These results were further confirmed by feeding the extract and its ash in half the previous doses, *i. e.*, 0.25 mg. Fe per day under which circumstances differences in efficiency would be accentuated because the amount fed is well below the optimum requirement. The results, however, were entirely similar to the first series.

It is concluded that the inorganic combination naturally present in spinach is better than any combination of inorganic salts we have been able to find, *i. e.*, iron and copper, iron and copper manganese, etc. Furthermore, it would seem that the solubility of at least some of the factors present may have a real significance. A complete qualitative and further analysis of this spinach extract ash are the next steps in the investigation and will be carried out in the near future.

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Studies on Stimulation in Nitella.

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Waves of negativity occurring spontaneously in *Nitella,* sometimes in regular rhythm, have been previously described.[1] Similar negative variations, passing down the cell, may be induced in a variety of ways, by touching, bending, by application of solutions or by small direct[2] potentials.[3] They resemble the action currents of muscle and nerve in form and magnitude but last longer and are propagated more slowly.

Nitella appears to follow in general the laws of stimulation of muscle and nerve but there are some interesting exceptions. For example, it is far from obeying the all or none law completely since the P.D. (normally positive[4] when the cell is in contact with dilute

[1] Osterhout, W. J. V., and Harris, E. S., PROC. SOC. EXP. BIOL. AND MED., 1928-29, xxvi, 124.

[2] The production of negative waves by induction shocks in *Nitella* was apparently first reported by Hörmann (Hörmann, G., Studien über die Protoplasmaströmung bei den Characeen, Jena, 1898).

[3] These are ordinarily effective at cathodal closing and anodal opening but may be induced at anodal opening by higher voltages.

[4] The protoplasm is regarded as positive when the positive current is outward, *i. e.*, when the positive current tends to flow from the inner surface through the protoplasm to the outer surface or from the outer surface through the electrometer and thence through an (imaginary) capillary passing through the protoplasm into the sap.

solutions) may disappear partially or completely at the stimulated spot, and conduction with a decrement occurs.[5] The normal P.D. returns in from 5 to 30 seconds. The electrical (polarization) resistance falls off simultaneously with the P.D. but it takes much longer to return to normal. The period during which recovery takes place constitutes the refractory period after which the cell may again be stimulated.

The stimulus is transmitted along the cell[6] at a rate of from 1 to 2 cm. per second and may pass across the "node" to a neighboring cell (or even through two aqueous contacts for a distance of half an inch or more to another cell, *e. g.*, to a cell from a different plant). The fact that it is unable as a rule to pass a spot whose positive P.D. has been (reversibly) lowered to a sufficient extent (*e. g.*, by an opposed potential or by the application of 0.05 M KCl) lends support to the idea that the wave of stimulation consists of a local current flowing outward through the protoplasm and through the cell wall to a neighboring spot which is less positive (and then back through the protoplasm and sap). Sufficient flow of current causes[7] the more positive spot to become less positive and to lose much of its electrical resistance, and a flow then starts from neighboring positive regions toward this spot and in this way the wave moves along the cell as postulated by the local action theory of nervous transmission.

Since recovery can take place while stimulation (chemical or electrical) continues it would seem that there are two opposing processes so that when the process producing a loss of P.D. (and of resistance) has reached a certain point the other automatically becomes dominant and restores the normal condition.

In many cases the negative wave has two maxima (*i. e.*, the stimulated spot becomes negative, then less negative, then more negative, after which the curve approaches zero[8]); this might be regarded as indicating that the inner layer of the protopolasm is affected before the outer. The behavior of the cell when the external solu-

[5] This does not necessarily mean that the protoplasm loses all its E.M.F. but only that the E.M.F. of the outer layer becomes equal to that of the inner.

[6] Waves due to cutting (death waves) travel much faster and produce irreversible changes: they differ in being able to pass a spot in contact with 0.1 M KCl or one killed by chloroform.

[7] This might be due to a structural change (production of leaks) or to an increase in conductivity (*Cf.* Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927-28, xi, 673). Ordinarily an outward flow of positive current is more effective than an inward flow.

[8] Approaching zero merely means that the stimulated spot is recovering its normal P.D.

tion is varied indicates that stimulation usually starts with the inner layer.[9]

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Bioelectrical Aspects of the All or None Law.

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If the all or none law holds, a muscle fibre or nerve fibre will always give the same response when stimulated so that the total electrical effect should be directly proportional to the number of fibres which react, provided the arrangement is in series rather than in parallel. But although the fibres (in a nerve or in such a muscle as the sartorius of the frog) may have a parallel arrangement they appear to behave as if they were in series since the current of action appears to increase with the number of stimulated fibres; so also cutting a muscle entirely in two gives more current of injury than when we cut only part way across.

This is explainable if we assume (as Gasser and Erlanger[1] have done in the case of nerves) that the inactive or uncut fibres act as shunts to the E.M.F. produced by those which are active or cut. Suppose, for convenience, that a group of 5 fibres may be repre-

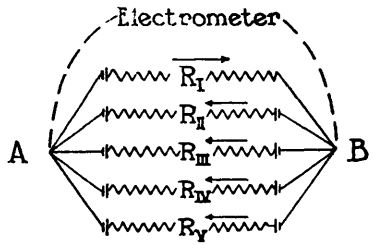

FIG. 1.

Hypothetical diagram to show electrical conditions in a group of fibres of which the uppermost (I) has been cut at the right end so that it produces the current shown by the arrows: the other fibres (II, III, IV, and V) produce no current (since the batteries in each are equal and opposite) but they act as shunts to I.

[9] Evidence will shortly be presented which indicates that the fibres of muscle and nerve may have a surface consisting of two layers, which is in harmony with the fact that the negative wave of nerve often shows two maxima and might afford a different interpretation from that given by Erlanger and Gasser (*Am. J. Physiol.*, 1927, lxxx, 522).

[1] Gasser, H. S., and Erlanger, J., *Am. J. Physiol.*, 1927, lxxx, 522.