Table 1. Inhibition by erythromycin (E) of polyphenylalanine synthesis in a cell-free ribosomal system from *E. coli*, strain Gratia. Erythromycin was used as the lactobionate derivative. The complete reaction system contained the following additions in a volume of 0.27 ml; in micromoles: adenosine triphosphate (ATP) (di-K salt), 2.0; guanosine triphosphate (GTP), 0.2; phosphoenolpyruvate (PEP) 1.2, tris 2.0 (*p*H 7.8); KCl 40.0; magnesium acetate, 6.0; mercaptoethanol, 29.0; in millimicromoles: L-phenylalanine C^{14}, 0.5, with a specific activity of 297 mc/mmole; in micrograms: transfer-RNA (*10*), 400; polyuridylic acid (polyU) [K salt, (*11*)], 25; the extract consisting both of ribosomes and enzymes contained 232 μg of bacterial RNA. The mixture was incubated for 20 minutes at 37°C, 5.0 ml of 10 percent trichloracetic acid were then added, the mixtures were heated at 95°C for 20 minutes, and the precipitates formed were washed with 1 percent trichloracetic acid by centrifugation until there was no detectable radioactivity in the washings. Finally the precipitates were collected on Millipore HA filters and the radioactivity was determined with a Nuclear-Chicago liquid scintillation counter. CS, complete system.

Additions	Radioactivity in total ppt. (counts/min)
CS	33,649
CS − ATP	2,762
CS − polyU	764
CS + 1.0 μmole E	14,815
CS + 1.5 μmole E	6,765

of precipitating RNA and certain synthetic polyribonucleotides (*9*). We infer that the mechanism of action of erythromycin may be related to that of chloramphenicol and may involve an interaction with one or several categories of RNA that mediate protein biosynthesis.

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Membrane Properties of Barnacle Muscle Fiber

Abstract. *Membrane properties of the giant muscle fiber of the barnacle* Balanus nubilus *were studied by controlling the ionic composition of the external and internal media. The resting potential decreases with increasing external K-concentration,* $[K^+]_{out}$, *and decreasing internal K-concentration,* $[K^+]_{in}$, *over a considerable range. Spike potentials are elicited when the internal calcium ions are removed, and the overshoot is determined by the ratio between* $[Ca^{++}]_{out}$ *and* $[K^+]_{in}$ *and not by the external or internal* $[Na^+]$.

Giant muscle fibers 1 to 2 mm in diameter have been described recently in the barnacle *Balanus nubilus* by Hoyle and Smyth (*1*). This preparation offers an excellent opportunity for studying membrane physiology by controlling the internal ionic composition of the fiber.

A resting potential of 70 to 80 mv was found with a 3*M* KCl-filled glass micropipette. Depolarization produced by an outward current through a separate micropipette even well above the threshold for mechanical response did not elicit a spike but gave only a graded potential, as is usual for crustacean muscle. The membrane became capable of producing an all-or-none spike when the Ca^{++} concentration inside the fiber was reduced. The internal solution was changed by the following two methods.

1) In the intracellular injection method, a single muscle fiber was isolated with a tendon on one end, and the other end was cut after it had been tied with a thread. With the fiber mounted horizontally on a glass plate, a glass injection pipette 0.2 to 0.5 mm in diameter was introduced longitudinally through a small incision made close to the cut end of the fiber (see diagram *A*, Fig. 1). The test solution was injected while the pipette was being advanced so that the solution was distributed over the whole length of the fiber up to the tendinous end. The injection was continued until the diameter of the fiber became 1.5 to 2.0 times the original diameter. This would presumably make the internal ionic composition of the treated fiber close to that of the injecting solution. The fiber apparently tolerates this inflation rather well. The first 5 mm from the incision was covered with paraffin oil. The rest of the fiber (1.0 to 1.5 cm) was immersed in barnacle physiological saline (*1*); the length of this portion was usually two to three times the space constant of the fiber. The membrane potential change was recorded with a micropipette close to the tendinous end while polarizing currents were applied through the injection pipette (Fig. 1*A*). When a Ca^{++}-binding agent was injected, such as K_2SO_4 solution with K salt of EDTA (ethylenedinitrilotetraacetic acid) or K salt of citrate solution ($[K^+] = 490$ m*M*, the osmotic pressure maintained by adding sucrose; and *p*H adjusted to 6.9), the membrane became capable of initiating an all-or-none spike as shown by Fig. 1, *C1* and *C*2.

2) In the sandwich method, a piece of membrane was used instead of a whole muscle fiber. First a Ca^{++}-binding agent was injected into an isolated muscle fiber as in the first method. Then the middle 1 cm of a single muscle fiber was isolated by cutting away both ends. This length of muscle fiber was opened by cutting the membrane longitudinally along one side. The opened membrane was placed between two thin glass plates in each of which a hole of about 1-mm diameter had been drilled. The inner side of each plate had been covered with vaseline to avoid leakage, and the two plates were adjusted so that the holes were superimposed (see diagram *B*, Fig. 1). Figure 1*F* shows the spike potentials that could be obtained when a K_2SO_4 solution with 10 to 20 m*M* K salt of EDTA was in contact with the internal side of the membrane. The solution on the external side contained 169 m*M* Ca in this case. This second method is advantageous when the observation of effects of different internal solutions is desired for the same fiber membrane, although a slow deterioration was usually found in such preparation. At the present stage it is not possible to remove the entire internal content of the fiber from the membrane, and so it is difficult to make the ionic concentration just inside the membrane identical to that of the test solution, even by the sandwich method. Most of the results described below were obtained by the first method.

The resting potential of the muscle fiber changed linearly with the logarithm of $[K^+]$ in the external saline, with a slope of 56 mv for a tenfold increase of $[K^+]_{out}$. The resting potential changed its sign from the negative

value to the positive value at about 200 mM of $[K^+]_{out}$. The K concentration in the fiber measured by the flame photometer ranged between 110 and 130 mM/kg of wet weight of fiber. Since the water content of the barnacle muscle fiber is about 77 percent of wet weight of fiber, these values correspond to 140 to 170 per liter of internal water. Therefore, the $[K^+]_{out}$ at zero resting potential does not differ much from the internal K^+ concentration.

The resting potential was observed when $[K^+]_{in}$ was altered by injection (changing the proportion of K_2SO_4 to sucrose in the internal test solution), and the K concentration in the fiber was examined by the flame photometer after each experiment. The resting potential decreased linearly with the logarithm of $[K^+]_{in}$ in the range below about 200 mM. Therefore, these results indicate that the resting potential is largely determined by the concentration gradient of K^+ across the membrane, as is generally believed for most excitable tissues.

However, the following lines of evidence may suggest that the resting potential does not behave in the manner predicted for a simple K electrode. The resting potential is rather insensitive to an increase of $[K^+]_{in}$ above about 200 mM. Furthermore, it even becomes significantly smaller when the $[K^+]_{in}$ is increased to more than 300 mM. The replacement of K_2SO_4 in the internal test solution with an osmotically equivalent amount of KCl results in a considerable decrease of the resting potential. A similar result is obtained for internal Br^- and I^- while no such decrease is found for acetate^{--} or citrate^{---}. So-called "hyperpolarizing rectification" (*2*) is found for Cl^-, Br^- and I^-. The results suggest that the resting membrane is also permeable to small anions in the aqueous solutions such as Cl^-, Br^-, and I^-.

The overshoot of the spike potential obtained from fibers treated internally with a Ca^{++}-binding agent increased with $[Ca^{++}]_{out}$ (Fig. 1, *D*1–3). The increment for a tenfold decrease of $[K^+]_{in}$ was 8 to 10 mv at lower Ca^{++} concentrations while it became 5 to 6 mv at higher Ca^{++} concentrations. The overshoot also increased with decrease of the $[K^+]$ inside the fiber. The increment for a tenfold decrease of $[K^+]_{in}$ was about 29 mv. The removal of Na from the external saline by replacing with Tris (tris(hydromethyl)aminomethane), choline, tetramethyl ammonium, or guanidine did not affect the spike. An increase of $[Mg^{++}]_{out}$ did not restore the spike potential in the absence of external Ca^{++}, but Sr^{++} and Ba^{++} had a restoring action in the absence of Ca^{++}. The results suggest that the membrane potential at the peak of the spike appears to be determined by transmembrane gradients of $[Ca^{++}]$ and $[K^+]$. Fatt and Ginsborg (*3*) have proposed a similar idea for the spike potential of crayfish muscle fibers as a result of their studies on the effect of externally applied tetraethyl ammonium, Sr, and Ba solutions. This interpretation is in contrast to the idea that in some other tissues, such as squid giant axon and frog skeletal muscle fiber, the spike potential is considered to be produced by a permeability increase of the membrane to Na^+, and therefore the overshoot is determined by the concentration gradient of Na^+ (*4*).

The possibility of a permeability change of the membrane to Ca^{++} has been studied in barnacle muscle fibers with radioactive Ca^{45}. The radioactivity of the muscle fiber was examined after immersing the fiber in radioactive saline for varying periods with and without stimulation. The estimated resting influx through the membrane was about 18 pmole/sec cm^2, and 50 to 150 pmole/cm^2 was exchanged during each spike. The electrical charge per spike carried by this amount of Ca^{++} per spike is about ten times that necessary to charge up the membrane capacity by 80 mv, so that the observed Ca influx could account for the charge transfer during the spike. Since the radioactivity was examined with the whole muscle

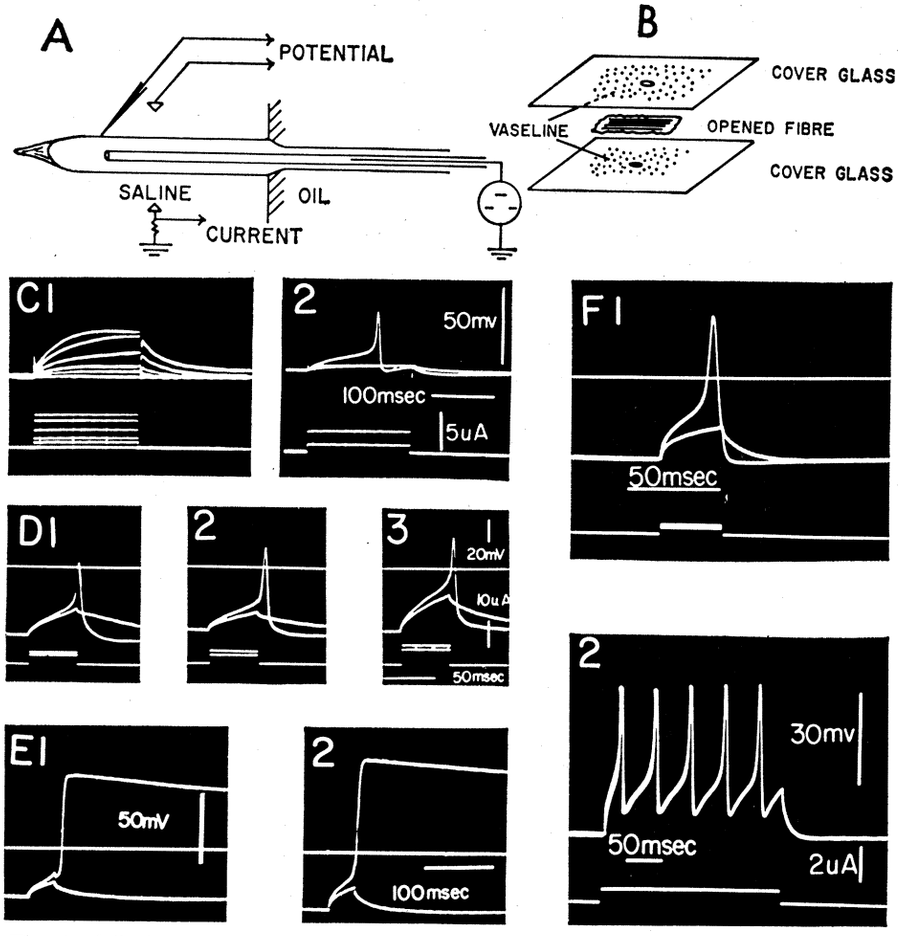

Fig. 1. (*A*) Arrangement for intracellular injection. (*B*) Arrangement for sandwich method. (*C*) Membrane potential changes (upper traces) of the same fiber associated with outward current pulses (lower traces), before (*C*1) and after (*C*2) the injection of K_2SO_4 solution with EDTA. Obtained in normal barnacle saline. (*D*) Effect of external Ca^{++} concentration on the spike potential of the fiber injected with K_2SO_4 solution with EDTA. $[Ca^{++}]_{out}$ is 20, 84, and 338 mM in *D*1, *D*2, and *D*3, respectively. (*E*) Spike potentials of a fiber injected with a solution containing no K (Na salt of EDTA and the osmotic pressure maintained by adding sucrose). $[Ca^{++}]_{out}$ is 20 and 84 mM in *E*1 and *E*2. (*F*) Spike potentials obtained with a sandwich preparation. $[Ca^{++}]_{out}$, 170 mM. The internal solution was K_2SO_4 solution with EDTA. The trace on the upper part of each record indicates the reference potential level.

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. 1*E*). 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 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; $Ca(NO_3)_2$, 1.0; $MgSO_4$, 0.25; NaH_2PO_4, 0.904; Na_2HPO_4, 0.048; *p*H 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.

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

No. of cells	Cell area (10^5 cm²)	Cell resistance (megohm)	Specific resistance (ohm cm²)		
			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	

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 0.1m*M*; 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 1m*M* and that of NaCl, 0.1m*M* (*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 cm²)	Increase (%)
1.0m*M* K^+	1535	
Changed to 0.1m*M* K^+	2480	62
1.0m*M* Ca^{++}	1380	
Changed to 10.0m*M* Ca^{++}	2210	60