

Terrestrial ages of irons would probably be reflected better by the C^{14}/Be^{10} ratio than by the absolute C^{14} content. Pending completion of Be^{10} determinations in the same specimens (12), we can use the C^{14} activity to obtain rough limits on the ages of the iron "finds." The activity in Odessa is not sufficient to be regarded as definite.

It has been shown previously that most iron "finds" contain no detectable Ar^{39} ($H = 325$ years) (13) and thus are at least about 1000 years old. The present results show that stone "finds" with terrestrial ages of thousands of years are not uncommon, and that some craters associated with iron meteorites (Henbury and most probably Odessa) have ages in the range of applicability of dating by C^{14} measurements.

Measurements of pertinent cross sections and production-rate ratios in accelerator targets are under way, and should provide a basis for more quantitative interpretation with respect both to the past cosmic-ray intensity and to the terrestrial ages of "finds" and associated impact phenomena (14).

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Measurement of Membrane Potentials in Neurospora

Abstract. Microelectrodes were used to record intracellularly from the filamentous fungus *Neurospora crassa*. Under standard conditions membrane potentials averaged 127 mv, inside negative. The potentials were potassium-sensitive, and depended upon the distance of the cells from the growing margin of the colony. In addition, the potentials were quickly reduced to about 30 mv in the presence of low concentrations of sodium azide or the polyene antibiotic nystatin.

The convenient size of *Neurospora* hyphae suggested that, unlike the cells of most other microorganisms, they might permit direct electrophysiological measurement of membrane properties. Such properties could then be correlated with cell growth and with the structure and permeability of the cell membrane, by using genetic and biochemical techniques not readily applicable to the tissues of higher organisms.

Neurospora hyphae are divided into "cells" by incomplete septa, and in growing colonies the cytoplasm streams continuously toward the advancing hyphal tips. Electron microscopic studies (1) have demonstrated the presence of a highly convoluted 75A unit membrane directly beneath the chitinous cell wall. Unlike the alga *Nitella*, *Neurospora* has no large central vacuole to complicate the interpretation of electrophysiological data (2).

In the present study, 1-day cultures of wild-type *N. crassa*, Perkins strain, mating type *a*, were grown at 25°C on minimal agar (3) covered with cellophane. Squares of cellophane with cells affixed were removed from the growth medium, washed, transferred to the recording chamber, and covered with the desired bathing fluid. Many hyphae reached diameters of 10 to 15 μ , and glass micropipettes with tip diameters less than 1 μ could be used to record intracellularly. The pipettes were filled with 3M KCl and had resistances between 20 and 35 megohms. All recordings were made with a direct-coupled amplifier. It was possible to keep individual cells impaled for more than half an hour without loss of membrane potential or cessation of normal cytoplasmic streaming. In general, however, cells were punctured in fairly rapid succession over a 30-minute period, and their membrane potentials were averaged to give the results discussed below. Usually the potentials were stable to within a few millivolts, but occasionally

spontaneous depolarizing deflections occurred which were 10 to 50 mv in amplitude and 0.05 to 2 seconds in duration. The nature of these deflections is unknown and is being investigated.

The magnitudes of the stable potentials were found to depend mainly on two experimental parameters: the ionic composition of the surrounding fluid, and the distance of the cells from the margin of the colony. In an arbitrary reference medium (4) and at the optimal distance (8 mm) behind the growing hyphal tips, the average membrane potential for 88 cells was 127 mv (standard deviation, ± 10 mv), inside negative relative to a reference electrode in the surrounding fluid. There was a gradual decline in average potential toward the center of the colony and, toward the

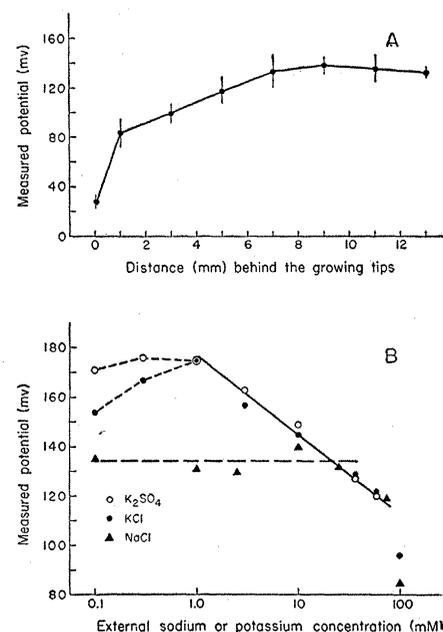


Fig. 1. *A*, Relationship between membrane potential and the location of cells in the colony. Points are averages for 5 to 15 cells measured in the reference medium (4). Vertical bars indicate \pm S.D. *B*, Effect of sodium and potassium on the membrane potential. Filled circles or triangles indicate variation of potassium chloride (\bullet) or sodium chloride (\blacktriangle) in the reference medium. Open circles denote the concentrations of potassium added as sulfate to a medium containing 25.3 mM sodium, as sulfate, and 1 percent sucrose. A new population of cells was studied in each medium, and each point represents the average potential for 5 to 15 cells located 8 mm from the growing tips. Standard deviations (not shown) were approximately ± 10 mv. In both *A* and *B* potentials are uncorrected for pipette tip potentials. The cells were negative inside, relative to a reference electrode in the surrounding fluid.

Table 1. Influence of metabolic inhibitors on membrane potentials in *Neurospora*.

Additions to reference medium	Concentration (molar)	Average potential* measured \pm S.D. (mv)
None		126 \pm 7
Sodium fluoride	10 ⁻²	124 \pm 7
Sodium azide	10 ⁻²	28 \pm 2
Sodium azide	10 ⁻³	29 \pm 8
Sodium azide	10 ⁻⁴	37 \pm 5
Sodium azide	10 ⁻⁵	78 \pm 19
Nystatin	2.2 \times 10 ⁻⁵	24 \pm 7
Nystatin	2.2 \times 10 ⁻⁶	19 \pm 9
Nystatin	2.2 \times 10 ⁻⁷	30 \pm 5

*All averages refer to measurements on 5 to 15 cells 8 mm from the margin of the colony.

periphery, a more abrupt drop to about 25 mv at the growing hyphal tips (Fig. 1A).

The influence of ionic environment has been explored for cells in the 8-mm region. When the components of the reference medium were varied singly, only potassium displayed a clear effect on the potential (Fig. 1B). Increasing the external potassium (by adding either KCl or K₂SO₄) from 1 to 60 mM produced a fall in the membrane potential which was linear with the logarithm of the potassium concentration and which had a slope of 32 mv per log unit. Correction for pipette tip potentials might increase this slope by 10 mv per log unit (5). Below 1 mM potassium, the relationship was no longer linear, which suggested that other ions might determine the membrane potential under these conditions. Indeed, in the absence of potassium the membrane potential was sodium-sensitive, with a slope of about 40 mv per log unit. In the reference medium containing 36.8 mM potassium, however, variations of NaCl or Na₂SO₄ between 0.1 and 60 mM had no measurable effect. Although media for the above experiments were not adjusted to constant total osmolarity, control measurements indicated that this was not necessary. Thus, essentially the same stable potentials were measured for cells in distilled water (222 \pm 15 mv) and for those in 200 mM sucrose (229 \pm 12 mv). The sharp decline in membrane potential produced by 60 to 100 mM NaCl or KCl was duplicated by high concentrations of sucrose, and was presumably an osmotic effect.

Among other ions tested, Mg⁺⁺, NH₄⁺, NO₃⁻, and H₂PO₄⁻ did not influence the membrane potential. Addition of 1 mM CaCl₂ to the reference medium raised the measured potentials from 130 to

175 mv, and noticeably stabilized the cells against mechanical injury. On the other hand, 10 mM (sodium) citrate, in the absence of added calcium, diminished the potentials from 130 to about 30 mv.

In an attempt to obtain further information concerning ionic contributions to the membrane potential, we assayed intracellular potassium and sodium by flame photometry. One-day cultures grown at 25°C in liquid minimal medium were found to contain approximately 160 mmole of potassium and less than 20 mmole of sodium per kilogram of cell water. These values are based on inulin determinations of extracellular space, and they are in agreement with previous potassium and sodium measurements for *Neurospora* (6). It is concluded that, under the present conditions, young *Neurospora* cultures concentrate potassium at least 4.3-fold from minimal medium, and may exclude sodium slightly.

Finally, the effects of several metabolic inhibitors were studied (Table 1). Sodium azide, at concentrations as low as 10⁻⁴ M, reduced the membrane potentials to about 35 mv; the maximum effect was already evident in the first cells measured, two minutes after the addition of inhibitor. In preliminary experiments sodium cyanide and dinitrophenol lowered the potentials with similar rapidity. Sodium fluoride (10⁻²M), however, had no apparent effect, even after 1 hour preincubation with the cells. The polyene antibiotic nystatin (7) proved to be the most potent compound tested, being maximally effective at a concentration of 2.2 \times 10⁻⁷M, again within 2 minutes. It has previously been suggested, on the basis of other types of evidence, that this antibiotic acts by damaging the selective permeability of the cell membrane (8).

The observations contained in this preliminary report are presently being extended. In addition, experiments are now in progress to correlate altered membrane properties in *Neurospora* with specific genetic mutations (9).

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4. The reference medium (36.8 mM KCl, 25.3 mM NaCl, and 1 percent sucrose) was designed as a simplification of minimal medium. Similar potentials were measured in both media, those in minimal medium being 129 \pm 10 mv for cells 8 mm behind the growing tips.
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Effect of Background Illumination on the Retinal Action Potential

Abstract. A study of the relation between the membrane potential and the amplitude of the slow component of the retinal action potential obtained from inside a single insect photoreceptor cell suggests that the slow component is equivalent to the change in the membrane potential caused by background illumination.

In recent years retinal action potentials have been recorded from single cells in the compound eye of the insect (1, 2). The retinal action potential presumably recorded from the inside of a photoreceptor cell or retinula cell is composed of two components, a fast transient or spike-like "on" response and a slow component maintained during illumination. This short report describes the effects of background illumination on the retinal action potential.

The materials used were the compound eye of a dragonfly, *Agriocnemis*. Methods involved were the same as reported elsewhere (3). Both the stimulating and background illumination obtained from tungsten lamps gave diffused light over the whole surface of the compound eye. Intensities of both lights were continuously varied by controlling the current through the lamps. This variation changed the spectral distribution of the light but probably did not affect the results of the experiment. Both intensity and duration of the stimulus were monitored by a photodiode and were displayed on the lower beam of an oscilloscope.

Figure 1A is a typical response from a single receptor cell obtained by intermittent stimulus light of 0.1-second