the time its mechanical activation is blocked by caffeine indicates that the effect of caffeine is not likely to be such a clamping action. Nicotine has been shown to act on the movement receptor in a graded manner close to the site of mechanotransduction and it is eminently reasonable to expect that clamping the membrane potential should antagonize both mechanical and chemical activation of the cell. It appears that caffeine must immobilize the mechanotransducer membrane so that it cannot change its permeability when subjected to a mechanical stimulus. No other instance is known of such an effect on a mechanoreceptor and systematic investigation of the effects of the structural relatives of caffeine on the crab PD may provide information regarding the molecular basis for mechanosensitivity. Why caffeine does not affect the crayfish PD organ cannot be stated with certainty; this may be due to a permeability barrier or to a species specific difference in the configuration of

the "receptor" molecules. The former is quite likely since the crayfish PD is buried in a mass of loose connective tissue which is impossible to remove completely without damage to the receptor cells. In comparison the crab PD is naked; and what little connective tissue adheres to it can be easily removed.

REFERENCES

1. Adrian, E. D., The Impulses Produced by Sensory Nerve Endings. Part 1, J. Physiol. (1926) 61:49-72.

2. Adrian, E. D., and K. Umrath, The Impulse Discharge from the Pacinian Corpuscle, J. Physiol. (1929) 68:139-154.

3. Adrian, E. D., and Y. Zotterman, The Impulses Produced by Sensory Nerve Endings. Part 2. The Response of a Single End Organ, J. Physiol. (1926) 61:151-171.

4. _____, The Impulses Produced by Sensory Nerve Endings. Part 3. Impulses Set Up by Touch and Pressure, J. Physiol. (1926) 61:456-483.

5. Katz, B., Depolarization of Sensory Terminals and the Initiation of Sensory Impulses in the Muscle Spindle, J. Physiol. (1949) 111:261-282.

6. Alexandrowicz, J. S., Muscle Receptor Organs in the Abdomen of Homarus vulgaris and Palinurus vulgaris, Quart. J. Micr. Sci., (1951) 92:163-199.

7. Florey, E., and E. Florey, Microanatomy of the Abdominal Stretch Receptors of the Crayfish (Astacus fluviatilis L.), J. Gen. Physiol. (1955) 39:69-85.

8. Wiersma, C. A. G., E. Florey and E. Furshpan, Effect of Acetylcholine on a Stretch Receptor of Crayfish, Fed. Proc., (1952) 11:172.

9. Wiersma, C. A. G., E. Furshpan and E. Florey, Physiological and Pharmacological Observations on the Muscle Receptor Organs of the Crayfish, Cambarus clarkii, Girard, J. Exp. Biol. (1953) 30:136-150.

10. Eyzaguirre, C., and S. W. Kuffler, Excitation in Nerve Cells of the Lobster Stretch Receptors, Biol. Bull. (1954) 107:310.

11. _____, Inhibitory Activity in Single Cell Synapses, Biol. Bull. (1954) 107:310-311.

12. _____, Processes of Excitation in the Dendrites and in the Soma of Single Isolated Sensory Nerve Cells of the Lobster and Crayfish, J. Gen. Physiol. (1955) 39:87-119.

13. _____, Further Study of the Soma, Dendrite and Axon Excitation in Single Neurons, J. Gen. Physiol. (1955) 39:121-153.

14. Krnjevic, K., and N. van Gelder, The Effects of Stretch on the Tension and Rates of Discharge of Crayfish Stretch Receptors, J. Physiol. (1960) 154:27P.

15. Alvarez-Buylla, R., and J. Ramirez de Arellano, Local Responses in Pacinian Corpuscles, Am. J. Physiol. (1953) 172:237-244.

16. Gray, J. A. B., and M. Sato, Properties of the Receptor Potential in Pacinian Corpuscles, J. Physiol. (1953) 122:610-636.

17. Loewenstein, W. R., Generator Processes of Repetative Activity in a Pacinian Corpuscle, J. Gen. Physiol. (1958) 41:825-845.

18. Loewenstein, W. R., and R. Altimirana-Orrego, The Refractory State of the Generator and Propagated Potentials in a Pacinian Corpuscle, J. Gen. Physiol. (1958) 41:805-824.

19. Loewenstein, W. R., and R. Rathkamp, The Site for the Mechano-electric Conversion in a Pacinian Corpuscle, J. Gen. Physiol. (1958) 41:1245-1265.

20. Loewenstein, W. R., The Generation of Electrical Activity in a Nerve Ending, Ann. N. Y. Acad. Sci., (1959) 81:367-387.

21. Bullock, T. H., Initiation of Nerve Impulses in Receptor and Central Neurons, Rev. Mod. Phys. (1959) 31:504-514.

22. Gray, J. A. B., Initiation of Impulses at Receptors, in Handbook of Physiology. Sec. 1: Neurophysiology, vol. 1, American Physiological Society, Washington D. C. (1959).

23. Boyd, I. A., and T. D. M. Roberts, Proprioceptive Discharges from Stretch Receptors in the Knee-joint of the Cat, J. Physiol. (1953) 122:38-58.

24. Cohen, M. J., The Response Patterns of Single Receptors in the Crustacean Statocyst, Proc. Roy. Soc., B (1960) 152:30-49.

25. Lippold, O. C. J., J. W. T. Redfearn and J. Vuco, The Effect of Sinusoidal Stretching on the Activity of Stretch Receptors in Voluntary Muscle and their Reflex Response, J. Physiol. (1958) 144:373-386.

26. Wiersma, C. A. G., and R. L. C. Pilgrim, Thoracic Stretch Receptors in Crayfish and Rocklobster, Comp. Biochem. Physiol. (1961) 2:51-64.

27. Wolbarsht, M. L., and V. G. Dethier, Electrical Activity in the Chemoreceptors of the Blowfly, 1. Responses to Chemical and Mechanical Stimulation, J. Gen. Physiol. (1958) 42:393-412.

28. Wiersma, C. A. G., and E. G. Boettiger, Unidirectional Movement Fibers from a Proprioceptive Organ of the Crab Carcinus maenas, J. Exp. Biol. (1959) 36:102-112.

29. Wiersma, C. A. G., Movement Receptors in Decapod Crustacea, J. Mar. Biol. Assoc., U. K. (1959) 38:143-152.

30. Barnes, T. C., Sensory Impulses in Crustacean Nerve, J. Physiol. (1930) 69:XXXII.

31. _____, Responses in the Isolated Limbs of Crustacea and Associated Nerve Discharges, Am. J. Physiol. (1932) 99:321-331.

32. Burke, W., An Organ for Proprioception and Vibration Sense in Carcinus maenas, J. Exp. Biol. (1954) 31:127-138.

33. Alexandrowicz, J. S., Further Observations on Proprioceptors in Crustacea and a Hypothesis about their Function, J. Mar. Biol. Assoc., U. K. (1958) 37:379-396.

34. Alexandrowicz, J. S. and M. Whitear, Receptor Elements in the Coxal Region of Decapod Crustacea, J. Mar. Biol. Assoc., U.K. (1957) 36:603-628.

35. Whitear, M., Chordotonal Organs in Crustacea, Nature (1960) 187:522-523.

36. _____, Personal communication.

37. Wiersma, C. A. G., Personal communication.

38. Tyler, A., Prolongation of Life-span of Sea Urchin Spermatozoa, and Improvement of the Fertilization-Reaction, by Treatment of Spermatozoa and Eggs with Metal-Chelating Agents (Amino Acids, Versine, DEDTC, Oxine, Cupron), Biol. Bull. (1953) 104:224-239.

39. Woodbury, J. W., and A. J. Brady, Intracellular Recording from Moving Tissues with a Flexibly Mounted Ultramicroelectrode, Science (1956) 123:100.

40. Wiersma, C. A. G., The Inhibitory Nerve Supply of the Leg Muscles of Different Decapod Crustaceans, J. Comp. Neurol. (1941) 74:63-79.

41. van Harreveld, A., A Physiological Solution for Fresh Water Crustaceans, Proc. Soc. Exp. Biol. Med., N. Y. (1936) 34:428-432.

42. Schlatter, M. J., Analysis of the Blood Serum of Cambarus clarkii, Pachygrapsus crassipes and Panulirus interruptus, J. Cell. Comp. Physiol. (1941) 17:259-261.

43. Inman, D. R., and P. Peruzzi, The Effects of Temperature on the Response of Pacinian Corpuscles, J. Physiol. (1960) 155:280-301.

44. Bazemore, A., K. A. C. Elliot and E. Florey, Factor I and γ-Aminobutyric Acid, Nature (1956) 178:1052-1053.

45. Edwards, C., and S. W. Kuffler, Inhibitory Mechanisms of Gamma Aminobutyric Acid on an Isolated Nerve Cell, Fed. Proc. (1957) 16:34.

46. Davenport, D., The Effects of Acetylcholine, Atropine and Nicotine on the Isolated Heart of the Commercial Crab, Cancer Magister Dana, Physiol. Zool. (1941) 12:178-185.

47. Roeder, K. D., and S. Roeder, Electrical Activity in the Isolated Ventral Nerve Chord of the Cockroach. 1. The Action of Pilocarpine, Nicotine and Acetylcholine, J. Cell. Comp. Physiol. (1939) 14:1-9.

48. Schallek, W., and C. A. G. Wiersma, The Influence of Various Drugs on a Crustacean Synapse, J. Cell. Comp. Physiol. (1948) 31:35-47.

49. Wiersma, C. A. G., and W. Schallek, Protection of Synaptic Transmission Against Block by Nicotine, Science (1947) 106:421.

50. _____, Influence of Drugs on Response of a Crustacean Synapse to Pre-Ganglionic Stimulation, J. Neurophysiol. (1948) 11:491-496.

51. Turner, R. S., W. A. Hagins and A. R. Moore, Influence of Certain Neurotropic Substances on Central and Synaptic Transmission in Callianassa, Proc. Soc. Exp. Biol. Med. (1950) 73:156-158.

52. Ellis, C. H., C. H. Thienes and C. A. G. Wiersma, The Influence of Certain Drugs on the Crustacean Nerve-Muscle System, Biol. Bull. (1942) 83:334-352.

53. Goodman, L. S., and A. Gillman, The Pharmacological Basis of Therapeutics, The Macmillan Co., New York (1955).

54. Wiersma, C. A. G., and E. Novitski, The Mechanism of the Nervous Regulation of the Crayfish Heart, J. Exp. Biol. (1942) 19:255-265.

55. Welsh, J. H., and H. H. Haskin, Chemical Mediation in Crustacea. III Acetylcholine and Autotomy in Petrolisthes armatus (Gibbes), Biol. Bull. (1939) 76:405-415.

56. Schallek, W., and C. A. G. Wiersma, Effects of Anti-Cholinesterases on Synaptic Transmission in the Crayfish, Physiol. Comp. Oecol. (1949) 1:63-67.

57. Prosser, C. L., Action Potentials in the Nervous System of the Crayfish. Effects of Drugs and Salts upon Synaptic Transmission, J. Cell. Comp. Physiol. (1940) 16:25-38.

58. Florey, E., An Inhibitory and an Excitatory Factor of Mammalian Central Nervous System and their Action on a Single Sensory Cell, Arch. Int. Physiol. (1954) 62:33-53.

59. _____, Further Evidence for the Transmitter Function of Factor I, Naturwiss. (1957) 15:424-425.

60. Cohen, M. J., The Function of the Receptors in the Statocyst of the Lobster Homarus americanus, J. Physiol. (1955) 130:9-34.

61. Pumphrey, R. J., Slow Adaptation of a Tactile Receptor in the Leg of the Common Cockroach, J. Physiol. (1936) 87:6P.

62. Barber, S. B., Chemoreception and Proprioception in Limulus, J. Exp. Zool. (1956) 131:51-73.

63. Echlin, F., and A. Fessard, Synchronized Impulse Discharge from Receptors in the Deep Tissues in Response to a Vibratory Stimulus, J. Physiol. (1938) 93:312-334.

64. Prosser, C. L., Action Potentials in the Nervous System of the Crayfish, J. Comp. Neurol. (1935) 62:495-505.

65. Walbarsht, M. L., Electrical Characteristics of Insect Mechanoreceptors, J. Gen. Physiol. (1960) 44:105-122.

66. Pringle, J. W. S., Proprioception in Limulus, J. Exp. Biol. (1956) 33:658-667.

67. Case, J. F., C. Edwards, R. Gesteland and D. Ottoson, The Site of Origin of the Nerve Impulse in the Lobster Stretch Receptor, Biol. Bull. (1957) 113:360.

68. Kuffler, S. W., Synaptic Inhibition Mechanisms, Properties of Dendrites and Problems of Excitation in Isolated Sensory Nerve Cells, Exp. Cell. Res. (1958) Suppl. 5, 493-519.

69. Arvanitaki, A., and N. Chalazonitis, Prototypes d'Interactions Neuroniques et Transmission Synaptic, Donnees Bioelectriques de Preparations Cellulaires, Arch. Sci. Physiol. (1949) 3:547-565.

70. _____, Potentials d'Activite du Soma Neuronique Geant (Aplysia), Arch. Sci. Physiol. (1955) 9:115-144.

71. Bullock, T. H., and C. A. Terzuolo, Diverse Forms of Activity in the Somata of Spontaneous and Integrating Ganglion Cells, J. Physiol. (1959) 138:341-364.

72. Arvanitaki, A., Recherches sur la response oscillatoire locale de l'axone geant isole de "Sepia," Arch. Int. Physiol. (1939) 49:209-256.

73. Brink, F., D. W. Bronk and M. G. Larabee, Chemical Excitation of Nerve, Ann. N. Y. Acad. Sci. (1946) 47:457-485.

74. Hagiwara, S., and N. Saito, Membrane Potential Change and Membrane Current in Supramedullary Nerve Cell of Puffer, J. Neurophysiol. (1959) 22:204-221.

75. Tauc, L., Etude de l'Activite elementaire des Cellules du Ganglion Abdominal de l'Aplysie, J. Physiologie (1955) 47:769-792.

76. Hodgkin, A. L., and A. F. Huxley, A Quantitative Description of Membrane Current and its Application to Conduction and Excitation in Nerve, J. Physiol. (1952) 117:500-544.

77. Cole, K. S., and R. F. Baker, Longitudinal Impedence of the Squid Giant Axon, J. Gen. Physiol. (1941) 24:771-788.

78. Cole, K. S., Rectification and Induction in the Squid Giant Axon, J. Gen. Physiol. (1941) 25:29-51.

79. Burkhardt, D., Effect of Temperature on Isolated Stretch-receptor Organ of the Crayfish, Science (1959) 129:392-393.

80. _____, Die Erregungsvorgange sensibler Ganglienzellen in Abhangigkeit von der Temperatur, Bio. Zentrbl. (1959) 78:22-62.

81. Eccles, J. C., The Properties of Dendrites, in D. B. Tower and J. P. Schade, ed., Structure and Function of the Cerebral Cortex, Elsevier, Amsterdam (1960).

82. Eccles, J. C., B. Libet and R. R. Young, The Behavior of Chromatolyzed Motoneurons Studied by Intracellular Recording, J. Physiol. (1958) 143:11-40.

83. Spencer, W. A., and E. R. Kandell, Electrophysiology of Hippocampal Neurons, IV. Fast Prepotentials, J. Neurophysiol. (1961) 24:272-285.

84. Tauc, L., Activites electriques fractionees observees dans des cellules ganglionnaire de l'Escargot (Helix pomatia), C. R. Acad. Sci., Paris (1955) 241:1070-1073.

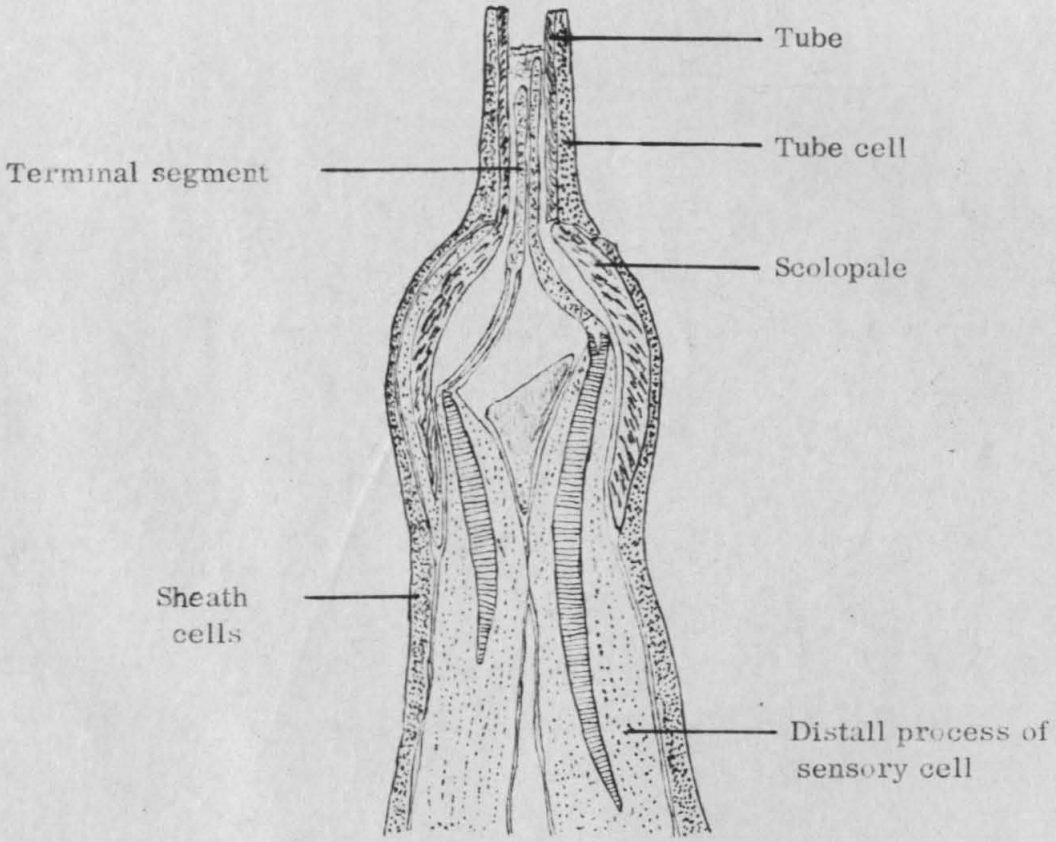
85. Lippold, O. C. J., J. G. Nicholls and J. W. T. Redfearn, A Study of the Afferent Discharge Produced by Cooling a Mammalian Muscle Spindle, J. Physiol. (1960) 153:218-231.

86. Lowenstein, O., and L. H. Finlayson, The Response of the Abdominal Stretch Receptor of an Insect to Phasic Stimulation. Comp. Biochem. Physiol. (1960) 1:56-61.

87. Pringle, J. W. S., and V. J. Wilson, The Response of a Sense Organ to a Harmonic Stimulus, J. Exp. Biol. (1952) 29:220-234.

APPENDIX

The ultrastructure of the distal processes of two neural elements in the PD organ of Carcinus. Notice the complex supportive structures and the dissimilarities in the structures of the two endings. Redrawn from Whitear (35).


Tube
Tube cell
Terminal segment
Scolopale
Sheath cells
Distall process of sensory cell