under the trade name Pectinol (*7*) in different concentrations and at different temperatures. Only one, namely, Pectinol 100 D, was found to be effective. *Brassica* roots were used because it was known that they produce abundant hairs in tap water (*2, 8*).

Following germination, when they had attained a length of 1 to 3 millimeters, the roots of ten seedlings were placed through small holes in pieces of stiff, paraffin-coated paper and floated in tap water in small beakers, at 30°C. At the end of 6 hours, the floats were transferred to a filtered solution of Pectinol 100 D.

Great difficulty was experienced in keeping the roots alive. However, by dusting the seeds with Orthocide prior to germination and by means of the method described previously (*3*), it was found possible to grow roots in a 0.75-percent flowing solution of Pectinol 100 D in distilled water at 30°C. At the end of about 16 hours, only about half of the roots showed signs of growth. These roots measured 5 to 15 millimeters, about half the length of control roots in tap water. All treated roots had an unusual appearance characterized by the lack of hairs, discoloration, plasmolysis, separation of epidermal cells, and abnormal sloughing of root-cap tissue. Some epidermal cells were free at one or both ends (Fig. 1 A), others were twisted out of position, and still others had dropped from the root, leaving empty gaps in the epidermis. The walls of the separated cells were thin but distinct and gave a positive test for cellulose. The deformities were such that they could be explained on the basis of enzyme action on the outer, cementing pectic layer, leaving the cellulose layer intact.

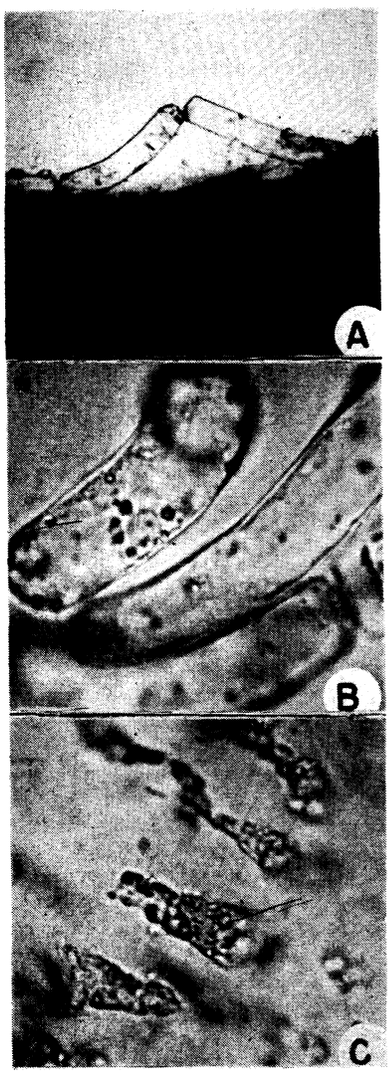

Fig. 1. *A*, epidermal cells of a root grown in a 0.75-percent flowing solution of Pectinol 100 D (×135). Separation of the cells indicates a change in the pectic layer. *B*, normal root-cap cells (×660). *C*, root-cap cells from the same treated root shown in *A*, showing complete dissolution of the cell walls (×660).

As in the earlier experiments with ammonium oxalate solutions (*3*), transfer of Pectinol-100-D-treated roots to a saturated solution of calcium sulfate resulted in resumption of normal growth, with the cessation of abnormalities and the immediate production of long hairs. That part of the root developed in the Pectinol 100 D solution remained unchanged. Some roots failed to respond, indicating irreparable injury to the apical meristem.

The condition of the root cap was of particular interest. In control roots, it formed a distinct, uniform covering over the root apex, the sloughed cells possessing healthy protoplasts and firm definite walls (Fig. 1B). In sharp contrast, the root caps of Pectinol-100-D-treated roots presented a discolored mass of partially or wholly macerated cells. In some cells the wall was thin, but distinct and definitely of cellulose, in others it was barely visible, and in still others it was dissolved completely (Fig. 1C).

The results of the experiments with pectic enzymes confirm earlier observations (*1–3*) of the occurrence of an outer layer of pectic material in the walls of the epidermal cells. If there were no definite cementing layer, it is inconceivable how individual cells could separate. The observations of recovered roots after removal to a calcium solution also corroborate the further view (*2–4, 8*) that hardening is the result of incorporation of calcium into the outer pectic layer of the elongating cell walls.

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11 July 1955

Response of the Slime Mold to Electric Stimulus

There are four fundamental properties of protoplasm of peculiar interest to the student of the nervous system. These are (i) that a change in the physical or chemical environment of protoplasm brings about an alteration in the phase boundary of the protoplasm that can be identified by (ii) the change that is propagated through its substance, usually with direction, (iii) that protoplasm possesses the property of integrating, coordinating, or correlating all the events that occur at the phase boundary, and (iv) that protoplasm reacts in a characteristic manner to this chain of events.

Although something is known about these four properties, the exact mechanisms involved have not yet been unraveled. However, students of the nervous system have presented a great deal of evidence concerning the nature of the physical and chemical changes in the environment necessary to stimulate a neurone. In addition, a good deal of information is available concerning the propagation of the stimulus through protoplasm. In this last case, the advent of electrometric techniques has made it possible to define, with considerable accuracy, quantitatively and qualitatively, the electric aspects of the propagated impulse.

Since the great complexity of the nervous system in vertebrates and also in invertebrates makes analysis of the mechanisms involved exceedingly difficult, an attempt has been made to approach this problem in a very much simpler but still very complex living organism, the slime mold, *Physarum polycephalum* (*1*). Tasaki and Kamiya (*2*) have reported that the slime mold will respond to a tap stimulus and to an electric stimulus in much the same manner. Using a very high input impedance direct-current amplifier coupled through a counter electromotive force to the direct-current amplifier of a Dumont 304H oscilloscope, similar responses were obtained by us with some very striking differences.

Figure 1 is a photograph of the oscilloscope trace following a tap stimulus. This response is a graded response. A weak stimulus is sufficient to start it; it

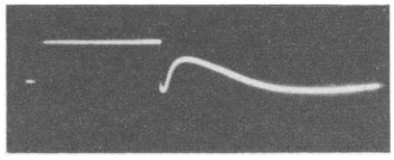

Fig. 1. Oscilloscope trace of response to tap stimulus.

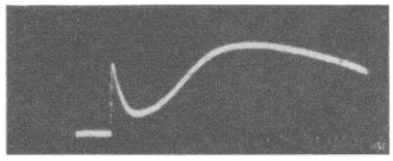

Fig. 2. Oscilloscope trace of response to electric stimulus.

disappears on repetition and when the plasmodium is killed. In addition, there is apparently an upper limit as the intensity of the blow is increased. The magnitude of the response may reach as high as 75 millivolts. There is good but not conclusive evidence that the magnitude of the response to a given strength of stimulus is significantly related to the vigor of the plasmodium. It can be demonstrated that this stimulus response is propagated along the thread.

In order to obtain a full picture of this response, the sweep speed of the oscilloscope was reduced to 2 seconds per inch by connecting a high capacitance across the sawtooth terminal and ground. Since the parameters of this response can be defined with considerable accuracy, it would seem that such a preparation makes an ideal test specimen for the assay of the effect of a variety of chemical agents on the activities of the protoplasm.

The attempt to stimulate the plasmodium electrically is fraught with many difficulties. The physical state of the environment of the plasmodium, which is determined in part by the electrolyte solution, the dimensions and placements of the electrodes, and the resistance and distributed capacity of the whole system in the absence of the plasmodium, produces pictures that are similar to a biological response. However, an electric response can be obtained, as is evidenced by Figure 2. Taken under the same conditions of calibration as the tap stimulus, the response shows qualitatively the same characteristics. Again the magnitude of the response would seem to be in some measure related to the vitality of the protoplasm, since a vigorous thread of rapidly streaming protoplasm gives a higher response than a thin thread with somewhat sluggish flow. Very rarely is the magnitude of the response anything like that following a tap stimulus.

The conditions necessary to produce an electric response to an electric stimulus seem quite different from those reported by Tasaki and Kamiya (*2*). The technique used in this study showed no evidence of a millisecond response, no matter what the voltage or current employed. Instead, it was found that a stimulus of at least 0.1-second duration seemed to be required. With such a duration, a response was obtained with a stimulus of 800 microamperes at 1.5 volts. Increasing the strength of the stimulus to 1 milliampere at 4.5 volts produced a graded response. Occasionally, this could be carried into higher levels. However, if the electric stimulus was increased very much, either the response failed completely, possibly because of a shock effect, or else it was obscured by the stimulus artifact.

The difficulty in getting a consistent response from the familiar electric set-up is the result of the simple fact that the protoplasmic thread is not a nerve fiber. A slime mold is capable of transmitting a stimulus just as is all living matter, but it differs from other tissues. For example, an excellent action potential of *Dionaea* was obtained by Stuhlman and Darden (*3*) with a maximum of 130 millivolts.

Tauc (*4*) attempted to find an action potential in *Physarum* but obtained only a spontaneous drop in the resting potential that was possibly owing to the formation of a membrane over the electrode.

The plasmodium of a slime mold is in reality "tissue" in the sense that it is an aggregation of centers of activity that are "cells" without walls. In short, it is not a homogeneous mass even though it is a continuous sheet of flowing protoplasm. Moreover, it moves—toward food, for example—as an integral whole and must therefore possess some means for transmitting stimuli from one point to another (*5*).

We may conclude, then, that a strand of one of the most primitive forms of life yields an action potential, both when it is stimulated electrically and when it is stimulated mechanically.

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5 July 1955

Regional Crossbedding and Petrology as Source Area Indicators

Basal Pennsylvanian sediments (Caseyville, Mansfield, Babylon sandstones, and equivalents) of the Eastern Interior Basin (Fig. 1) are separated by 500 to 1200 miles from possible source areas such as the Canadian shield and inferred Paleozoic uplands to the east. This geographic separation, together with mineralogic maturity and lithologic uniformity of the sandstones, makes it difficult if not impossible to determine source areas from regional variation of gross lithology. This is especially true because appreciable extrapolation beyond present basin limits is required. Our solution to this problem involves regional measurement of crossbedding and regional sedimentary petrology to determine the location and composition of probable source areas.

In order to have fullest confidence in our conclusions, it was necessary to study basal Pennsylvanian sandstones of the Michigan Basin (Parma sandstone) and portions of the Appalachian Basin (Sharon and Lee sandstones) in addition to the sandstones of the Eastern Interior Basin. Inclusion of these areas led to more definitive conclusions about Eastern Interior Basin sediment sources and provided the essential key to a sediment source interpretation of the basal Pennsylvanian of much of the northeastern part of the United States.

More than 950 measurements of crossbedding in 340 outcrops were obtained from more than 1000 miles of linear basal Pennsylvanian outcrop in the North Central States. Statistical analysis (hierarchical case of the analysis of variance) provided measures of reliability for average directions of sediment transport and also segregated total variability of crossbedding direction into small scale (within an outcrop), intermediate scale (within a 6- or 12-mile interval), and large scale (between 6- or 12-mile intervals along the outcrop belt). The order of variability within an outcrop is much less than it is within an intervals and within an interval it is greater than it is between intervals along the outcrop belt.

Regionally, crossbedding direction is very uniform. Crossbedding points south-southwest in all areas studied except in western Illinois, where it points southeast. The crossbedding is interpreted as accurately reflecting sediment transport on the regional slope from the source area toward the area of greatest subsidence and crustal instability (Ouachita trough). Excluding western Illinois, a general southwestward tilt of the craton in the North Central States is implied.

Because of the orthoquartzitic char-