water at pH 8, and the suspension filtered. The active protein was found in the colorless filtrate. This procedure was repeated twice in order to remove completely the inactive protein. Crystallization was accomplished by adding slowly, with stirring, a solution containing 1 cubic centimeter of glacial acetic acid in 20 cubic centimeters of 0.5 saturated ammonium sulfate to a solution of the protein containing sufficient ammonium sulfate to cause a faint turbidity. Small needles about 0.03 millimeters long appeared immediately and crystallization was completed in an hour. Crystallization may also be caused by the addition of a little saturated ammonium or magnesium sulfate to a solution of the protein in 0.001 N acid. Several attempts to obtain crystals by dialyzing solutions of the protein gave only amorphous material. To date a little more than 10 grams of the active crystalline protein have been obtained.

Although it is difficult, if not impossible, to obtain conclusive positive proof of the purity of a protein, there is strong evidence that the crystalline protein herein described is either pure or is a solid solution of proteins. As yet no evidence for the existence of a mixture of active and inactive material in the crystals has been obtained. Tobacco-mosaic virus is regarded as an autocatalytic protein which, for the present, may be assumed to require the presence of living cells for multiplication.

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ACTION POTENTIALS DURING HIGH AND LOW FREQUENCY STIMULATION OF MEDULLATED NERVE

IT has been shown by Hill and his collaborators (Feng and Hill,[1] Bugnard,[2] Hill,[3] Bugnard and Hill[4]) that when the frequency of a stimulus applied to frog medullated nerve is increased above about 500 shocks per second at room temperature there is a falling off in the total response as measured either by the heat produced or the accompanying electric potential. At a frequency of 2,500 one-way or 5,000 two-way shocks per second the response is only about 10 per cent. of that at the optimal frequency. Recently Cattell and Gerard[5] made the observation that a high frequency stimulus, itself producing a very small response, does not prevent the nerve from responding to a stimulus of lower frequency, applied above, at or below the electrodes giving the high frequency stimulation. These results indicated that the decreased effectiveness of high-frequency excitation involves a local change occurring at the stimulating electrodes. In the light of these observations Bugnard and Hill[6] have made a further analysis of the problem, interpreting the results on the basis of refractory period, summation and post-excitatory changes in irritability. All the experiments mentioned above were carried out with a technique which measured only the total response over a period of time and have the drawback that they do not permit observations of the individual action potentials in relation to the various combinations of stimulation frequencies, a deficiency which has had to be supplied by inference. We have therefore made a series of similar experiments, recording the details of the potential picture on the cathode-ray oscillograph. Individual responses were then directly observed under different experimental conditions with the following results.

1. The effect of increasing shock frequency. As the stimulation rate is progressively increased successive supermaximal shocks fall within the refractory period of the preceding responses and the individual action potentials therefore become smaller. Then there follows the well-known alternation in magnitude, and finally, with frequencies above 1,000 per second, only small irregular responses occur. This last state is the potential picture of the phenomenon originally described as "inhibition" by Bugnard. If the intensity of the stimulus is increased greater responses can be obtained.

2. The ability of the rapidly stimulated or "inhibited" nerve to conduct superimposed impulses. If, against a background of high frequency stimulation, extra stimuli of the same or lower intensity are applied through different electrodes, there are produced responses to these extra stimuli which are transmitted through the region of high frequency stimulation, as was observed by Cattell and Gerard. These responses can be reduced in magnitude by increasing sufficiently the intensity of the background stimulation; an effect which is presumably due to increased background activity, as described in the preceding paragraph.

3. The ability of the nerve to respond to superimposed stimuli. Not only can the "inhibited" nerve be stimulated at electrodes other than those carrying the high frequency background, but extra stimuli applied through the same electrodes are also capable of producing a response. (Indeed, in this case a curious phenomenon has been regularly observed: following the response to the extra stimulus there is an increased

1 T. P. Feng and A. V. Hill, *Proc. Roy. Soc. B*, 113: 366, 1933.

2 L. Bugnard, *Jour. Physiol.*, 80: 441, 1934.

3 A. V. Hill, Suppl. SCIENCE, Vol. 79, 9, 1934.

4 L. Bugnard and A. V. Hill, *Jour. Physiol.*, 83: 383; 394, 1935.

5 McK. Cattell and R. W. Gerard, *Jour. Physiol.*, 83: 407, 1935.

6 L. Bugnard and A. V. Hill, *Jour. Physiol.*, 83: 416, 1935.

responsiveness to the background stimuli. This increase has been seen to last as long as 50 milliseconds.)

The extra stimulus can be applied in either of two ways: (*a*) electrically in the same direction as the background stimulation or (*b*) in the opposite direction. Under both conditions a response can be produced. In the first case the response varies in height as the extra stimulus varies in its time relations with respect to the stimuli of the high frequency background. This indicates that the response is dependent upon the summation of stimuli and accords with the observations of Bugnard and Hill.[6] When the two sets of stimuli are opposed in direction a full-sized response is produced with great regularity. Obviously summation of stimuli plays no part here, and the probable factors concerned will be discussed below.

Discussion

We can, therefore, present the following potential picture which describes the decreased effectiveness of stimuli applied at rates on the order of 2,500 per second: (1) The response to these high rates of stimulation is largely but not completely abolished. (2) This decreased response is partly due to a decreased excitability at the stimulating electrodes, since the response can be increased by raising the strength of the high frequency stimuli or by summating an extra shock with the background excitation. (3) The decreased excitability which obtains at the high frequency electrodes is not present elsewhere on the nerve, since extra stimuli of normal strength applied through other electrodes can produce practically full-sized responses. Furthermore, the decreased excitability at the high frequency electrodes is localized in the region of the cathode, since an extra just supermaximal shock applied at the electrodes carrying the high frequency stimulus, but reversed in electrical sense, produces almost a full response.

From the foregoing picture the decreased effectiveness of high rates of stimulation can be explained on the basis of two factors: refractoriness and cathodal depression, as suggested by Bugnard and Hill.[6] At any one time most of the fibers are refractory to successive stimuli of the series. The ineffective stimuli, therefore, produce a cathodal depression such as was described by Gildemeister[7] and by Erlanger and Blair.[8] Our observations are in accord with the conclusions of Bugnard and Hill[6] (p. 424) that "shocks which are 'ineffective' owing to falling in the refractory period, nevertheless depress the excitability and extend the refractory state,"[9] and also that extra shocks applied through the same electrodes and in the same sense electrically are effective only when they summate with background stimuli. Bugnard and Hill, however, conclude that an extra shock "in the opposite electrical sense, raises the excitability and shortens the refractory state" (p. 424) and postulate that such a shock does not itself excite but enables the next following background stimulus to become effective. Although such a condition may exist in alternating high frequency stimulation, it does not occur when an extra reversed shock is applied against a background of unidirectional stimulation as in the present experiments. Oscillograph records of the time relations show, on the contrary, that the large response following the extra stimulus is produced by the latter and not by the succeeding background stimulus. We have furthermore tested, both at the anode and at the cathode, the excitability of the nerve immediately after 30 or more seconds of high frequency stimulation. At the cathode the response to a maximal testing shock does not reach full height until after 10 to 12 seconds, while at the anode there has been almost no change in excitability and the response to the testing shock is nearly maximal immediately after the background stimulation is removed. We therefore conclude that with high rates of stimulation the local depression is confined to the region of the cathode, so that an extra reversed stimulus is effective because the anodal region is not significantly depressed.

[7] M. Gildemeister, *Pflüger's Arch.*, 124: 447, 1908.

[8] J. Erlanger and E. A. Blair, *Am. Jour. Physiol.*, 99: 108, 1931.

Summary

Observations of individual action potentials during high frequency stimulation show that the failure to excite, in so far as it is not due to refractoriness, depends upon a localized depression at the cathode which may last for a considerable period after the stimulation has been discontinued. Excitability of all other regions of the nerve, including that at the high frequency anode, is not significantly altered.

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[9] Bugnard and Hill use the term ''refractory state'' for a local decrease in excitability owing to a combination of the effects of refractory period and local excitatory change.

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