FACILITATION IN SEA ANEMONES

I. THE ACTION OF DRUGS

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(With Four Text-figures)

INTRODUCTION

In a series of papers on the nerve net of the Actinozoa, Pantin (1935*a*, *b*, *c*, *d*) showed that the decisive factor in the neuromuscular activity of these animals is an extreme development of facilitation. This is seen most readily in the transmission of excitation from the nerve net to certain muscles, in particular the marginal sphincter of *Calliactis parasitica* and the longitudinal mesenteric muscles of *Metridium senile*. These muscles do not respond to a single electrical stimulus applied to the nerve net, but a second stimulus following soon after causes an immediate contraction. This means that a single stimulus which has no visible effect leaves behind it an effect which facilitates the transmission of a second impulse to the muscle. This facilitating effect of a stimulus dies away gradually, and it is the interval between stimuli, or the amount of facilitation, which determines the size of the response. This contrasts sharply with the mechanism in vertebrate skeletal muscle, where the strength of the stimulus determines the size of the response.

Analysis of the process of facilitation was begun by Ross & Pantin (1940) in a study of the action of ions and other substances on the response of anemones to stimulation. They concluded that their results could be explained best if it were assumed that two distinct processes exist in the transmission of excitation from nerve to muscle in anemones: (1) a process of facilitation or sensitization of the neuromuscular junction, and (2) a process of excitation which is ineffective unless sensitization has already taken place.

It is possible that either or both these processes are chemical in origin. Cholinergic and adrenergic systems of transmission are known to occur in phyla other than the Chordata. Substances such as acetylcholine, choline esterase and adrenaline are found in animals from most phyla. The widespread sensitivity of animals to acetylcholine, adrenaline and related substances suggests that chemical systems of junctional transmission occur in most animals. It has been known for many years that coelenterates are sensitive to drugs (Romanes, 1885). It seemed likely, therefore, that a study of the effects of drugs on the facilitated responses of sea anemones might throw some light on the mechanism of facilitation.

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METHODS

The sea anemone, *Calliactis parasitica*, was used in the experiments. As in Pantin's (1935) work on this animal, the responses of the marginal sphincter muscle were recorded on a smoked drum by means of a light spring lever. The stimuli consisted of condenser discharges delivered through non-polarizable Ag/AgCl electrodes. A relay of the type described by Hall & Pantin (1937) enabled the condenser to be charged and discharged at a frequency controlled by a metronome in the circuit.

The effect of a drug on the anemone was observed in the following way. First a series of responses in natural sea water was recorded. Then a quantity of the drug being tested was introduced into the sea water and any immediate effects on the behaviour of the animal noted. Records of the response to stimulation were taken at frequent intervals thereafter so that any changes in the size and character of the response could be detected. Animals stimulated for corresponding periods in natural sea water can be regarded as controls. *Calliactis* can be stimulated once every 5 min. for 5–6 hr. before any decline in the size or speed of the response becomes apparent. This point has been kept in mind in analysing the results. In no experiment has the anemone been stimulated intensely enough to cause the augmented responses associated with the onset of fatigue (Ross & Pantin, 1940).

RESULTS

The drugs used in the experiments can be divided into four groups: (1) drugs which affect *cholinergic* nerve endings in the vertebrates; (2) drugs which affect *adrenergic* nerve endings in the vertebrates; (3) drugs which affect vertebrate nerve and muscle in various ways but which do not belong to groups (1) or (2); (4) substances which have been detected

in actinians and other marine invertebrates and which might have some physiological function in these animals.

There are certain features of the drugs' effects which should be mentioned before the action of individual drugs is described. It was soon discovered that drugs affect anemones only at relatively high concentrations, usually not below $1 : 10^4$ (compare acetylcholine exciting leech muscle at $1 : 5 \times 10^8$). Drugs also act more slowly on actinians than on other animals. Whereas a few seconds or at most a few minutes is usually long enough to produce the full effect on vertebrates, most drugs exert their maximum effect on *Calliactis* only after 1–2 hr. Long exposure to any drug eventually leads to a depression of the response, even when the drug causes enhanced responses at first. This general depression cannot be distinguished easily from specific depressant effects which some drugs seem to produce. In describing the results, only those drugs that have an early depressant effect, i.e. appearing within 2 hr. at concentrations of $1 : 10^4$, and whose action is wholly depressant, have been regarded as specific depressant agents.

One interesting feature of the action of drugs on sea anemones is the resistance shown by these animals to drugs which have toxic effects on other animals. In every case the anemones were returned to natural sea water after the experiments, which usually lasted for several hours. Not once did an experiment cause the death of an animal, and the effects of the treatment had always passed off within 24 hr.

Group 1

Drugs tested in this group were acetylcholine and a few of the many substances which act on cholinergic junctions in the vertebrates, viz. eserine, atropine, nicotine and curare. Since Dale & Loewi and their colleagues showed the role of acetylcholine as the transmitter of excitation at parasympathetic nerve endings and at the endings in voluntary muscle in the vertebrates, a number of tests have been carried out to see if this chemical mechanism also exists in the invertebrates. Among the effectors which respond to acetylcholine are the cephalopod stomach (Ungar, 1936), the cephalopod heart (Kruta, 1936), the longitudinal muscles of the leech (Minz, 1932), the earthworm gut (Wu, 1939) and the crustacean heart (Welsh, 1939). The most notable failure of acetylcholine in the invertebrates is in the leg muscles of *Carcinus* (Katz, 1936). Acetylcholine has been detected in extracts of cephalopod ganglia (Bacq, 1935), holothurian longitudinal muscles (Bacq, 1935) and crustacean heart (Welsh, 1939). Choline esterase, the agent which destroys acetylcholine in the vertebrates as soon as the muscle is excited (Loewi & Navratil, 1926), has been found in most invertebrate tissues with the exception of the sphincter of *Calliactis* (Bacq & Nachmansohn, 1937).

Acetylcholine and eserine. Acetylcholine bromide (B.D.H.) was tested on *Calliactis* in concentrations ranging from $1 : 10^6$ to $1 : 10^3$ in sea water. Fig. 1 A shows a typical result at a concentration of 1 : 1500 at *p*H 6·5. It is evident that no significant change occurs in the size or form of the response. In a few experiments a slight increase in the size of the response was observed between 15 and 30 min. after the drug was introduced, but this effect was no larger than increases which sometimes occur in untreated animals. After very long exposure, 4 hr. or more at $1 : 10^4$, the response becomes weaker, but this is probably the general depressant effect mentioned above.

As Bacq & Nachmansohn (1937) have shown that *Calliactis* sphincter contains negligible quantities of choline esterase, the failure of acetylcholine cannot be attributed to its destruction by this enzyme in the animal. Moreover, when acetylcholine is applied with eserine to protect it from the enzyme, both drugs at concentrations of $1 : 10^4$, the records obtained are similar to those in Fig. 1 A, where the effect of acetylcholine alone is shown. Eserine alone is also ineffective.

Acetylcholine causes no direct response of any part of the anemone with the exception that a concentration of $1 : 10^4$ will dilate the mouth (Pantin & Pantin, 1943). However, many other substances do this, so it cannot be regarded as a specific acetylcholine effect.

These experiments, therefore, give no evidence of any specific action by acetylcholine on *Calliactis*. This result and the absence of choline esterase in *Calliactis* strongly suggest that there can be no step in junctional transmission in anemones involving the liberation of acetylcholine at the nerve endings. Before deciding this point, however, the effects of some of the other drugs of the group should be considered.

Atropine. Atropine interrupts parasympathetic transmission in the vertebrates and abolishes the parasympathomimetic action of acetylcholine. It acts by preventing acetylcholine from reaching and exciting the effector cells. There are records of an action by atropine on certain coelenterates. Romanes (1885) found that it caused convulsive swimming movements in the jellyfish *Sarsia*. Moore (1917) was able to increase the rate of pulsation in *Gonionemus* by atropine. On *Metridium* he found that $1 : 2 \times 10^3$ atropine in sea water produced contractions of the tentacles and spasmodic longitudinal contractions of the whole animal.

Atropine enhances slightly the responses of *Calliactis* during the first hour at $1 : 10^4$ (*p*H 8·3). Later a gradual reduction in the size of the response sets in and after 3 hr. the steps in the staircase become indistinct and the whole response is very slow and prolonged. Both these effects can be seen in Fig. 1 B. At the same time the threshold of stimulation rises to about twice its original level in a typical experiment.

action of atropine at 1 : 10^3 is qualitatively the same, but the whole process is speeded up and the depressant effect appears shortly after half an hour. Before this happens there is a period when multiple responses or 'after-discharges' (Pantin, 1935*c*) tend to occur frequently. However, atropine does not cause spontaneous contractions of the sphincter or any other part of the animal.

Although atropine has a depressant effect on *Calliactis*, this effect is hardly parallel to its action on the vertebrates, where it abolishes the response quickly and completely. Complete abolition of the response of *Calliactis* only occurs after 5 hr. exposure to atropine at 1 : 10^3. At lower concentrations the response is not abolished completely and the depression develops so gradually that it is difficult to regard it as a matter of importance.

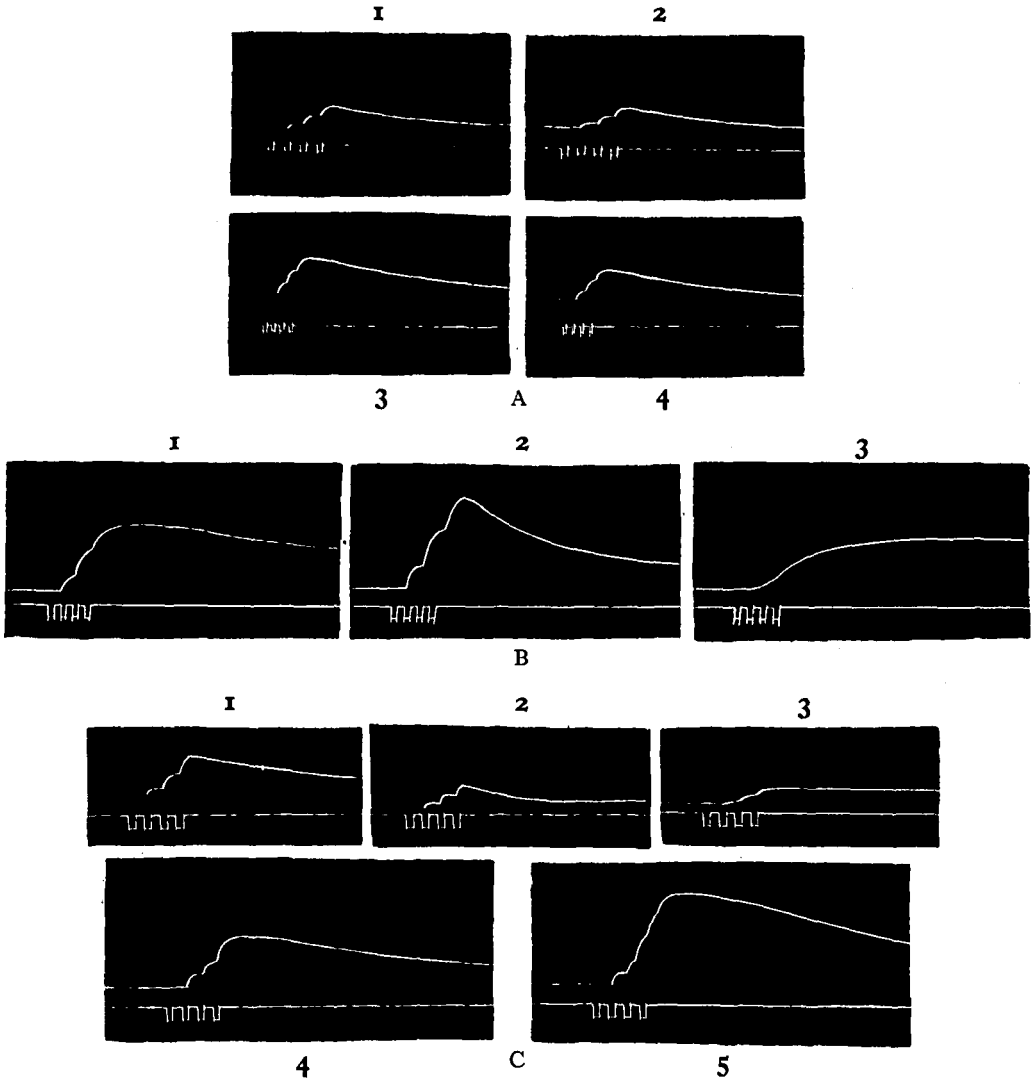

Fig. 1. Responses of *Calliactis* sphincter. A. Action of acetylcholine. Four stimuli at 1 in 1 sec. (1) in natural sea water; (2) after 155 min. in acetylcholine (1 : 1500). Four stimuli at 1 in 0·5 sec. (3) in natural sea water; (4) after 115 min. in acetylcholine (1 : 1500). B. Action of atropine. Four stimuli at 1 in 1 sec. (1) in natural sea water; (2) after 45 min. and (3) after 150 min. in atropine (1 : 10^4). C. Action of nicotine. Four stimuli at 1 in 1 sec. (1) in natural sea water; (2) after 180 min. and (3) after 250 min. in nicotine (1 : 10^4); (4) in natural sea water; (5) after 45 min. in nicotine (1 : 10^3).

Curare. In vertebrates curare blocks the passage of impulses across the neuromuscular junctions in voluntary muscle by preventing acetylcholine from reaching and exciting the muscle. It has no such effect on *Calliactis*. After 3–4 hr. exposure to curare (Merck) at 1 : 10^4, the response is unchanged except for the general depressant effect. It is interesting to

recall that Katz (1936) observed that curare has no effect at the neuromuscular junctions in the leg muscles of *Carcinus*.

Nicotine. Nicotine (B.D.H.) at a concentration of $1 : 10^4$ (*p*H 8·3) produces no effect on *Calliactis* for about 3 hr., but after that the response gradually diminishes in size. Larger doses ($1 : 10^3$) bring about a decline in the speed and size of the response after about 30 min. At this concentration, however, nicotine causes spontaneous contractions of the whole animal beginning about 5 min. after the drug is introduced and occurring frequently in the first half hour of the experiment. These contractions are usually maintained for some time, and it is often 5 min. before relaxation of the sphincter is complete. In the same period the responses to electrical stimuli frequently take the form of 'after-discharges' or multiple responses (Pantin, 1935*c*). In one experiment with nicotine at $1 : 10^3$, 'after-discharges' occurred in two out of three cases. Fig. 1 C shows the depressant action of nicotine at $1 : 10^4$ and an example of 'after-discharge' at $1 : 10^3$.

In vertebrates nicotine at first excites and then paralyses the autonomic ganglia and the nerve endings in voluntary muscle. These effects seem to resemble the effects observed on *Calliactis* where a period of spontaneous contractions is followed by a strong depressant action. However, tests on animals anaesthetized with magnesium show that the spontaneous contractions are due to impulses arising peripherally. When the anemone is immersed in a mixture containing equal portions of sea water and $0{\cdot}4M$ $MgCl_2$, which according to Ross & Pantin (1940) anaesthetizes the sense organs in a few minutes, spontaneous contractions do not occur if the animal is then exposed to $1 : 10^3$ nicotine. It is therefore unlikely that this excitatory effect of nicotine on *Calliactis* is due to a specific action on the neuromuscular junctions as in the vertebrates.

Similarly, the depression of the facilitated response which occurs with nicotine appears to be of the general rather than the specific type. At $1 : 10^4$ its onset is so gradual that after 4 hr. the response is still one-half the normal size. It seems, therefore, that the similarity between the action of nicotine on vertebrates and on *Calliactis* is only superficial.

To sum up. Acetylcholine, eserine and curare fail to have any effects at all on *Calliactis*; atropine and nicotine fail to have effects that are truly analogous to those they cause at cholinergic junctions in the vertebrates. In view of these results and the absence of choline esterase in *Calliactis* spincter, it seems unlikely that the transmitting mechanism in anemones is the same as at those junctions where acetylcholine functions as the chemical transmitter.

Group 2

Included in the second group of drugs are adrenaline, epinine, ephedrine, tyramine, tryptamine, cocaine, ergotoxine and 933 F. All these drugs act at sympathetic nerve endings in the vertebrates, and adrenaline, or some substance closely resembling adrenaline, is regarded as the chemical transmitter of the sympathetic system.

Few tests have been made on invertebrates with this class of drugs. Adrenaline has a depressant action on the holothurian cloaca (Wyman & Lutz, 1930), but it excites the cephalopod heart (Kruta, 1936) and stomach (Ungar, 1936). On the earthworm gut (Wu, 1939) adrenaline causes a contracture of the oesophagus, and strong doses ($1 : 10^5$) inhibit, while weaker doses ($1 : 10^7$) excite the 'crop and gizzard' preparation. Little work has been done on the occurrence of these substances in the invertebrates apart from the work of Henze (1929), who found large quantities of tyramine in the 'salivary glands' of cephalopods, and Bayer & Wense (1936), who reported that adrenaline can be detected in *Paramecium*.

Adrenaline. Adrenaline (adrenalina B.P., B.D.H.) was tested on *Calliactis* in concentrations varying from $1 : 10^6$ to $1 : 3 \times 10^3$ in sea water. Fig. 2 A shows a typical result at $1 : 10^4$. It is clear that adrenaline, the most active sympathomimetic drug in the vertebrates, has no effect on the anemone. Adrenaline is unstable in an alkaline medium, and the tests were carried out in acid sea water. Fig. 2 A shows a result at *p*H 5·4, and this accounts for the depression of the response that appears in the record. Effects of this kind are obtained when anemones are exposed to acid sea water for some time (Ross & Pantin, 1940).

Adrenaline, like acetylcholine, does not cause spontaneous contractions, 'after-discharges' or changes in excitability. It also has no effect on *Calliactis* when it is administered in conjunction with cocaine, which sensitizes tissues to the action of adrenaline in the vertebrates (Fröhlich & Loewi, 1910). Finally, the possibility that the failure of adrenaline is due to its destruction in the tissues has been ruled out almost entirely by tests (carried out by Dr H. Blaschko) which showed that *Calliactis* does not possess the amine oxidase that destroys adrenaline in various other animal tissues (Blaschko, Richter & Schlossmann, 1937).

Ephedrine. Ephedrine acts like adrenaline in the vertebrates, but because it is a more stable substance its effect is less transitory. Ephedrine hydrochloride (B.D.H.) was tested on *Calliactis* at concentrations of $1 : 5 \times 10^3$ and $1 : 10^3$. At the lower concentration there is no change in the response and no effect on behaviour or excitability. At $1 : 10^3$ ephedrine has an inhibiting effect after 2 hr. At first the response becomes progressively smaller, but after $3\frac{1}{2}$ hr. the steps of the staircase become obliterated and eventually several stimuli may be necessary to cause a response. This effect is so slow in developing that it must be regarded as an example of the general depressant action already mentioned. At any rate

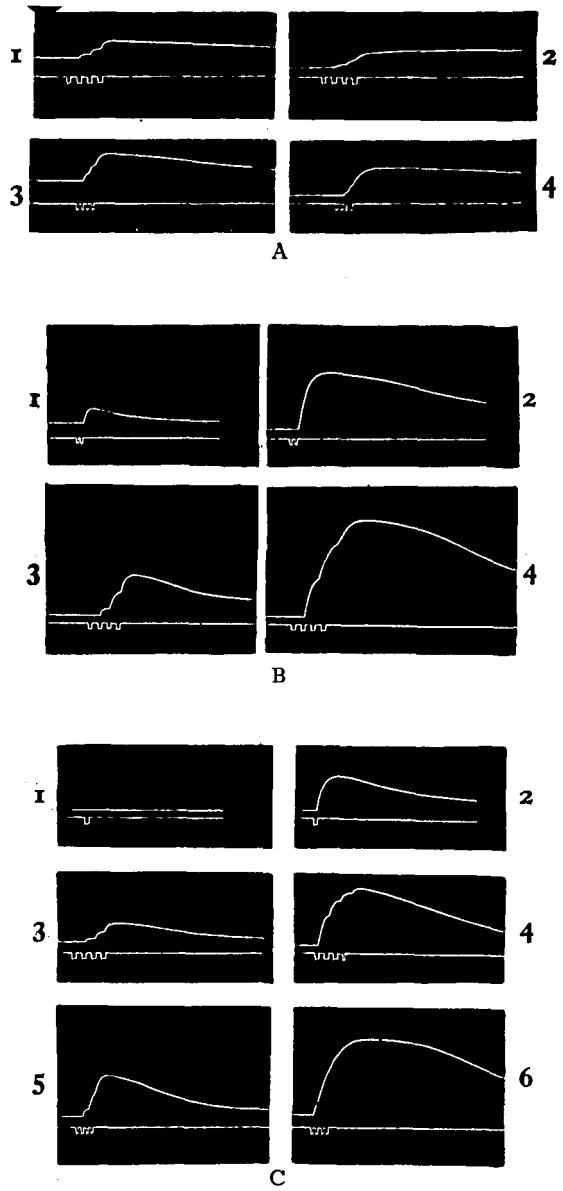

Fig. 2. Responses of *Calliactis* sphincter. A. Action of adrenaline. Four stimuli at 1 in 1 sec. (1) in natural sea water; (2) after 120 min. in adrenaline ($1 : 10^4$; *p*H 5·4). Four stimuli at 1 in 0·5 sec. (3) in natural sea water; (4) after 120 min. in adrenaline ($1 : 10^4$; *p*H 5·4). B. Increase in size of response with tyramine. Two stimuli at 1 in 0·5 sec. (1) in natural sea water; (2) after 87 min. in tyramine ($1 : 10^4$). Four stimuli at 1 in 1 sec. (3) in natural sea water; (4) after 84 min. in tyramine ($1 : 10^4$). C. Responses to single stimuli with tyramine. One stimulus (1) in natural sea water; (2) after 50 min. in tyramine ($1 : 10^4$). Four stimuli at 1 in 1 sec. (3) in natural sea water; (4) after 43 min. in tyramine ($1 : 10^4$). Four stimuli at 1 in 0·5 sec. (5) in natural sea water; (6) after 115 min. in tyramine ($1 : 10^4$).

it is clear that ephedrine has no effects on *Calliactis* analogous to its sympathomimetic effects in the vertebrates.

Epinine. Epinine (hydrochloride, Burroughs Wellcome) is even less effective than ephedrine on *Calliactis*. It has no effect at all at $1 : 10^4$ in sea water, and only very slightly depressant effects at $1 : 10^3$ (*p*H 8·3).

Tyramine. Unlike the three drugs of the group whose effects have just been described, tyramine proved to have important effects on the anemone. It was tested not only because it is another of the substances with an action like adrenaline in the vertebrates but also because it is a substance of some importance in marine invertebrates. Like adrenaline, it excites the heart (Kruta, 1936) and stomach (Ungar, 1936) of cephalopods. Sereni (1930) has shown that tyramine causes expansion of the chromatophores of cephalopods. As tyramine is produced in the 'salivary glands' of these animals (Henze, 1929) and removal of these glands causes permanent expansion of the chromatophores, Sereni (1930) put forward the view that tyramine exerts a humoral control over the chromatophores.

On *Calliactis*, tyramine hydrochloride (B.D.H.) first enhances considerably the size of the response. This effect develops rapidly. Fig. 2 B shows records taken 84 and 87 min. after the anemone was exposed to tyramine ($1 : 10^4$). The size of the enhancement is of the order of 3–4 times the size of the original response in natural sea water, and at this concentration it usually lasts for several hours.

In addition to enhancing the responses, tyramine may permit responses to single stimuli at any time after 30 min., thus altering the most distinctive feature of the facilitated response in *Calliactis*. Three examples of this effect are shown in Fig. 2 C occurring between 40 min. and 2 hr. In these records there are responses to the second, third and fourth stimuli as well as the first, but the first response is bigger than the succeeding ones.

At first the single-shock responses caused by tyramine appear very erratically. Before 2 hr. they occur in about 1 in 10 responses to stimuli. Between 2 and 3 hr., however, there is a period of about 30 min. duration when the effect occurs much more frequently. Afterwards the effect disappears completely, but not because the tyramine has been used up, as it is still effective on another animal. Eventually after 6 hr. exposure at $1 : 10^4$, and earlier at higher concentrations, the response deteriorates as the effects of fatigue and the general depressant action begin to operate.

In an effort to overcome the fortuitous character of the responses to single stimuli, cocaine was used in conjunction with tyramine in view of the sensitizing action of cocaine used in conjunction with adrenaline in the vertebrates (Fröhlich & Loewi, 1910). When the two drugs were applied together at concentrations of $1 : 10^4$ more consistent results

were obtained. In a typical test lasting 4 hr. two out of five responses occurred on the first stimulus, compared with about 1 in 10 with tyramine alone. This interaction of cocaine and tyramine on *Calliactis* recalls the sensitizing effect of cocaine used with adrenaline in the vertebrates.

Tyramine has no other effects on *Calliactis*. It does not alter the threshold of stimulation; it does not cause 'after-discharges'; it does not affect the behaviour of the animal, and spontaneous contractions have not been observed. This absence of the effects associated with a peripheral action suggests that the effect of tyramine on the size and character of the response is due to an action on the neuromuscular junctions themselves where the facilitation mechanism is located (Pantin, 1935*a*).

The effectiveness of tyramine suggests that the mechanism of transmission in *Calliactis* may be related in some way to the mechanism operating at adrenergic junctions in the vertebrates. However, it must be pointed out that the action of tyramine on the anemone is very different from the action of tyramine or adrenaline in the vertebrates. In the latter case these drugs are able to cause direct responses of the effectors with sympathetic innervation without nervous stimulation at all. In *Calliactis* there is no direct response to tyramine, and the effect of the drug is only revealed by certain changes that occur in the response to stimulation. Probably we are dealing here with a fundamental difference between these two neuromuscular systems which is reflected in the totally different character of the drug effects.

Tryptamine. Tryptamine or beta-indolethylamine (hydrochloride, Roche) has effects on *Calliactis* similar to those caused by tyramine. It increases the size of the response to stimulation and later permits responses to occur to single stimuli. Tryptamine is considerably less potent than tyramine in causing both these effects, and as with tyramine, no other change accompanies these effects on the response. There are no spontaneous contractions, no 'after-discharges' and no changes in excitability. Therefore, the tryptamine effect also suggests that a mechanism related to the mechanism of transmission at adrenergic junctions exists in anemones. But we have to note the fact that these effects are limited to changes in the response to stimulation and that direct effects of the type caused by tryptamine in the vertebrates do not occur in *Calliactis*.

Oxytyramine, tyrosine, hordenine and dorpan. Four substances which closely resemble tyramine in chemical composition, oxytyramine (hydrochloride, Merck), tyrosine (B.D.H.), hordenine (sulphate, Merck) and dorpan (Roche) were tested on *Calliactis* at concentrations between $1 : 10^4$ and $1 : 10^3$. None of them increased the size of the responses or caused responses to single stimuli. Except for slight depressant effects by tyrosine and oxytyramine they were completely ineffective.

Isoamylamine, ethylamine and oxyethylamine. The effects of a number of simpler amines on *Calliactis* were examined. Isoamylamine (hydrochlorate, Roche) had effects like those of tyramine, doubling the size of the response after 90 min. at a concentration of $1 : 5 \times 10^3$ and causing occasional responses to single stimuli. Ethylamine (hydrochloride, B.D.H.) had no effect at $1 : 10^4$ and had a depressant effect after 45 min. at $1 : 10^3$. Oxyethylamine or colamine (hydrochloride, Roche) was completely ineffective on *Calliactis* at concentrations of $1 : 10^4$ and $1 : 10^3$.

Cocaine. In vertebrates cocaine sensitizes tissues to the action of adrenaline and potentiates the response to sympathetic stimulation. It has been seen already that cocaine shows up the response to tyramine in a manner resembling its sensitizing effect in the vertebrates. Cocaine also enhances the response of *Calliactis* to stimulation. Fig. 3 A shows contractions which are about twice the normal size after 90 min. in cocaine at $1 : 10^4$. This result is similar to the augmentory action of cocaine on the nictitating membrane of the cat (Rosenblueth & Rioch, 1933). It must be noted, however, that cocaine does not cause responses to single stimuli

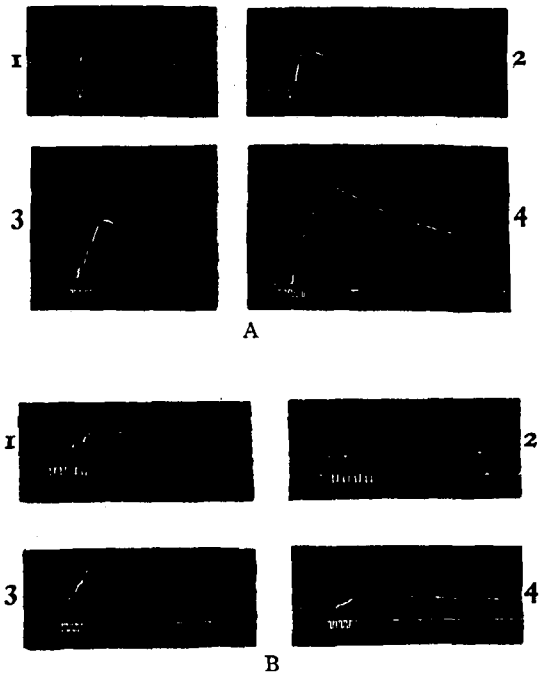

Fig. 3. Responses of *Calliactis* sphincter. A. Action of cocaine. Two stimuli at 1 in 0·5 sec. (1) in natural sea water; (2) after 90 min. in cocaine ($1 : 10^4$). Four stimuli at 1 in 0·5 sec. (3) in natural sea water; (4) after 90 min. in cocaine ($1 : 10^4$). B. Action of ergotoxine. Four stimuli at 1 in 1 sec. (1) in natural sea water; (2) after 150 min. in ergotoxine ($1 : 10^5$). Four stimuli at 1 in 0·5 sec. (3) in natural sea water; (4) after 150 min. in ergotoxine ($1 : 10^5$).

that it has no effect on the behaviour of the anemone. It is particularly interesting that the well-known local anaesthetic action of cocaine does not appear in tests on *Calliactis*.

Ergotoxine. Ergotoxine abolishes the response of vertebrate smooth muscles to adrenaline and to stimulation through the sympathetic system. It also acts on certain invertebrates. Bacq (1932) reported that ergotamine paralyses the peripheral mechanism of the chromatophores of cephalopods and also suppresses the adrenaline effect on these structures. Wu (1939) found that ergotoxine antagonizes the action of adrenaline on the earthworm gut, but it has no effect in the absence of adrenaline.

Ergotoxine reduces considerably the size of the response of *Calliactis* to stimulation. Fig. 3 B shows the effect of ergotoxine (ethanesulphonate, B.D.H.) at $1 : 10^5$ after $2\frac{1}{2}$ hr. and the reduction of the response to approximately one-quarter its initial size. This depressant action recalls the action of ergotoxine in the vertebrates, although the complete suppression of the response was never observed. It is unlikely that this ergotoxine effect is an example of the general depressant action observed with most drugs after several hours, because ergotoxine was applied here in a relatively dilute solution, the drug not being available in a more soluble form.

Tests were also made to see if there is any interaction between ergotoxine and tyramine on *Calliactis*. When the anemone is exposed to ergotoxine ($1 : 10^5$) for 3 hr. and then placed in sea water containing tyramine at $1 : 10^4$, responses to single stimuli do not occur, and the enhancement of the response that occurs is slight compared to the big increases recorded with tyramine alone. Thus there is an interaction between ergotoxine and tyramine in *Calliactis* that resembles the interaction between ergotoxine and adrenaline in other animals.

Although ergotoxine reduces the size of the response in *Calliactis*, the second stimulus of a series continues to be the first effective stimulus. Responses delayed beyond the second stimulus were not observed. In this respect the ergotoxine effect resembles the depressant effects of carbon dioxide and magnesium on the neuromuscular junctions in *Calliactis* (Ross & Pantin, 1940).

933F. Bacq & Fredericq (1934) showed that the nictitating membrane of the cat ceased to respond to adrenaline after 933 F (piperidinomethylbenzodioxane), although the response to stimulation of the post-ganglionic fibres remained unaffected. This effect raised difficulties for the simple theory of chemical transmission at sympathetic junctions and was widely discussed (Monnier, 1936; Cannon & Rosenblueth, 1937).

933 F has important effects on *Calliactis*. For the first hour at $1 : 10^4$ (*p*H 8·3), the response and behaviour of the anemone remain unchanged, but after 2 hr. a steady increase in the size of the response becomes apparent. By 3 hr. this enhancement is very great (Fig. 4 A), exceeding that caused by calcium and potassium (Ross & Pantin, 1940) and bigger even than the tyramine enhancement. In Fig. 4 A the height of a single response on the smoked record at an interval of 1 sec. is increased almost ten-fold and approaches the limit of contraction of the muscle.

Between 3 and 4 hr. at $1 : 10^4$ another effect appears. As with tyramine, responses to single stimuli occur, at first occasionally, but between 4 and 5 hr. almost without fail. Fig. 4 B shows two examples of this effect. The size of these responses to single stimuli varies considerably. At times they almost amount to a complete closure of the sphincter, and in most cases they are bigger than the responses to single stimuli caused by tyramine. However, frequent stimulation seems to cause a falling off in the size of these responses and sometimes leads to a temporary return to the normal condition with responses occurring on the second stimulus.

After 5 hr. the responses to single stimuli disappear and the size of the response begins to decline. Eventually the depressant effects associated with fatigue and long exposure set in (6–8 hr.).

Following a response to a single stimulus with 933 F a period of inexcitability occurs. This effect, which did not occur with tyramine, is shown in Fig. 4 B (2) where all stimuli after the first fail to cause a response. The duration of this inexcitable period varies from one experiment to another, but it is usually about 15–20 sec. before even a slight response can be obtained. Fig. 4 B (3) shows a state of partial recovery after an interval of about 40 sec. No explanation of this effect can be advanced, but it would seem that, in causing responses to single stimuli, 933 F in some way exhausts the response mechanism temporarily.

These effects on the response are the only 933 F effects observed on *Calliactis*. Spontaneous contractions and 'after-discharges' do not occur more often than usual even when the effect on the response is at its maximum, and the behaviour of the anemone remains normal throughout the tests. Thus 933 F, like the other effective drugs discovered, exerts its effect on the response to stimulation, without causing responses itself. It should be pointed out that in the case of 933 F there is no similarity between the nature of its effect on *Calliactis* and on vertebrate tissues which respond to it.

The effectiveness of a few drugs of this group immediately suggests that a process, related in some respects to the chemical process at adrenergic nerve-endings, exists in *Calliactis*. The ineffectiveness of adrenaline and other substances shows that there are certain differences in the chemical nature of these processes. But it is important to note that although certain sympathomimetic substances are effective, they do not by themselves cause responses as in the vertebrates. Therefore, it is likely that a different type of hypothesis from the chemical theory of

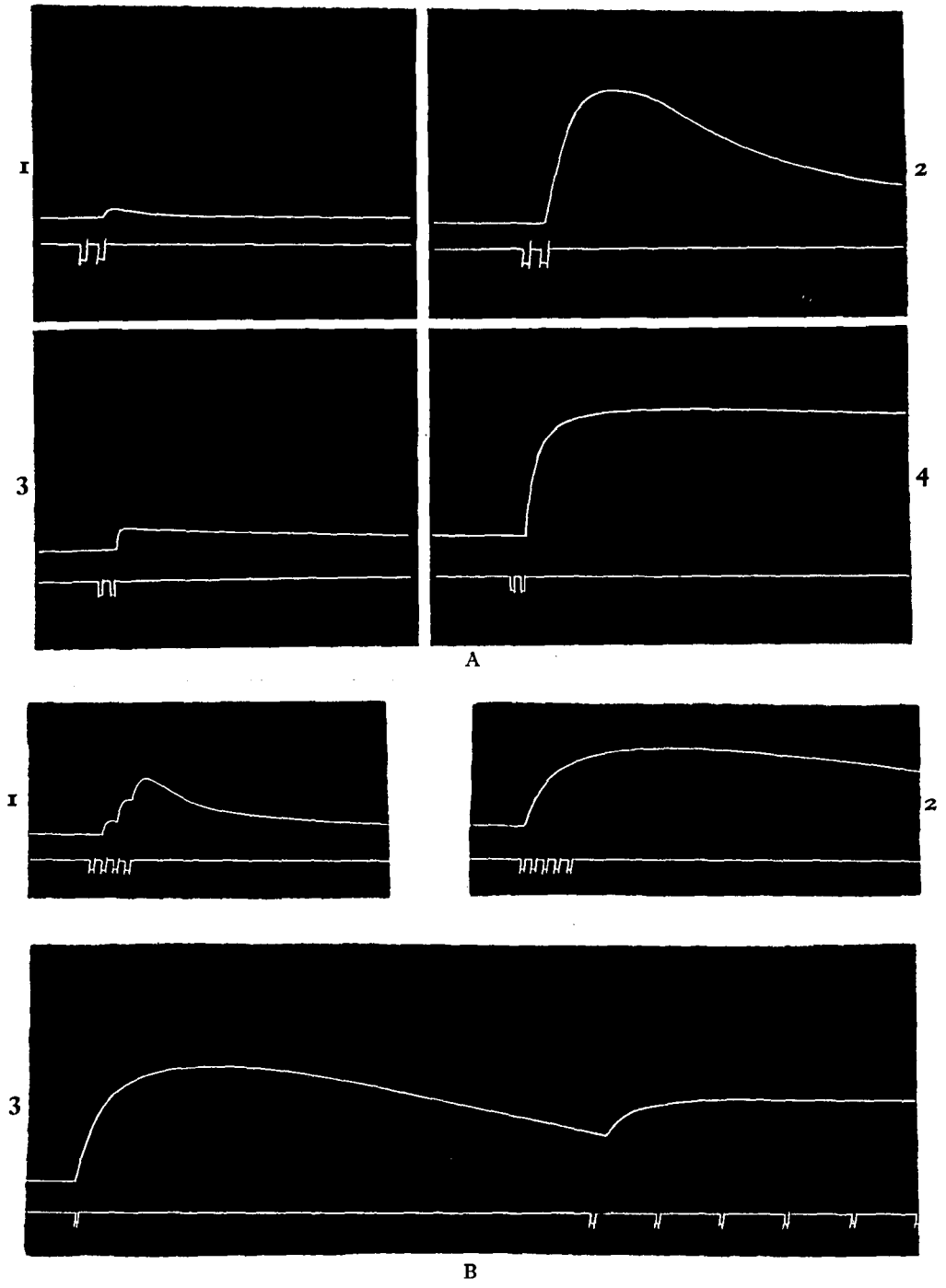

Fig. 4. Responses of *Calliactis* sphincter. A. Increase in size of response with 933 F. Two stimuli at 1 in 1 sec. (1) in natural sea water; (2) after 210 min. in 933 F ($1 : 10^4$). Two stimuli at 1 in 0·5 sec. (3) in natural sea water; (4) after 180 min. in 933 F ($1 : 10^4$). B. Responses to single stimuli with 933 F. (1) four stimuli at 1 in 1 sec. in natural sea water; (2) five stimuli at 1 in 1 sec. after 240 min. in 933 F ($1 : 10^4$); (3) one stimulus after 270 min. in 933 F ($1 : 10^4$) followed by second stimulus 40 sec. later and successive stimuli at intervals of 5 sec.

smission will have to be advanced to explain these effects.

Group 3

Certain other drugs which affect vertebrate nerve and muscle, but are not specifically associated with cholinergic and adrenergic mechanisms of transmission, were tested on *Calliactis*, viz. strychnine, histamine, pituitrin, guanidine and veratrine.

Strychnine. In the vertebrates strychnine sensitizes the reflex centres of the central nervous system and exaggerates the normal reflex response. Romanes (1885) found that strychnine stops the pulsations of the jellyfish *Cyanea* and brings about a tonic contraction of the muscles. Moore (1917) reported that strychnine has no effect on *Aurelia* but it excites starfish, shrimps and cephalopods. He concluded from this that strychnine only affects the more highly organized nervous systems.

Nothing resembling the excitant effect on vertebrates was observed in tests with strychnine on *Calliactis*. It did not cause spontaneous contractions and at $1 : 10^4$ it had no effect on the response. At $1 : 10^3$ the response diminished gradually and after 3 hr., when the size of the response had been greatly reduced, several stimuli were necessary to cause a response. Like similar effects with magnesium this is probably due to the breakdown of through conduction in the nerve net (Ross & Pantin, 1940).

Histamine. Histamine causes most vertebrate smooth muscles to contract, and this is attributed to a direct action on the muscle cells rather than to junctional effects. Histamine has no such effect on *Calliactis*. At $1 : 10^4$ the response remains unchanged. At $1 : 10^3$ the response is delayed beyond the second stimulus after $2\frac{1}{2}$ hr., and as this is accompanied by a big increase in the threshold the effect is probably due to inexcitability.

Pituitrin. Pituitrin, which like histamine is a general stimulant of vertebrate smooth muscles, is completely ineffective on *Calliactis*. The tests were carried out with 0·5 and 2·5 c.c. of pituitary extract (Parke Davis), i.e. 5 and 25 i.u., in 500 c.c. of sea water.

Guanidine. Guanidine has an excitant effect on many vertebrate smooth muscles, especially on the wall of the alimentary tract. Sereni (1928) reported that it has a stimulating action when applied to the peripheral nerves of cephalopods. Moreover, guanidine derivatives are well known constituents of tissues from lower invertebrates including the Actinozoa (Kutscher & Ackermann, 1933). Guanidine (nitrate, B.D.H.) was tested on *Calliactis* at concentrations of $1 : 10^4$ and $1 : 10^3$. Only general depressant effects were observed.

Veratrine. Veratrine causes a characteristic contracture of vertebrate skeletal muscle after stimulation. On *Calliactis*, veratrine causes a marked reduction in the speed and size of the contractions. After $2\frac{1}{2}$ hr. at $1 : 10^4$, the response to a pair of stimuli is only one-tenth its original size. This is too marked a decline to be a general effect.

The anemone shows a more marked direct response to veratrine than to any other drug tested. At $1 : 10^3$, spontaneous contractions occur frequently. However, there is evidence that this is due to a peripheral action rather than to a direct action on the muscles. When veratrine was tested on animals that had been exposed to magnesium to anaesthetize the sense organs, spontaneous contractions did not occur.

In general, these five drugs have not had effects that resemble their effects on vertebrates. The failure of general stimulants of vertebrate smooth muscle like histamine, pituitrin and guanidine is important in showing the difference between the general pharmacological properties of the two systems.

Group 4

Tests were carried out on a few substances which have been detected in anemones or other marine invertebrates. Some of the drugs in the other groups could also be placed in this category, viz. tyramine, histamine and guanidine. Three other substances found in marine invertebrates were tested on *Calliactis*: tetramethylammonium hydroxide, which has been extracted from *Actinia equina* and is believed to be the poison contained in the nematocysts (Ackermann, Holtz & Reinwein, 1923); trimethylamine oxide, which is found in the muscles of many marine animals (Kutscher & Ackermann, 1933); betaine hydrochloride, which is related to γ-butyrobetaine detected in *Actinia equina* (Ackermann, 1927). The only effect obtained from these substances was a marked depressant action by trimethylamine oxide. After 90 min. at $1 : 10^3$ this substance abolishes the response altogether. No other drug is so effective as a depressant agent.

CONCLUSION

The experiments described above have shown that the neuromuscular system of the sea anemone *Calliactis parasitica* is sensitive to certain drugs which have sympathomimetic effects on the vertebrates. There is no evidence of any action by drugs of the parasympathomimetic group such as acetylcholine.

One feature of the results has been the absence of any direct effects by the drugs. Out of more than twenty-five drugs tested, only nicotine and veratrine caused spontaneous contractions, and these appeared to be due to a peripheral and not a muscular action. Moreover, the drugs whose effects on the response are most marked, tyramine, tryptamine and 933 F, are singularly without effect in causing direct contractions of the animals. It is possible, however, that this absence of direct effects is related to the conditions of the experiments. We are working here with tissues that do not possess a vascular system as in the vertebrates, and drugs cannot be introduced

suddenly and at high concentrations at the particular spot where the effect is to be observed. The slow development of the responses to single stimuli caused by the effective drugs may be significant in this connexion. It is necessary, therefore, to bear in mind the limitations of the experiments and the delayed action of the drugs, although it seems unlikely that transmission by a single chemical step can occur in anemones in view of the absence of direct effects by the drugs.

The action of the effective drugs on *Calliactis* is different from that on vertebrates. Instead of direct excitation of the muscles by the drugs, the effects observed are confined to enhancement and sensitization. The latter effect takes the form of responses to single stimuli to which the anemone does not respond under normal conditions.

As a result of the action of ions on sea anemones, Ross & Pantin (1940) suggested that there are two processes in neuromuscular transmission: (1) a process of sensitization or facilitation which must precede (2) a process of excitation of the muscle. There was evidence that the first of these processes was chemical in nature, and the hypothesis was advanced that a 'facilitator' liberated at the nerve endings carried out the sensitization process. Some, but not all, the sensitization phenomena observed with certain drugs can be explained on this hypothesis.

On the 'facilitator' hypothesis three effects would be expected when an anemone is exposed to a substance with sensitizing properties: (1) enhancement of the response, (2) responses to single stimuli, (3) delayed decay of facilitation. The enhancing properties of the drugs tyramine, tryptamine and 933 F have already been noted and clearly agree with the expected result. It is a fact also that the same drugs cause responses to single stimuli, thus fulfilling the essential requirement of a sensitizing drug. However, there is a fortuitous element in this effect. The magnitude, duration and time of appearance of the responses to single stimuli vary within wide limits, and the effect appears and disappears abruptly without any transitional stages. These features of the effect would not be predicted by the hypothesis.

Additional experiments were carried out to test the third point, the effect of the sensitizing drugs on the decay of facilitation. If such an effect occurs, it should appear in the period of enhanced responses before the responses to single stimuli occur. Table 1 shows the result obtained when the effect of tyramine on the decay of facilitation was examined. Clearly, no significant change has occurred. The response to the second stimulus of a pair dies away when the interval between the stimuli is about 3 sec., exactly as in the normal animal. Tests with 933 F gave the same result. In this respect the effective drugs behave in the same limited way as calcium and potassium (Ross & Pantin, 1940), which enhance the response without affecting the rate of decay of facilitation.

Table 1

Interval between stimuli sec.	Height of response to four stimuli (*Calliactis*) mm. on smoked record	
	In natural sea water	After 90–110 min. in tyramine ($1:5\times10^3$)
0·5	19·0	35·0
1·0	12·5	23·0
1·5	5·0	10·5
2·0	2·4	5·5
2·4	0·6	1·2
2·6	0·2	0·5
2·8	Trace	0·2
3·0	0	Trace
3·2	0	0

It is clear that the effects on *Calliactis* of drugs like tyramine only partly correspond with the effects expected from truly sensitizing drugs on the facilitator hypothesis. We are left, therefore, with a rather complicated picture. The actinian neuromuscular system shows certain affinities with the sympathetic system of the vertebrates in the class of substances which affect the transmission of excitation to the muscles. But the similarity ends there. The mode of action of the substances on anemones shows that the transmitting system in these animals is very different from the direct chemical system in the vertebrates. These differences are only partly explained in terms of the facilitator hypothesis, which explained satisfactorily the special character of the response to stimulation and the action of ions on *Calliactis*. It must be left to future investigations to explain the anomalous features of the drug effects and provide a clearer picture than we have at present about the mechanism of facilitation.

SUMMARY

1. The action of a number of drugs which affect the neuromuscular systems of vertebrates has been examined on the sea anemone, *Calliactis parasitica*. In contrast to their action in vertebrates, no drugs directly cause contraction in the muscles of the anemone.

2. Some drugs of the group which act at cholinergic junctions in the vertebrates, including acetylcholine itself, were ineffective on *Calliactis*. Atropine and nicotine, of the same group, had depressant effects.

3. Some drugs of the group which act at adrenergic junctions in vertebrates, including adrenaline itself, were ineffective on *Calliactis*. On the other hand, tyramine, tryptamine and 933 F enhanced and sensitized the response to nervous excitation. Thus responses to single stimuli occurred, whereas normally responses only follow the second and

subsequent stimuli. Cocaine enhanced and ergotoxine depressed the responses of *Calliactis* in a manner analogous to their effects in vertebrates.

4. No significant effects were observed with other substances, including strychnine, veratrine, histamine, betaine and tetramethylammonium hydroxide.

5. The limitation of the drug effects to enhancement and sensitization supports the view that neuromuscular transmission in *Calliactis* cannot be a simple chemical process as in the vertebrates. In general the results support the hypothesis that there is a separate process of sensitization which must precede the excitation of the muscle in these animals. On the other hand, the drugs which have sensitizing effects on *Calliactis* do not possess all the properties that would be expected of a natural 'sensitizer' or 'facilitator' carrying out this process of sensitization at the nerve ending.

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