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Role of Lipids in the *Neurospora crassa* Membrane

II. Membrane Potential and Resistance Studies; the Effect of Altered Fatty Acid Composition on the Electrical Properties of the Cell Membrane

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Summary. The effect of doubling the saturated fatty acid content on the electrophysiology of *Neurospora crassa* membranes was studied. Intracellular membrane input resistance (R_m) and potential (E_m) were measured for wild-type (*w/t*) and *cel*$^-$ (Tween 40) organisms as a function of temperature. Over the 0 to 40 °C temperature range studied, mean E_m values of both *w/t* and *cel*$^-$ (Tw 40) organisms increased from -160 to -210 mV. This difference is greater than that expected from Nernst potential considerations, indicating an active component of E_m. This active component is insensitive to a doubling of the saturated fatty acid content. R_m exhibits a temperature dependence and hysteresis. Averaged data indicate an increase in R_m with decreased temperature. The slope of the temperature dependence varies among individual hyphae. Above 17.5 °C *cel*$^-$ (Tw 40) hyphae averaged greater than 70% higher values of R_m than *w/t*. Below 17.5 °C *w/t* R_m data divided into low and high temperature dependence groups, while *cel*$^-$ data exhibited a low temperature dependence. The results are discussed in relation to gel-liquid crystal phase transitions, membrane fluidity, and the contribution of fatty acid structure to membrane electrical properties.

Recent investigations have led to the proposal and support of the hypothesis that membrane fatty acid composition is important in controlling membrane physiology. Mainly, indirect evidence links alterations of fatty acid composition with changes in ion permeability and enzyme activity (Van Dijck, Goldbach & Van Deenen, 1973; Haslam, Spithill,

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Linnane & Chappell, 1973; Papahadjopoulos, Jacobson, Nir & Isac, 1973; Rottem, Cirillo, DeKruyff, Shinitzky & Razin, 1973; Davis & Silbert, 1974; Wojtczak, 1974). These fatty acid-induced alterations of membrane phenomena have been attributed to either: (1) the alteration of membrane architecture, i.e. the alteration of relationships between protein and lipid membrane components, or, (2) alteration of the physical state, i.e. lipid fluidity, of the cell membrane. If, in fact, changes in membrane fatty acid composition do alter ion permeability or the activity of enzymes responsible for the maintenance of ionic gradients across the cell membrane, then differences in electrical properties of cell membranes with altered fatty acid composition should result. Moreover, such differences in the electrical properties of the cell membrane should be detectable by *in vivo* electrophysiological techniques. Consequently, the current group of experiments was undertaken to determine if the alteration of the fatty acid composition of the cell membrane would alter membrane resistance or resting membrane potential. Changes in membrane resistance would indicate alteration of either the size or number of membrane pathways through which ions carry current, while changes in membrane potential not attributable to ionic equilibria would indicate alteration of enzyme activities associated with metabolically maintained components of the resting membrane potential.

An investigation into the electrophysiological consequences of altered membrane lipids requires an organism suited to both electrophysiological technique (i.e. microelectrode penetration) and alteration of membrane lipid composition (preferably by genetic and media manipulation). The breadmold, *Neurospora crassa*, uniquely fulfills both these requirements (Slayman, Lu & Shane, 1970; Henry & Keith, 1971). Moreover, *Neurospora* is known to have the majority of its extremely large resting membrane potential under metabolic control (Slayman, 1965*b*) assuring the possibility of distinguishing between the membrane potential contributions made by altered enzyme activity and changes in diffusion pathways.

The first paper in this series (Friedman, 1977) reported the physical properties of the phospholipids of wild-type (*w/t*) *Neurospora crassa* membranes and compared them to the more saturated membrane phospholipids of the *cel*$^-$ *Neurospora* organism grown on Tween 40. It was shown that the saturated fatty acid content of the Tw40 organism is roughly twice that of the *w/t* organism (*see* Table 1). This doubling of the saturated fatty acid content of the membrane was shown to raise the gel-liquid crystal phase transition of the extracted phospholipids by 20 °C. Nevertheless, the phospholipid phase transitions for both *w/t* and *cel*$^-$ occur

Table 1. Fatty acid composition of *Neurospora* phospholipids (mole per cent)[a]

Fatty acid methyl ester	*w/t* (22 °C)	*Cel*$^-$ (Tw 40) (34 °C)
16:0	14.0	39.3
16:1	2.6	1.7
16:2	1.6	–
18:0	1.5	2.3
18:1	6.1	10.2
18:2	58.7	35.0
18:3	15.5	11.5
% Saturated	15.5	41.6
% Unsaturated	84.5	58.4
% 16-C	18.2	41.0
% 18-C	81.8	59.0

[a] Modified from Friedman (1977).

below 0 °C. In addition, it was shown that intact *Neurospora* do not exhibit a phase transition detectable by differential scanning calorimetry.

This second paper reports the results of membrane resistance and membrane potential measurements on *w/t* and *cel*$^-$ *Neurospora* as a function of temperature in an effort to determine if a doubling of membrane saturated fatty acid content alters the electrophysiological behavior of the *Neurospora* membrane.

Materials and Methods

Wild-type *Neurospora crassa* (strain RL 3-8A) and the fatty acid-requiring *cel*$^-$ strain (FGSC165) were grown on sterile, scratched cellophane discs in Petri plate cultures as described previously (Slayman, 1965*a*; Friedman, 1977). Vogel's minimal media (Vogel, 1956) was supplemented with 2% (w/v) sucrose and 1.5% agar for the growth of the wild-type organism at 22 °C. *Cel*$^-$ organisms were grown at 34 °C on the same medium with Tween 40 (polyoxyethylene sorbitan monopalmitate) added.

For electrophysiological experiments *Neurospora* hyphae growing on cellophane were excised from Petri plate cultures with scalpel and forceps and placed on the undersurface of a metal-reinforced coverglass (5 × 15 mm). The coverglass was then positioned on a stainless steel support such that the *Neurospora*-containing cellophane was sandwiched between the coverglass and the stainless steel support with the *Neurospora* facing downward into a solution-containing chamber (Fig. 1).

Care was taken to insure the uniformity and physiological condition of hyphae selected for impalement. Impaled hyphae were taken from approximately 1 cm behind the growing edge of the plate culture. Impaled segments did not contain branches. Both voltage-recording and current-passing electrodes were placed in the same hyphal segment. Hyphae exhibiting an initial membrane potential of less than 100 mV were rejected.

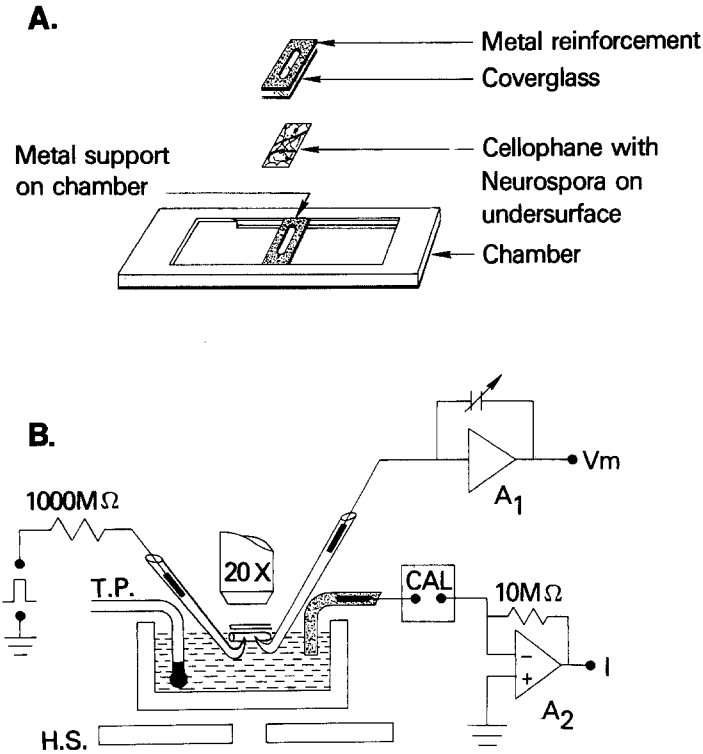

Fig. 1. Experimental set-up for electrophysiology. (*A*) *Neurospora* hyphae grown on cellophane were suspended facedown into a fluid-filled chamber. (*B*) The chamber was placed under a compound microscope equipped with a heating stage (*H.S.*). Bath temperature was monitored with a temperature probe (*T.P.*) placed in the bath. A high impedance, capacitance neutralized amplifier (A_1) was used to monitor membrane potential (V_m), capacitance being adjusted by "squaring up" a calibration pulse (*CAL*) inserted between an indifferent electrode and an operational amplifier (A_2) in a negative feedback configuration which served as a current monitor (*I*). Current was injected into hyphae with a second microelectrode connected to a physiological stimulator through a 1000 MΩ resistor

Electrophysiological measurements were made while *Neurospora* hyphae were bathed in an aqueous solution containing (in mM): 10 Tris, 20 NaCl, 10 KCl, 1 $CaCl_2$ and 85 sucrose, buffered with HCl to pH 8. Bath temperature was controlled by a fluid-cooled microscope stage (Cambion Model 806-1036-01) used in conjunction with an automatic temperature servo control system (Cambion Model 809-3001-01, Cambridge Thermionic Corp., Cambridge, Massachusetts). Bath temperatures were monitored by placing a small thermometer probe in the bath (Yellow Springs Model 520 used in conjunction with Yellow Springs thermometer model 15-176D, Yellow Springs, Ohio). The thermometer's millivolt output was digitized with an analog-to-digital converter (Analog Devices AD 2003, Analog Devices, Norwood, Mass.). The bath temperature was photographed in digital form simultaneously with the oscilloscope display.

Electronic instrumentation was similar to that used by Naitoh, Eckert and Friedman (1972) as diagrammed in Fig. 1. Hyphae were intracellularly stimulated with depolarizing

current pulses delivered through hooked, glass capillary microelectrodes filled with 2.8 M KCl and having tip diameter of less than 1 μ. Membrane potentials (V_m) of hyphae as well as "IR drops" caused by injecting depolarizing currents were measured with a second intracellular microelectrode placed in the same hypha at a distance of roughly 100 μ from the first. A Bioelectric Instruments NF1 neutralized-capacitance amplifier was used for recording membrane potentials and responses to current passage. Capacitance neutralization was achieved by "squaring up" a calibration pulse (delivered by a Stoelting Model CA5 calibrator) inserted between virtual ground of the current-measuring amplifier (Analog Devices 40J) and the bath. A 2.8 M KCl agar bridge connected the bath to the summing junction of the current-measuring amplifier through the calibrator. The outputs of the neutralized-capacitance amplifier (V_m) and of the current-measuring amplifier (I) were displayed on a dual beam oscilloscope (Tektronix R5103N) and photographed with a Grass kymograph camera.

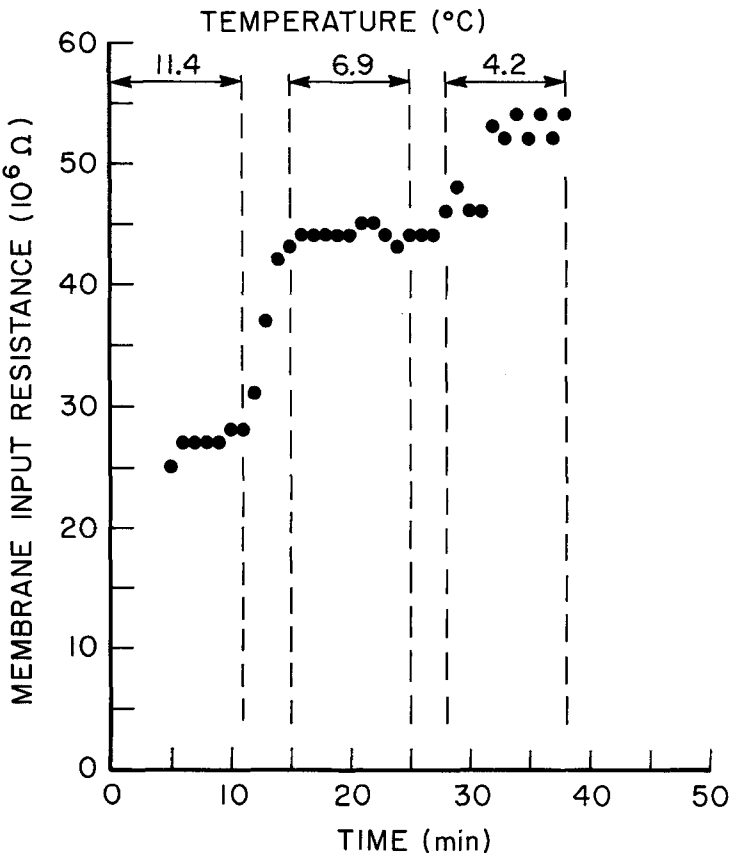

Fig. 2. "Stepping" of membrane resistance as a function of temperature. Input resistance increases in direct response to decrease in temperature of the bathing medium. Resistance values are relatively constant as a function of time at a given temperature. Data plotted are from one experiment on a *w/t* organism

Results

Stepping

Initial experiments were undertaken to determine if it is possible to detect a change in electrophysiological parameters as a function of temperature and, if so, what interval is necessary for such a change to occur. A series of "stepping" experiments was performed in which *Neurospora* hyphae were impaled at a known temperature, allowed to equilibrate at that temperature for 10 min, subjected to a temperature change, followed by 10 min of equilibration at that new temperature, and so on. During the equilibration periods, measurements were made of membrane potential and membrane resistance. This process was continued on individual hyphae for as long as recordings could be obtained from the initial intracellular penetrations. The membrane resistance data of one such experiment is plotted in Fig. 2, and indicates that resistance is temperature dependent. Membrane resistance increases with decreased temperature and achieves a steady-state value within a few minutes of temperature change.

In contrast, similar plots of membrane potential *vs.* time failed to

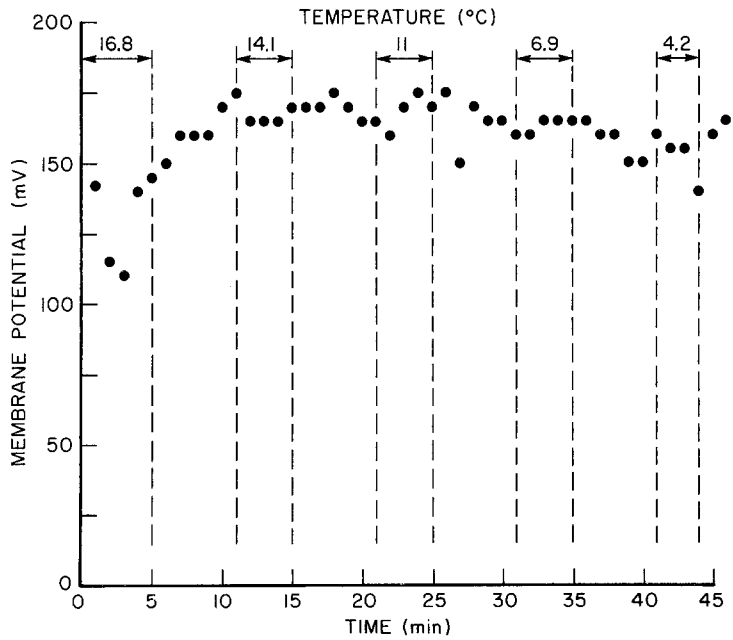

Fig. 3. Membrane potential as a function of time and temperature. Gradual changes in temperature (~1 °/min) do not dramatically change membrane potential values. Changes in membrane potential values as a function of time are also not evident

demonstrate a temperature dependence discernable by eye for individual hyphae (i.e. there was no "stepping"). Fig. 3 demonstrates that: (1) membrane potential values undergo "spontaneous" fluctuations at constant bath temperatures, and (2) gradual changes in the bath temperature of individual hyphae do not produce dramatic temperature-dependent changes in membrane potential.

These results differ from those of Slayman (*personal communication*) who has observed that changes in temperature cause depolarization of hyphal membranes followed by slow, time-dependent membrane repolarization. These changes in membrane potential seem to result from a sudden shift in temperature (20–30 °C in less than 1 min). In the present group of experiments, the use of a gradual temperature change ($\sim 1°$/min) has avoided this problem.

Membrane Potential vs. Time

Although it is not obvious from the visual inspection of individual "stepping" experiments, the averaged data of many such experiments

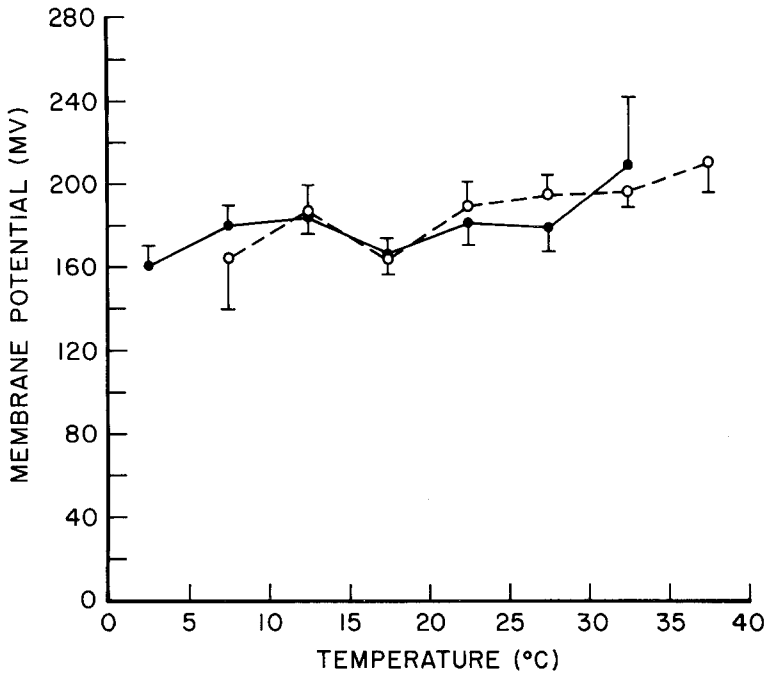

Fig. 4. Membrane potential as a function of temperature. Membrane potential values for all *w/t* experiments were averaged for 5 °C intervals, mean values (●) and 95% confidence limits plotted. *Cel*$^-$ (Tw 40) data were handled similarly (○)

indicate a strong temperature dependence of membrane potential. When hyphae are maintained at a given temperature for 5 min and then either heated or cooled (at a rate of 1°/min) to a new temperature, a plot of mean temperature values for 5 °C intervals can be obtained, as shown in Fig. 4. As indicated, mean values of intracellular membrane potential span −160 to −210 mV for the 0 to 40 °C temperature range studied. These values of membrane potential are in agreement with previously reported values (Slayman *et al.*, 1970). The averaged membrane potential values for *w/t* and *cel*$^-$ (Tw 40) hyhae lie close to each other throughout the 0–40 °C temperature range. The data demonstrate that a difference of 40 °C in bath temperature produces a 50-mV difference in resting membrane potential. A 20-mV difference would be expected on the basis of equilibrium (Nernst) potential considerations.

Membrane Resistance vs. Temperature

Membrane resistance data were collected simultaneously with the membrane potential data. In some experiments it was possible to obtain

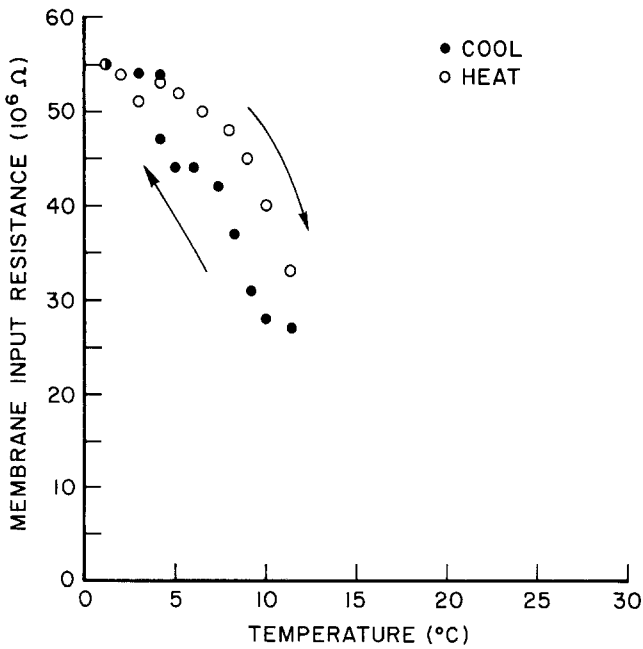

Fig. 5. Hysteresis of membrane resistance as a function of temperature. When the temperature of the bathing medium is lowered to near 0 °C, and then raised, hyphal membrane resistance values for both heating and cooling portions of the experiment are in close agreement. Data plotted are of one experiment on a *w/t* organism

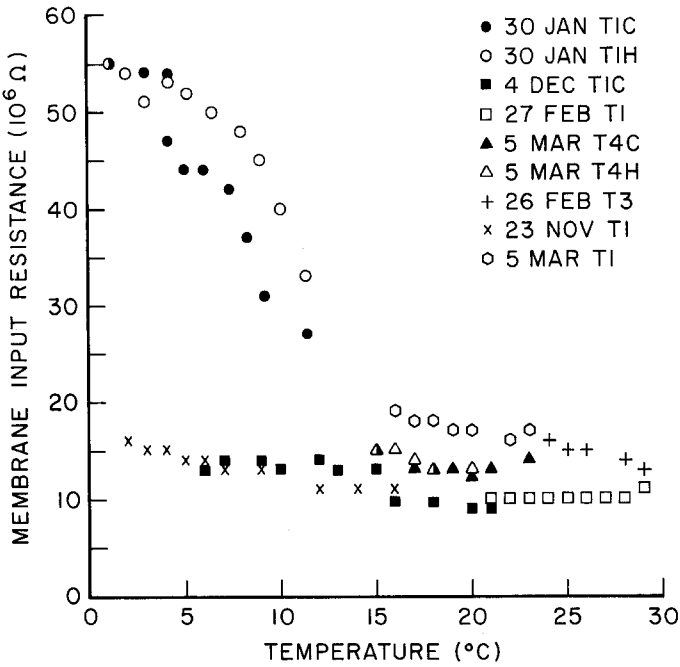

Fig. 6. Plot of membrane resistance *vs.* temperature for some *w/t* hyphae. In most cases membrane resistance increased as temperature decreased, but not always. The slope of the temperature dependence was variable

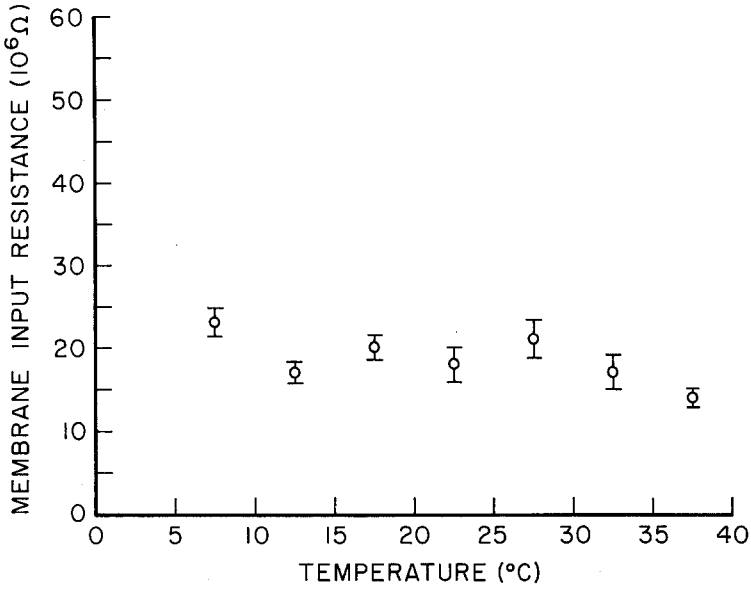

Fig. 7. Relationship between membrane resistance and temperature for *cel*$^-$ (Tw 40) *Neurospora*. Data of all *cel*$^-$ (Tw 40) experiments were averaged for 5 °C intervals, mean values and 95% confidence limits plotted

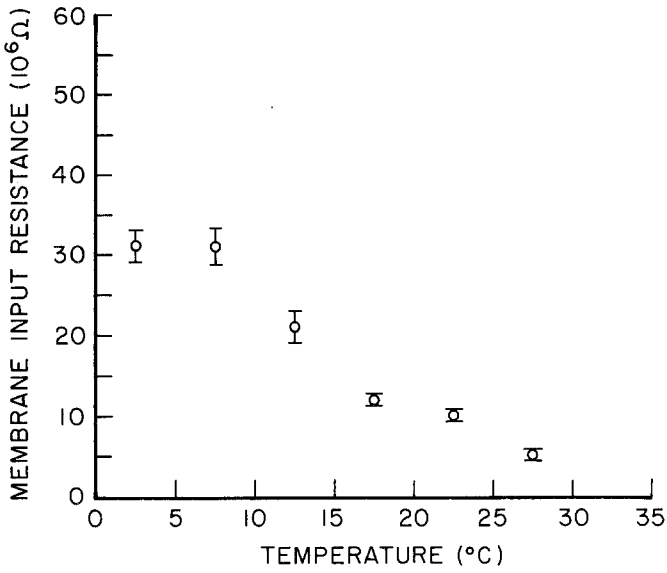

Fig. 8. Relationship between membrane resistance and temperature for *w/t Neurospora*. Data of all *w/t* experiments were averaged for 5 °C intervals, mean values and 95% confidence limits plotted

data on a single hypha during a temperature change of 15 °C and to continue collecting data during a subsequent return of temperature to its initial value. One such experiment is shown in Fig. 5, and indicates the close agreement of membrane resistance values during the heating and cooling portions of an experiment. Most hyphae were found to have higher membrane resistances at low temperatures, but this was not always the case as demonstrated in Fig. 6 for *w/t*. Similar findings were found by Murray (1966) in *Aplysia* visceral ganglion cells. When all membrane resistance data obtained from *cel*$^-$ (Tw 40) are averaged for 5 °C intervals and plotted as a function of temperature, a gradual increase in membrane resistance with decrease in temperature is seen throughout the 5–40 °C temperature range studied (Fig. 7).

The relationship between membrane resistance and temperature shown by *w/t Neurospora* hyphae is more complex. When all the *w/t* membrane resistance data are averaged for 5 °C intervals and plotted as a function of temperature, a sigmoid-shaped curve is generated (Fig. 8), showing a gradual increase in membrane resistance with decrease in temperature from 35 °C to 15 °C, a region of sharper increase in membrane resistance from 15 °C to 7 °C, and a plateauing of membrane resistance values from 7° to 0 °C. Alternatively, the membrane resistance

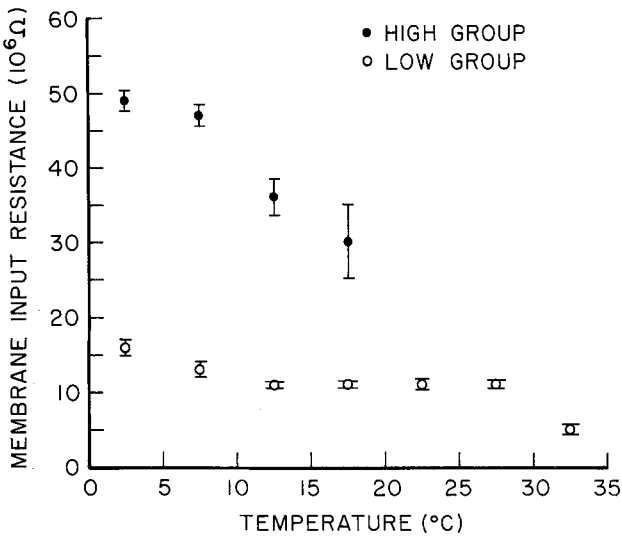

Fig. 9. Relationship between membrane resistance and temperature for *w/t* data considered as two groups, one group having a larger temperature dependence than the other. The data is the same as used in Fig. 6, but assigned to either "High Resistance" or "Low Resistance" groups. Data for each group were averaged for 5 °C intervals, mean values and 95% confidence limits plotted

experiments performed on *w/t Neurospora* can also be interpreted as representing two groups of hyphae, one group having a gradual increase in membrane resistance with temperature decrease throughout the 0 to 35 °C region monitored, and a second group having a larger dependence of membrane resistance on temperature in the 0 to 15 °C region. Plots of some individual experiments from each group are shown in Fig. 6. If *w/t* data are treated as two groups, the data of each group being averaged for 5 °C intervals and plotted as a function of temperature, the curves shown in Fig. 9 result. Comparing Fig. 7 with Fig. 8 or Fig. 9 indicates that the more saturated *cel*$^-$ (Tw 40) hyphae appear to have higher membrane resistance than *w/t*, but a similar temperature dependence above ~17.5 °C.

Discussion

The intent of the present study was twofold: (1) to determine if a relationship exists between membrane phase transitions and electrical properties of the cell, and (2) to determine the effect of altered membrane fatty acid composition on cellular electrophysiological parameters.

Although *Neurospora* is uniquely suited to address these problems, some concern should be expressed about the ability of intracellular technique to accurately reflect *Neurospora* cell membrane phenomena. The multibranched structure of *Neurospora* hyphae, and the presence of a hyphal cell wall are potential sources of electrophysiological complexity. These have been shown, however, not to introduce significant artifact into *Neurospora* electrophysiological data (Slayman, 1965a, b).

A more troublesome source of electrophysiological artifact is the phenomenon of septal plugging. Both Slayman (*personal communication*) and the author have observed transient increases (<3 min) in experimentally measured "IR" drops of *Neurospora* hyphae. These increases are attributable to temporary blockages of the openings between septae and the cell membrane. Such blockages increase the longitudinal resistance of the hyphae, and, if undetected, could lead to spurious conclusions about membrane resistance. To exclude such artifactual increases in longitudinal resistance from the data analysis presented here, transient increases in measured "IR" drops of $\leqq 3$ min have not been considered. Thus, by eliminating from consideration the major source of instability of longitudinal resistance, it is possible to assume for the purposes of the following discussion that the longitudinal resistance of *Neurospora* hyphae remains relatively constant, and small in relation to membrane resistance.

Further support for the belief that intracellular technique accurately reflects *Neurospora* membrane phenomena stems from the similarity of *Neurospora* electrophysiological data with that of other membranes: (1) The increase in membrane resistance with decrease in temperature (Fig. 2) is a phenomenon which occurs in both biological and synthetic membranes. Hope and Aschberger (1970) report the temperature dependence of membrane resistance in both marine and freshwater algae, and Krasne, Eisenman and Szabo (1971) demonstrate the dependence of conductance (the reciprocal of resistance) on temperature in planar bilayer membranes. (2) The temperature-induced hyperpolarization of resting membrane potential in excess of that accountable to diffusional processes (Fig. 4) occurs in neurons (Gorman & Marmor, 1970) and algae (Hope & Aschberger, 1970). (3) The temperature dependence of resting membrane potential measured for *Neurospora* is within the range of published values for plant and animal cells (Table 2).

Previous literature would predict a relationship between phase transition and electrical properties of the cell membrane (Fredericq, Bacg & Florkin, 1971; Krasne *et al.*, 1971; DeKruyff, DeGreet, Van Eyk,

Table 2. Published values of change in resting membrane potential with change in temperature

Organism	Author	ΔRMP (mV/°C)
Plant Cells		
Chara coralina	Hope & Aschberger (1970)	1.5
Griffethsia pulvinata	Hope & Aschberger (1970)	0.65
Nitella	Hogg, Williams & Johnston (1968)	1.6
Nitella (in the light)	Spanswick (1972)	2.5
Nitella (in the dark)	Spanswick (1972)	0.97
Nerve Cells		
Anisodoris	Gorman & Marmor (1970)	2.0
Aplysia	Marchiafava (1970)	1.2
Aplysia	Murray (1966)	−4 to +2.3
Carcinus[a]	Dorai Raj & Murray (1962)	0.9
Helix[a]	Kerkut & Ridge (1962)	2.0
Lobster[a]	Dalton & Hendrix (1962)	1.0
Other		
Hela	Borle & Loveday (1968)	0.32
Purkinje Fiber, dog Ht.[a]	Trautwein *et al.* (1953)	3.0

[a] Taken from Murray (1966).

Demel & Van Deenen, 1973; Overath & Trauble, 1973; Papahadjopoulos *et al.*, 1973; Rottem *et al.*, 1973). Demonstrating this relationship in *Neurospora* will be more difficult than initially hoped because of the occurrence of the phospholipid phase transitions below 0 °C (Friedman, 1977). Increasing the long-chain, saturated fatty acid content of the cell membrane may raise the phase transition above 0 °C. The electrophysiological data presented here are consistent with the calorimetry data indicating the occurrence of *Neurospora* phospholipid phase transitions below 0 °C. No abrupt changes in electrophysiological properties were observed in the 0–40 °C temperature range studied, nor was there a displacement to higher temperatures of the electrophysiological properties of *cel*$^-$ (Tw 40) as might be expected from the increased saturated fatty acid content of its membranes.

With regard to direct effects of membrane fatty acid composition on electrophysiological parameters, previous work suggests that both passive and enzyme-mediated transport mechanisms can be influenced by the fatty acid content of the membrane. The permeability of K ions into liposomes, *E. coli*, and *Mycoplasma* increases with increased unsatu-

ration (Scarpa & deGier, 1971; Van Deenen, 1972; Davis & Silbert, 1974). Active transport of ions into mitochondria (Haslam *et al.*, 1973) and sarcoplasmic reticulum (Fiehn & Hasselbach, 1970) have also been shown to be dependent on the presence of unsaturated fatty acids. Since both active and passive components of membrane potential exist in *Neurospora* (Slayman, 1965*b*), it is possible to consider the effect of altered membrane fatty acid composition on both active and passive components of *Neurospora* membrane potential.

The electrophysiological data presented here indicate that doubling the saturated fatty acid content of the membrane does not alter the membrane potential nor the relationship between membrane potential and temperature. Since the predominant component of *Neurospora*'s membrane potential, ~170 of 200 mV, is metabolically controlled (Slayman, 1965*b*), the electrophysiological data suggest that the enzymes responsible for the active maintenance of the resting membrane potential are insensitive to a doubling of saturated fatty acid content. Possibly, there exists a critical concentration of saturation above or below which changes in membrane transport would be seen. Such critical levels have been postulated for *E. coli* (Davis & Silbert, 1974) and yeast mitochondria (Haslam *et al.*, 1973). It is possible that the supramaximal permissible amount of saturation was not achieved in *cel*$^-$ (Tw 40).

With regard to the influence of fatty acid saturation on *in vivo* passive membrane electrical properties, data presented here indicate a large, temperature-dependent increase in membrane input resistance in some *w/t* organisms (Figs. 6, 9) which was not seen in the more saturated *cel*$^-$ (Tw 40) organisms. This large increase in membrane input resistance was seen in hyphae derived from five different cultures over a period of 45 days. Thus, the large increase in membrane input resistance is an infrequent but reproduceable occurrence. If one assumes that the cytoplasmic resistivity of *Neurospora* is similar to that of nerve and muscle (del Castillo & Machne, 1953; Carpenter, Hovey & Bak, 1975), the data presented here suggest a relationship between unsaturation and increased *in vivo* membrane resistance. Possibly more extreme alteration of fatty acid saturation will yield a more dramatic and consistent change in membrane resistance.

Thus far, studies addressing the problem of the relationship between membrane fatty acid composition and membrane permeability have focused on fatty acid saturation. Quite possibly other structural parameters of fatty acids have as great or greater influence. It is known, for example, that incorporation of the branched-chain fatty acid, phytanic acid, pro-

duces osmotic fragility and abnormal growth in *Neurospora* (Brody & Allen, 1972) and causes Refsum's disease in humans (Steinberg, Vroom, Engel, Cammermeyer, Mize & Avigan, 1967). The relationship between other structural properties of fatty acids and electrophysiological parameters would appear to be a subject worthy of future investigation.

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References

Borle, A.B., Loveday, J. 1968. Effects of temperature, potassium and calcium on the electrical potential difference in Hela cells. *Cancer Res.* **28**:2401

Brody, S., Allen, B. 1972. The effects of branched chain fatty acid incorporation into *Neurospora crassa* membranes. *J. Supramolec. Struct.* **1**:125

Carpenter, D.O., Hovey, M., Bak, A. 1975. Resistivity of axoplasm. II. Internal resistivity of giant axons of squid and *Myxicola*. *J. Gen. Physiol.* **66**:139

Davis, M.T., Silbert, D.F. 1974. Changes in cell permeability following a marked reduction of saturated fatty acid content of *E. coli* K 12. *Biochim. Biophys. Acta* **373**:224

DeKruyff, B., DeGreef, W.J., Van Eyk, R.V.W., Demel, R.A., Van Deenen, L.L.M. 1973. The effect of different fatty acid and sterol composition on the erythritol flux through the cell membrane of *Acholeplasma laidlawii*. *Biochim. Biophys. Acta* **298**:479

DeKruyff, B., Van Dijck, P.W.M., Goldbach, R.W., Van Deenen, L.L.M. 1973. Influence of fatty acid and sterol composition on the lipid phase transition and activity of membrane-bound enzymes in *Acholeplasma laidlawii*. *Biochim. Biophys. Acta* **330**:269

del Castillo, J., Machne, X. 1953. Effect of temperature on the passive electrical properties of the muscle fibre membrane. *J. Physiol. (London)* **120**:431

Fiehn, W., Hasselbach, W. 1970. The effect of phospholipase A on the calcium transport and the role of unsaturated fatty acids in ATPase activity of sarcoplasmic vesicles. *Eur. J. Biochem.* **13**:510

Fredericq, H., Bacq, Z.M., Florkin, M. 1971. Chemical control of the lipid phase transition in black lecithin films. *Arch. Int. Physiol. Biochim.* **83**:139

Friedman, K.J. 1977. Role of lipids in the *Neurospora crassa* membrane. I. Influence of fatty acid composition on membrane lipid phase transitions. *J. Membrane Biol.* **32**:33

Gorman, A.L.F., Marmor, M.F. 1970. Contributions of the sodium pump and ionic gradients to the membrane potential of a molluscan neurone. *J. Physiol. (London)* **210**:897

Haslam, J.M., Spithill, T.M., Linnane, A.W., Chappell, J.B. 1973. The effects of altered membrane lipid composition on cation transport by mitochondria of *Saccharomyces cerevisiae*. *Biochem. J.* **134**:949

Henry, S.A., Keith, A.D. 1971. Saturated fatty acid requirer of *Neurospora crassa*. *J. Bacteriol.* **106**:174

Hogg, J., Williams, E.J., Johnston, R.J. 1968. The temperature dependence of the membrane potential and resistance in *Nitella translucens*. *Biochim. Biophys. Acta* **150**:640

Hope, A.B., Aschberger, P.A. 1970. Effects of temperature on membrane permeability to ions. *Aust. J. Biol. Sci.* **23**:1047

Krasne, S., Eisenman, G., Szabo, G. 1971. Freezing and melting of lipid bilayers and the mode of action of nonactin, valinomycin and gramicidin. *Science* **174**:412

Marchiafava, P.L. 1970. The effect of temperature change on membrane potential and conductance in *Aplysia* giant nerve cell. *Comp. Biochem. Physiol.* **34**:847

Murray, R.W. 1966. The effect of temperature on the membrane properties of neurons in the visceral ganglion of *Aplysia*. *Comp. Biochem. Physiol.* **18**:291

Naitoh, Y., Eckert, R., Friedman, K. 1972. A regenerative calcium response in *Paramecium*. *J. Exp. Biol.* **56**:667

Overath, P., Trauble, H. 1973. Phase transitions in cells, membranes and lipids of *E. coli*. Detection by fluorescent probes, light scattering, and dilatometry. *Biochemistry* **12**:2625

Papahadjopoulos, D., Jacobson, K., Nir, S., Isac, T. 1973. Phase transitions in phospholipid vesicles. Fluorescence polarization and permeability measurements concerning the effect of temperature and cholesterol. *Biochim. Biophys. Acta* **311**:330

Rottem, S., Cirillo, V.P., DeKruyff, B., Shinitzky, M., Razin, S. 1973. Cholesterol in *Mycoplasma* membranes. Correlation of enzymic and transport activites with physical state of lipids in membranes of *Mycoplasma mycoides var. capri* adapted to grow with low cholesterol concentrations. *Biochim. Biophys. Acta* **323**:509

Scarpa, A., DeGier, J. 1971. Cation permeability of liposomes as a function of chemical composition of lipid bilayers. *Biochim. Biophys. Acta* **241**:789

Slayman, C.L. 1965*a*. Electrical properties of *Neurospora crassa*. Effects of external cations on the intracellular potential. *J. Gen. Physiol.* **49**:69

Slayman, C.L. 1965*b*. Electrical properties of *Neurospora crassa*. Respiration and intracellular potential. *J. Gen. Physiol.* **49**:93

Slayman, C.L., Lu, C.Y.-H., Shane, L. 1970. Correlated changes in membrane potential and ATP concentrations in *Neurospora*. *Nature (London)* **226**:274

Spanswick, R.M. 1972. Evidence for an electrogenic ion pump in *Nitella translucens*. I. The effects of pH, K^+, Na^+, light and temperature on the membrane potential and resistance. *Biochim. Biophys. Acta* **288**:73

Steinberg, D., Vroom, F.Q., Engel, W.K., Cammermeyer, J., Mize, E.C., Avigan, J. 1967. Refsum's Disease. A recently characterized lipidosis involving the nervous system. *Ann. Intern. Med.* **66**:365

Van Deenen, L.L.M. 1972. Permeability and topography of membranes. *Chem. Phys. Lipids* **8**:366

Vogel, H.J. 1956. A convenient growth medium for *Neurospora* (Medium N). *Microb. Genet. Bull.* **13**:42

Wojtczak, L. 1974. Effect of fatty acids and acyl-CoA on the permeability of mitochondrial membranes to monovalent cations. *FEBS Lett.* **44**:25