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EFFECTS OF HIGH HYDROSTATIC PRESSURE
ON NEUROMUSCULAR TRANSMISSION IN SHALLOW-LIVING
AND DEEP-LIVING CRUSTACEANS

by

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To Rich Harbison

For his support from the beginning

And for his good advice

But mostly for his friendship.

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Effects of High Hydrostatic Pressure on Neuromuscular Transmission
in Shallow-Living and Deep-Living Crustaceans

by

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ABSTRACT

The effects of high hydrostatic pressure on excitatory neuromuscular transmission in shallow- and deep-living crustaceans were compared. Pressure caused depression of the amplitude of excitatory junctional potentials (e.j.p.s) at the neuromuscular junction in the shallow-living crab, Libinia emarginata. A pressure of 100 atm depressed the e.j.p. amplitude by about one-half.

In the deep-sea crab, Geryon quinquedens, which ranges to a depth of 2000 m (or 200 atm pressure), adaptations to high pressure were observed in two different types of muscle fibers: 1) In fibers with e.j.p.s that showed high levels of facilitation, the magnitude of pressure-induced depression decreased with increasing frequency of nerve stimulation; i.e., there was a pressure-induced increase in facilitation. Also a pressure-induced increase in the duration of the falling phase of the e.j.p. was observed which served to increase the level of depolarization resulting from summation of the e.j.p.s at high frequencies of nerve stimulation. In these highly facilitating fibers the physiologically significant frequencies that cause appreciable contraction are probably high. At high frequencies the pressure-induced increases in facilitation and summation together served to completely counteract the depressive effect of pressure, and the net depolarization attained during a train of nerve stimulation was relatively unaffected by pressures up to at least 200 atm.

2) Fibers with e.j.p.s showing low levels of facilitation may undergo significant contraction at low frequencies of nerve impulses where neither facilitation nor summation play a significant role. The amplitude of e.j.p.s recorded from this fiber-type in the deep-sea crab were, on the average, unaffected by pressures to 200 atm. The e.j.p.s of some of these fibers showed depression, but others were amplified under pressure.

The results of experiments with the lobster, Homarus americanus, which ranges to a depth intermediate between Libinia and Geryon were in many respects intermediate between the results obtained with the two species of crab. Studies of the effect of pressure on isometric tension developed by whole muscles in Homarus and Geryon were consistent with the results of the studies of the e.j.p.; pressure depressed the rate of rise of tension in Homarus and had little effect in Geryon.

The results of this work provides a physiological basis for the observation that shallow-living animals are generally immobilized by pressures in excess of 200 atm.

Experiments were performed in an attempt to elucidate the mechanism underlying the pressure-induced depression of e.j.p. amplitude. Results were suggestive that the depression of e.j.p. amplitude reflects a pressure-induced decrease in the number of quanta of transmitter substance released by the nerve endings.

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INTRODUCTION

The biological effects of high hydrostatic pressure have been investigated for a considerable time. The field has been reviewed in two recent monographs (Zimmerman, 1970; Sleight and Macdonald, 1972). The effects of pressure on whole animals, on muscle physiology, on cell physiology, and on biochemical systems have been among the major areas of interest.

There have been few investigations into the neurophysiological effects of high pressure, but observations of the behavior of a wide variety of animals subjected to high pressure suggest that the nervous system is a significant site of the action of pressure. In general, animals become hyperactive and sometimes exhibit seizures in the pressure range to 200 atm and are immobilized at greater pressures.

Pressure affects many biological processes in drastic ways. Even so, organisms live at the greatest ocean depths which exceed 10,000 m, or 1000 atm pressure (pressure increases approximately 1 atm for every 10 m of depth). Representatives of most groups of marine animals have been found at depths exceeding 6000 m (see Bruun, 1957; Wolff, 1960; and Zenkevitch and Birstein, 1956).

Profound adaptations must be present in animals that live at great depths. This thesis is a comparative study of the effects of high hydrostatic pressure on transmission at the crustacean neuromuscular synapse in shallow-living and deep-living crustaceans. It was undertaken in order to determine whether there are effects of pressure on synaptic transmission and to

elucidate some mechanisms of adaptation of this system in animals that live at high pressure in the deep-sea.

Before presenting my work, I will review some of the previous work on the biological effects of high pressure. The literature is extensive, and this review is not an exhaustive one. I have emphasized those aspects that I think are most relevant to the interpretation and significance of my findings. I will then discuss the crustacean neuromuscular system in some detail to provide the neurophysiological framework within which I interpret the results of my work.

Some Biological Effects of High Hydrostatic Pressure

Mechanisms of the effects of pressure on biological systems

A few general comments will first be made about the kinds of mechanisms that might be expected to underlie the biological effects of pressure. What usually comes to mind first when one thinks of biological effects of pressure are effects involving gases; e.g., nitrogen narcosis, decompression sickness (bends), or gross mechanical damage to gas-filled organs (e.g., lungs or swimbladders). Such gas-related effects are not the subject here; the concern is, rather, with the biological effects of high hydrostatic pressure on the liquid and solid phases that make up the organism.

Hydrostatic pressure can change the kinetics of chemical and biochemical reactions (see Hamann, 1963; and Johnson and Eyring, 1970), either decreasing or increasing the reaction rate. These effects presumably reflect volume changes that accompany the formation of activated complexes.

In equilibrium processes, high pressure will favor the state of the system with smaller volume; e.g., ions tend to be more soluble at high pressure because they exert an electrostrictive force on the water in their hydration shell, pulling the water molecules in close to occupy a smaller volume (see Hills, 1972). This effect, itself, may have biological implications, and it is easy to imagine that many biological processes involve equilibria between states of different volume. Also, it is conceivable that many of the complex molecular structures involved, for example, in enzyme systems and biological membranes, may have open structures which deform

under pressure and change their properties.

Effects of pressure on whole animals

Studies of the responses of intact aquatic animals to high hydrostatic pressure were conducted by Regnard in the late nineteenth century and by Ebbecke in the early twentieth century (see Flügel and Schlieper, 1970). In general, pressures of 100-200 atm caused aquatic animals to become hyperactive, and seizures were observed in some. Exposure to pressures exceeding 200 atm resulted in immobility and death.

The same pattern was observed in more recent studies of the effects of pressure on shallow-living marine animals, but deep-living animals did not, in general, become hyperactive, and greater pressures were required to immobilize and kill them. Macdonald (1971) studied the effects of high pressure on a shore amphipod, and Macdonald, Gilchrist, and Teal (1972) studied the effects on various oceanic plankton animals, mostly crustaceans. The shore amphipod, Marinogammarus marinus, showed increased locomotor activity at 50-100 atm. Locomotor activity declined, and violent dorso-ventral flexing occurred at about 150 atm. At 200 atm the pleopod beat frequency was reduced by about 40%, and at 500 atm complete immobility ensued. Recovery was poor upon decompression from 500 atm. Similar responses were observed in a surface-living (0-400 m) euphausiid, but in several species of deeper-living (to about 1200 m) copepods, exposure to 500 atm pressure only reduced locomotor activity, and recovery upon decompression was good. No hyperactivity was observed in the copepods. A

similar difference in the reaction of shallow- and deep-living ostracods was observed. Several species of ostracod that occur to depths of 1000 m were subjected to 500 atm pressure. They were immobilized, and recovery was poor. Pressures of 200-500 atm reduced the swimming activity of the deep-living ostracod, Gigantocypris mülleri (260-2300 m), and most recovered after decompression from 500 atm. Resistance to the effects of high pressure in such experiments seems to be a feature of animals that live at depth in the ocean.

Klystra, Nantz, Crowe, Wagner and Saltzman (1967) studied the effects of high hydrostatic pressure in mice breathing oxygen-saturated, fluoro-carbon liquid. Trembling of the limbs and uncoordinated movements were observed at 50-80 atm. Such effects were absent in isolated limbs and caudal to spinal transection, and the authors attributed the effects of pressure to direct effects upon the central nervous system. Most other investigators who have studied the reaction of intact animals to high pressure have also pointed out the likelihood that some of the effects they have observed result from the action of pressure on the nervous system.

Neurophysiological effects of pressure

There have been several studies of the effects of high hydrostatic pressure on the conduction of action potentials in nerve. Grundfest and Cattell (1935) and Grundfest (1936) studied the effects of high pressure on compound action potentials in frog motor nerves, and Spyropoulos (1957 a&b) and Tasaki and Spyropoulos (1957) studied the effects in single motor axons

of frogs and toads and in the giant axon of the squid.

In the early work on compound action potentials, the preparations were bathed directly in mineral oil. Spyropoulos (1957a) found differences in the effects of pressure dependent on whether preparations were bathed in mineral oil or saline, which heats a great deal less than mineral oil when compressed, and he attributed the differences to the effects of temperature changes in the preparations bathed in mineral oil. There is also the possibility that all of the work on conduction in nerve may include artifacts due to pH changes accompanying changes in pressure. Most buffers used in biological work are pressure-sensitive, with the exception of Tris buffer which is relatively insensitive to pressure (Disteche, 1972). However, in recent work on the effects of helium pressure on squid axons, Henderson and Gilbert (personal communication) found no differences between the effects on preparations bathed in bicarbonate and Tris-buffered saline, showing that pH changes are not an important source of artifact in that system.

The most prominent effect of hydrostatic pressure on conduction in nerve was an increase in the duration of the action potential. The action potential in the single nerve fiber of the toad increased in duration about 1.5-fold at 170 atm pressure and about 5-fold at 680 atm (Spyropoulos, 1957a). Most of the increase resulted from an increase in the duration of the falling phase of the action potential. In squid axon (Spyropoulos, 1957b) 272 atm increased the duration of the falling phase of the action potential by about 50%, the rising phase by about 25%. In both the toad and squid axons there was little effect upon the action potential amplitude. In squid axon, the

decrease in membrane impedance during the action potential was about doubled in duration and its maximal amplitude was somewhat reduced ($<15\%$). Pressure had little or no effect on the resting potential in the squid axon. The membrane resistance was increased slightly by pressures below about 340 atm and decreased irreversibly by greater pressures. Membrane capacitance did not change (within 20%). Conduction velocity was decreased slightly (10-15%) at 340 atm.

In some of the different nerve preparations studied, pressure has been reported to reduce the firing threshold, cause repetitive firing in response to a single stimulus, and cause spontaneous firing.

Recent work of Henderson and Gilbert (personal communication) on the effects of helium pressure on the squid axon is largely in agreement with the work of Spyropoulos. Helium pressure prolonged the duration of the action potential without substantially affecting the amplitude. Voltage-clamp experiments showed that the prolongation of the action potential resulted from a pressure-induced slowing of the rise-time of both the early inward and late outward currents, with little effect on the maximum value of the currents.

Concurrent with the work reported in this thesis, Kendig (personal communication) has studied the effects of helium pressure on nerve conduction and synaptic transmission in the superior cervical ganglion of the rat. Helium pressure slightly slowed the conduction velocity and increased the duration of compound action potentials in the pre-ganglionic nerve, and below 137 atm, had little effect on the amplitude. Above 137 atm, the amplitude was depressed in some preparations.

Kendig found that potentials recorded from the post-ganglionic nerve were affected by helium pressure. The amplitude of potentials arising from excitatory synaptic transmission were depressed by pressure (which is consistent with the results of the work reported in this thesis). Those arising from inhibitory transmission were affected irregularly; in some experiments the potential decreased under pressure, but in others it increased or was unchanged.

Effects of pressure on muscle tension

Much work was done, mostly in the 1920's and 30's, on the effect of pressure on the contraction of muscle. The effects varied depending upon conditions such as temperature, fatigue, and whether single twitches or the response to tetanic stimulation was measured. The twitch response of frog gastrocnemius muscle to sciatic nerve stimulation was increased at 100 atm pressure at room temperature, but was decreased by this pressure at low temperature or in muscle fatigued by prior tetanic stimulation (Cattell and Edwards, 1928 and 1932). Higher pressure (408 atm) caused depression even in the twitch response in fresh muscle at room temperature. The contraction-relaxation period was greatly prolonged at pressures causing depression. At low temperature, the development of tension in response to tetanic stimulation was more slowly developed under pressure, but, in contrast to twitches, was only slightly depressed in amplitude.

Cardiac atrial muscle contracting rhythmically in response to sinus venosus discharges was affected similarly to twitches in frog gastrocnemius

muscle (Edwards and Brown, 1934). Isometric tension was increased several fold at room temperature and 204 atm pressure, but began to diminish at 408 atm and was blocked at 884 atm. There were some changes in the electrocardiogram, but the authors judged these insufficient to account for the effects on tension which they attributed to a direct action of pressure on the contractile mechanism.

Work on the effect of pressure on myosin ATPase activity suggest that the contractile mechanism is directly affected by pressure. Guthe (1957) found that at pH levels below 7.3, myosin ATPase activity was depressed by pressures up to 500 atm, but at higher pH, pressure increased the activity. He attributed the effect of pressure at low pH to a direct action on the rate-limiting step and at high pH to an opposing of the reversible denaturation of the enzyme.

Effects of pressure on cell structure and physiology

Pressure has been shown to disrupt cytoplasmic structure, pinocytotic vesicles and channels, and microtubules in various unicellular organisms (Landau, Zimmerman, and Marsland, 1954). At 25°C, pressures of 68-204 atm interfered with pseudopodia formation and locomotion in Amoeba proteus. At 304-408 atm the cells became spherical. At lower temperatures, these effects were observed at lower pressures. Centrifugation experiments showed that the cytoplasm was less "viscous" at high pressure or low temperature, with the effect of a 10°C decrease in temperature about equal to 136 atm pressure. Although the contractile vacuole stopped functioning

at high pressure, the rounding-up of the cell was accompanied by no change or by a slight decrease in cell volume, and therefore could not be attributed to osmotic effects.

Landau and Thibodeau (1962) found pinocytotic channels and Golgi complexes to be absent in specimens of Amoeba proteus fixed for electron microscopy at 544 atm pressure. These structures were easily observable in specimens fixed at 1 atm.

Tilney, Hiramoto, and Marsland (1966) described pressure-induced disorganization of microtubules in Actinosphaerium nucleofilum. Pressures of 272-408 atm caused the shortening of axopodia and the disintegration of microtubules. Some reorganization occurred after 10-20 min at pressure. At 544 atm, axopodia and microtubules completely disappeared, and no adaptational recovery was observed. Similar pressure-induced disorganization of microtubules has been observed in other systems; e.g., in meiotic spindles and in microtubules assembled in vitro from rabbit-brain tubulin (Salmon, 1975 a&b).

Crustacean Neuromuscular Physiology

The motor innervation of crustacean limbs and the nervous control of muscle tension

The muscles of the walking legs of decapod crustaceans were used in the experiments reported in this thesis. They are innervated by a very small number of nerve fibers (see Wiersma and Ripley, 1952), some of which are excitatory (i.e., delivering a transmitter substance that initiates a chain of events in the muscle that results in contraction), and some fibers that are inhibitory (i.e., blocking contraction). I deal only with excitatory neuromuscular transmission. The stretcher muscle of the propodite and the opener muscle of the dactylopodite, are innervated by a single excitatory axon which branches and innervates every fiber in each muscle. I deal exclusively with these two muscles. The excitatory axon can be stimulated separately from the inhibitory axon(s), and the response of the membrane potential to the transmitter released at the neuromuscular junction can be recorded from any of the muscle fibers.

Fig. 1 is an example of the responses recorded with an intracellular electrode from a fiber in the stretcher muscle of Homarus. The depolarization of the membrane in response to a nerve impulse is termed an excitatory junctional potential (e.j.p.). In contrast to an action potential, the e.j.p. is not actively conducted along the fiber, but is localized to the vicinity of the nerve ending. However, because the nerve endings are distributed along the entire length of crustacean muscle fibers (Fatt and Katz, 1953a), e.j.p.s

Fig. 1. e.j.p.s recorded from a fiber in the stretcher muscle of Homarus.

The upper trace shows the form of the e.j.p. The middle and lower traces are trains of e.j.p.s elicited by stimulation at frequencies of 1/sec and 10/sec respectively. Note changes in scale. These records, as well as all of the records presented in this thesis, have been traced from the original Polaroid photographs.

2mv
└
40msec

1mv
└
10sec

4mv
└
1sec

can be recorded at any point. The amplitude and duration of the e.j.p.s varies considerably from one muscle fiber to another, and in many fibers the amplitude is strongly dependent upon the frequency of stimulation. This frequency-dependence is illustrated in Fig. 1. At a stimulation frequency of 10/sec the e.j.p. was initially small, but grew larger with continued stimulation until a plateau was reached. At a stimulation frequency of 1/sec the amplitude remained low (note change of scale). The growth in e.j.p. amplitude at high frequencies of stimulation is termed facilitation. It has been shown, in crustaceans, to result from an increase in the amount of transmitter released from the nerve endings with each successive nerve impulse (Dudel and Kuffler, 1961).

Another important property of e.j.p.s is summation. When the stimulation is frequent enough to elicit e.j.p.s before the previous ones have decayed back to base-line, the e.j.p.s will summate to a level of depolarization greater than the amplitude of a single e.j.p. In Fig. 1, at a stimulation frequency of 10/sec, the rise in base-line indicates the presence of summation.

The magnitude of the depolarization above a certain minimum value controls the tension developed by a muscle fiber (Orkand, 1972); the processes of facilitation and summation integrate the pattern of arrival of nerve impulses into graded depolarizations and contractions of the muscle fiber. The amplitude, duration, and degree of facilitation of the e.j.p. vary widely from fiber to fiber in a given muscle so that some fibers will be undergoing contraction at relatively low frequencies of nerve impulses while others will

be recruited only at higher frequencies.

Physiology of transmitter release

It is generally believed that transmitter substance is released from nerve endings in discrete "packets" of similar size. This feature of transmitter release was first shown at the frog neuromuscular junction (Fatt and Katz, 1952; del Castillo and Katz, 1954), and subsequently at mammalian (Boyd and Martin, 1956; Liley, 1956) and crustacean (Dudel and Kuffler, 1961a) junctions.

In frog muscle, Fatt and Katz observed spontaneous, randomly occurring, low-level depolarizations similar in form to the larger e.j.p.s^{*} evoked by nerve stimulation. I will refer to these as miniature e.j.p.s^{*} (m.e.j.p.s). Fatt and Katz pointed out that variations in the amplitude of e.j.p.s during a train of nerve stimulation seemed to be distributed in discrete unit increments similar in magnitude to the size of the randomly occurring m.e.j.p.s. They hypothesized that the e.j.p. is built up of the summed effect of a number of "packets" (or quanta) of transmitter released simultaneously, and that the m.e.j.p.s result from the random leak of single quanta.

* A different terminology is usually employed when referring to vertebrate neuromuscular junctions; i.e., end-plate potentials (e.p.p.s) and m.e.p.p.s, rather than e.j.p.s and m.e.j.p.s which are traditionally used when referring to crustacean junctions. For simplicity I use the crustacean terminology for both systems.

del Castillo and Katz (1954) developed a statistical theory of transmitter release based on this hypothesis which satisfactorily explained many features of transmission. The elements of the theory are as follows:

- 1) Transmitter is released in discrete quanta containing similar amounts of transmitter substance.
- 2) Each quantum of transmitter released interacts with the muscle membrane causing a similar unit increase in its ionic conductance.
- 3) The release of quanta caused by the arrival of the nerve impulse is probabilistic, and can be described by the equation:

$$m = n\bar{p} \quad (1)$$

where m is the average number of quanta released, n is the number of quanta available for release, and $\bar{p}$ is the average of the probabilities of release of each of the available quanta.

- 4) There is always some small probability of release which accounts for the spontaneous m.e.j.p.s, which result from the release of a single quantum.
- 5) The arrival of a nerve impulse at the nerve ending greatly increases the probability of release for a brief time causing the simultaneous release of a number of quanta.

In order to test their theory, del Castillo and Katz devised several ways to determine m experimentally; i.e., to count the number of quanta released in response to a nerve impulse. It was assumed that p for each of the n quanta was small ($\ll 1$). Then, from equation (1), the number of quanta

released by each nerve impulse during a train of nerve impulses should be distributed in accordance with Poisson's law, and:

$$\frac{N_x}{N} = \frac{m^x}{x!} \exp(-m) \quad (2)$$

Where N is the total number of nerve impulses, and N_x is the number of responses evoked by the release of x number of quanta (0, 1, 2, 3, etc.). In preparations of vertebrate muscle, observations of the e.j.p. must be made with transmitter release partially blocked by high external $[Mg]$, low external $[Ca]$, or curare, so that the e.j.p. amplitude is below the threshold for initiating an action potential. Transmission can be blocked with high $[mg]$ or low $[Ca]$ to the extent that a significant fraction of the nerve impulses do not elicit an e.j.p. Then, equation (2) applied to the special case where $x=0$ reduces to equation (3), and m can be calculated simply by dividing the number of transmission failures by the total number of nerve impulses.

$$\frac{N_0}{N} = \exp(-m) \quad (3)$$

The only assumption involved in calculating m from experimental data with equation (3) that is not integral to the theory is that $p \ll 1$; i.e., that the statistics of release are Poisson. Since transmission was strongly depressed in their experiments by the alterations in $[Ca]$ and $[Mg]$ del Castillo and Katz concluded that this assumption was likely to be valid.

A second way in which del Castillo and Katz determined m rests on the hypothesis that the m.e.j.p.s are due to the release of single quanta of transmitter. Making the additional assumption that the quantum

depolarizations sum linearly in building up the e.j.p. (see p. 34), m can then be calculated from equation (4).

$$m = \frac{\text{mean amplitude of the e.j.p.s}}{\text{mean amplitude of the m.e.j.p.s}} \quad (4)$$

In using this equation, it is not assumed that the release statistics are Poisson. It would also be valid if p was not $\ll 1$ and the statistics of release were consequently binomial.

These two methods of calculating m each stem from different aspects of the theory, are based on the measurement of different quantities, and involve different assumptions. del Castillo and Katz found that the values of m calculated from experimental data with these two methods agreed closely, which strongly supports the validity of the theory.

Using m calculated from equation (4), they calculated the expected frequencies for values of x greater than zero from equation (2). This required correction for the variation in size of the quantum response. For this purpose a Gaussian curve was fitted to the distribution of the m.e.j.p.s, and x times the mean and variance of this curve was used to plot the expected distribution around the Poisson classes. For the classes at low values of m there was good agreement between experimental and theoretical curves.

del Castillo and Katz calculated m in a third way, from the coefficient of variation (CV = standard deviation/mean) of the e.j.p. amplitude. If the statistics of release are assumed to be Poisson, then for a train of e.j.p.s:

$$CV = \sqrt{1/m} \quad (5)$$

In this calculation, linear summation of the quantum depolarizations is also assumed. They found that m calculated from the CV agreed closely with the other two methods when transmission was depressed to a low level.

At higher levels of transmission ($m > 10$) the proportion of failures of transmission was too low to permit calculation of m by equation (3), and there was a discrepancy between m calculated by equations (4) and (5); m calculated by dividing the mean e.j.p.-amplitude by the mean m.e.j.p.-amplitude predicted a CV larger than was actually observed. del Castillo and Katz suggested several explanations for this discrepancy, one of which was non-linear summation of the quantum depolarizations. If the e.j.p. amplitude is a significant fraction of its theoretical maximum amplitude (i.e., the reversal potential minus the resting potential (see Martin, 1955)), then summation will not be linear; each additional quantum will add slightly less depolarization in building up the e.j.p. Then the mean e.j.p.-amplitude divided by the mean m.e.j.p.-amplitude would be an underestimate of the true value of m and would predict a CV (equation (5)) that would be too large. Even the true m would predict a CV that was too large, because non-linear summation would cause a reduction in the amplitude of the fluctuations of the e.j.p. Martin (1955) provided a correction factor for the effects of non-linear summation (see p. 34) and found that if both m and the CV measured at high levels of transmission were corrected for non-linear summation there was no discrepancy between experiment and theory.

Dudel and Kuffler (1961a) performed a similar analysis of e.j.p.s at the crayfish neuromuscular junction with analogous results. Since e.j.p.s do

not usually elicit action potentials in crustacean muscle, and since e.j.p.s of small amplitude can be produced in many fibers by stimulating at a low frequency, it is not necessary to block transmission in crustacean preparations. There is a major difficulty however; nerve endings are distributed widely over the surface of the muscle fibers in crustaceans, and an intracellular electrode records the composite of activity at near and distant endings. The contribution to the recorded e.j.p. of quanta released at distant nerve-endings will be attenuated relative to the contribution of quanta released at nearby endings. This spatial complication in crustacean muscle makes an analysis as described above for the frog impossible with conventional intracellular recording techniques. For their analysis of the e.j.p. in crustacean muscle, Dudel and Kuffler used an extracellular electrode placed near a single nerve-ending. Such an electrode records the voltage-drop due to synaptic current presumably produced solely by the nearby ending. Spontaneous miniature potentials of fairly constant amplitude were observed as well as occasional failures of transmission at low frequencies of nerve stimulation. The results of their analysis were analogous to those of del Castillo and Katz with the frog. Similar results have been obtained in a wide variety of different crustaceans; cf. Frank (1973) in the lobster.

Response of the muscle membrane to transmitter release

This section is concerned with the electrical properties of the muscle fiber that affect the amplitude and form of the e.j.p. The e.j.p. results from a brief increase in the ionic conductance of the muscle membrane at the

sites of transmitter release. Current flows into the fiber, spreads along the interior of the fiber, and leaves by crossing the non-synaptic membrane. The change in membrane potential is slowed by the effect of the membrane capacitance. I will first consider a simplified situation in which the flow of current persists long enough to allow the voltage change to reach the steady state. The effects of the membrane capacity will be considered subsequently.

A circuit analog describing the effect of a steady current, entering the muscle fiber at the site of a nerve ending, on the steady voltage at that site is presented in Fig. 2 (from Martin, 1955).

Fig. 2

The electromotive force (V_o) that drives the synaptic current, in series with the transverse resistance between the inside and the outside of the muscle fiber ($1/G$), is short circuited by a resistance ($1/mg$) that appears as the result of transmitter released by the nerve ending. Current then flows through the circuit, depolarizing the membrane. V_o is the difference between the resting potential and the reversal potential of the e.j.p., or just the resting potential if the reversal potential is assumed to be zero (see Taraskevich, 1971). Each quantum of transmitter is assumed to contribute an equal increment of conductance (g).

The e.j.p. amplitude ($\bar{v}$) is the voltage-drop across $1/G$, and since the current through $1/G$ must equal the current through $1/mg$:

$$\frac{\bar{v}}{V_0 - \bar{v}} = \frac{mg}{G} \quad (6)$$

Equation (6) rewritten for the voltage change resulting from the release of a single quantum of transmitter ($\bar{v}_1$) is:

$$\frac{\bar{v}_1}{V_0 - \bar{v}_1} = \frac{g}{G} \quad (7)$$

Since $\bar{v}_1 \ll V_0$ then:

$$\frac{\bar{v}_1}{V_0} \approx \frac{g}{G} \quad (8)$$

Combining (6) and (8):

$$m = \frac{\bar{v}}{\bar{v}_1} \left(1 - \frac{\bar{v}}{V_0}\right)^{-1} \quad (9)$$

Equation (9) shows that if the e.j.p. amplitude is a small fraction of V_0 , the depolarizing effect of the individual quanta sum approximately linearly, and m can be calculated by dividing the e.j.p. amplitude by the amplitude of the miniature potentials. If the e.j.p. amplitude is a significant fraction of V_0 , then summation is less than linear, and the quantum content for a given e.j.p. amplitude is greater by the factor $(1 - \bar{v}/V_0)^{-1}$. Martin (1955) computed the corrected values of m in this way for the frog neuromuscular junction at high levels of transmission ($m > 10$). He used the same correction factor to compute a corrected CV:

$$CV(\text{corrected}) = CV(\text{measured}) \times (1 - \bar{v}/V_o)^{-1} \quad (10)$$

He found the corrected values of m and CV to be related as predicted from Equation (5).

The passive electrical properties of cable-like cells such as muscle and nerve fibers have been described quantitatively (Hodgkin and Rushton, 1945; Fatt and Katz, 1951 and 1953b). The steady voltage impressed upon the membrane in response to steady current injected into a muscle fiber at a point is given by the following equation (see Fatt and Katz, 1951):

$$V = \frac{I}{2} \sqrt{r_m r_i} \quad (11)$$

where r_m is the transverse resistance of the membrane X unit length (ohm-cm), and r_i is the longitudinal resistance of the fiber/unit length (ohm/cm). The term $\frac{1}{2} \sqrt{r_m r_i}$ is the effective resistance between the inside and the outside of the fiber at a point (termed the input resistance). This treatment assumes a cable of infinite length; i.e., several space constants long in both directions (see Fatt and Katz, 1951) from the point of current applications. In shorter fibers the current and voltage profiles will be distorted.

The time course of the voltage change for a rectangular pulse of current is determined by the capacitance and resistance of the muscle membrane:

$$\tau_m = r_m c_m \quad (12)$$

Where τ_m is the membrane time constant and c_m is the membrane capacitance/unit length of muscle fiber (uF/cm). The membrane time constant can be measured as the time of rise of the voltage to 0.85 of its maximal amplitude

for a rectangular current-pulse delivered at the same point where voltage is measured (see Hodgkin and Rushton, 1946).

If the fiber diameter is known all of the constants given above can be expressed relative to the surface area of the fiber rather than the length and are written with capital letters. Fatt and Katz (1953b) reported the following approximate values for the membrane constants in crab muscle:

$$C_m = 40 \mu\text{F}/\text{cm}^2$$

$$R_m = 100 \Omega \text{cm}^2$$

$$R_i = 75 \Omega \text{cm}$$

$$\tau_m = 5 \text{ msec}$$

Based on these values, the input resistance for a fiber with a diameter of 200μ is roughly 10^4 ohms.

MATERIALS AND METHODS

Experimental animals

The effects of high hydrostatic pressure on neuromuscular transmission were compared in three species of marine decapod crustaceans: the shallow-living spider crab, Libinia emarginata, the relatively deep-living red crab, Geryon quinquedens, and the lobster, Homarus americanus, which ranges to an intermediate depth. Williams (1965), reviewing the marine decapods of the Carolinas, reports the depth range of Libinia as the shore to about 50 m and occasionally as deep as 140 m. Smith (1887) reports catching a specimen off Cape Hatteras at a depth of 160 m. In the part of its range north of Cape Cod, Smith (1879) regarded this species as confined to depths between the shore and about 30 m. Schroeder (1955) reported the maximum depth of occurrence of lobsters off the eastern U.S. coast as about 520 m. They have been reported to occur to a depth of at least 600 m by McRae (1960). Specimens of Geryon have been retrieved off the eastern U.S. coast from depths as great as 2000 m (Tanner, 1886; Richard Haedrich, unpublished data) and as shallow as 300 m (Tanner, 1886; Schroeder, 1955). There is a small, shallow population in the Gulf of Maine that ranges from about 100 to 300 m in depth (Roland Wigley, personal communication). The maximum depth of occurrence of these species may be somewhat underestimated, as populations tend to be sparse near the maximum depth and, therefore, may have eluded discovery. The differences between the three species is so wide that errors

of this kind are not apt to be significant. Pressure increases by about 1 atm for every 10 m of depth; Libinia ranges to a maximum pressure of about 16 atm, Homarus to about 60 atm, and Geryon to about 200 atm.

The lobsters used in this study were obtained from a local fish market, and the spider crabs from the supply department of the Marine Biological Laboratory at Woods Hole. Red crabs were obtained from two sources: those used in experiments during the winter of 1973-74 were collected by trawling from the R/V KNORR (cruise 35) in the Hudson Canyon area in November. The following winter, red crabs were collected with lobster pots in December from a commercial fishing vessel, the MARS (High Seas Corporation, Fall River, Massachusetts), operating off the southern New England coast. In both cases the crabs were taken from a depth of about 500 m.

All the animals were maintained at 1 atm pressure in aquaria supplied with flowing seawater at the ambient seawater temperature. Experiments with Geryon were performed only during the winter because this deep-water species always lives at low temperatures in nature. Libinia and Homarus are both eurythermal species. Experiments with Homarus were performed both in winter and summer, and the experiments with Libinia were performed in the spring.

Physiological solutions

The ionic composition of the blood of Geryon has been determined (William De Paul, unpublished data). There was wide variability in the ionic composition especially in the case of Ca which ranged 13-30 mM.

Geryon saline made up on the basis of De Paul's data had the following composition (mM): 405 Na, 20 K, 20 Ca, 30 Mg, and 525 Cl. Homarus saline was made according to the determinations of Cole (1941) and contained: 460 Na, 15 K, 26 Ca, 8 Mg, 527 Cl, and 8 SO₄. The most marked difference between these two salines is the higher Mg content of the Geryon saline. Most of the experiments with Geryon were performed using Geryon saline, but a few were performed using Homarus saline. About half of the experiments with Libinia were performed using Homarus saline, and half using Geryon saline.

The saline solutions were buffered with 10 mM Tris buffer (Trizma base and HCl, or Tris Maleate and NaOH), which was used because the pH of Tris-buffered solutions is practically unaffected by pressure (Disteche, 1972). The temperature coefficient of Tris is large ($\text{dpH/dT} = -0.028$, Bates, 1961), and the pH was adjusted to 7.4 (7.3-7.5) for the temperature at which the experiments were conducted.

Recording and stimulating apparatus

A conventional microelectrode recording system was used. Glass microelectrodes filled with 3M KCl (resistance, 2-15 megohms) were connected to a WPI model M 701 solid-state electrometer by means of a silver-silver chloride pellet. A silver-silver chloride reference electrode connected the saline bath to ground via a 3M KCl agar bridge. Electrical responses were monitored with a Tektronix 502 oscilloscope and photographed with a Polaroid camera. A suction electrode connected to a Grass S4 stimulator

through an isolation unit was used for nerve stimulation. Pulses had a duration of 0.1 msec and were about 3-10 V in amplitude at the output of the isolation unit. In some experiments current, supplied by a constant-current source (New, 1972) driven by pulses from the stimulus isolation unit, was injected into the muscle fiber through a second microelectrode..

Preparation

The opener muscle of the dactylopodite and the stretcher of the propodite were used in these experiments. The dactylopodite is the most distal segment of the decapod walking leg. The dactylopodite articulates with the next segment, the propodite, which contains the two muscles that move the dactylopodite, the opener and closer muscles. The stretcher and bender muscles, which are responsible for propodite movements, are contained within the next segment proximal to the propodite, the carpopodite. Proximal to the carpopodite is the meropodite. Axons in the meropodite were stimulated in some preparations (see below).

In both lobsters and crabs, a single excitatory axon branches and innervates both the opener and stretcher muscles, providing their only excitatory innervation (Wiersma and Ripley, 1952). In lobsters, the opener is innervated by only one inhibitory axon, and a separate inhibitory axon supplies the only inhibitory innervation to the stretcher. In addition to these two inhibitory axons, crabs have a common inhibitory axon that innervates both muscles.

In the lobster experiments several methods were used to isolate and stimulate the excitatory axon separately from the inhibitory axon. One

method made use of the fact that the nerve fibers running through the meropodite are organized into several distinct bundles, and the inhibitory axon innervating the opener is located in a separate bundle from the excitatory axon (see Kravitz, Kuffler, Potter, and van Gelder, 1963; and Kravitz, Kuffler, and Potter, 1963) so that the excitatory axon could be stimulated alone simply by stimulating the bundle in the meropodite containing it. When this method of axon stimulation was used, muscle fibers of the opener were exposed by removing a small piece of the shell from the dorsal surface of the propodite, adjacent to the joint with the dactylopodite.

An alternative method of preparing the opener muscle and its excitatory axon was sometimes used (see Kravitz, Kuffler, and Potter, 1963). The muscle was exposed by removing the ventral part of the shell of the propodite and the closer muscle. The excitatory and inhibitory axons innervating the opener muscle run together on the exposed surface; they can be followed proximally by dissecting into the carpopodite, where they separate, the inhibitory axon joining a bundle of other nerve fibers, and the excitatory axon innervating the stretcher muscle. The excitatory axon was dissected from the surface of the stretcher muscle and stimulated.

In experiments with the stretcher muscle of the lobster, the opener was exposed in the above manner and the nerve was dissected from its surface and stimulated, i.e., stimulation was antidromic. Fibers of the stretcher were exposed by removing a small piece of the shell of the carpopodite adjacent to the joint with the propodite.

The stretcher muscle was used in all of the crab experiments. In crabs, isolating the excitatory axon from the inhibitory axons is more complicated because all the nerve fibers in the meropodite form a single bundle, and because a common inhibitory axon innervates both the stretcher and opener. In about one-third of the preparations of Geryon the bundle in the meropodite was teased with fine needles into progressively smaller filaments, each of which was electrically stimulated to test for the presence of the excitatory axon innervating the opener and stretcher. This was continued until a filament remained which would produce contraction of both the opener and stretcher when stimulated and appeared under the dissecting microscope to contain only a single axon. Inhibitory junctional potentials (i.j.p.s) were never observed in preparations made in this manner.

In about two thirds of the preparations of the stretcher muscle in Geryon and all of the preparations of Libinia, the nerve was dissected from the surface of the opener muscle and stimulated antidromically. In both Geryon and Libinia, i.j.p.s resulting from stimulation of the common inhibitor were observed in some of the fibers of some of the preparations made in this manner. They consisted of hyperpolarizations that were of smaller amplitude and longer duration than the e.j.p.s and added to the e.j.p.s, shortening the duration of the depolarization and usually producing an after-hyperpolarization. The threshold voltage for eliciting i.j.p.s was usually greater than that for e.j.p.s, so that appropriate adjustment of the stimulus intensity allowed the excitatory axon to be stimulated separately. Another way to separately stimulate the excitatory axon in these preparations was sometimes used. The nerve

was teased into two filaments, each of which was stimulated separately; the filament not causing contraction of the stretcher was cut. In most of the preparations where this was done, no i.j.p.s were observed, but they were observed in a few, indicating that the common inhibitory axon and the excitatory axon remained together. In such cases further dissection was usually not possible (the remaining filament was too fine), and the inhibitor was eliminated by keeping the stimulating voltage below its threshold.

Equipment and procedure for microelectrode recording at high pressure

A pressure vessel rated for a maximum pressure of 500 atm was specially constructed for this work. It was designed by Barry Walden of the Department of Ocean Engineering of the Woods Hole Oceanographic Institution, and was built jointly by the Corey Tool Company of Braintree, Massachusetts and the instrument shop at Woods Hole. It is cylindrical with a 6 in. diameter bore, 18 in. deep. It is closed by a plug sealing on a 45° angled face with an "O" ring. The plug is held in place by a nut threaded into the bore. The vessel and plug are ARMCO 22-13-5 stainless steel, and the nut is AMPCO 18 centrifugally cast aluminum-bronze. The plug is penetrated by ten electrical leads (Mecca 2006 high pressure electrical penetrators). The vessel and hydraulic system were filled with light mineral oil. Pressure was applied with a Black Hawk hand pump which caused an increment of about half an atm/stroke and was measured with a Heise Bourdon tube gauge. Aminco 1/4 in. high-pressure tubing, fittings and valves were used.

The pressure vessel was mounted vertically in a thermally insulated 55-gallon drum that served as a water bath, which was cooled by means of a coil connected to a refrigeration unit.

The preparation was held in a saline-filled experimental chamber that was placed inside the mineral oil-filled pressure vessel. Two chambers of different design were used. The primary function of these chambers was to permit manipulation of the microelectrode, while having sufficient mechanical stability to allow the chamber to be moved into position inside the pressure vessel with the electrode in place inside a muscle fiber. The first chamber designed provided for excellent mechanical stability, but allowed recording with only one microelectrode. This chamber (Fig. 3) is a half-cylinder of Plexiglas, 8 in. long and 4 in. in diameter. The crustacean leg was held by rubber bands and dental periphery wax to a Plexiglas shelf (not shown). The opening of the chamber has a Plexiglas flange with a rubber gasket. A Plexiglas lid is held in place and sealed against the gasket with stainless steel clamps that are fastened to the flange with wing screws. A rubber diaphragm in the bottom wall of the chamber insured that no difference in pressure developed across its walls.

A single micrometer drive from a Narashige UM-6 ultra-micromanipulator mounted on the lid at an angle of 45° was used to advance the microelectrode. A small hole drilled in each end of the micrometer drive allowed the mineral oil to flow into the air spaces in the mechanism during compression. With a hole at only one end, oil passing by the dovetail caused movement. The microelectrode penetrated the chamber lid through a hole lined with a

Fig. 3. The experimental chamber (internal details are not shown). It is made from a half cylinder of Plexiglas 8 in. long and 4 in. in diameter. The microelectrode is aimed at the muscle by adjusting the position of the Plexiglas lid which is then secured by tightening the clamps around its edge. After a muscle fiber has been impaled, the sealed chamber is picked up and placed on end in the pressure vessel.

rubber-tubing seal to minimize loss of saline. A length of Mecca high pressure cable fitted with a shield driven at the same potential as the micro-electrode connected the electrode to one of the penetrators in the pressure-vessel plug.

After many pressure cycles the insulation of the Mecca penetrators would break down slightly. This resistance was checked frequently using the electrode test circuit of the electrometer and if measurable (< 1000 megohms), the penetrator was replaced.

Nerve stimulation was accomplished with a conventional suction electrode into which the cut end of the nerve was drawn. The entire suction electrode arrangement was contained within the experimental chamber. Soldered connections were cast in epoxy. A small hole in the tubing connecting the suction electrode and syringe prevented the nerve from being blown out of the suction electrode by the expansion of small air bubbles upon decompression.

Muscle fibers were impaled in the following manner: The microelectrode was inserted backwards through the hole in the lid of the experimental chamber and clamped to the electrode carrier. The chamber was filled to the top with saline, and the lid placed in position. While viewing with a dissecting microscope, the microelectrode was aimed at the muscle by adjusting the position of the lid and advanced to the surface of the muscle with the micrometer drive. The chamber lid was then clamped in place. No attempt was made to pick out precisely the muscle fiber to be impaled, as the chamber lid would usually move slightly when the clamps were tightened. The

micrometer drive was advanced until a resting potential was recorded; the nerve was then stimulated to elicit e.j.p.s. Most experiments were done with fibers on the surface of the muscle, but deeper fibers were used occasionally.

A crane rigged over the opening of the pressure vessel was used to lift the nut and plug, both of which hung suspended when the pressure vessel was open. Two 1/4-in. diameter stainless steel bars extend from the inside surface of the plug. The experimental chamber was placed on Plexiglas supports attached to these bars.

In its position on the support bars, the experimental chamber stands on end. A wing screw and "O" ring plugging a hole in the top end was removed, and saline was syringed into the hole to remove most of any remaining air. There were always some bubbles adhering to surfaces that could not be removed. If 1 ml of air remained trapped inside the 600-ml experimental chamber (a clear overestimate), then the dissolved oxygen would increase about 5%, nitrogen 12%, and carbon dioxide 0.1% if all of the air went into solution during compression. It is assumed that this was inconsequential. This calculation is based on the amount of dissolved gases in seawater in equilibrium with air at 1 atm and 10°C (Sverdrup, Johnson, and Fleming, 1942).

Finally, a small amount of mineral oil was added to the pressure vessel to insure complete filling after final assembly. The chamber and plug were then lowered into place followed by the nut which was screwed down tight.

This method worked very well. With only reasonable care, the micro-electrode tip remained in the muscle fiber during all the manipulation entailed. Successful recordings were made with this method aboard a diesel ship under full power!

In some experiments two microelectrodes were inserted into the same muscle fiber, one for current injection and one for recording. This required two independently movable electrodes with precise control of position in all three planes of space. A second experimental chamber was designed for these experiments; it consisted of a Plexiglas dish 4 in. in diameter with a tightly fitting cover made of 1/2 in. Plexiglas. Two Narashige UM-6 ultra-micromanipulators were mounted on the cover; both entered the dish through a 1-1/8 in. diameter hole in the cover. When this chamber was placed in the pressure vessel, mineral oil floating on top of the saline filled this hole. The lead to the current electrode was provided with a shield driven from V_{out} of the constant-current source. The insulation of the high pressure penetrators through which current was passed and voltage recorded were checked frequently to insure that they provided immeasurably high resistance.

Because the electrodes were not as rigidly supported in this chamber, it had to be handled delicately, but still worked quite well. Nevertheless the additional problem of keeping two electrodes in the same muscle fiber made these experiments comparatively difficult. Experiments with a single electrode were quite easily performed; the less rigid support of the electrode in this chamber being more than compensated by the ease and precision with

which the electrode could be moved, and this chamber was used for many of the e.j.p. experiments involving a single electrode.

Measuring isometric tension at high pressure

In some experiments, the isometric tension developed by the stretcher muscle in response to nerve stimulation was measured. For this purpose a photoelectric tension transducer was constructed based on a design by Meiss (1974). The transducer consisted of a housing containing a penlight bulb illuminating two photodiodes (Texas Instruments 1N2175) mounted side by side. Light reached the photodiodes by passing through a 1 mm wide slit in a vane attached to a leaf spring. The vane and photodiodes were arranged such that movement of the spring would cause the level of illumination of one of the diodes to increase and the other to decrease. The diodes were connected in series with nine volts across them and the voltage at a point between them was monitored by the oscilloscope. The transducer was mounted on the lid of the double-electrode chamber and a heavy thread used to connect the leaf spring to the propodite of the crustacean leg. The tendon connecting the bender muscle of the propodite was cut in most of these experiments.

Leaf springs of different stiffnesses were used to vary the sensitivity of the transducer. The response of the transducer was calibrated in air with one of the springs used, by hanging weights on the spring. Fig. 4 shows that the response was linear through the entire range. Then the effects of immersion in mineral oil were tested. A weight was first hung on the spring and the transducer was lowered into the pressure vessel so that the weight, but not

Fig. 4. Relation between the output of the tension transducer (volts) and the weight (grams) applied to the leaf spring. Measurements were made in air.

the transducer was immersed in the mineral oil. Tension could be taken off the spring by lifting the weight with a piece of string attached to it, and the change in voltage caused by the weight was recorded. Then the transducer was lowered into the mineral oil, and the voltage change caused by the weight was measured again. Immersion of the transducer in oil caused its sensitivity to small movements of the leaf spring to be reduced by about a factor of 10 (see Fig. 5). The response was linear to about 0.5-0.75 V, and all the experiments (except a few which are noted) were performed with the transducer operating in a range well below this. At higher voltages (greater movements of the leaf spring) the slope of the relation shown in Fig. 5 became much steeper, the sensitivity being reduced much less than by a factor of 10. The loss in sensitivity to small movements was attributed to light scattering by the mineral oil.

Paradigm of pressure application

A standard paradigm for the application of pressure (sometimes with slight modifications) was used. A series of different pressures was tested with control measurements at 1 atm made immediately before and after each measurement at pressure. The usual test-pressures were 50, 100, 150, and 200 atm, but higher pressures were occasionally used. The application of pressure required from about 2-10 min depending on its magnitude, and decompression was accomplished in a minute or two. Measurements were made immediately after the desired pressure had been reached, and the preparation was decompressed immediately after completing the measurements which

Fig. 5. The effect of immersion of the photoelectric transducer in mineral oil. The ordinate is the transducer output (volts) with the weight only immersed in mineral oil, and the abscissa is the output with both weight and transducer immersed.

usually required 5-10 min.

Temperature considerations

Temperature can be a confounding variable in pressure experiments because compression is accompanied by heating and decompression by cooling. In some experiments, the temperature of the saline was monitored by a thermistor mounted in the experimental chamber and recorded with a chart recorder. The adiabatic heating of seawater elevated from 1 atm to 200 atm pressure causes an increase in temperature of 0.1°C (von Arx, 1962). The change in the temperature of the saline in these experiments was greater because compression is not carried out in a thermodynamically reversible manner and because mineral oil heats a great deal more upon compression than saline. After the desired pressure was reached, the saline temperature continued to rise slowly due to heat flow from the mineral oil. After decompression the temperature continued to fall slowly. The maximum rise in temperature incurred during these experiments when 200 atm pressure was applied and held was about 1°C .

Often the experimental chamber was a few degrees warmer than the mineral oil when it was first placed into the pressure vessel. Equilibration required an hour or more during which time the saline temperature slowly fell. Measurements made at 1 atm during this time served as controls for the effects of temperature. Also, part or all of the heat generated by compression goes into reducing the rate of fall of the temperature, so compression can be accomplished with little or no rise in temperature. The results of both these

methods indicate that the pressure effects reported here are uncontaminated by temperature effects (see Results).

RESULTS

General Effects of Pressure on e.j.p. Amplitude and Duration

Observations were made of the effects of high hydrostatic pressure on e.j.p.s recorded from fibers of the stretcher muscle in Libinia, the opener and stretcher in Homarus, and the stretcher in Geryon. For the purpose of analysis it proved useful to divide the fibers into two classes on the basis of the magnitude of facilitation shown by their e.j.p.s. If the amplitude of the e.j.p.s elicited by nerve stimulation at 10/sec was more than twice the amplitude at 1/sec, the fiber was termed 'highly facilitating'; when this ratio was two or less, the fiber was said to be 'poorly facilitating'. This ratio will be referred to as the "facilitation ratio" (Fe). Fibers of both classes were observed in all of the muscles studied except the opener in Homarus where only highly facilitating fibers were found.

In all three species, high pressure caused depression of the amplitude of e.j.p.s recorded from highly facilitating fibers. In many of these fibers, the depression was less at higher frequencies of nerve stimulation. Depression was also observed in the poorly facilitating fibers except for some in Geryon where an increase in e.j.p. amplitude at high pressure was observed. There were differences between species in the magnitude of the pressure-induced depression and in its dependence on the frequency of nerve stimulation. In addition to its effect on amplitude, pressure caused an increase in the duration of the e.j.p.s which also varied in magnitude between species. In this

section I discuss the major effects of pressure in general terms illustrated by data selected from a few experiments. More complete presentation of the data, along with the drawing of comparisons between species, is left for subsequent sections.

Fig. 6 shows e.j.p.s recorded from a highly facilitating fiber in the stretcher muscle of Homarus at stimulation frequencies of 1 and 10/sec, and pressures of 1 and 100 atm. At a frequency of 1/sec, 100 atm pressure resulted in a reversible reduction of e.j.p. amplitude to about 0.60 of the amplitude at 1 atm. At a frequency of 10/sec the effect was more complicated. The e.j.p.s summated and appeared as serrations superimposed on a fairly constant level of depolarization. The amplitude of the e.j.p.s (measured from the trough to the following crest; referred to subsequently as "phasic amplitude") was depressed by pressure to about 0.67 of the amplitude at 1 atm; that is, the magnitude of depression was somewhat less at 10/sec than at 1/sec. In addition to this effect, the background level of depolarization resulting from summation was increased under pressure. This was found to be the result of a prolongation of the duration of the falling phase of the e.j.p. The effect of pressure on the e.j.p. duration is shown for a different fiber in Fig. 7. In terms of their effects on the level of depolarization achieved during a train of stimulation (the average amplitude measured from the resting potential baseline to the crests of the e.j.p.s) the depression of e.j.p. amplitude and the increase in its duration oppose one another. In the fiber in Fig. 6, the resulting effect of pressure on the level of depolarization

Fig. 6. e.j.p.s recorded from a highly facilitating fiber of the stretcher muscle in Homarus at stimulation frequencies of 1 and 10/sec and pressures of 1 and 100 atm. The top and bottom traces are e.j.p.s at 1 atm before compression and after decompression respectively.

1 ATM

1/sec

10/sec

100 ATM

1 ATM

1mv
10sec

4mv
1sec

Fig. 7. Effect of pressure on the duration of the e.j.p. in a fiber in the stretcher muscle in Homarus. The two e.j.p.s of larger amplitude were recorded at 1 atm before and after compression to 100 atm, and the smaller amplitude e.j.p. was recorded at 100 atm pressure. This composite was traced from separate photographs of single e.j.p.s, evoked by stimulation at 1/sec.

reached during the train of stimulation at 10/sec was nearly zero. High pressure had no systematic effect on the resting potential of the muscle fibers in any of the species tested. Compression and decompression were frequently accompanied by small changes in the resting potential (typically ± 5 mV or less) which probably resulted from small movements of the micro-electrode; sometimes the electrode was dislodged from the muscle fiber during changes in pressure.

Controls for the effects of temperature changes

Since compression is accompanied by heating and decompression by cooling, it is possible that temperature changes could influence the observed effects. Control experiments for the influence of changes in temperature were performed by making measurements at 1 atm and under pressure while the temperature of the preparation was falling slowly (see p. 56). Fig. 8 shows the effects of pressure on the phasic e.j.p.-amplitude in such an experiment; e.j.p. amplitude is plotted against time, during which the temperature fell and high pressure tests were made. The fiber was a highly facilitating one from the opener muscle in Homarus. Each circle represents the mean phasic amplitude of 20 or more e.j.p.s. Open circles represent measurements made at 1 atm; filled circles represent measurements made at high pressure. The fiber was tested at 1, 5, and 10/sec initially and after each change in pressure, and the temporal sequence of the high pressure tests was 50, 100, 150, and 200 atm. At the start of the experiment, the saline bathing the

Fig. 8. Effect of pressure on the phasic amplitude of e.j.p.s recorded from a highly facilitating fiber of the opener muscle in Homarus. The fiber was tested at stimulation frequencies of 1, 5, and 10/sec initially and after each change in pressure. The circles represent the mean of about 20 e.j.p.s recorded at 1 atm (open circles) and high pressure (filled circles). The e.j.p. amplitude is plotted against the time, during which high pressures were tested in the following sequence: 50, 100, 150, and 200 atm.

- ejp. Amplitude at 1 ATM
- ejp. Amplitude at High Pressure

preparation was at room temperature, and the pressure vessel and mineral oil were at 18°C. During the experiment the saline temperature fell about 5°C, without any systematic effect upon the phasic e.j.p.-amplitude at 1 atm.

No increase in temperature occurred upon compression to 50 or 100 atm.

Upon compression to 150 and 200 atm, an increase in temperature of about 0.5°C and 0.8°C respectively was observed. It was concluded that the temperature changes encountered in these experiments did not significantly influence the effects of changes in pressure on the amplitude of the e.j.p.

In this experiment the time of decay of the e.j.p. from peak to half-amplitude ranged 26-28 msec at 1 atm and was 28 msec at 50 atm, 30 msec at 100 atm, 32 msec at 150 atm, and 34 msec at 200 atm; high pressure slightly prolonged the duration of the falling phase of the e.j.p., and the temperature changes occurring in this experiment had no significant effect.

Comparative Effects of Pressure on e.j.p. Amplitude
in Libinia, Homarus, and Geryon

Several physiological characteristics of the muscle fibers used in these studies are summarized in Table 1; the temperatures at which the experiments were performed and the temperature at which the animals were acclimated at the time of the experiments are also given. There were no significant differences in the recorded values of resting potential among the three species, or in the different fiber-types, except that poorly facilitating, Geryon fibers appeared to withstand impalement less well than the others. Note that at low frequencies of stimulation the e.j.p.s of highly facilitating fibers were usually smaller than those of the poorly facilitating fibers, and the poorly facilitating fibers tended to have e.j.p.s of longer duration. It should also be noted that the acclimation temperature varied considerably between species. The experiments with Geryon were performed only with cold acclimated animals, and occupied most of the time during the winter of the two years of this study. Experiments with Homarus were done mostly during the warmer months, although some were performed during the winter, concurrent with the Geryon experiments, to control for differences in acclimation temperature. All of the Libinia experiments were done in the spring and early summer. Note also that experiments with the opener in Homarus were performed at warmer temperatures than experiments with all of the other muscles.

Table 1. Some physiological characteristics of the muscle fibers, the temperature (T) at which the experiments were conducted, and the temperature (T_a) at which the animals were acclimated. (Fe is the facilitation ratio (see text), $T_{1/2}$ is the time of decay of the e.j.p. from peak to half-amplitude in msec. The resting potential and e.j.p. amplitude are in mV, and the temperatures are in °C.)

Species	Muscle	Fe	Resting Potential*		e.j.p. Amplitude†			
			Initial	Final	$\leq 2/\text{sec}$	7-10/sec	$T_{1/2}$	T
<u>Libinia</u>	Str	> 2	Mean Range	52.4 40-72	1.3 0.5-2.9	3.3 1.0-8.4	59 28-100	8.8 7.3-11.3
			(N)	(9)	(5)	(13)	(13)	(13)
<u>Libinia</u>	Str	≤ 2	Mean Range	56.4 44-70	5.7 2.3-12.7		104 26-250	8.1 6.8-10.5
			(N)	(3)	(6)		(6)	(6)
<u>Homarus</u>	Op	> 2	Mean Range	60.5 55-71	1.1 0.17-4.1	3.2 0.71-0.60	26 12-42	15.3 11.0-18.0
			(N)	(6)	(8)	(6)	(8)	(8)
<u>Homarus</u>	Str	> 2	Mean Range	51.0 46-54	2.1 0.64-5.0	4.3 1.6-7.4	65 34-100	7.3 4.5-10.0
			(N)	(4)	(5)	(4)	(5)	(6)
<u>Homarus</u>	Str	≤ 2	Mean Range	54.3 50-64	7.7 2.6-16.2		126 80-190	7.2 5.0-11.0
			(N)	(6)	(7)		(7)	(7)

Table 1 (cont'd)

Species	Muscle	Fe	Resting Potential*		e.j.p. Amplitude [†]			
			Mean Range (N)	Initial Final	≤2/sec	7-10/sec	T _{1/2}	T _a
<u>Geryon</u>	Str	> 2	62.2 45- 80 (18)	52.2 40- 60 (14)	1.33 0.29- 5.5 (18)	6.2 2.0- 19.5 (11)	58 15- 150 (19)	7.5 3.5- 11.0 (19)
<u>Geryon</u>	Str	≤ 2	59.7 50- 75 (11)	45.4 33- 60 (8)	3.75 1.3- 6.5 (11)	87 10- 250 (10)	7.2 4.8- 10.3 (10)	1.8 0.2- 4.2 (11)

* Resting potentials were measured upon insertion of the electrode at the start of the experiment and at the end of the experiment upon withdrawal.

† The e.j.p. amplitude was measured at a frequency of nerve stimulation of ≤ 2/sec for all fiber types and also at 7-10/sec in the fibers where Fe > 2.

The effect of pressure on the phasic amplitude of the e.j.p. in each species is shown in Figs. 9-12. For each fiber tested, an index of the effect of pressure on the e.j.p. amplitude (v_p^*) was calculated in the following manner:

$$v_p^* = \frac{\bar{v}_p}{(\bar{v}_a + \bar{v}_b)/2}$$

where $\bar{v}_p$ is the mean e.j.p.-amplitude at a given high pressure.

$\bar{v}_a$ is the mean e.j.p.-amplitude at 1 atm after decompression.

$\bar{v}_b$ is the mean e.j.p.-amplitude at 1 atm before compression.

The mean amplitudes were usually calculated from a sample of 20 e.j.p.s., but in some cases where the amplitude fluctuations were very small, the mean was calculated from a sample of 10. A train of about 40 stimuli was given, and the amplitudes of the last 20 e.j.p.s were measured; facilitation was usually maximal by the twentieth stimulus (see Fig. 6).

In Figs. 9-12, the mean of the values of v_p^* of all the fibers tested in a particular category

$$\bar{v}_p^* = \frac{\sum_{i=1}^N v_{Pi}^*}{N}$$

is plotted as a function of pressure. The error bars mark off $\pm$ one standard error, and the straight lines were calculated by least squares linear regression fitted to the individual values of v_p^* . Poorly facilitating fibers were tested at a stimulation frequency of 1/sec or less; some highly facilitating fibers were tested at low frequency (1-2/sec), some at high frequency (7-10/sec) and some at both. The frequency range over which the highly

Fig. 9. Effect of pressure on the phasic amplitude of the e.j.p. in fibers of the stretcher muscle in Libinia. In trains of summing e.j.p.s at 7-10/sec, the phasic amplitude was measured from trough to peak. $\bar{v}_p^*$ is an index of the amplitude at pressure relative to the amplitude at 1 atm (see text for details of its calculations). Fibers where F_e (the e.j.p. amplitude at 10/sec divided by the e.j.p. amplitude at 1/sec) was two or less were tested at a stimulation frequency of 1/sec or less and are plotted separately from fibers with a larger F_e stimulated at low frequency (1-2/sec) and high frequency (7-10/sec). The sample sizes for each fiber type and frequency, at each pressure, are given in parentheses below the abscissa, as well as the number of preparations used. The error bars represent ± 1 standard error and are absent for sample sizes of 1 and when included within the points. Straight lines were calculated by least squares, linear regression of the individual values of v_p^* .

	PREPS					
□ Low Fe	(6)	(3)	(6)	(5)	(4)	(5)
○ High Fe 1-2/sec	(5)	(4)	(4)	(4)	(2)	(7)
■ High Fe 7-10/sec	(13)	(8)	(13)	(7)	(5)	(5)

Fig. 10. Effect of pressure on the phasic amplitude of the e.j.p. in fibers of the opener muscle in Homarus. See legend of Fig. 9 for details.

○ High Fe 1-2/sec (8)

■ High Fe 10/sec (6)

(6)

(8)

(3)

(1)

PREPS

(7)

(4)

(6)

(4)

(2)

(5)

Fig. 11. Effect of pressure on the phasic amplitude of the e.j.p. in fibers of the stretcher muscle in Homarus. See legend of Fig. 9 for details.

	PREPS				
□ Low Fe	(7)	(4)	(6)	(4)	(7)
○ High Fe 1/sec	(5)	(3)	(5)	(1)	(5)
■ High Fe 10/sec	(4)	(2)	(4)	(1)	(4)

Fig. 12. Effect of pressure on the phasic amplitude of the e.j.p. in fibers of the stretcher muscle in Geryon. See legend of Fig. 9 for details.

□ Low Fe	(10)	(7)	(10)	(8)	(8)	PREPS (9)
○ High Fe $\leq 2/\text{sec}$	(17)	(6)	(12)	(16)	(8)	(15)
■ High Fe 7-10/sec	(10)	(4)	(9)	(10)	(6)	(10)

facilitating fibers were tested was chosen because at frequencies greater than 7-10/sec contraction of the fibers tended to disturb the electrode placement, and frequencies below 1-2/sec often produced responses too small to be easily measurable. The data for the various conditions are plotted separately. The sample sizes (N) for each test-pressure are given in parentheses below the abscissa as well as the number of preparations used. The recovery of the original e.j.p.-amplitude after decompression was good (see Table 2), with a few exceptions which may have resulted from damage of the muscle fiber membrane caused by movement of the microelectrode.

The influence of facilitation ratio on the pressure effect

On the average there was little effect of pressure on the e.j.p. amplitude in the poorly facilitating fibers in Geryon (Fig. 12). However, the e.j.p.s of some of the poorly facilitating fibers in Geryon were strongly depressed by pressure, and others showed an increase in amplitude. This is shown in Fig. 13 where v_p^* for each of the poorly facilitating fibers is plotted against pressure. In three of the fibers, there was a large pressure-induced increase in e.j.p. amplitude at lower pressures, but a smaller or no increase at higher pressures. All of the other fibers tested in this study showed a pressure-induced depression of e.j.p. amplitude.

In Libinia, the poorly facilitating fibers were slightly less depressed than the highly facilitating fibers. Highly facilitating fibers in all three species were examined to determine if v_p^* depended upon the facilitation ratio. No correlation was found.

Table 2. Reversibility upon decompression of the effect of pressure on the phasic e.j.p.-amplitude. $\bar{v}_a/\bar{v}_b$ is the average amplitude after decompression divided by the average amplitude before compression. Fe is the Facilitation ratio (see text). S.D. is the standard deviation, and N is the sample size.

Species	Muscle	Fe	Freq. (/sec)		Pressure (atm)			
					50	100	150	200
<u>Libinia</u>	Str	≤2		$\bar{v}_a/\bar{v}_b$	<u>0.99</u>	<u>1.00</u>	<u>1.05</u>	<u>1.08</u>
				S.D.	0.06	0.08	0.04	0.13
				(N)	(3)	(6)	(5)	(4)
<u>Libinia</u>	Str	> 2	1-2	$\bar{v}_a/\bar{v}_b$	<u>0.95</u>	<u>1.12</u>	<u>1.02</u>	<u>1.07</u>
				S.D.	0.12	0.17	0.02	0.06
				(N)	(4)	(5)	(3)	(2)
<u>Libinia</u>	Str	> 2	7-10	$\bar{v}_a/\bar{v}_b$	<u>0.97</u>	<u>1.00</u>	<u>1.06</u>	<u>1.01</u>
				S.D.	0.11	0.09	0.09	0.09
				(N)	(8)	(13)	(7)	(6)
<u>Homarus</u>	Op	> 2	1-2	$\bar{v}_a/\bar{v}_b$	<u>1.01</u>	<u>0.94</u>	<u>0.97</u>	<u>0.91</u>
				S.D.	0.13	0.07	0.08	
				(N)	(6)	(8)	(3)	(1)
<u>Homarus</u>	Op	> 2	7-10	$\bar{v}_a/\bar{v}_b$	<u>1.05</u>	<u>0.98</u>	<u>0.94</u>	<u>1.00</u>
				S.D.	0.09	0.11	0.03	0.01
				(N)	(4)	(6)	(4)	(2)
<u>Homarus</u>	Op	≤2		$\bar{v}_a/\bar{v}_b$	<u>1.01</u>	<u>0.95</u>	<u>0.94</u>	
				S.D.	0.04	0.06	0.03	
				(N)	(4)	(6)	(4)	
<u>Homarus</u>	Str	> 2	1-2	$\bar{v}_a/\bar{v}_b$	<u>1.04</u>	<u>0.91</u>	<u>0.97</u>	
				S.D.	0.05	0.11		
				(N)	(3)	(5)	(1)	

Table 2. (cont'd)

Species	Muscle	Fe	Freq. (/sec)		Pressure (atm)			
					50	100	150	200
<u>Homarus</u>	Str	> 2	7-10	$\bar{v}_a/\bar{v}_b$	<u>0.99</u>	<u>0.96</u>	<u>1.04</u>	
				S.D.	0.03	0.10		
				(N)	(2)	(4)	(1)	
<u>Geryon</u>	Str	≤ 2		$\bar{v}_a/\bar{v}_b$	<u>1.00</u>	<u>1.03</u>	<u>0.96</u>	<u>0.98</u>
				S.D.	0.09	0.08	0.03	0.05
				(N)	(7)	(11)	(9)	(8)
<u>Geryon</u>	Str	> 2	1-2	$\bar{v}_a/\bar{v}_b$	<u>0.96</u>	<u>0.94</u>	<u>1.00</u>	<u>0.98</u>
				S.D.	0.07	0.14	0.11	0.08
				(N)	(6)	(13)	(17)	(9)
<u>Geryon</u>	Str	> 2	7-10	$\bar{v}_a/\bar{v}_b$	<u>1.07</u>	<u>1.15</u>	<u>1.01</u>	<u>1.09</u>
				S.D.	0.09	0.27	0.04	0.13
				(N)	(4)	(9)	(10)	(6)

Fig. 13. Effect of pressure on e.j.p. amplitude in each of the poorly facilitating fibers in Geryon. v_p^* is an index of the e.j.p. amplitude at pressure for each fiber relative to its amplitude at 1 atm (see text for the details of its calculation).

Frequency-dependence of the effect of pressure on the phasic amplitude of the e.j.p. in highly facilitating fibers

In some highly facilitating muscle fibers the magnitude of the pressure-induced depression was less at higher frequencies of nerve stimulation than at lower frequencies. This frequency-dependence is shown clearly for Geryon in Fig. 12, and there is the suggestion of such an effect in Fig. 10 for the opener muscle in Homarus. Of the eight fibers in Geryon tested at 100 atm pressure at both high and low frequency, v_p^* was greater at high frequency for each fiber. The average difference was substantial, $\bar{v}_{100}^*$ at low frequency equal to 0.70 and at high frequency, 0.89; this can be looked at as a pressure-induced increase in facilitation by a factor of 1.27. At 200 atm facilitation was increased by 1.71. In five of six fibers in the opener in Homarus tested at both frequencies, v_{100}^* was greater at the higher frequency (in one fiber it remained about the same). Averaging all six fibers, $\bar{v}_{100}^*$ was 0.46 at low frequency and 0.64 at high frequency. This amounted to a pressure-induced increase in facilitation by a factor of 1.39. In Homarus stretcher muscle, all of the three fibers tested at both frequencies showed less depression at high frequency, but the difference was smaller; $\bar{v}_{100}^*$ was 0.71 at low frequency and 0.79 at high frequency, or an increase in facilitation by a factor of 1.11. In the four fibers tested in Libinia v_p^* did not depend on the frequency, $\bar{v}_{100}^*$ at low frequency was 0.48 and at high frequency was 0.47.

Relation between v_p^* and e.j.p. phasic amplitude

In each of the species tested, the data were examined for any correlation between the e.j.p. amplitude and v_p^* . The only clear relationship found was in the poorly facilitating fibers in Geryon where v_p^* decreased as the amplitude increased. This relationship is presented in Fig. 14.

Summary of the effects of pressure on the phasic amplitude of the e.j.p. in poorly and highly facilitating fibers

The following summarizes the facilitation- and frequency-dependence of the effects of pressure on e.j.p. amplitude: 1) Pressure caused a reduction in e.j.p. amplitude except in some of the poorly facilitating fibers in Geryon where it caused an increase. 2) The poorly facilitating fibers in Geryon were also exceptional in that v_p^* was correlated (negatively) with e.j.p. amplitude. 3) e.j.p.s of the poorly facilitating fibers in Libinia showed somewhat less depression than e.j.p.s of the highly facilitating fibers in this animal. 4) In the highly facilitating fibers in Geryon and in the opener in Homarus, the magnitude of pressure-induced depression was less at high frequencies of stimulation than at low frequencies. This effect appeared to be much smaller in the stretcher in Homarus and was not observed at all in Libinia.

Species differences in the effect of pressure on the phasic amplitude of the e.j.p.

Figs. 15-17 are plots of the same data given in Figs. 9-12, reorganized to show species differences more clearly. The error bars have been omitted.

Fig. 14. The relation of the effect of pressure and the e.j.p. amplitude in poorly facilitating fibers in Geryon. $\bar{v}_{100}^*$ is $\bar{v}_p^*$ at 100 atm pressure. Stimulation frequency at 1/sec.

Fig. 15. Species differences in the effect of pressure on the e.j.p. amplitude in highly facilitating fibers at low stimulation-frequency. Plots are of the same data in Figs. 9-12 (see legend of Fig. 9 for details). Error bars have been omitted.

						PREPS
■ <i>Geryon</i> Str	(17)	(6)	(12)	(16)	(8)	(15)
□ <i>Homarus</i> Str	(5)	(3)	(5)	(1)	(0)	(5)
○ <i>Homarus</i> Op	(8)	(6)	(8)	(3)	(1)	(7)
● <i>Libinia</i> Str	(5)	(4)	(4)	(4)	(2)	(7)

Fig. 16. Species differences in the effect of pressure on the phasic amplitude of the e.j.p. in highly facilitating fibers at high stimulation-frequency. Plots are of the same data in Figs. 9-12 (see legend of Fig. 9 for details). Error bars have been omitted.

						PREPS
■ <i>Geryon</i> Str	(10)	(4)	(9)	(10)	(6)	(10)
□ <i>Homarus</i> Str	(4)	(2)	(4)	(1)	(0)	(4)
○ <i>Homarus</i> Op	(6)	(4)	(6)	(4)	(2)	(5)
● <i>Libinia</i> Str	(13)	(8)	(13)	(7)	(5)	(5)

Fig. 17. Species differences in the effect of pressure on e.j.p. amplitude in poorly facilitating fibers. Plots are of the same data in Figs. 9, 11, and 12 (see legend of Fig. 9 for details). Error bars have been omitted.

■ <i>Geryon</i> Str	(10)	(7)	(10)	(8)	(8)	PREPS
□ <i>Homarus</i> Str	(7)	(4)	(6)	(4)	(0)	(7)
● <i>Libinia</i> Str	(6)	(3)	(6)	(5)	(4)	(5)

The effect of pressure in highly facilitating fibers at low stimulation-frequency for the three species is shown in Fig. 15. The stretcher in Libinia and the opener in Homarus both show a pressure-induced depression of e.j.p. amplitude, with $\bar{v}_{100}^*$ about 0.5 for both. The stretcher in Homarus showed slightly less depression ($\bar{v}_{100}^*$ about 0.6). Geryon stretcher showed the least depression, with $\bar{v}_{100}^*$ about 0.7. The experiments with Geryon and the stretcher in Homarus were performed using cold acclimated animals (see Table 1), and those with Libinia and the opener in Homarus were performed with animals acclimated to warmer temperatures, and it may be that cold acclimation confers some resistance to pressure-induced depression of e.j.p. amplitude.

At high frequencies of stimulation a difference in the magnitude of pressure-induced depression shown by the highly facilitating fibers became apparent between Geryon and the stretcher in Homarus. This is shown in Fig. 16. The e.j.p.s in Geryon showed considerably less depression ($\bar{v}_{100}^* = 0.91$) than those of the stretcher in Homarus ($\bar{v}_{100}^* = 0.72$). Although the number of experiments on the stretcher in Homarus was small, this difference in $\bar{v}_{100}^*$ was significant at the 0.05 level ($t = 2.54$, $df = 11$). Likewise, a difference appeared with high frequency of stimulation between the opener in Homarus and the stretcher in Libinia, with the opener in Homarus showing less pressure-induced depression ($\bar{v}_{100}^* = 0.64$) than the stretcher in Libinia ($\bar{v}_{100}^* = 0.46$). The difference in $\bar{v}_{100}^*$ was significant at the 0.2 level ($t = 2.66$, $df = 17$). These differences arise from the frequency-dependence of the pressure effect in Geryon and in the opener in Homarus. They

probably cannot be ascribed to differences in acclimation temperature, as the experiments with Geryon and the stretcher in Homarus were both performed with cold acclimated animals, and the experiments with both Libinia and the opener in Homarus were performed with warm acclimated animals (see Table 1).

The species differences between the effect of pressure on e.j.p. amplitude in the poorly facilitating fibers is shown in Fig. 17. In Geryon there was no depression on the average ($\bar{v}_{100}^* = 1.06$), and the magnitude of depression in Libinia ($\bar{v}_{100}^* = 0.63$) was somewhat greater than in the stretcher in Homarus ($\bar{v}_{100}^* = 0.76$). A possible effect of cold-acclimation cannot be ruled out for this difference in depression between Homarus and Libinia. However, in an effort to get further information as to whether such a mechanism might be important, the data from each of the species was examined to see if v_{100}^* correlated with acclimation-temperature. No correlation was found. The range of acclimation-temperatures within the species was smaller than that between the species (see Table 1), and this can only be taken as suggestive that such a mechanism is not operating.

Effects of Pressure on the Duration of the e.j.p. and on the Depolarization
Achieved During a Train of Stimulation at High Frequency

High pressure produced a large reversible increase in the duration of the falling phase of the e.j.p. in muscle fibers from Homarus and Geryon (see Fig. 7). In Libinia there was a much smaller increase which peaked at pressures up to 100 atm and turned into a slight average decrease at 200 atm. These effects are shown in Fig. 18. $T_{1/2p}$ is an index of the effect of pressure on the time of decay of the e.j.p. from peak to half-amplitude and was calculated in a manner analogous to the amplitude indices in the previous figures. In Libinia, $T_{1/2}$ at 1 atm for the fibers plotted averaged 86 msec ranging 30-250. For Homarus $T_{1/2}$ averaged 100 (34-190), and for Geryon it was 87 (10-185) at 1 atm.

At high frequencies of stimulation where successive e.j.p.s were summing, this pressure-induced increase in e.j.p. duration resulted in an increase in the depolarization from base-line. In Fig. 19 the effect of pressure on the net level of depolarization from the resting potential achieved during a train of high frequency stimulation is plotted for highly facilitating fibers of Libinia, the stretchers in Homarus, and Geryon. In all of the fibers plotted here the e.j.p.s were summing at the frequency tested at 1 atm. The net level of depolarization from base-line was estimated by adding the average phasic amplitude of the last 10-20 e.j.p.s to the amplitude of the depolarization resulting from summation, measured from the trough before the last e.j.p. to the potential level to which the membrane returned within 0.5 sec after

Fig. 18. The effect of pressure on the decay time of the e.j.p. from peak to half-amplitude. $\bar{T}_{1/2p}^*$ is an index of the effect of pressure calculated analogously to $\bar{v}_p^*$.

	1	50	100	150	200	PREPS
■ <i>Geryon</i> Str	(16)	(7)	(10)	(12)	(7)	(11)
□ <i>Homarus</i> Str	(7)	(4)	(6)	(4)	(0)	(6)
○ <i>Libinia</i> Str	(13)	(9)	(13)	(7)	(4)	(7)

Fig. 19. Effect of pressure on the net depolarization (resting potential to e.j.p. peak) achieved during a train of stimulation at 7-10/sec. Only fibers whose e.j.p.s were summing at 1 atm were included in this figure. $\bar{D}_p^*$ is an index of the effect of pressure on the depolarization calculated analogously to $\bar{v}_p^*$.

						PREPS	
■	<u>Geryon</u> Str	(6)	(4)	(5)	(6)	(4)	(6)
□	<u>Homarus</u> Str	(2)	(2)	(2)	(1)	(0)	(2)
○	<u>Libinia</u> Str	(10)	(8)	(9)	(5)	(3)	(6)

stimulation was stopped (see Fig. 6). Sometimes the resting potential did not return fully to its original level in this time, but remained slightly depolarized. In these cases, recovery was often completed after several seconds. This delayed recovery probably represents a contraction artifact, which contributes some small uncertainty to the measurements of the level of depolarization. However, the species differences shown in Fig. 19 are much too large to be significantly affected by this measurement problem. It is clear in Fig. 19 that in Geryon and Homarus where the e.j.p. duration was greatly prolonged by pressure, there was very little depression of the depolarization achieved during a train of summing e.j.p.s. In Libinia where pressure had little effect upon e.j.p. duration, however, the depression was substantial. Comparison of Fig. 19 with Fig. 16, where the pressure-induced depression of the phasic amplitude of the e.j.p. is plotted, shows that for Geryon and Homarus the pressure-induced increase in duration just about compensates for the pressure-induced depression of e.j.p. amplitude, with the result that the net level of depolarization achieved is practically unaffected by pressure. In Libinia the effect of pressure on e.j.p. duration is small and does not make up for the depression of phasic amplitude, so the level of net depolarization achieved is strongly depressed.

The Effect of Pressure on the Input Resistance and Time Constant
of the Muscle Fibers

The input resistance and time constant were measured in four muscle fibers in the opener in Homarus. Pulses of inward current were passed through one microelectrode while measuring the magnitude of the steady hyperpolarization and the time of rise to 0.85 (see p. 35) of this magnitude with a second microelectrode. The applied current passed $5 \times 10^{-8} - 2 \times 10^{-7}$ amps and caused hyperpolarizations ranging 2.8-22 mV at 1 atm. Records from one experiment in which a fiber was subjected to 200 atm pressure is shown in Fig. 20. Pressure caused a reversible increase in both the steady potential and the time of rise. In Fig. 21 an index (calculated in the usual manner) of the effect of pressure on the input resistance is plotted for each of the muscle fibers tested. The input resistance at 1 atm averaged 1.5×10^5 ohms, ranging $4 \times 10^4 - 4.4 \times 10^5$ ohms. These values of input resistance are in accord with those reported for crustacean muscle fibers (see p. 36 and Fatt and Katz, 1953b). The resting potentials at the start of these experiments averaged 64 mV (53-70 mV). Generally the reversibility upon decompression was good. Pressure caused an increase in input resistance in all of the muscle fibers. The magnitude of the increase was about 1.2-fold at 100 atm and 1.5-fold at 200 atm. One fiber was tested at pressures up to 500 atm where the input resistance increased by more than 3-fold.

Pressure also caused an increase in the time constant of the membrane response to inward pulsed current in these Homarus fibers. Time constants

Fig. 20. Effect of pressure on the response of the membrane potential of a fiber of the opener muscle in Homarus to hyperpolarizing current pulses. The upper trace in each pair represents the current pulse which was 5×10^{-8} amps, and the lower trace is the response of the membrane potential.

10mv |
100msec

Fig. 21. Effect of pressure on the input resistance of fibers in the opener muscle in Homarus. R_p^* is an index of the effect of pressure calculated in an analogous manner to v_p^* .

averaged 40 msec (23-80 msec) at 1 atm. The increase in time constant with pressure is evident in the record shown in Fig. 20 and is plotted for all of the fibers tested in Fig. 22. The time constant increases with pressure by a somewhat larger factor than the input resistance. A pressure of 100 atm increased the input resistance by a factor of 1.24 (± 0.05 , standard deviation) and the time constant by a factor of 1.64 (± 0.42). If the increase in input resistance and time constant resulted solely from a pressure-induced increase in the membrane resistance the time constant should be proportional to the square of the input resistance (i.e., $\bar{\tau}_p = \bar{R}_p^2$). Since $R = \frac{1}{2} \sqrt{r_m r_i}$ and the time constant $\tau_m = r_m C_m$ (see Introduction pp. 35-36 for theoretical treatment and definitions). Calculating the expected change in time constant from the observed change in input resistance in this manner, $\bar{\tau}_{100} = 1.54$ would be expected. The observed value agrees fairly well with this prediction.

Also, if one compares the effect of pressure on the time constant for Homarus with the duration of the falling phase of the e.j.p. given in Fig. 18, it is seen that the effect of pressure is roughly of the same magnitude for both.

The effect of pressure on input resistance was tested in three muscle fibers in the stretcher in Geryon. However, technical difficulties precluded getting good measurements of the time constant in these fibers. The current passed ranged 1×10^{-8} - 5×10^{-8} amps causing hyperpolarizations ranging 5.0 - 6.6 mV at 1 atm. The input resistance at 1 atm averaged 3.4×10^5 (1.3×10^5 - 6×10^5) ohms. Fig. 23 shows the effect of pressure on input resistance. There was a pressure-induced increase in input resistance in two of the fibers of Geryon (although in one of these the effect decreases

Fig. 22. Effect of pressure on the time constant of muscle fibers in the opener in Homarus. γ_p^* is an index of the effect of pressure calculated in an analogous manner to v_p^* .

Fig. 23. Effect of pressure on the input resistance of muscle fibers in the stretcher muscle in Geryon. R_p^* is an index of the effect of pressure calculated in an analogous manner to v_p^* .

substantially between 100 and 150 atm). In the third fiber pressure had little effect upon the input resistance.

Effect of High Pressure on Isometric Tension
in the Stretcher in Homarus and Geryon

The species differences in the effect of pressure on e.j.p. amplitude reported above suggest that pressure may cause a reduction in the tension developed in response to nerve stimulation by the muscles of shallow-living crustaceans while having little effect upon tension development in the deep-living crab, Geryon. Such a finding would help tie the results reported above, together with the behavioral observations of pressure-induced paralysis in shallow-living crustaceans reported by others (see p.16). Because only a small sample of fibers, mostly from the surface of the muscles, were used in the e.j.p. studies, and because the composition of the muscles (in terms of fibers differing in the extent to which they show facilitation and summation) is unknown, it was not possible to predict precisely what effect pressure would have on contraction. Such a prediction is also made difficult because of the possibility that pressure may affect excitation-contraction coupling and/or the contraction mechanism (see pp. 20-21) and that these effects might vary with the depth-capability of the species.

Even though effects of pressure on contraction could not be uniquely interpreted as resulting from pressure effects on the e.j.p.s, it was felt that the results of tension measurements under pressure might be highly suggestive of such an interpretation, thereby helping to establish the relevance of the observed effects of pressure on the e.j.p. to the behavior of the animals. For this reason, the isometric tension developed by the whole muscle

was examined at various pressures and frequencies of nerve stimulation in the stretcher in Homarus and Geryon.

Figs. 24 and 25 are records of the effect of pressure on the tension developed at a stimulation frequency of 20/sec in a preparation of Homarus and Geryon, respectively. The rise of tension at 1 atm of the Homarus muscle illustrated in Fig. 24 was quite complicated; initially tension developed rapidly, after about 1 sec its rate of rise declined, but accelerated again after about three seconds and continued to rise rapidly until nerve stimulation was terminated. A second phase of rising tension was observed in some of the muscles of both Homarus and Geryon, and was blocked by high pressure in both species (e.g., Fig. 24). Some muscles in both species also behaved in the fashion shown in Fig. 25, where tension rose to a plateau which was maintained until stimulation terminated.

There is evidence that short (<1 sec) bursts of motor nerve impulses at frequencies of 15-20/sec are involved in walking movements in crabs (Atwood and Walcott, 1965). Because short bursts are involved it is probably the initial rate of rise of tension at a given frequency of stimulation that is relevant to the behavior of the animal and of interest here. Also, it is the initial rate of rise of tension that should reflect, at least in part, the rise of depolarization of the muscle fibers. After several seconds of stimulation, depolarization should reach a steady level (provided transmitter is not depleted) and subsequent changes in tension would be expected to reflect changes in the excitation-contraction coupling mechanism or the contraction mechanism. For these reasons, I deal only with the effects of pressure on

Fig. 24. The effect of pressure on the isometric tension developed by a stretcher muscle in Homarus in response to nerve stimulation at 20/sec. The upper trace of each pair is the voltage output of the tension transducer, and the lower trace marks the onset and termination of stimulation.

1 ATM

150 ATM

1 ATM

0.4 mv |
4 sec

Fig. 25. The effect of pressure on the isometric tension developed by a stretcher muscle in Geryon in response to nerve stimulation at 20/sec. The upper trace of each pair is the voltage output of the tension transducer, and the lower trace marks the onset and termination of stimulation.

0.2 mv |
2 sec

the initial rate of rise of tension.

In these experiments the initial rate of rise of tension was maintained for at least 1 - 2.5 sec after the onset of stimulation at 10-50/sec and for at least 5 sec at a stimulation frequency of 5/sec; the effect of pressure on the isometric tension developed within these time periods (i.e., on the initial rate of rise of tension) in stretcher muscles of Homarus and Geryon are shown in Figs. 26 and 27. The index of tension, $\bar{T}_p^*$, was calculated in the usual manner.

Figs. 26 and 27 show that pressure caused a strong depression of the initial rate of rise of tension in Homarus but only slight depression in Geryon. The magnitude of depression did not depend on the frequency of nerve stimulation in the range of 5 - 50/sec. However, in three preparations of Geryon the effect of pressure on isometric tension developed at frequencies of stimulation of 1 - 2/sec was tested, and the results were very different. Pressure resulted in a large increase in tension, with $\bar{T}_{150} = 6.9$ (range 1.76 - 14.7). The actual value is probably exaggerated; a very weak spring was used to measure these small tensions, and in two of the three preparations the increase in tension under pressure put the response of the tension transducer outside of its linear range (see p. 50).

Fig. 26. Effect of pressure on isometric tension of the stretcher muscle in Homarus. T_p^* is an index of the effect of pressure on the initial rate of rise of tension in each preparation, analogous to v_p^* .

Fig. 27. Effect of pressure on isometric tension of the stretcher muscle in Geryon. $\bar{T}_p^*$ is an index of the effect of pressure on the initial rate of rise of tension, analogous to $\bar{v}_p^*$.

Other Effects of Pressure

Spontaneous activity in the motor nerve

In several experiments with each of the species tested, pressure caused spontaneous firing of the nerve. Pressures greater than 150 atm were usually required to elicit this spontaneous activity. Fig. 28 shows a record from a fiber of the stretcher in Homarus in which spontaneous e.j.p.s were observed at 175 atm pressure. The activity usually ceased after a short time (less than a minute); additional small increments of pressure (several atmospheres) would sometimes produce another period of spontaneous activity, but usually of shorter duration. When present, this behavior occurred early in the life of the preparation and usually disappeared during the course of the experiment. There was some variation among species, the spontaneous activity occurring frequently in the stretcher in Homarus, but only rarely in Geryon stretcher, Homarus opener, and Libinia stretcher.

Action potentials in the muscle fibers

Muscle action potentials in response to nerve stimulation were never observed in the muscle fibers at 1 atm pressure, but in one muscle fiber in Geryon and one in Libinia, action potentials were observed at pressure. Records from the Geryon fiber are shown in Fig. 29. When the nerve was initially stimulated at 7/sec and 200 atm pressure, the muscle fiber responded with an action potential after a few stimuli and then with progressively smaller local responses at regular intervals. A second train of stimuli

Fig. 28. Spontaneous e.j.p.s recorded from a fiber of the stretcher in
Homarus at 175 atm pressure.

2mv |
└── 100msec

Fig. 29. e.j.p.s and action potentials recorded from a fiber of the
stretcher in Geryon. The stimulation frequency was 7/sec.
The temporal sequence in which these records were taken was
from top to bottom.

200 ATM

200 ATM

1 ATM

100 ATM

50 ATM

1 ATM

20mV
2 sec

resulted in an action potential but no local responses. At 1 atm, after decompression, only e.j.p.s were present, and upon subsequent compression to 100 atm the action potential occurred again. Decompression to 50 atm caused the action potential to be replaced by a small local response, and a smaller local response was observed after final decompression to 1 atm.

Mechanism of the Pressure-Induced Depression of e.j.p. Amplitude

A preliminary attempt was made to determine if the pressure-induced depression of e.j.p. amplitude was due to a reduction in the number of quanta of transmitter released by the nerve endings. To this end a comparison was made between the effect of pressure, and the effect of changes in the frequency of nerve stimulation, on the amplitude and coefficient of variation of intracellularly recorded e.j.p.s. This is an unconventional way of estimating changes in quantum content and can only provide suggestive evidence. It was used because the conventional way of measuring quantum content at crustacean neuromuscular junctions involves extracellular recording of the synaptic currents near a single nerve-ending. In making extracellular recordings it is crucial that the electrode position remains stable within a few microns (see Dudel and Kuffler, 1961), and it seemed unlikely that such precise stability could be maintained in these pressure experiments.

The rationale for the method of estimating changes in the quantum content used in this study is as follows: The variation in amplitude of the e.j.p.s during a train of nerve stimulation presumably arises from variations in the number of quanta of transmitter released and, in the case of intracellularly recorded e.j.p.s in crustacean muscle fibers, possibly from variations between the spatial distribution of the quanta released by each nerve impulse; nerve endings are distributed widely over the surface of crustacean muscle fibers (Fatt and Katz, 1953a), and quanta released at endings close to an intracellular electrode will make a greater contribution

to the recorded depolarization than quanta released at distant endings (see p. 32). Under conditions uncomplicated by the effects of the spatial distribution (various vertebrate muscle preparations or extracellularly recorded e.j.p.s in crustaceans) the coefficient of variation ($CV = \text{standard deviation} / \text{mean e.j.p. amplitude}$) can be used to directly calculate the quantity m , the mean number of quanta making up the e.j.p.s, by the relation $CV = \sqrt{1/m - 1/n}$, where n is the total number of quanta available for release (see Fatt and Katz, 1954). To calculate m from this relation it must be assumed that the statistics of release are Poisson so that $m \ll n$, thereby reducing the relation to $CV = \sqrt{1/m}$ (see p. 30 and del Castillo and Katz, 1954). It must also be assumed that the quantum depolarizations sum linearly to build up the e.j.p. Experimental conditions can be arranged under which these assumptions are valid. In the case of intracellularly recorded e.j.p.s in crustacean muscle, m cannot be calculated from the CV because variations between the spatial distribution of the quanta released by each impulse make some unknown contribution to the magnitude of the CV.

It is possible, however, to get at the relation between m and the CV in an indirect manner, experimentally. It has been shown in a variety of crustacean muscles that the facilitation of e.j.p. amplitude resulting from increased frequency of nerve stimulation results primarily from an increase in m (c.f. Dudel and Kuffler, 1961b; Frank, 1973). Therefore, measurements of how the e.j.p. amplitude and the CV vary with the frequency of nerve stimulation can be used to determine experimentally how these two parameters should vary together when m is changed. If identical pressure-induced

changes in e.j.p. amplitude are accompanied by identical changes in m . Then, by analogy with the effects of frequency, it is suggested that the pressure-induced depression of e.j.p. amplitude results purely from a pressure-induced depression of m . This was, in fact, the observed result.

Fig. 30 shows records comparing the effects of nerve-stimulation frequency and pressure on the amplitude of e.j.p.s recorded from a fiber of the Homarus opener muscle (the same muscle fiber that is described in Fig. 8). Some e.j.p.s recorded at a frequency of 5/sec and 1 atm pressure are shown in the middle trace. The mean amplitude was 3.92 mV, and the CV was 0.08 ($N = 200$ for all the measurements in this experiment). Reducing the frequency of stimulation to 1/sec resulted in a reduction of the e.j.p. amplitude to 1.00 mV and an increase in the CV to 0.16 (top trace). When a virtually identical reduction in amplitude was achieved by applying 200 atm pressure rather than by reducing the frequency, a virtually identical increase in the CV accompanied it (bottom trace). At a stimulation frequency of 5/sec and 200 atm pressure, the mean e.j.p.-amplitude was 1.04 mV and the CV was 0.15, in good agreement with the hypothesis that the pressure-induced reduction in e.j.p. amplitude results primarily from a decrease in the number of quanta of transmitter released.

Upon withdrawal of the electrode at the conclusion of the experiment the resting potential was recorded at 71 mV. Taking this value as V_0 (see p. 33), i.e., assuming the e.j.p.-reversal potential to be zero (Taraskevich, 1970), the measured CV's were corrected for the effect of non-linear summation.

Applying the correction did not significantly change any of the values of the

Fig. 30. Records from the muscle fiber of Fig. 8. Note changes in scale.

1/SEC
1 ATM

5/SEC
1 ATM

5/SEC
200 ATM

CV, and it was concluded that summation of the quantum depolarizations was linear.

The interpretation of this experiment rests partly on the validity of the assumption that, when the frequency of nerve stimulation is changed, the e.j.p. amplitude and the CV change as functions only of the variables m , n , or $\bar{p}$ which compose the basic equation of the statistics of release, $m = n\bar{p}$. Where $\bar{p}$ is the average of the probabilities of release of each quanta (see p. 28). If this assertion is valid, then the interpretation rests solely on the extent to which the observed similarity between the effects of frequency-changes and pressure-changes can be taken as an indication that the mechanisms of both effects are identical. These issues are considered in the Discussion section (p. 141).

An experiment similar to the one reported above was performed with a highly facilitating fiber from the stretcher muscle of Geryon. At 1/sec and 1 atm pressure, the mean e.j.p.-amplitude in this fiber was 5.59 mV, and the CV was 0.08 ($N=200$ for all these measurements). At a frequency of 0.4/sec, the mean amplitude was reduced to 2.22 mV and the CV increased to 0.12. At 1/sec and 200 atm pressure, the amplitude was 2.03 mV and the CV was 0.12. The resting potential was 60 mV at the end of this experiment, and here also, correction for non-linear summation made no significant difference in the result. These results also agree well with the hypothesis that the pressure effect on e.j.p. amplitude results from a pressure-induced depression in the number of quanta of transmitter released.

DISCUSSION

Adaptive mechanisms of highly facilitating fibers

The deep-sea crab appears to have several mechanisms that can make up for the pressure-induced depression of e.j.p. amplitude. Highly facilitating fibers have small e.j.p.s and show little contraction at low frequencies of stimulation. At high frequencies the processes of facilitation of e.j.p. amplitude and summation of the e.j.p.s combine to yield greater depolarization and, therefore, contraction. These are presumably the functionally significant frequencies. In Geryon, which lives to a depth of about 2000 m (200 atm), the phasic e.j.p.-amplitude was depressed less at higher frequencies of stimulation (7-10/sec) than at lower frequencies (1-2/sec); i.e., there was a pressure-induced enhancement in facilitation (see p. 85). Also, the duration of the falling phase of the e.j.p. was prolonged by pressure (Fig. 18) which served to increase the net level of depolarization achieved at frequencies high enough to result in summation. The pressure-induced enhancement in facilitation and the pressure-induced increase in the duration of the falling phase of the e.j.p.s can be regarded as adaptive mechanisms which oppose the pressure-induced depression of e.j.p. amplitude. In Geryon where the e.j.p.s were summing at high frequencies of nerve stimulation, the net depolarization achieved during a train of stimulation was nearly unaffected by pressure up to at least 200 atm (Fig. 19); i.e., the two adaptive mechanisms completely made up for the depression of e.j.p. amplitude over the entire range of pressure at

which this animal is found in nature. At low frequencies of stimulation where neither adaptive mechanism is operative, the depolarization (the phasic e.j.p.-amplitude) at 150 atm pressure was depressed to almost one-half that at 1 atm (Fig. 15).

Neither adaptive mechanism appeared to be present in the highly facilitating fibers in Libinia, a shallow-living crab found at pressures less than 20 atm. The e.j.p. amplitude at low frequencies of stimulation was depressed by pressure to a somewhat greater extent than in Geryon (Fig. 15), and no pressure-induced enhancement in facilitation (p. 85) and little or no increase in the duration of the falling phase of the e.j.p. (Fig. 18) was observed. The net depolarization attained during a train of high frequency stimulation where e.j.p.s were summing was depressed at 150 atm to slightly less than one-half its level at 1 atm (Fig. 19).

In Homarus which lives to a depth intermediate between Libinia and Geryon (i.e., about 60 atm pressure), the phasic amplitude of e.j.p.s from the highly facilitating fibers of the opener and stretcher showed responses to pressure intermediate between the responses of the two crabs. At low frequencies of stimulation the pressure-induced depression of e.j.p. amplitude (Fig. 15) was similar to that in the stretcher of Libinia (somewhat greater than in Geryon), but, as in Geryon, there was a pressure-induced enhancement in facilitation (p. 85) which served to reduce the effect of pressure upon the phasic amplitude of the e.j.p. at high frequencies of stimulation. The magnitude of pressure-induced depression of phasic amplitude shown by the highly facilitating fibers of Homarus (both opener

and stretcher) at high frequencies of stimulation was intermediate between that shown by Geryon stretcher and Libinia stretcher (Fig. 16).

Measurements of the effect of pressure on the duration of e.j.p.s recorded from Homarus muscle (Fig. 18) and on the net depolarization achieved during a train of stimulation at high frequency (Fig. 19) were made in only a few fibers in the stretcher muscle. As in Geryon, pressure prolonged the duration of the e.j.p., and the consequent increase in summation at high frequencies of stimulation served to increase the net level of depolarization achieved during a train. As in Geryon, this increased summation, combining with the pressure-induced enhancement in facilitation, served to nearly completely counteract the depressive effect of pressure; that is, the net depolarization achieved during a train of stimulation at high frequency was nearly unaffected by pressure. However, the effect of pressure on net depolarization was tested in only two fibers, only one of which was subjected to pressure greater than 100 atm (namely 150 atm), and it is possible that pressure (especially in excess of 100 atm) may have some depressive effect on the net depolarization in Homarus.

That acclimation to low temperatures may confer some resistance to high pressure cannot be ruled out as a possible mechanism for some of the species differences described above. Also, it is clear that the apparent adaptations of Geryon were not the result of short-term acclimation to high pressure. Specimens were obtained from a depth at the shallow end of the range of the species (about 50 atm pressure) and they were maintained in aquaria at 1 atm for many months while the experiments were conducted.

A pressure-induced enhancement in facilitation and prolongation of the duration of the falling phase of the e.j.p. were not confined only to species that are found in the deep-sea. The effect of pressure on e.j.p.s in four highly facilitating fibers from a single preparation of the claw of the fiddler crab, Uca sp., was observed. Uca is an amphibious intertidal crab. The effects of pressure on e.j.p. amplitude, facilitation, and e.j.p. duration in this animal were similar to its effects in Homarus. This preparation proved a difficult one to perform. Pressure experiments were performed with only the four fibers mentioned here, and the study of Uca was discontinued.

Adaptive mechanisms of poorly facilitating fibers

Poorly facilitating fibers usually have larger e.j.p.s at low frequencies of stimulation than the highly facilitating fibers, and, by definition, they show little facilitation at high frequency. Physiological frequencies of nerve impulses for contraction of these fibers in the behaving organism may include frequencies that are quite low, where neither facilitation nor summation play an important role. In Geryon the poorly facilitating fibers showed no average depression at pressures to 200 atm (Fig. 17). This lack of an average effect resulted because some of these fibers showed an increase in e.j.p. amplitude, while others showed depression (Fig. 13). A pressure-induced enhancement of e.j.p. amplitude was never observed in poorly facilitating fibers of Libinia or Homarus. Pressure caused considerable depression of e.j.p. amplitude in both these species (Fig. 17). This difference in behavior could not be attributed to acclimation to cold,

as the Homarus experiments were also performed with cold-acclimated animals. The pressure-induced enhancement in e.j.p. amplitude may represent a species specific adaptation to pressure in Geryon.

Effect of pressure on muscle contraction

There was a considerable pressure-induced decrease in the initial rate of rise of isometric tension in whole stretcher muscles of Homarus at nerve-stimulation frequencies of 5-50/sec (Fig. 26), but in Geryon only a slight pressure-induced depression was observed at these frequencies (Fig. 27). This is consistent with the different depth ranges of the two species and with the differences observed in the effects of pressure on the electrical response of their muscle fibers to nerve stimulation. It supports the notion that the species-differences in the effect of pressure on e.j.p. amplitude and duration that were observed are relevant to the behavior of the animals. Also significant was the observation that pressure caused an increase in the isometric tension developed by the stretcher in Geryon at low (1-2/sec) stimulation frequencies (p. 120). Presumably, only the poorly facilitating fibers would be contracting at low frequencies, and some of these fibers showed an increase in e.j.p. amplitude at high pressure which was probably responsible for the increase in tension at low frequency.

Mechanism of the pressure-induced depression of e.j.p. amplitude

Above I have reported experiments showing that a given reduction of e.j.p. amplitude produced by a decrease in the frequency of nerve stimulation or an increase in pressure is accompanied by a similar increase in

the coefficient of variation (see p. 133). It was argued that this result suggests that high pressure causes a reduction in the number of quanta of transmitter released with each nerve impulse. The validity of this interpretation rests upon the validity of the following two suppositions:

1) that the relationship between the e.j.p. amplitude and the CV, as both change in response to changes in the frequency of nerve stimulation is affected only by m and n , the variables that control the number of quanta released; 2) that the analogy between the effects of frequency and pressure can be taken to mean that both agents operate through similar mechanisms. The validity of these two suppositions is the subject of this section.

It has been shown in a variety of crustaceans that the increase in e.j.p. amplitude occurring in response to an increase in frequency is due to an increase in m (c.f. Dudel and Kuffler, 1961b; Frank, 1973). A decrease in the CV in response to increased frequency could result directly from changes in m or n ($CV = \sqrt{1/m - 1/n}$; no assumption that release is Poisson is required since this equation is also valid for binomial release). In the case of intracellularly recorded e.j.p.s, a decrease in the CV could also result from a decrease in the variability between the spatial distribution of the quanta released by each nerve impulse, but this would presumably be a function of m and n for each release site. The primary source of possible error here is non-linear summation (see p. 133), which would cause the CV to decrease with increasing e.j.p. amplitude and could be the sole cause of the observed changes in CV. Applying Martin's correction factor for non-linear summation (p. 35) to the

CV's measured in these experiments did not significantly change them (p. 133), and it was concluded that summation in these experiments was linear.

The second question is whether pressure also affects the e.j.p. amplitude and CV by a reduction in the number of quanta released. Mechanisms that could affect e.j.p. amplitude are apparent from consideration of equation 6 (p. 34) which is written out again for convenience:

$$\frac{\bar{v}}{V_o - \bar{v}} = \frac{mg}{G} \quad (6)$$

Where m is the number of quanta of transmitter released, g is the conductance of the muscle membrane produced by a single quantum of transmitter, G is the transverse conductance of the muscle membrane ($1/G$ is the input resistance), V_o is the electromotive force driving the synaptic current (equal to the difference between the resting potential and the reversal potential of the e.j.p.), and $\bar{v}$ is the mean e.j.p.-amplitude during a train of nerve stimulation.

A pressure-induced decrease in $\bar{v}$ could result from an increase in G (i.e., a decrease in the input resistance). It was shown, however, that pressure increases the input resistance of the muscle fibers; e.g., 200 atm pressure increased the input resistance about 1.5-fold in Homarus fibers. This introduces a complication. According to equation (6), a 1.5-fold pressure-induced increase in input resistance would cause the e.j.p. amplitude to increase by the same factor, and the mechanism responsible

for the net depression of e.j.p. amplitude would be working against this effect. It was shown for a Homarus muscle fiber (p.133) that a 4-fold decrease in e.j.p. amplitude, achieved by either reducing the frequency or increasing the pressure (200 atm), was accompanied by a doubling of the CV (which is consistent with the relation $CV = \sqrt{\frac{1}{m}}$). If the pressure-induced depression of e.j.p. amplitude resulted from a reduction in the number of quanta released, working against a 1.5-fold increase in amplitude caused by the pressure-effect on the input resistance, then it would take a 6-fold reduction in quanta to cause the 4-fold net reduction in e.j.p. amplitude. If this is so, then the 4-fold pressure-induced reduction in e.j.p. amplitude should have been accompanied by a 2.45-fold increase in the CV (from the relation $CV = \sqrt{\frac{1}{m}}$), not the approximately 2-fold increase that was observed.

Actually, the 2.45-fold increase in the CV predicted from the above considerations is an upper limit. Equation (6) applies to the steady state, ignoring possible effects of the membrane capacitance on the e.j.p. amplitude. Increasing the input resistance (assuming the increase reflects an increase in membrane resistance) will increase the membrane time constant (equation (12), p. 35) and slow the rate of rise of the e.j.p. If the steady-state potential is not reached during the e.j.p., then an increase in input resistance by a given factor will cause a smaller increase in the e.j.p. amplitude. (Compare the relative amplitudes of the potential change at 1 atm and at pressure produced by hyperpolarizing current-pulses in Fig. 20. The steady-state potential at 200 atm is much greater than at 1 atm, but early

during the rise of potential (say at 25 msec) the difference is quite small.) Because of this consideration the two results discussed here (the parallel between the effects of frequency and pressure on the e.j.p. amplitude and the CV, and the finding that pressure causes an increase in the input resistance of the muscle fibers) are not necessarily at odds with one another. These considerations do, however, underline the fact that the method used in this study to try to identify the mechanism responsible for the pressure-induced depression of e.j.p. amplitude is an imprecise one and can only offer suggestive evidence.

Equation (6) shows that a decrease in V_0 or g could be responsible for the pressure-induced decrease in e.j.p. amplitude. However, no change in the CV would be expected as a direct consequence; these changes might affect non-linear summation, which could indirectly alter the CV, but this has already been shown to be an unimportant effect.

It is conceivable that pressure may, by some mechanism unrelated to the number of quanta released, cause an increase in the CV and a decrease, say, in the sensitivity of the muscle fiber to the neurotransmitter. In this case the parallel changes in the e.j.p. amplitude and CV accompanying both frequency and pressure changes would be fortuitous. But the hypothesis that pressure and frequency both affect the number of quanta of transmitter released is more parsimonious and, therefore, more attractive.

Behavior of the poorly facilitating fibers in Geryon

The effect of pressure on e.j.p. amplitude in the poorly facilitating fibers in Geryon was unique in two respects. 1) In some cases pressure

caused an increase in e.j.p. amplitude (p. 80). 2) The effect of pressure in the poorly facilitating fibers was correlated with the e.j.p. amplitude (Fig. 14): small e.j.p.s became larger under pressure, and large e.j.p.s were depressed.

One can only speculate about the mechanisms that may be involved in these effects. Evidence described above suggested that the pressure-induced depression of e.j.p amplitude results from a pressure-induced depression of the release of transmitter. Furthermore pressure caused an increase in the input resistance of the muscle fibers, which might cause the e.j.p. amplitude to increase. It may be that in the poorly facilitating fibers that show a pressure-induced increase in e.j.p. amplitude, any decrease in transmitter release is more than compensated by an increase in input resistance of the muscle fibers.

General significance of this work

Before biological investigations of the oceans established that organisms do live under hundreds of atmospheres of pressure, biologists thought the possibility unlikely (see Murray and Hjort, 1912). It has been a long time since then, and almost nothing is known of the physiological adaptations of deep-sea organisms that allow them to exist under high pressure; this is largely because deep-sea animals in suitable condition for physiological work are not easily obtained. The red crab, Geryon, is a fortunate exception.

Also fortunate is the fact that the crustacean neuromuscular junction is a common preparation used by neurophysiologists for the study of synaptic transmission. Much is known about its physiology.

Techniques were developed to permit recordings to be made at high pressure of the electrical response of the muscle membrane to nerve stimulation. It was found that pressure caused depression of neuromuscular transmission, which can account for the paralysis observed in many species of shallow-living crustaceans subjected to high pressure (see Macdonald, 1971; and Macdonald, Gilchrist, and Teal, 1972).

Pressure-induced increases in the processes of facilitation and summation were implicated as adaptive mechanisms permitting the neuromuscular junction of the deep-sea crab to function normally over the pressure-range in which it lives in nature. These adaptations involve playing off some of the effects of pressure against others (at the physiological level), the net effect being an insensitivity to pressure over a broad range. Such a mechanism would be suitable for the vertically migrating midwater animals which undergo large changes in pressure over relatively short periods.

Pressure may have similar effects on other synapses and the adaptive mechanisms suggested here might be important in the nervous system of all deep-sea animals; the same processes of facilitation and summation of local membrane responses that control the tension developed by the crustacean muscle are important components for integration of synaptic influences in the nervous system of all animals.

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AUTOBIOGRAPHICAL SKETCH

I was born on March 30, 1946 and spent my early childhood in Newark, New Jersey and my later childhood through high school in a suburb, Union. My first interest in anything academic was aroused in a sophomore biology class in high school. As a senior, I took an advanced biology class, and I was accepted into the biological sciences program at Rutgers University. The summer before starting at Rutgers I had misgivings about declaring a major so early and transferred into the liberal arts program.

At college I developed an interest in physiological psychology and majored in psychology. From the summer following my freshman year through my senior year I worked as a research assistant, first in a laboratory doing experiments on hoarding in mice, and later doing data analysis for a social psychologist studying drinking (alcohol) in teenagers. During this time I was becoming more interested in physiology, less in behavior.

When a junior, I received a National Science Foundation summer fellowship to work in the Psychology Department at the University of California at Riverside. While there, I became acquainted with the Physiology Department at U.C.L.A. where I was accepted for graduate school the following year. I returned to Rutgers for my senior year, did an honors research project in physiological psychology, which was really my first "research experience" (and which I thoroughly enjoyed and later published).

The first year and a half at U.C.L.A. involved a lot of courses in large classes with medical students. After my senior "research experience" that

was very hard to take. I got involved in research again during the second year, but a lot of things had happened, and I was not happy with it. Part of the reason was that I had developed some interest in ecology because of contact with Jared Diamond, a part-ecologist in the physiology department. I also developed a strong interest in marine animals through SCUBA diving. Anyway, for a lot of reasons I decided to quit U.C.L.A. and try something else; I did not exactly know what. When I told Nat Buchwald, my major professor, of my decision, he suggested that I try graduate school in biological oceanography. Since I had not majored in biology and lacked a lot of course background, it had not occurred to me that I could get in. Buchwald said that he thought I could and that a person with a background such as mine might be able to make a contribution by combining aspects of both physiology and oceanography. I applied and was accepted into the M.I.T./W.H.O.I. Joint Program in Biological Oceanography.

I did not take Buchwald's comment about combining fields very seriously at the time; I thought I might forget about physiology and do something entirely different, but it turned out that he was right.

After being adrift at the Woods Hole Oceanographic Institution for about a year, searching for a thesis project, I came to the conclusion that my taste really was for physiology. I approached John Teal with the idea of coming into his laboratory to do a thesis in one of his areas of interest, high-pressure physiology. When I read about this field I realized that high pressure must have significant effects on the function of nervous

systems, that very little research had been done in that direction, and that my background was well suited to it. I spent the next year equipping a laboratory and developing techniques, and the following two years doing the experiments reported in this thesis.