

fiber rather than the internal content alone, the observed radioactivity might include that of Ca ions adsorbed on the membrane surface.

The rate of fall of the spike potential decreased as $[K^+]_{in}$ was reduced, and finally the spike became very prolonged. The peak of the spike was followed by a plateau potential which often lasted more than 10 seconds when a K^- -free solution was injected (Fig. 1E). Voltage clamp experiments showed that the delayed rectifying current was marked at high $[K^+]_{in}$ but was diminished when $[K^+]_{in}$ was decreased. This rectifying current corresponds to the K current in the squid axon (5), and this change in the current appears to correspond to the change of the rate of fall of the spike in the absence of the clamp.

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Electrical Resistance of Cell Membranes of *Avena* coleoptiles

Abstract. *The cell membrane resistance to direct current was measured in single cells for the first time in a higher plant tissue, oat coleoptiles (Avena sativa). On the assumption that the current density over the cell surface was uniform, a mean value of about 1300 ohm-cm² was found for cells in an external nutrient medium containing 1 mmole each of K⁺, Na⁺, and Ca⁺⁺ per liter. As expected, either decreasing K⁺ concentration or increasing Ca⁺⁺ concentration increased the resistance.*

Membrane potential differences of 70 to 170 mv (the vacuole being negative) have been shown to be related, under certain conditions, to passive fluxes of cations and to differential permeability toward cations in single cells of the *Characeae* (1) and in

Table 1. Membrane resistance of *Avena* cells in the standard nutrient medium.

No. of cells	Cell area (10 ² cm ²)	Cell resistance (megohm)	Specific resistance (ohm cm ²)		
			Range	Mean	S.E.
18	17-61	1.2- 7.2	300-2230	910	150
15	18-70	2.5- 9.0	500-2800	1480	210
8	21-50	2.0-11.0	690-3750	1520	400
Mean of series				1300	

angiosperm seedling tissues, coleoptiles, and root cells (2). When this is so, there should be a close relation between the electrical properties of the membranes and the passive fluxes across them. This is so in *Chara australis* (3). The theoretical relations between these quantities have been discussed in detail for plant cells by Briggs, Hope, and Robertson (4) and Dainty (5).

As yet, no valid measurements of the membrane resistance in single cells of higher plants have been made (6). This report summarizes some preliminary observations made on *Avena* coleoptile cells of the resistance between the vacuole and external medium.

Segments of coleoptiles of *Avena sativa* var. Algerian were cut from 4- to 5-day-old plants grown in a nutrient medium containing (in millimoles per liter): KCl, 1.0; Ca(NO₃)₂, 1.0; MgSO₄, 0.25; NaH₂PO₄, 0.904; Na₂HPO₄, 0.048; pH 5.6 to 5.8. Most of the measurements were made with segments irrigated by this medium. A measure of cell-membrane resistance was obtained by passing a measured current between a micro salt bridge inserted into the vacuole and an external Ag/AgCl electrode and noting the change in potential difference between a second pair of electrodes, one inserted into the vacuole, the other in the external medium (1, 7). The microelectrodes inserted into the cell were, as previously described (2), about 1 μ in diameter at the tip and the cells ranged from 20 to 70 μ in diameter. The resistance (R) measured was that across both the plasmalemma and tonoplast, and was the gross resistance for unit area:

$$R = (\Delta E/I) A$$

where ΔE was the change in potential difference, I the current and A the area of the cell surface, calculated as a right cylinder; R was not corrected for internal resistance of the sap or for possible variation in current density through the surface. Another source

of error lay in the possibility that two microelectrode insertions close together caused some damage and loss of resistance at the puncture sites. The measurements showed considerable variability which had no close relation to either the potential difference or the size of the cell. It is possible that the variability of the present data is real and that R may vary appreciably among the cells of higher plant organs.

The results from several sets of experiments are summarized in Tables 1 and 2. In Table 1 the perfusing solution was the nutrient mixture already given. Although the range of resistance values was large, the mean resistance of three series of measurements was 1300 ohm cm². In other experiments, Table 2, resistance was measured in single cells before and after decreasing the K⁺ concentration in the standard nutrient mixture from 1.0 to 0.1mM; this change increased the resistance by about 60 percent. Other experiments gave a similar increase in resistance resulting from a tenfold increase in Ca⁺⁺ concentration (Table 2).

The membrane resistance of 1300 ohm cm² is similar to the 3000-7000 ohm cm² observed in *Chara australis* when the concentration of K⁺ was 1mM and that of NaCl, 0.1mM (1). The effect of variation in the concentration of K⁺ is also analogous to that in

Table 2. The increase in membrane resistance of single cells induced by lowering K⁺ concentration or increasing Ca⁺⁺ concentration in the standard nutrient medium. Means of six cells.

Solution	Specific resistance (ohm cm ²)	Increase (%)
1.0mM K ⁺	1535	
Changed to 0.1mM K ⁺	2480	62
1.0mM Ca ⁺⁺	1380	
Changed to 10.0mM Ca ⁺⁺	2210	60

Chara but the change in resistance is not as large.

This effect is consistent with the concept that electric resistance is largely determined by passive fluxes of K^+ ions in *Avena* cells. Evidence for the dependence of the potential difference on K^+ has been found (2, 8). The effect of increased Ca^{++} might supposedly cause a decrease in permeability to K^+ ions and hence an increase in R . However, high concentration of Ca^{++} causes hyperpolarization in *Avena* cells (8) to about 150 mv, corresponding to no reasonable ratio of activity. It appears that the well-known enhancement of ion accumulation by Ca^{++} (9) may be a result of an effect on an active transport mechanism. Briggs (10) has suggested several ways by which an active transport system might modify the potential difference compared with that resulting in purely passive fluxes.

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Guanine: Formation during the Thermal Polymerization of Amino Acids

Abstract. *The action of heat on a mixture of amino acids was studied as a possible abiological pathway for the synthesis of purines and pyrimidines. Guanine was detected. This result is significant in the context of chemical evolution.*

The thermal polymerization of amino acids has been extensively investigated by Fox and his co-workers (1). The striking results that have been obtained seem to indicate that heat may have been a possible source of energy for the synthesis of protein under abiological conditions.

The heating of amino acids ordinarily results in tars and other pyrolytic matter (2). In the presence of large proportions of aspartic acid or glutamic acid, however, copolymeric peptides are formed. Anhydropolymers, consisting of the 18 amino acids usually present in proteins, can be obtained. The properties of these preparations are similar to those of protein (3).

The main purpose of our investiga-

tion was to see whether some of the nucleic acid constituents could be formed during the thermal polymerization of amino acids. Such a result is, perhaps, not to be altogether unexpected, since amino acids are intermediates in the biosynthetic pathways leading to the formation of purines and pyrimidines. Guanine, which occurs both in DNA and RNA, was identified in the reaction products obtained by heating the amino acids together.

The proteinoid was prepared according to the method of Fox. Two parts of glutamic acid, two parts of aspartic acid, and one part of an equimolar mixture of the remaining 16 amino acids were heated together in a stream of nitrogen for 6 hours at 180° to 200°C.

The resulting mixture was then hydrolyzed with 6N HCl at 105°C immediately after its preparation. The hydrolyzate was evaporated under vacuum to a very small volume and neutralized with ammonium hydroxide. Thin-layer chromatography and paper chromatography were used for the analysis.

The technique of thin-layer chromatography is illustrated in Fig. 1. The supporting medium was a layer of silica gel G, 0.5 mm thick (4). The solvent was a mixture containing water-saturated butanol (99 percent) and ammonium hydroxide (1 percent). A portion of the reaction mixture was streaked along the origin of the plate. A mixture containing approximately 25 μ g of each of the bases, adenine, guanine, cytosine, and uracil, was applied along the origin. In a typical run, the solvent ascended 15 cm along the plate in 4 hours. The bulk of the free amino acids was separated from the bases in this manner.

The thin-layer chromatography plates were then dried and the right marginal area in which the bases would appear was sprayed with 2,7-dichlorofluorescein. In ultraviolet light the bases were located as dark absorbing areas against a green fluorescent background (5).

A strip of silica gel corresponding to each of the standards was scraped off the chromatography plate and was eluted with 10 ml of 0.01N formic acid. The eluted material was evaporated to a small volume (200 μ l) and a two-dimensional chromatograph on Whatman No. 4 paper was made. The solvents were a mixture of propanol, ammonia, and water (6:3:1 by volume), and butanol, propionic acid, and water (14:9:10 by volume). Shadowgrams of these chromatograms were prepared by laying the chromatograms over Kodagraph contact paper and shining an ultraviolet light above the chromatogram (6). The ultraviolet

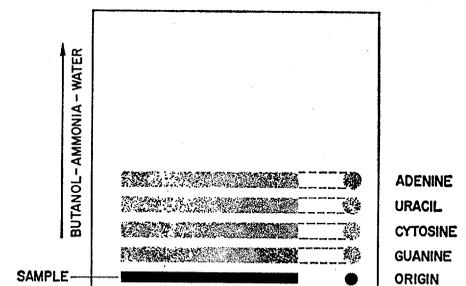


Fig. 1. Technique of thin-layer chromatography.