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THE ACTION POTENTIALS IN *MAIA* NERVE BEFORE AND AFTER POISONING WITH VERATRINE AND YOHIMBINE HYDROCHLORIDES.

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In a previous communication [Cowan, 1933] the effect of veratrine on the action potentials in nerve trunks from the walking legs of *Maia squinado* was described. Using a sensitive galvanometer of 3·8 sec. period, it was found that the initial deflection due to a single shock was increased about twentyfold, as compared with that in unpoisoned nerve trunks, and the resulting "retention" of action potential or "after negativity" was also greatly increased both in magnitude and duration, up to half an hour elapsing before the galvanometer spot returned to the base line. With the instrument available it was possible to study the course of the disappearance of the "retention," but the question of whether the initial deflection is due to a repetitive response or to some alteration in the size or duration of the action potential wave, particularly the high voltage components, was left unanswered. Some experiments with a valve amplifier and an oscillograph were begun primarily to settle this question, but they led us to investigate also the action potentials in unpoisoned nerves and in nerves poisoned with yohimbine hydrochloride.

Tait and Gunn [1908] and Tait [1909] showed that yohimbine salts prolong both the absolutely and relatively refractory periods of medullated nerve, and Waller [1910], using a Thomson galvanometer of about 1 sec. period, showed that yohimbinized frog's nerve when subjected to tetanic stimulation gave "a series of fugitive negative variations in which the positive after deflection was particularly evident." We are not aware that any experiments have been done on the effect of yohimbine salts on the action potentials in non-medullated nerve; consequently in addition to making oscillographic records we studied the slower after-potentials with a sensitive Downing galvanometer.

Apparatus, materials and method.

(a) *For the oscillographic records of the action potentials.*

The nerve was suspended in moist air in a paraffin-wax chamber which was closed by a glass cover, and the action current was led off to a three-stage amplifier by electrodes consisting of platinum plates. A monophasic response was obtained by crushing the peripheral end of the nerve as it lay on the electrode and putting on it a drop of isotonic potassium chloride solution. A diagram of the nerve (Fig. 1) shows the dispositions of the leading-off electrodes and of the platinum-wire stimulating electrodes.

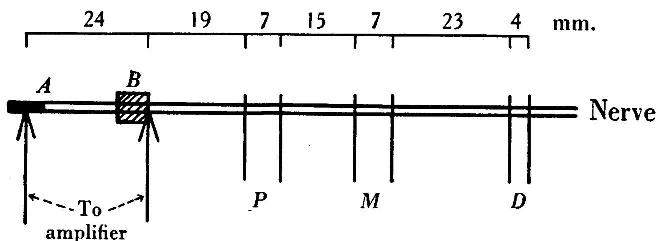

Fig. 1. The positions of the leading off and stimulating electrodes. *P*, the peripheral stimulating electrodes. *M*, the medial stimulating electrodes. *D*, the distal stimulating electrodes. *A*, the region depolarized by isotonic potassium chloride solution. *B*, platinum plate.

A resistance-capacity coupled amplifier was employed, the circuit of which is shown in Fig. 2. In the absence of proper equipment, we had to improvise an oscillograph from a moving-iron loud-speaker unit. This necessarily required a considerable amount of power in the last stage of the amplifier, and, no adequate batteries being available, we decided to use the mains. The anode current for the power stage of an amplifier with long time constant cannot ordinarily be supplied from the mains, owing to the relatively high resistance of the rectifying valve (and smoothing chokes, if used). The variations in the anode current resulting from the application of a signal to the grid bring about variations in the voltage supplied by the rectifier, and so introduce distortion. These variations cannot easily be eliminated by a decoupling circuit, when the time constants of the interstage couplings are long. The use of a push-pull power stage, however, obviates this difficulty, since the total anode current is independent of the signal voltage applied to the grids, provided that the valves are adequately matched and correctly biassed. In this circuit, also, the standing anode current is not passed through the oscillograph; if this were done in our case, the oscillograph reed became magnetically saturated and, of course, behaved anomalously. Since a transformer cannot be used if a long time constant is desired, a paraphase circuit must be used to introduce the necessary phase displacement of the signal applied to the grid of one of the power valves.

The anode current for the intermediate amplifying and paraphase stages may also be supplied from the mains, since the variations in the current taken by these stages is negligible compared with that taken by the power stage. A small mains ripple was at first

apparent in our oscillograph, when a 10,000 ohm resistance was connected across the input terminals; this was largely balanced out by choosing a suitable value for the anode resistance in the paraphase stage, a ripple with an amplitude of about 0·5 mm. (corresponding to 0·1 millivolt applied to the input terminals) being left in to serve as a time marker. The input to the two power valves was equalized by adjustment of the grid potentiometer which fed the paraphase valve. The anode current of the first amplifying stage was supplied by dry batteries not so much on account of the ripple introduced, as on account of the large and random fluctuation of the mains voltage. The filaments of all except the power valves were run off accumulators; the filaments of the second and paraphase stages could have been run off the mains without introducing ripple, but not that of the first stage.

The amplifier was shielded by mounting all except the power stage on an earthed copper plate. The grids of the first and second valves and the electrode leading from the uninjured part of the nerve were all earthed.

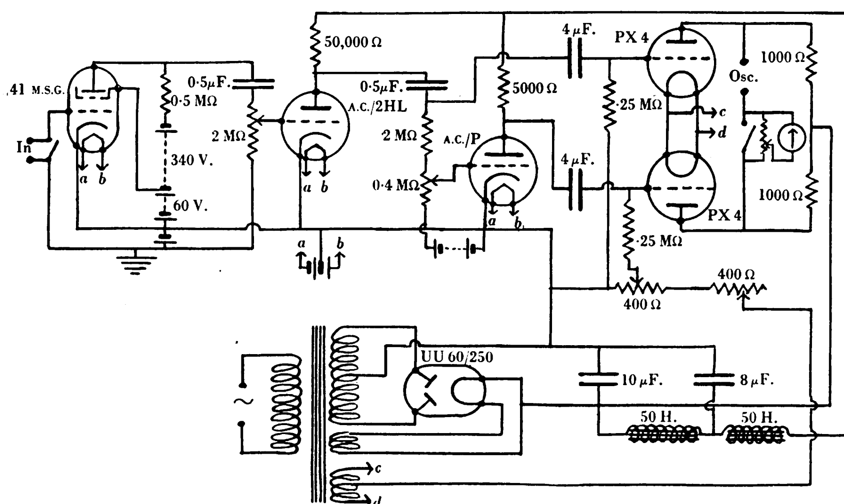

Fig. 2. A circuit diagram of the amplifier.

An Ormond moving-iron loud-speaker unit (resistance 1000 ohms) was adapted to serve as an oscillograph. The unit was dismantled and the rod which was intended to serve as a connection between the loud-speaker cone was removed and the reed filed down to about half its former thickness in order to increase the possible amplitude of movement, and the unit was re-assembled. On the reed as near as possible to the speech coil was mounted an ophthalmoscope lens of 1 m. focal length with its back surface silvered. As nearly as we could judge, the period of mechanical vibration of the moving part of this improvised oscillograph was about 0·3–0·5 msec. Calibration by condenser discharges indicated that the reed took about 0·5–1 msec. to reach the maximum deflection that we could use without overloading the power valves. Before the

oscillograph a vertical slit illuminated by a 6 volt 24 watt head-lamp bulb was so arranged that a sharp image was obtained at a distance of about $4\frac{1}{4}$ m. The horizontal movements of this image were recorded on fast ciné bromide paper using a Cambridge falling-plate camera in the usual way. The overall sensitivity of the amplifier and oscillograph was about 6·5 mm. per millivolt. Since the sensitivity of the oscillograph itself was about 0·45 mm. per volt, this indicated a total voltage amplification of about 14,500.

The stimuli, usually single condenser discharges, were applied to the nerve at different distances from the peripheral end by three pairs of platinum wire electrodes (Fig. 1). The condenser which had a capacity of 1·0 microfarad was charged to a potential of 20 volts, the shunt resistance had a value of 1500 ohms and the series resistance a value of 3000 ohms. We had intended to use optimal stimulation [Hill, 1932] and the strength of stimulus which we employed certainly would have been just maximal at 17° C. [Beresina and Feng, 1933], but a subsequent examination of our action current records has led us to conclude that it was not maximal at 12° C. The importance of this matter is discussed later.

At first the stimulator picked up a good deal of interference and conveyed it to the amplifier causing the oscillograph mirror to vibrate. Presumably this was by virtue of some capacitative effect, since the wires of the lighting circuit of the room in which we worked were imperfectly shielded. The trouble was eliminated by enclosing the whole of the stimulating apparatus, except for the Morse key and necessary leads, in an earthed copper box. Since the leading-off electrode from the uninjured part of the nerve went to the earthed side of the amplifier input, it was necessary to insulate the apparatus from the box because, if this were not done, leakage from the stimulating circuit to earth produced large artefacts on the records when the key was depressed. The use of a plate 1 cm. long as the earthed leading-off electrode also helped to prevent the stimulating currents from leaking down the nerve past this electrode and so reaching the "live" lead to the amplifier. However, we did not quite eliminate this stimulus escape because it served as a convenient method of signalling the moment of application of the stimulus on the records which were made to determine the velocity of the nerve impulse.

After these adjustments had been made the performance of the whole apparatus was examined. The time constant was determined from oscillograph records made when known small E.M.F.'s of 1, 2 and 3 millivolts were applied to the input terminals. The E.M.F.'s were obtained

by opening a key short-circuiting part of a rotary potential divider in series with the 10,000 ohms across the amplifier. The mean value of the time constant was 0·15 sec. From these experiments, others in which a known steady E.M.F. was applied to the amplifier input for a short time, and records made when condensers of known capacities were discharged through known resistances we concluded that we should be justified in measuring the records of the action potential spikes to within about $\pm 0{\cdot}1$ mm. along the time axis and to $\pm 0{\cdot}5$ mm. along the potential axis, *i.e.* to within $\pm 0{\cdot}5$ msec. and $\pm 0{\cdot}10$ millivolt, respectively; records of later events however would need either correction or comparison with controls.

The nerves used were dissected by Levin's [1927] method. After being allowed to rest for an hour in aerated sea water a nerve was mounted, a few drops of isotonic potassium chloride solution were put on the peripheral end to allow monophasic recording, and then a few test stimuli were given. After a further quarter of an hour to permit recovery from any effects of the mounting process a series of four or five records was made, and on the same piece of bromide paper one or two records were made when a known calibration E.M.F. was thrown into the circuit by opening the key short-circuiting part of a rotary potential divider in series with the nerve. Two or three series of records having been taken, isotonic potassium chloride solution was dropped on to the nerve between the stimulating and leading-off electrodes in order to be certain that there were no physical artefacts other than the small amount of stimulus escape needed as a signal in the determinations of the velocity of the nerve impulse.

The nerves, which were treated with veratrine or yohimbine hydrochloride, were transferred at the end of the first half-hour's washing in sea water to sea water to which had been added one of these substances in the desired concentration. In the veratrine solutions a concentration of 1 in 25 millions was used, and in the yohimbine solutions a concentration of 1 in 20,000. After half an hour in one of these solutions the nerve was taken out, mounted, and isotonic potassium chloride solution dropped on the peripheral end. With the veratrinized nerves the test stimuli were omitted and recording was begun immediately and about 20 min. were allowed between each stimulus when full recovery was desired. Yohimbinized nerves were treated in the same way as normal nerves. Every nerve at the end of an experiment was blocked with isotonic potassium chloride solution and control records were made.

(b) *For the study of the slow components of the action potential wave in yohimbinized nerve.*

For this work a Downing galvanometer of sensitivity $2 \cdot 7 \times 10^{-10}$ amp. at 3 m. and period 0·67 sec. was used critically damped, and the movements of the image of an illuminated slit which was placed before the galvanometer mirror were recorded with the falling-plate camera. Time marks were obtained on the records by allowing a metronome to interrupt the light beam at 1 sec. intervals. The nerve was mounted in a paraffin-wax chamber and the monophasic action potentials were led off to the galvanometer through calomel half cells, the injury potential being partly balanced in a manner similar to that described elsewhere [Cowan, 1934]. Single shocks, which were given by a stimulator similar to that used for the oscillographic experiments, were applied by platinum wire electrodes, and since neither of the electrodes going to the galvanometer was earthed, it was possible to obviate all stimulus escape by interposing between the stimulating and action current electrodes a platinum wire connected to earth.

The nerves were prepared and treated in the way already described. A series of records of the action potentials in normal and yohimbinized nerves was made, each experiment being ended by blocking the nerve between the stimulating and leading-off electrodes with isotonic potassium chloride solution and making control records to detect artefacts.

NORMAL NERVES.

Results.

Inspection of the oscillograph records (Pl. I, 1) shows that the time occupied by the high-voltage components of the action potential complex becomes greater (0·03–0·07 sec.) as the stimulating electrodes are moved to positions further from the leading-off electrodes, and it is fairly clear that there are present at least two impulses which travel in the nerve trunk with different velocities. In order to evaluate the two principal velocities we proceeded in the following way. On each curve the distance between the signal mark, due to the stimulus, and the beginning of each deflection, due to an action current impulse, was measured. The time interval (t) represented by this distance comprises the latent period (l) of the nerve plus the time taken by the impulse in question to travel with a velocity v, a distance s from the point of excitation to the more central

of the two leading-off electrodes. Thus, for a shock from the proximal stimulating electrodes,

$$t_p = (l + s_p/v) \text{ sec.}$$

where $s_p = 19$ mm.; similarly for a shock from the medial electrodes

$$t_m = (l + s_m/v) \text{ sec.},$$

where $s_m = 41$ mm., and for the distal electrodes

$$t_d = (l + s_d/v) \text{ sec.},$$

where $s_d = 71$ mm.

From the above equations it is possible to calculate v and l. Different values of the velocity of the faster impulse calculated by this method lay between 2·65 and 2·30 m. per sec. with a mean value of about 2·5 m. per sec. at 12° C. Owing to the relatively slow speed of the recording paper our records were not sufficiently good to permit the latent period of the nerve to be calculated with any accuracy from the above equations, but they suggest that the latent period was probably not greater than 0·5 msec. The maximum spike voltages observed in different nerves ranged from 0·9 to 3·1 millivolts,and the usual value in a good preparation was 2·8–3·0 millivolts. The duration of the rising phase of the spike was from 3 to 5 msec. Because of the long duration of the "retention" or after-potential due to the fast impulse it was practically impossible to perceive in the records the beginning of the spike of the slower impulse. In many cases we attempted to get over this difficulty by measuring the interval from the signal marking the moment of stimulation to the attainment of the second peak, thus including the duration of the rising phase of the slower spike as well as the latent period of the nerve in each measurement and then in the calculation by the method already given these two were treated as a single term. The velocities of the slower impulse so found ranged from 0·75 to 1·20 m. per sec. and the mean value was about 1·0 m. per sec. The spike voltages in different nerves lay between the limits of 0·5 and 2·8 millivolts, and the average nerve in good condition gave about 1·5 millivolts.

Discussion.

If it is assumed that Lucas's [1908] value of $Q_{10} = 1{\cdot}79$ for the temperature coefficient of the velocity of the nerve impulse holds for non-medullated nerve, then our result of 2·5 m. per sec. for the faster impulse in *Maia* nerve is in agreement with those reported at higher temperatures by Carlson [1906] who used nerve-muscle preparations from an American spider crab, probably at 22–25° C.; by Barnes [1930] who determined the velocities of motor impulses in *Cancer pagurus* with a Matthews'

oscillograph; and by Monnier and Dubuisson [1931] who found a value of 5 m. per sec. when they examined in detail with a cathode-ray oscillograph the impulses set up by electrical stimulation of isolated nerve trunks from *Callinectes sapidus* Rathbun, at 22–23° C. If the same temperature coefficient applies to the slower impulse, then our value for *Maia* nerve is a little high compared with that found by Monnier and Dubuisson in their experiments at 22–23° C. (1·5 m. per sec.), but it is possible that the difference may be connected with the difference in the species of animals used. Similarly, and as would be expected at the lower temperature, the remaining quantities—the latent period (upper limit), the duration of the rising phase of the faster impulse, and the duration of the whole high voltage complex—are of the same order of magnitude, but greater than those reported by Monnier and Dubuisson, thus indicating that, although there may be minor differences in these quantities, due to difference in species, temperature is the main factor governing their size.

Besides the other papers mentioned above there is a recent one by Lullies [1933] in which he has attempted to correlate the velocities of the impulses observed in nerve trunks from the Mediterranean variety of *Maia* with the anatomical structure of the nerve, *e.g.* diameter of fibres, and presence or absence of a medullary sheath. He was able to observe three different impulses in the nerve trunks and to relate these impulses to three different sets of fibres which could be distinguished on microscopic examination of cross-sections of the trunk. However, in the velocity measurements he used a string galvanometer with the relatively long period of 1/70 sec. and in the light of the papers quoted and our own results with *Maia* nerve there seems little doubt that his values of about 3·1 m. per sec. and 1·3 m. per sec. at 23–25° C. for the two faster impulses are rather low.

Monnier and Dubuisson did not find the third slow impulse which Lullies observed, nor did we find it. Now, Monnier and Dubuisson's, Lullies's and our own results all have one striking feature in common—that is the small spike voltages observed. On the one hand, with induction shocks for stimulation of the nerve trunks, Monnier and Dubuisson in their experiments and Lullies in his, observed spike voltages of about 1 millivolt for the faster impulse and much smaller ones for the slower impulse; on the other hand, with low voltage condenser stimuli of relatively long duration, we obtained spikes of about 3 millivolts for the faster impulse and about $1\frac{1}{2}$ millivolts for the slower impulse. Yet, none of these spike voltages is more than a small part of the injury poten-

tial (30 millivolts [Cowan, 1934]), whilst in frog's medullated nerve the spike voltage from monophasic leads approaches closely to the injury potential [Rosenberg, 1927, 1929]). From the evidence given later in this paper in the section on veratrinized nerve and in an earlier paper [Cowan, 1933], there is no doubt that it is possible for the spike voltages to be very nearly equal to the injury potentials in *Maia* nerve; therefore we conclude that in these experiments our stimuli were not maximal, and that there was a good deal of short circuiting by inactive fibres. We cannot say how effective our stimuli were, but obviously we failed to stimulate the slowest set of fibres, which according to Lullies may comprise up to one-third of the cross-section of a *Maia* nerve trunk.

VERATRINE POISONED NERVES.

Results.

In Pl. I, 2, are the records of two typical responses from a veratrinized nerve. In spite of the prolonged after-potential the spike due to the second impulse can still be perceived. The velocities of the two impulses (2·5 and 0·9 m. per sec.) and their spike voltages (2·7 and 2·1 millivolts) do not differ from those observed in normal nerves, and the duration of the rising phase of the faster impulse is unchanged.

From a first inspection of the records the after-potential appears immediately the maximum spike voltage has been attained and from comparison with a control record, which indicates the correction necessary owing to the effect of the discharge of the coupling condensers of the amplifier, to continue at about the same level for at least 450 msec. However, a closer examination shows that the records differ from the control in two respects. The first difference is more marked in the record of the response to a shock at the proximal stimulating electrodes: it is made clearer if the part of the record representing the after-potential is plotted with a more open scale of ordinates (Fig. 3), and if on the same figure the control curve is also plotted after having its ordinates multiplied by a factor so as to make it start at the same point as the after-potential curve. The after-potential curve lies above the control, and at about 125 msec. after the moment of stimulation a "hump" begins and lasts for a further 100 msec. The second difference between the after-potential and control curves is seen in both of the responses—during the after-potential and superimposed upon it are small rapid potential waves. In order to test whether these were due to a repetitive response with partial fusion of the impulses, we made records of the response to a

second stimulus given within a few minutes of the first, and records of the response to tetanic stimulation. Pl. I, 3, shows the responses to two single shocks, the lower given within $6\frac{1}{2}$ min. of making record *P* of Pl. I, 2, and the upper about a minute later, *i.e.* whilst the nerve was showing a large "retention." The spikes are indistinguishable from those given by normal nerve and the after-potential show practically no prolongation. After a further 20 min. had been allowed for recovery the

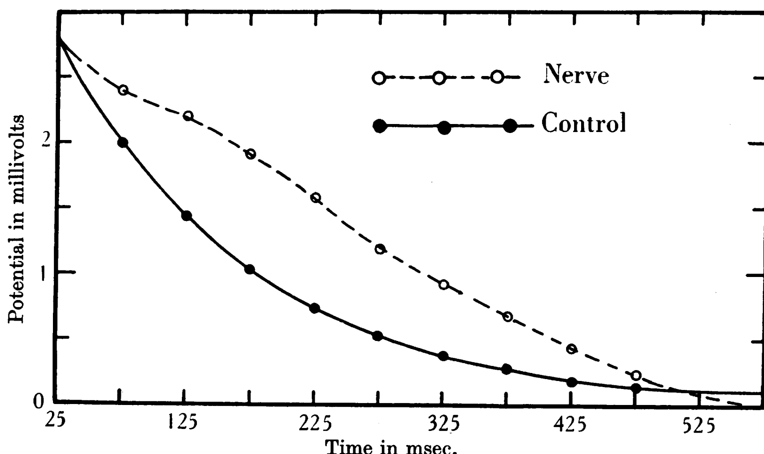

Fig. 3. Part of a record (Pl. I, 2*P*) of the after-potential in a veratrinized nerve, plotted on an enlarged ordinate scale. The point at which the curve starts is marked by an arrow on the original record. The control starting at the same point shows the purely physical effect due to the discharge of the coupling condensers in the amplifier.

nerve again gave a typical veratrine response, although the after-potential was not quite so well developed as in the earlier records. Pl. II, 4 is part of a record of the response given by an unpoisoned nerve to tetanic stimulation at a frequency of 25 per sec., and Pl. II, 5, is part of a record of the response given by a veratrinized nerve to tetanization. After the first impulse there is little difference between the responses from normal and veratrinized nerve; certainly in veratrinized nerve there is no sign of fusion of the impulses.

Discussion.

Judging from the spike voltages, the amount of short-circuiting, due to our failure to stimulate the slowest group of fibres, must have been as great in the veratrinized nerves as in the normal nerves already discussed. However, the main result of our oscillographic study has been to show

that the characteristic response of veratrinized *Maia* nerve is not repetitive, but it is due to a greatly prolonged after-potential which appears immediately the maximum spike voltage has been attained, and continues for at least 450 msec. These observations, when linked with the earlier galvanometric experiments of one of us [Cowan, 1933], lead to the conclusion that the after-potential, starting immediately after the attainment of the spike, may last for a total period of half an hour. In the galvanometric experiments at 17° C. the stimuli were maximal, and the "retentions" or after-potentials were usually nearly equal to the injury potentials (*e.g.* injury potential 27·4 millivolts and "retention" 22·6 millivolts): these experiments are part of the evidence mentioned earlier that, had the stimuli been maximal in the oscillographic experiments, much larger spike voltages would have been obtained.

Whilst in their essential points our results agree with Graham and Gasser's [1931] oscillographic analysis of the action potentials in frog's medullated nerve incompletely poisoned with veratrine, with Fromherz's [1933] galvanometric experiments with frog's nerve more completely poisoned, and with Garten's [1899] study, by means of the capillary electrometer, of the action potentials in the non-medullated olfactory nerve of the spike after poisoning with veratrine, they also illustrate how much slower the recovery process is in *Maia nerve.*

Two further interesting points arise from our experiments. The first is the "hump" in the after-potential. Two explanations of this are possible: it may be that veratrine lowers the stimulation threshold of the third group of fibres [Lullies, 1933] in the nerve trunk, allowing an impulse to be propagated with the low velocity of 0·13–0·10 m. per sec.; alternatively, the "hump" may be a delayed rising phase of the after-potential such as has been described by Graham and Gasser [1931; see also Gasser, 1934] in veratrinized frog nerve. Because of the long duration of the "hump" and the absence of any sign of a spike of a third wave, we would reject the first possibility in favour of the second. In the records from unpoisoned nerves there is no clear evidence of a rising phase of the after-potential, but in view of the limitations of our apparatus as compared with the cathode-ray oscillograph, this is not surprising. It is also interesting to note that the corrected capillary electrometer records which Garten [1899, Pl. V] gives of the action potentials in the slow (conduction velocity 0·2 m. per sec. according to Nicolai, 1901) non-medullated pike nerve after poisoning with veratrine show a distinct rising phase of the after-potential. The second point concerns the rapid potential wavelets which we found superimposed on the after-

potential; they were not observed by Graham and Gasser [1931] in frog nerve nor were they usually observed in the pike olfactory nerve by Garten [1899], although one of his published records shows some oscillations. Similar wavelets in the electrical response of frog's muscle have however been studied by Hoffmann [1912] who has shown that poisoning with weak doses of veratrine gives a prolonged response of an oscillatory character; while treatment with stronger doses gives a smooth non-oscillatory response [see also Lamm, 1911, 1912].

Yohimbine poisoned nerves.

(a) *Results obtained with the amplifier and oscillograph.*

In Pl. II, 6, are given some typical records from yohimbine poisoned nerves. Again, two impulses can be distinguished. The velocity of the faster impulse, the duration of its rising phase and the spike voltage do not differ from those observed in unpoisoned nerve. Almost immediately the spike has attained its maximum voltage it is followed by a positive after-potential which develops a voltage up to two-thirds of that of the spike. In the records a descending E.M.F. can be seen to occur immediately after the spike maximum, but the rapid rate of decrease suggests that the ordinary negative after-potential, which is observed in unpoisoned nerve, is largely suppressed. Later and superimposed upon the positive after-potential is the spike of the second impulse, and this in its turn is succeeded by another positive after-potential which reinforces that remaining from the first impulse. The second phase of after positivity lasts from 0·1 to 0·2 sec.

Owing to the low solubility of yohimbine hydrochloride in sea water 1 in 20,000 was the maximum concentration which we could use without increasing the acidity very considerably. Weaker solutions merely gave smaller positive after-potentials, without any other change in the shape of the response.

In a further experiment, after mounting a normal nerve in the usual way, we made a number of records and then applied a solution of yohimbine hydrochloride in sea water in the neighbourhood of the leading-off electrode to the amplifier from the uninjured part of the nerve. After about 3 min. we were able to make records of the characteristic yohimbine response.

(b) *Results obtained with the Downing galvanometer.*

Pl. II, 7, gives a record of the yohimbine response and Pl. II, 8, gives a record of the action current in an unpoisoned nerve for comparison.

The galvanometer was sufficiently rapid to record ballistically both the spike and the positive after-potential in yohimbinized nerve, and what is more important, it was possible to detect a low voltage negativity following the positive after-potential. This small negative after-potential, which continued for more than 15 sec., was of about the same magnitude as the low-voltage component of the ordinary after-potential in unpoisoned nerve.

We attempted to increase the size of the positive after-potential by tetanizing the poisoned nerve for half a minute before the test shock, but this produced no measurable effect on the galvanometer deflections.

Discussion.

Waller's [1910] experiments on frog nerve poisoned with yohimbine and our experiments on crab nerve, also poisoned with yohimbine, give us no reason to believe that the positive after-potentials observed differ from the smaller ones which have obtained in unpoisoned frog nerve. That we have been able to produce positive after-potentials by treating the nerve in the neighbourhood of the lead from the uninjured part with yohimbine hydrochloride solution, indicates that these potentials are not due to activity in the depolarized region. The point is of interest in view of the controversy [for references see Gerard, 1932] which has been in progress, although recently Gasser [1934] has withdrawn his former opinion that such positive after-potentials in unpoisoned nerve were artefacts.

The positive potentials which have been reported in unpoisoned frog nerve after single stimuli by Amberson and Downing [1929] seem to have been smaller than those which have been reported by other workers [Garten, 1910; Sochor, 1912; Woronzow, 1924] in unpoisoned frog nerve which had been subjected to tetanization. Woronzow noted this influence of tetanization in enhancing the positive after-potential, and suggested that it depends in some way on the fact that tetanization produces a negativity of a more stable kind than is produced by a single impulse. It seems to us that, according to Woronzow's suggestion, the "retention" set up in *Maia* nerve by tetanization should be followed by an after-positivity much greater than that which can be obtained in frog nerve. But, as far as we are aware, positive after-potentials have never been observed in *Maia* nerve. Levin [1927] looked for them, but could not find them.

After consideration of our own experiments on yohimbinized *Maia* nerve we suggest a rather different explanation of the data mentioned

above. In our experiments the suppression of the negative after-potential was a very noticeable feature, and we were unable to increase the positive after-potentials in poisoned nerves by preliminary tetanization. Now tetanization of unpoisoned nerve at any reasonable frequency also means a partial suppression of the normal after-potential. This suppression of the normal after-potential rather than previous negativity seems to favour the development of positive after-potentials.

Summary.

1. An amplifier incorporating a paraphase-fed push-pull output stage is described; this could be driven from the A.C. supply mains without introducing appreciable distortion, so that a large high-tension battery was unnecessary.

2. In nerves from the walking legs of *Maia squinado* there are at least two sets of fibres in which impulses are conducted with very different velocities. At 12° C. the velocity of the faster impulse is about 2·5 m. per sec. and that of the slower impulse about 1·0 m. per sec.

3. In *Maia* nerves poisoned with veratrine a prolonged after-potential begins immediately after the spike maximum has been attained and gradually disappears in half an hour. A "hump" which appears in the records at about 125 msec. after the moment of stimulation probably indicates that veratrine produces a delayed and long-continued rising phase in the after-potential.

4. The poisoning of *Maia* nerve with yohimbine hydrochloride causes the spike potential to be succeeded by positive after-potentials which may last up to 0·2 sec. Since these positive potentials can be produced by treatment of the uninjured part of the nerve, they are not due to changes in the depolarized region.

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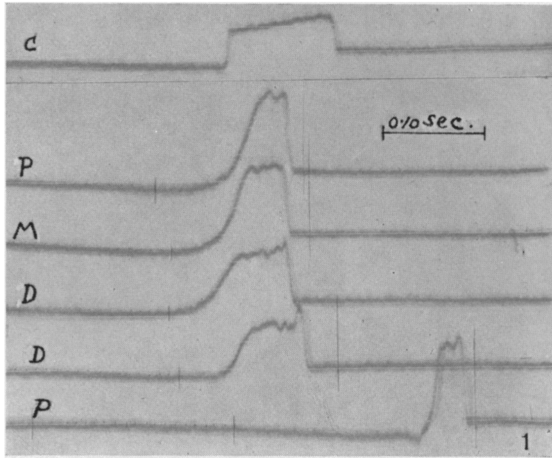

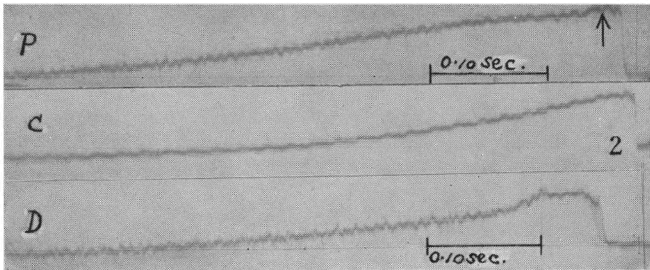

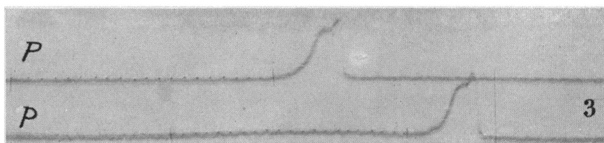

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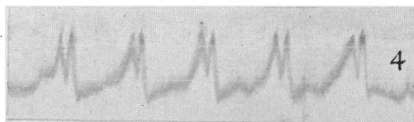

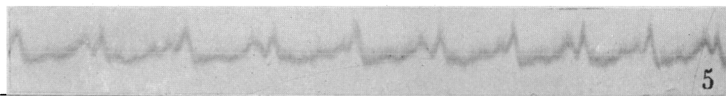

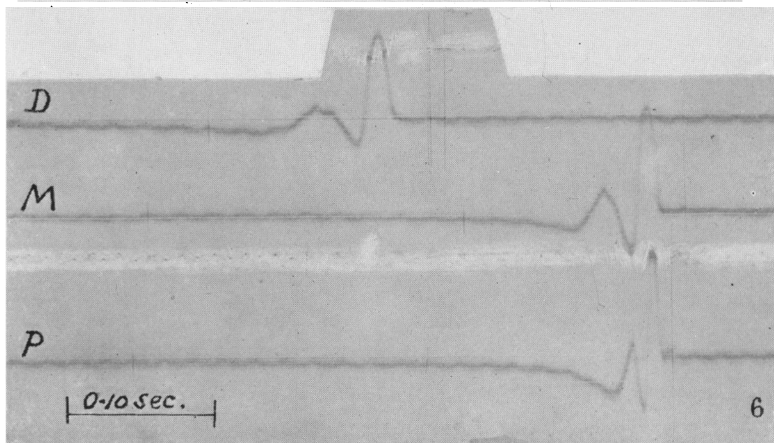

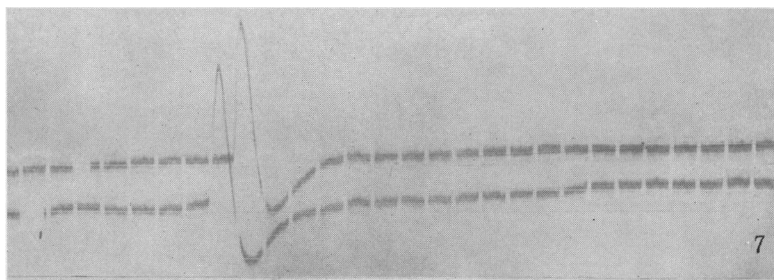

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EXPLANATION OF PLATES I AND II.

PLATE I.

1. Records of the action potentials in normal nerve. The letters *P*, *M* and *D* indicate that shocks were given at the peripheral, medial and distal stimulating electrodes. *C*, is a control made by applying an E.M.F. of 1 millivolt to the amplifier with the nerve in circuit.
2. Records of the action potentials in veratrinized nerve in response to stimuli at the proximal (*P*) and distal (*D*) electrodes. *C* is a control, 2 millivolts to the amplifier input, showing the course of the discharge of the coupling condensers of the amplifier. The arrow at 25 msec. in the record *P* indicates the point from which the enlarged curve, given in Fig. 3, begins.
3. The action potentials given by the same nerve which was used for the above records (2), but made whilst it was exhibiting a considerable "retention."

PLATE II.

4. Part of a record of the action potentials in an unpoisoned nerve which was tetanized at a frequency of 25 per sec.
5. Part of a record of the action potentials in a veratrine poisoned nerve, also during tetanization.
6. The action potentials in a yohimbine poisoned nerve in response to single shocks at the peripheral (*P*), medial (*M*) and distal (*D*) stimulating electrodes.
7. Two photographic records of the deflections of a Downing galvanometer produced by the action currents in yohimbine poisoned nerve in response to single shocks. The gaps are at 1 sec. intervals.
8. Two records made under the same conditions as (7), but with an unpoisoned *Maia* nerve. The gaps are at 1 sec. intervals.