Electrical Response of a Slime Mold to Mechanical and Electrical Stimuli

By

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With 6 Text-figures

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During a series of experiments to determine the course of the rhythmic change in the electromotive force generated in the myxomycete plasmodium, commonly known as the slime mold, Kamiya and Abe (1950) recently found that a weak mechanical shock applied to one of the lead electrodes elicits invariably an electrical response which causes a quick deflection in the galvanometer. This electrical response had apparently such a short deflection that it was impossible to figure out its temporal configuration by means of a freely suspended reflecting galvanometer. The present investigation was undertaken with a view to elucidate the nature of this electrical response and the mechanism by which it is released by the slime mold, *Physarum polycephalum.*

The results we obtained in this investigation indicated very clearly that the electrical response of the slime mold disobeys the all-or-none law which is known to govern the propagated electrical response of both animal and plant cells under ordinary experimental conditions. It was further shown that this "action potential" of the slime mold is always accompanied by a simultaneous change in the electric impedance of the protoplasm, similar to the electrical response in other cells (Cole and Curtis 1938, 1939; Tasaki and Mizuguchi 1949).

At present, we are not yet in a position to give any adequate explanation to the experimental results we have obtained. Neither could we correlate this response with any other known activity of the slime mold. In this paper, therefore, the data obtained are presented objectively, without any preconceived ideas as to the nature of processes involved in the response.

Method

In most cases a strand of myxomycete plasmodium was suspended. according to the method of Kamiya and Abe, in the air of the moist glass chamber between the small masses of protoplasm spreading on the

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surface of the two non-polarizable electrodes (Zn-$ZnSO_4$-agar) E_0 and E_2 in Fig. 1.

To record the action potentials and the impedance changes of the slime mold, we have made use of several different types of electrical circuits. In the later stages of the present investigation, the circuit shown diagrammatically in Fig. 1 was generally used. The main part of this circuit consists of a low frequency alternating current Wheatstone bridge. The bridge current from the transformer T in the figure was an A.C. of 50 cycles

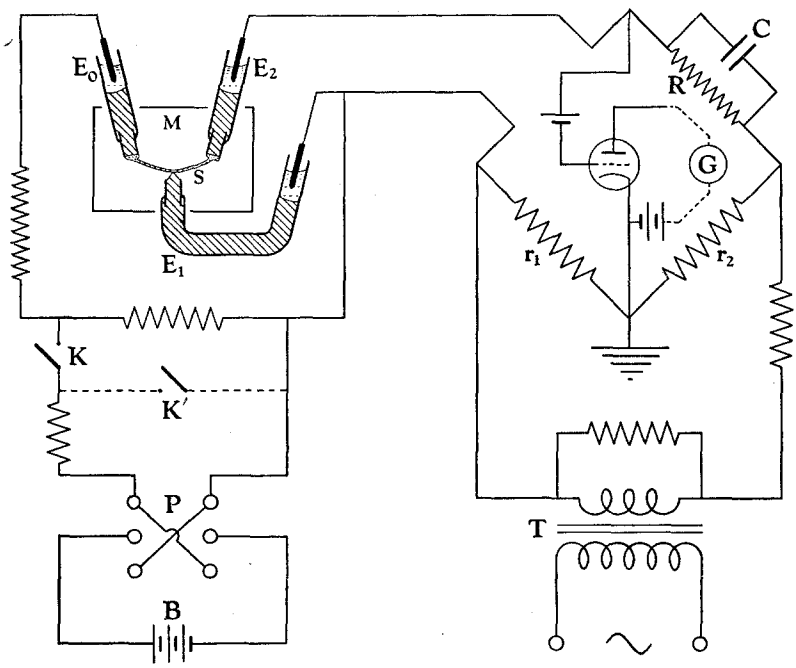

Fig. 1. Electrical circuit used for recording the electrical responses of *Physarum polycephalum*. *S*: Battery for electrical stimulation. *P*: Current reverser. *K* (and *K'*): Contacts operated either manually or by means of a Helmholz pendulum. *T*: Transformer serving as the alternating current source of the Wheatstone bridge. *G*: Oscillograph. As regards the numerical values of *r*'s, *R* and *C*, see the text.

per sec. Its strength was kept as low as possible because we were afraid that a strong bridge current might bring about possible changes in the state of the protoplasm. In all cases the A.C. voltage between the electrodes E_1 and E_2 was not allowed to exceed 50 millivolts; it was generally between 15 and 30 millivolts.

The detector system was a two-stage direct-coupled amplifier and oscillograph of the Duddel type, similar to that used by Tasaki and Fujita in their studies on the effect of chemicals upon the electrical properties of the *Nitella* cell (unpublished). In the output circuit of this amplifier was connected an electrical filter (a *L*-*C* tank circuit tuned to the frequency of the bridge current), and this served to separate the A.C. com-

ponent of the input voltage from the D.C. component. It was possible, by means of this special device and by using two elements of the oscillograph, to record the action potentials and the impedance changes simultaneously on the same film. The sensitivity of the vibrator of the oscillograph used for recording the action potential was approximately 7.5×10^{-5} ampere per mm. and a constant voltage of 1 millivolt applied to the input of the amplifier caused in this vibrator a deflection of 5 mm. The sensitivity of the A.C. vibrator was 1.5×10^{-6} ampere per mm.

The electrical stimulus was in general a short rectangular current pulse of the duration between 0.2 and 100 milliseconds, obtained by operating the keys K and K' in the diagram of Fig. 1. A three-electrode arrangement was used to reduce the shock artifact. Between the battery circuit and the stimulating electrode E_0, a constant resistance of about 10 megohms was generally connected; this was done to check the effect of the electrical activity of the protoplasm under the stimulating electrode E_0 entering into the potential and impedance records. As the resistance of the strand of protoplasm suspended between the electrode was well below 1 megohm in most cases, this high resistance served further to control the strength of the stimulating current.

The main difficulty in the present investigation lies in the fact that both the electromotive force and the impedance of the protoplasm were slowly but constantly changing so that a steady condition of the preparation could not be obtained. Under the conditions of our experiment, the autonomous rise and fall of the electromotive force of the slime mold, which is known to be intimately correlated with the protoplasmic flow (Kamiya and Abe 1950), varied mostly within 15 millivolts. The resistance of the strand between the electrodes was generally about 5.0 megohm, and it varied rhythmically up to 20 per cent of the mean value. The parallel reactance of the preparation varied also considerably from sample to sample, and from time to time in one and the same individual. At the frequency of 50 cycles per sec., it was generally greater than 10 times the parallel resistance and showed a very pronounced rhythmical variation. But, as the period of the rhythmical variation of these quantities was fairly long (the period being 2 to 3 minutes), it was possible, in practice, to change the known arms of the Wheatstone bridge in such a manner that the bridge was continuously balanced.

Results

1. The time process of the electrical response

As has been stated above, the electrical response of the slime mold could be induced most readily by a light mechanical shock applied to one of the lead-off electrodes with which the plasmodium was suspended. When a light tap is given through the tip of a glass rod to the electrode consisting of a glass tube filled with agar, a transient variation in the electromotive force was elicited from the protoplasm which tends to make the stimulated locus negative to the resting region of the protoplasm. The rising phase of this "action potential" is very short as compared with its falling phase,

which decayed approximately exponentially at the time constant of 1 to 1.5 seconds.

The most remarkable feature of this "action potential" is that the size of the response depends pronouncedly upon the strength of the shock applied. Within a certain limit, a stronger stimulus induced a greater response. When the first mechanical shock was relatively weak and the response was correspondingly small, the second, stronger shock applied at a short interval after the first caused a greater response which was superposed upon the first response if the interval was short enough. When a series of mechanical shocks of approximately the same strength was given to the slime mold, the responses were found to accumulate upon one another and to approach a steady potential level. The peak value of the negative variation amounted often to about 10 millivolts.

It was found possible to release similar responses by electrical means. In our observation on the effect of electrical stimulus upon the slime mold, we generally used the tripolar arrangement as shown in Fig. 1. When the impedance changes accompanying these responses were not observed simultaneously, the alternating current was not supplied to the Wheatstone bridge. But, as we have ordinarily kept the resistance R in the known arm of the bridge at about 1 megohm, the observed potential should be slightly smaller than the voltage actually developed by the protoplasm.

We found at the onset of our experiment that the strength of current required to evoke a response in the slime mold varies remarkably according to the area with which the stimulating electrode is kept in contact with the protoplasm and also according to the direction of the stimulating current. The threshold strength of the current, or the strength required to elicit the smallest perceptible response in the galvanometer, decreased as the contact surface diminished. This indicates that a certain definite surface density of current is required to induce a response. When the plasmodium was allowed to spread on the surface of the agar electrodes, the threshold became very high and it seemed practically impossible to elicit a response by electrical means. In the following observations, therefore, we always took precaution to bring the strand of protoplasm in contact with the middle electrode E_1 (Fig. 1) only several minutes before the start of the experiment, so that the protoplasm had no time to spread over this electrode.

With the experimental arrangement of Fig. 1, it is evident that electric responses of the plasmodium are observed only when the region of the protoplasm under or around the middle, common electrode E_1 is thrown into action by the stimulating current. The region of the stimulating electrode E_0 is practically insulated from the lead-off electrodes; and, furthermore, a mass of protoplasm is spreading over the surface of the electrode E_0. A point of interest in this experiment is that a stimulating current flowing outwards through the surface of the protoplasm under consideration is decidedly more effective in eliciting a response than a current flowing in the opposite direction. In the experiment of Fig. 2, the stimulating current was withdrawn after a period of about 0.1 second, and there seems to be a

possibility that an anodal current induces a response, just as in the vertebrate nerve, on its withdrawal.

The question now arises as to whether or not the process involved in the electrically induced response is the same as that caused by mechanical means. To test this point, we have examined the effect of mechanical stimuli applied at varying intervals before and after an electrical stimulus. In all cases it was clearly demonstrated that responses induced by the two different means were superposed upon one another in the same manner as two responses elicited by one and the same method were (Fig. 3). This

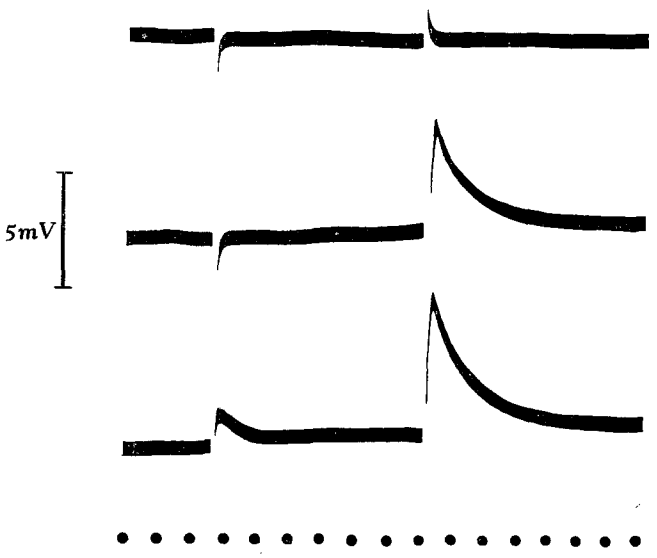

Fig. 2. The relation between the direction of the stimulating current and the size of the response. Stimulus artefacts on the left were caused by brief current pulses (about 0.1 sec. in duration) applied with the common (stimulating and lead-off) electrode E_1 connected to the anode of the battery. Responses on the right column were obtained with stimulating currents of the opposite sign. Strenght of the currents were 1 (top), 2 (middle) and 3 (bottom) microamperes. The time marks are 1 second apart.

undoubtedly indicates that the process underlying these responses is common in these two cases.

2. The strength-duration relation

Although the type of electrical response before us was found to be considerably different from those recorded in *Nitella* cells or vertebrate nerve fibers, it was shown possible to determine the strength-duration relation on this material taking the smallest perceptible response as index of excitation. For controlling the duration of the stimulating voltage, two knock-over keys of a Helmholz pendulum (K and K' in Fig. 1) were used. In this experiment, the high resistance connected between the battery circuit and

the stimulating electrode E_0 was short-circuited and, as a consequence, the stimulus strength was expressed in volts instead of amperes.

In spite of the fact that in the slime mold the state of the material is constantly changing according to a rhythmic pattern, the relation between the threshold voltage (v) and the time (t) of passage of the stimulus can be determined in a fairly reproducible manner with an accuracy of about 10 per cent. The observed data fit very well with the well-known Weiss' formula $v = b\ (1 + k/t)$, where b represents the rheobasic voltage and k the chronaxie. In the last column of Table 1 are shown the values calculated by this formula for different durations, in which b and k are 8.2 volts and 1.0 millisecond respectively. It was a great surprise to us to find

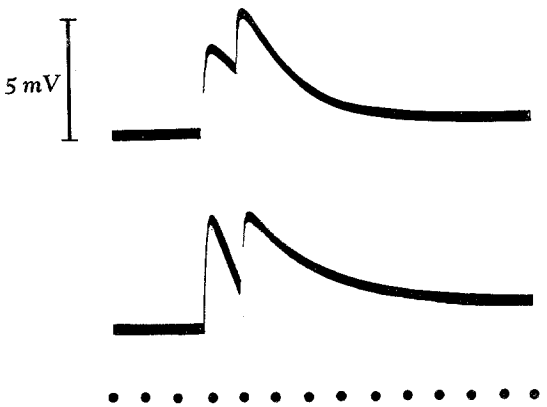

Fig. 3. Top: An electrically induced electrical response followed by a mechanically induced response. Bottom: A mechanically induced response followed by an electrically induced response. Resistance R in the circuit of Fig. 1 was 1 megohm, and $r_1 = r_2 = 0$. No bridge A.C. was applied. Time: 1 sec.

Table 1. The strength-duration relation just sufficient for inducing the smallest perceptible response in a myxomycete plasmodium

Duration	Strength (observed)	Strength (calculated)
msec.	volts	volts
0.22	42	45.9
0.44	28.5	27.1
0.66	21	20.7
0.88	18	18.4
1.32	14	14.5
1.76	12	12.9
2.20	13	12.0
3.30	10.5	10.7
4.40	9.5	10.0
5.50	10	9.7
6.60	9.5	9.5

that the chronaxie for this material is as short as 1 millisecond at room temperatures (for chronaxie values of various cells or tissues, cf. Heilbrunn's text-book [1943], pp. 503—504). The rheobase was found to be in general between 2 and 10 volts.

On several occasions, the strength-duration relation for the slime mold was determined by means of condenser discharges. In that case, the contact K in Fig. 1 was replaced by a condenser of which the capacity could be varied from 0.001 up to 10 microfarads, and the stimulus was started by opening the key K' manually. The data obtained obeyed again the hyperbolic law $v = b\ (1 + h/RC)$, where RC stands for the decay constant of the stimulating voltage and h for the constant which can be converted into the chronaxie by multiplying socalled Lapicque factor 0.35 (see Lapicque, 1926, p. 324). The value of chronaxie determined by this method agreed well with that obtained by rectangular voltage pulses.

3. The change in the electric impedance accompanying the response

It has been shown by Cole and Curtis (1938, 1939), Tasaki and Mizuguchi (1949) and others that the change in the electromotive force in *Nitella* cells and nerve fibers accompanies, like a shadow, a simultaneous change in the electric impedance. It seemed therefore worthwhile examining the resistance of this material for an A.C. following electrical and mechanical stimulation. Impedance measurements by the ordinary A.C.

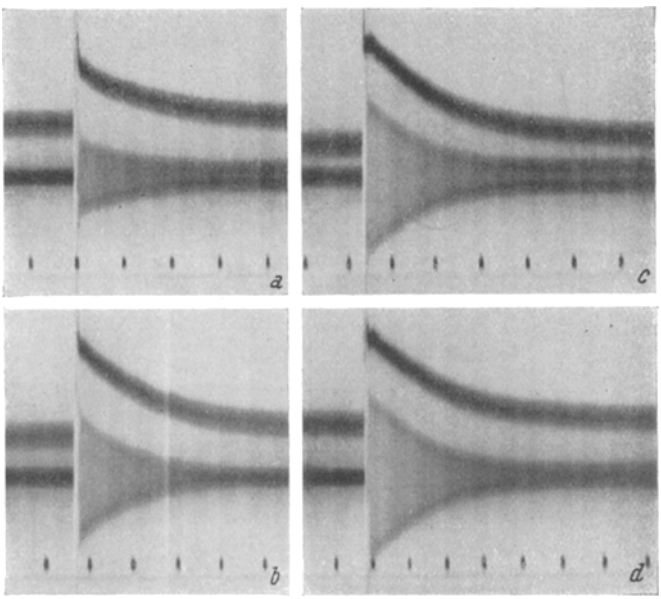

Fig. 4. The relation between the strength of the stimulating current and the size of the response. Potential and impedance changes were recorded simultaneously. Strengths of the current in microamperes are 5.0 (a), 7.5 (b), 10.0 (c) and 12.5 (d). The alternating current Wheatstone bridge was balanced at $r_1 = 30$ ohms, $r_2 = 20$ ohms, $R = 0.5$ megohms and $C = 10^{-10}$ farad. Time: 1 sec.

Wheatstone bridge method revealed immediately that, whenever there was an "action potential", there was a change in the impedance.

We next proceeded to examine the correlation between the action potential and the impedance change. The method we adopted was to amplify the action potential and the bridge A.C. with one and the same amplifier and then to separate these two types of response from each other by means of an electrical filter (Tasaki and Fujita, unpublished). By this method we examined the change in the electrical properties of the protoplasm around the middle electrode E_1 in Fig. 1.

All the records we have obtained indicated very clearly that there is a close parallelism between the impedance change and the action potential. These two different types of exponentially decaying processes always showed the same time-constant of decay. When the stimulus was made

stronger, both types of response increased their magnitude in proportion (Fig. 4). When a number of electric shocks were delivered in succession, the two types of response gave summation curves of exactly the same temporal configuration (Fig. 5).

Close parallelism between the impedance change and the action potential was demonstrated further with mechanical stimuli applied to the middle electrode E_1 in Fig. 1. At this region, the surface of the protoplasm kept in contact with the electrode had a relatively small area. When mechanical stimuli were applied to the slime mold through the grid electrode E_2 where the protoplasm was spreading over a wide area, parallelism between the two types of electrical response did not seem very perfect. As we were interested for the time being only in the correlation between the action potential and the impedance change, we did not try to modulate the frequency of A.C. except 50 cycles.

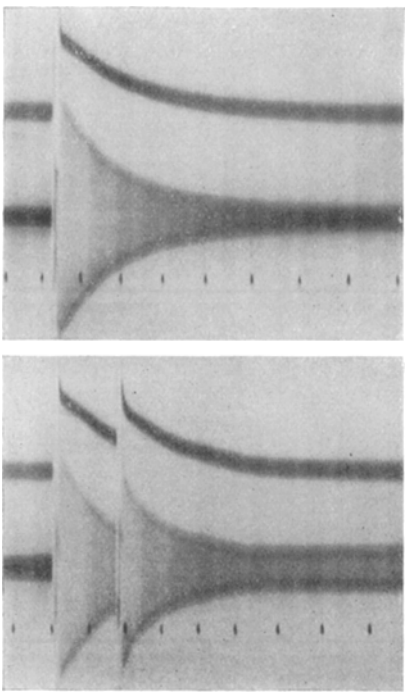

Fig. 5. Responses induced by a single (top) and double (bottom) rectangular current pulses. The strength of stimuli was in all cases 3 microamperes, and the duration was about 0.1 sec. The bridge was balanced at approximately $r_1 = 30$ ohms, $r_2 = 40$ ohms, $R = 1$ megohm and $C = 4 \times 10^{-10}$ farad. Time: 1 sec.

On several occasions, we tried to determine to what extent both the parallel resistance and the reactance vary during activity. The known arms of the balanced bridge were made to be so altered that it was no longer balanced at rest but it would be balanced, in the course of many trials, at some particular moment after stimulation. It was found by this method that, immediately after the onset of response, the parallel resistance decreased by 2–10 per cent and the reactance increased by 15–50 per cent, giving a resultant impedance loss of 2–10 per cent (Fig. 6). With A.C. of 50 cycles per sec., the parallel capacity of the preparation at rest was, under the conditions of our experiment, below 10^{-9} farad which corresponds to about 3 megohms in reactance. The resistance was generally between 0.3 and 0.6 megohms.

Discussion

By the experiments stated above, it was shown that the "action potential" of the myxomycete plasmodium accompanies, similar to that of *Nitella* cells and of nerve fibers, a simultaneous change in the electric impedance. This response, however, differs from that in *Nitella* and in nerve in the

following respects: (a) The size of the response in the slime mold increases, within a certain limit, as the strength of the stimulus increases, while in *Nitella* and nerve the response is released in all-or-none manner. (b) The response in the slime mold shows summation when two stimuli are applied at a short interval, while in *Nitella* and in nerve the absolutely refractory period lasts until the activity comes to an end. (c) The duration of the negative variation of the plasmodium is about 2,000 times as long as the chronaxie, whereas in *Nitella* and nerve it is only several, ten times the chronaxie.

The properties of the plasmodium's electrical activity mentioned in (a) and (b) above seem to resemble the response of a curarized or refractory muscle fiber to motor nerve stimulation (see e.g. Kuffler 1942, Fig. 8; Katz 1942). In a number of cases, we have examined whether or not the response is transmitted along the strand from the site of stimulation. But we could find no sign of transmission.

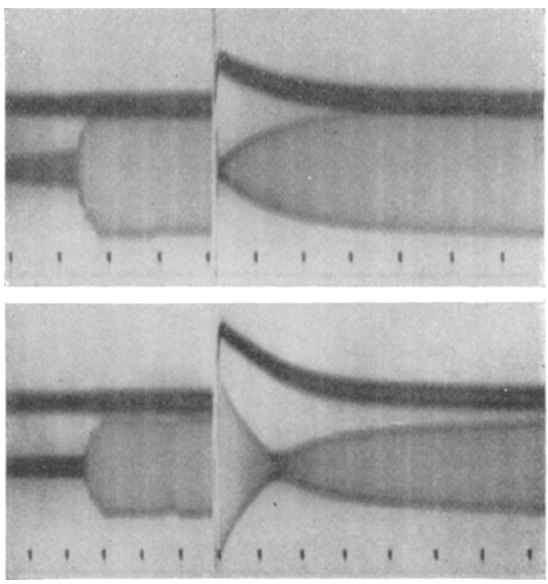

Fig. 6. Electric impedance during activity. The bridge was first balanced at rest, then the resistance and the capacity of the known arm of the bridge were altered and a stimulus was delivered. Parallel resistance and reactance at rest were, in the case of the upper record, 0.28 and 2.2 megohms respectively; the resistance was decreased by 2 per cent and the capacity was decreased by 15 per cent prior to application of the stimulus. In case of the lower record, the parallel resistance and reactance at rest were 0.29 and 2.1 megohms respectively, and the resistance and the capacity were decreased by approximately 2 and 12 per cent respectively.

We also tried to find microscopically visible changes, if any, in the protoplasm when it is thrown into action. A small part of the protoplasm midway the suspensed strand is laid on a thin sheet of agar which adheres to the side wall of the moist glass chamber and which works as a nonpolarizable common electrode E_1. With such a preparation of the material it is possible to observe through the microscope the protoplasm at the very site where the action potential is elicited while the magnitude of the elicited action potential is ascertained by the oscillograph. Thus the vigorous flow of endoplasm back and forth along the connecting strand can be seen most clearly. But no cessation of flow whatsoever is seen to take place at that locus of the strand from which the action potential is ascertained to have been evoked, nor did we detect any sign of microscopically

observable changes in protoplasm at the moment of response. This the fact that the protoplasmic streaming in *Nitella* is stopped temporarily when the cell is thrown into action is not shared by the slime mold protoplasm. This is probably due to the fact that the local stimulation of the submerged *Nitella* cell rapidly propagates to the entire cell while in myxomycete plasmodium the region of response is limited mostly to that locus where the protoplasm is directly stimulated from outside. Even though the action potential exerted some effect on the protoplasmic flow, the intracapillary flow of the endoplasm in the plasmodium would be under control of a region which does not take part in the electrical response.

Seifriz and Epstein (1941) observed that a mechanical shock applied to the plasmodium of *Physarum polycephalum* by a falling drop of water immediately "sets" the protoplasm bringing about the suspension of flow which they call "shock anesthesia". This shock is, however, far more drastic than the mechanical shock applied by us. In order to throw the slime mold protoplasm into electrical activity by a mechanical means, only a slight tap on the electrode is sufficient. Moreover, Kamiya and Abe (1950) observed that the action potential of the slime mold is sometimes evoked even spontaneously with varying intervals and varying intensity under constant environment. We can say that the electrical response does not necessarily accompany the hindrance of the protoplasmic flow.

Summary

1. The electrical response released by the slime mold, *Physarum polycephalum*, through mechanical and electrical stimuli disobeys the all-or-none law.

2. A stimulating current flowing outwards through the surface of the protoplasm is more effective in evoking a response than a current flowing in the opposite direction.

3. The process underlying electrical responses is the same irrespective of the means of stimulation, either mechanical or electrical.

4. The strength-duration relation agrees well with the Weiss' formula $v = b\ (1 + k/t)$, in which b (rheobase) is 2–10 volts and k (chronaxie) is approximately 1 millisecond.

5. The "action potential" of the slime mold accompanies a simultaneous change in the electric impedance of the protoplasm. There is always a close parallelism between them.

6. Protoplasmic flow can proceed unhindered at the moment when electrical responses are induced.

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