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Passive Electrical Properties of Halobacterium Species

I. Low-Frequency Range

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Abstract. The electrical conductivity of suspensions of two species of Halobacterium was measured at low A. C. frequency. The results obtained from *Halobacterium halobium* suspensions show that the bacteria act as non-conducting particles. In contrast, the cells of a Halobacterium obtained from the Dead Sea (*Halobacterium marismortui*) had an apparently high conductivity which can be explained partly in terms of the cell-membrane being pierced by pores through which ions can move freely and partly in terms of highly concentrated cell ions, all of which are mobile.

Key words: Conductivity – Halobacterium.

The extremely halophilic bacteria grow in media containing NaCl at concentrations approaching saturation. The cells are characterized by concentrations of potassium which approach those of the outside Na^+, the outside K^+ concentration being around 1 mM. In *H. marismortui*, the cells contain large amounts of potassium even when the metabolic rate is undetectably low, and even though the cell-membrane allows rapid exchange of ions (Ginzburg et al., 1971). The mechanism whereby potassium is retained within the cell is not, therefore, immediately obvious, and it seems possible that the mobility of this ion may be lower inside the cell than outside. In contrast, there seems no reason to doubt that the coll sodium and chloride are in thermodynamic equilibrium with these ions in the outside medium.

It was decided to measure some passive electrical parameters of *H. marismortui*, to see whether such measurements could throw light on the membrane permeability and on the state of the cell ions.

Electrical measurements on suspensions of cells such as erythrocytes, yeast and some species of bacteria have shown that at frequencies below 100 KHz the cell-membranes behave as insulators; at high frequencies the insulating properties are lost, and it becomes possible to measure directly the electrical conductivity and dielectric properties of the inner contents (Fricke, 1933; Fricke and Curtis, 1934; Fricke et al., 1956). In this paper, we describe measurements of the electrical con-

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ductivities of two species of Halobacterium at low frequency. The bacteria were suspended in 3.5 M or 4 M NaCl. To the best of our knowledge, these are the first conductivity measurements to be made on biological material at such high salt concentrations. As a control, we have determined the electrical conductivity of Bakers' yeast at low and high salt concentrations. Yeast can withstand high NaCl concentrations without loss of viability (Ørskov, 1945). Our results at low NaCl concentration are comparable to many similar measurements made by earlier workers (e.g. Fricke and Curtis, 1934), and serve as a basis of comparison with the measurements at high salt concentration.

Theory

A general equation for the conductivity of spheres suspended in a conducting medium was derived by Maxwell (1873), and was later generalized by Fricke (1924) to apply to all spheroids in suspension:

$$\frac{k - k_1}{k + xk_1} = p\,\frac{k_p - k_1}{k_p + xk_1}. \tag{1}$$

k = specific conductivity of suspension
k_1 = specific conductivity of suspending solution
k_p = specific conductivity of spheroid particles
p = volume fraction of particles in suspension
x = shape factor, dependent on axial ratio of particle and on $k_1 : k_2$

The equation assumes that the particle is electrically homogeneous.

Maxwell (1892) also considered particles consisting of a core and an interfacial surface layer with properties markedly different from both the interior and exterior phases. This treatment was used by Cole (1928) and by Fricke and Curtis (1936) to determine the surface conductance of spherical particles consisting of a shell with conductivity k_3 and a core with conductivity k_2:

$$\frac{k - k_1}{k + xk_1} = p\,\frac{\bar{k}_2 - 1}{\bar{k}_2 + xk_1}. \tag{2}$$

$\bar{k}_2$ = effective conductivity of particle

$$\bar{k}_2 = k_3\,\frac{(2\,k_3 + k_2)\,(a + t)^3 - 2\,(k_3 - k_2)a^3}{(2\,k_3 + k_2)\,(a + t)^3 + (k_3 - k_2)a^3}. \tag{3}$$

a = radius of particle
t = thickness of shell

When $k_3 = 0$, i.e. the shell is impermeable, then Equation (1) must be used.

Let us consider three models.

Model 1. $k_3 = 0$ i.e. the shell of the particle is non-conducting. This is equivalent to a cell, the membrane of which is practically impermeable to ions. Under such conditions

$$p = \frac{x(k_1 - k)}{k + k_1 x}. \tag{4}$$

Model 1 can be said to hold when p, as obtained from conductivity measurements and Equation (4), agrees with p as measured by other methods (e.g. centrifugation in calibrated tubes; microscopic counting).

Model 2. The shell of particle has a finite conductance, i.e. $k_3 \neq 0$. The core itself is, however, bounded by a non-conducting layer so that the conductivity of the core is effectively zero, i.e. $k_2 = 0$. This is equivalent to a cell with an impermeable, non-conducting membrane surrounded by a conducting wall. If $t \ll a$, it follows from Equation (3) that:

$$\bar{k}_2 = \frac{2\,tk_3}{a}. \tag{5}$$

An estimate of k_3 can be made from the concentrations and mobilities of the ions in the shell:

$$k_3 = \sum c_3^i\, u_3^i\,. \tag{6}$$

c_3^i = concentration of ith mobile ion in the shell
u_3^i = mobility of ith mobile ion in the shell

If the cell-wall is at equilibrium with the outside medium, there must be a Donnan equilibrium between the wall and outside, and hence c_3 can be calculated (Overbeek, 1956; Carstensen et al., 1965):

$$c_3^+ + c_3^- = c_3^f \left[1 + \frac{(2\sum c_1)^2}{c_3^f}\right]^{1/2}. \tag{7}$$

c_3^+ = mobile cations in shell
c_3^- = mobile anions in shell
c_3^f = fixed charges in shell
c_1 = mobile ions in outside solution

Two extreme cases can thus be envisaged. In the first, when $c_1 > c_3^f$ then $\sum c_3^+ + \sum c_3^- = 2\sum c_1$. Thus the concentration of free ions in the Donnan phase is more or less equal to that of the free ions in the outer solution. In the second case, when $c_3^f \gg c_1$, the concentration in the shell is almost independet of the ion concentration in the outer solution, and may almost equal c_3^f or be much higher than c_1. In such cases there is a Donnan accumulation of ions. Should u_3^+ and u_3^- be roughly similar to the ion mobilities in the outer medium, then $k_3 = k_1$ (in case 1) and $k_3 \gg k_1$ (in case 2). This latter case was found in *E. coli* and *Micrococcus lysodeikticus* by Carstensen (1967).

Model 3. Both k_2 and k_3 have finite values. This is equivalent to a cell with conducting contents surrounded by a permeable, conducting membrane. Thus k_2 represents the conductivity of the cell-body while k_3 is the conductivity of the cell-membrane. When $a \gg t$ Equation (3) can be simplified:

$$(a + t)^3 = a^3 + 3\,a_t^2$$

and

$$\frac{\bar{k}_2}{k_3} = \frac{2\,k_3 t + k_2 a}{k_3 a + k_2 t} \tag{8}$$

and solving for k_3:

$$k_3 = \frac{1}{4}\left(\frac{a}{t}(k_2 - \bar{k}_2)^2 + 8\, k_2\bar{k}_2\right)^{1/2} - \frac{a}{t}\,(k_2 - \bar{k}_2)\,. \tag{9}$$

When $k_3 \ll k_2$, then $\frac{t/a}{k_3} = 1/\bar{k}_2 - 1/k_2\,.$ (10)

Methods

Materials

Yeast was purchased in compressed form from Paca Ltd., Ramat Gan, Israel. Portions of yeast were dispersed in a large excess of NaCl solution (40 mM or 4 M) and were equilibrated overnight at 20° C. The following morning the suspension was centrifuged, and the yeast cells resuspended in fresh solution so as to have 30–40% of the total volume occupied by cell material.

The *H. halobium* strain used was given by Professor A. D. Brown to Dr. Yair Heimer who kindly gave us a culture. It was grown in 4 M NaCl with Bacto-peptone and Bacto-yeast autolysate and resembled in essence a medium described by Brown (1963). Two litres of freshly-grown culture were centrifuged and the sedimented bacteria resuspended in 20 ml of the original medium by gentle mixing with a magnetic stirrer. Conductance and volume-fraction measurements were made on this suspension, which was then centrifuged to obtain the supernatant on which conductance measurements were also made. The Dead Sea Halobacterium used was isolated in 1965. It is identical in appearance and biochemical characteristics with *H. marismortui* (Bergey's Manual, 4th Edition). (Personal communications of Drs. Moshe Mevorach and B. Elazari-Volcani.) The method of culture has been described (Ginzburg et al., 1970). Experimental methods for determination of conductance and volume fraction were the same as for *H. halobium*. In addition, samples were taken from the suspension and supernatant for determination of K, Na and protein.

Apparatus

Measurements of electrical resistance (series resistance) were made with a General Radio impedance bridge (model 1608-A) fitted with an external power source (Monsanto signal source model 303A). Suspensions and solutions, the resistances of which were to be measured, were poured into a cell built according to the specifications of Pauly and Schwan (1966) and modified by the boring of holes through the walls of the cup. The cell was inverted into a 20-ml beaker containing the substance to be measured, and stood inside a water-jacketed vessel through which a stream of water flowed at the desired temperature. The temperature of the water was controlled by a Masterline 2160 Bath and Circulator (Forma Scientific, Marietta, Ohio).

Cell-Volume Fraction

This was routinely determined by use of hematocrit capillary tubes. From the pellet volumes thus obtained, there had to be subtracted the volume of medium trapped between the cells composing the pellet (the trapped volume). This parameter could not be measured directly in the capillary tubes, the volume of which is too small for the purpose. Instead, it was measured indirectly by making, on one and the same cell-suspension, two sets of measurements of pellet volume, one with the hematocrit capillary tubes and the other with larger calibrated tubes which held enough material to permit the accurate measurement of trapped volume. The pellet volumes being measurable in both types of tube, it was possible to *calculate* the trapped volume in hematocrit-tube pellets from the trapped volume *measured* in the larger tubes. After an accurate determination of trapped volume had been made, the value obtained was used to correct pellet volumes measured by means of hematocrit tubes in all subsequent experiments.

The method used for the determination of trapped volume is essentially the same as that described by Solomon (1952) for erythrocytes: a cell-suspension is centrifuged in the presence of a marker substance too large to penetrate the cells and marked either by radioactivity or, as in the present case, by a distinctive color. The marker used was Blue Dextran, a Sephadex product with a molecular weight of 2 million and absorbing light strongly at 62 nm. After centrifugation of the cell-suspension with marker in a calibrated tube, the volume of the pellet is determined, the supernatant is scrupulously removed and the pellet is suspended in a small known volume of medium without marker. After a second centrifugation, the absorption of the supernatant can be used to calculate the volume of marked medium that must have been trapped within the pellet. The volume of marked medium is then expressed as a percentage of the total pellet volume.

The results of these experiments are shown in Table 1. The accuracy of the cell-volume fraction measurements depends upon the agreement between replicate hematocrit tubes (less than 2% variation between replicates) and on the trapped volume determination (for variability see Table 1). It can be calculated that the cell-volume fraction can be determined with a standard deviation of better than 3% of the mean (calculated according to Wilson, 1952).

Table 1. Measurements of volume of medium trapped within pellets after centrifugation of cell suspensions. Figure in brackets refers to number of determinations

Organism	Trapped volume as % of total volume	
	Large tubes	Microhematocrits
Yeast	26.3 + 0.6 (10)	27.5 + 0.7 (6)
H. halobium	22.1 + 0.13 (5)	27.8 + 1.0 (4)
H. marismortui	28.4 + 0.2 (10)	28.8 + 1.4 (8)

Table 2. Parameters required for calculation of resistance of cell suspensions. The shape factor is calculated from the axial ratio and from Figure 5 in Fricke (1924)

Organism	Shape factor	Cell-wall thickness (nm)	Volume of wall as % total cell volume
Yeast in 40 mM NaCl	2	70 ± 10	6
Yeast in 4 M NaCl	2	70 ± 10	6
H. halobium	1.7	13 – 15	5–10
H. marismortui	1.9	15	5–10

Cell Dimensions

a) Axial Ratio. This is needed to compute the shape factor, from Figure 5 in Fricke (1924). This figure is a graphical representation of the shape factor for prolate spheroids. The yeast cells were assumed to be spheres; the axial ratio is therefore 1. The cell axes of the Halobacterium species were measured on photomicrographs. The ratio of length to breadth is 1 : 3.2 for *H. halobium* and 1 : 3.5 for *H. marismortui* (mean of 40 cells). The shape factors are given in Table 2.

b) Ratio of Cell-Wall Thickness to Radius. This factor is needed for the calculation of the proportion of the cell-volume occupied by the wall. The width of the wall was measured on electron-micrographs. These were specially prepared from freeze-etched samples of yeast taken from the batch used for the conductivity measurements. The results are included in Table 2 and agree well with the results quoted by Moor and Muhlethaler (1963). The yeast cell-volume was determined from the packed cell-volume divided by the number of cells in the suspension. The volume of a single cell being known, its radius could be calculated. The calculation of the cell radius is dependent on the accuracy to which the trapped-volume fraction is known. Assuming outside limits of 20 and 30% for this fraction, the radius of a single cell could lie between 3.20 and 3.06 μ. The fraction of the cell-volume occupied by wall material would then vary between 2.2 and 6.6%. A figure of 6% is obtained when the best estimate of the trapped volume is used.

The widths of the cell-envelopes of the Halobacterium species are taken from Kirk and Ginzburg (1972). The cell-volumes were measured on photomicrographs. However, it is only possible to make a rough estimate of the fraction of the cell-volume occupied by cell-envelope (Table 2).

Results

It was necessary to establish that measurements of single-celled suspensions would give the same results as had been obtained by other workers working with different instruments, and furthermore, whether it would be possible with concentrated salt solutions – up to 4 M – to make sufficiently accurate measurements for the conductivity of Halobacterium cells to be calculated.

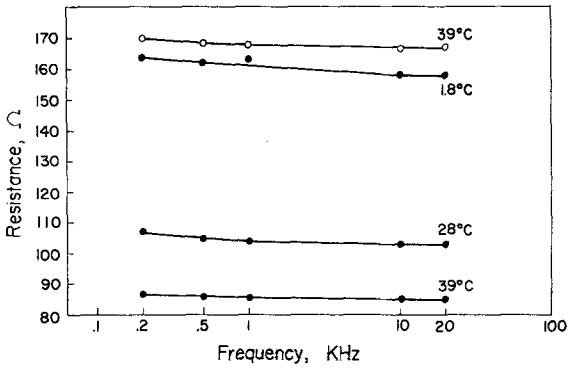

Fig. 1. Resistance of 40 mM NaCl (●—●) and of yeast suspended in 40 mM NaCl (○—○) plotted against frequency in kHz

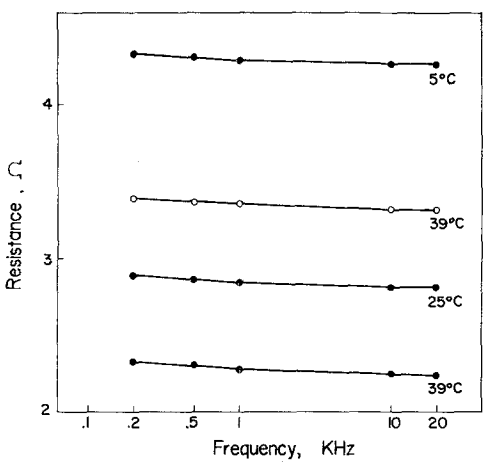

Fig. 2. Resistance of 4 M NaCl (●—●) and of yeast suspended in 4 M NaCl (○—○) plotted against frequency in kHz

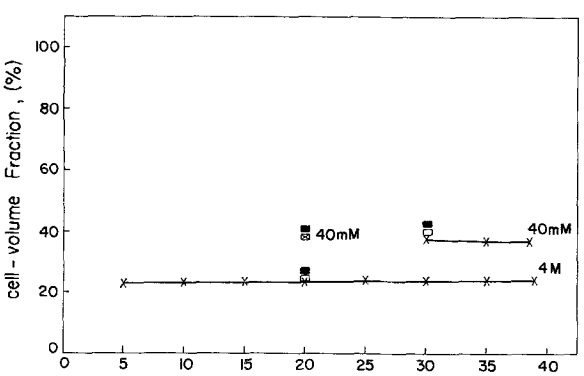

Fig. 3. Volume fraction of cell suspension occupied by yeast cells as calculated from conductivity measurements of suspensions and of supernatants, from Equation (1): ×—×. □ ■: volume fraction measured by centrifugation; □ is corrected for the volume occupied by the cell-wall. *t*: temperature in °C of conductivity measurements

Table 3. Volume fraction of cell suspension occupied by cells of *H. halobium* as calculated from conductivity measurements of suspensions and of supernatants. Measurements at 20 kHz. Cell-volume fraction measured by centrifugation method is also given. The resistance was measured not less than four times on each sample

Age of culture (h)	Temperature (°C)	Resistance, ohms		Cell volume fraction	
		Supernatant	Suspension	Conductivity (%)	Centrifugation (%)
17	20.2	3.33	4.68	19.5	14.9 ± 0.5
	40.5	2.48	3.46	19.1	14.9 ± 0.5
18	20.0	3.28	3.80	9.3	9.8 ± 0.3
	39.0	2.43	2.81	9.3	9.8 ± 0.3
24	20.0	3.25 ± 0.01	3.85 ± 0.05	11.0	9.2 ± 0.3
48	20.0	3.31 ± 0.05	4.04 ± 0.14	12.6	9.8 ± 0.3
48	20.0	3.04 ± 0.06	3.58 ± 0.02	10.4	10.4 ± 0.3
48	20.0	3.24 ± 0.02	4.00 ± 0.00	12.4	20.9 ± 0.6

For the above reasons, preliminary measurements were made on suspensions of yeast cells. The first suspending solution to be used was 40 mM NaCl. The resistance of suspensions and of the supernatants obtained after centrifuging off the cell were measured at frequencies from 0.2–20 KHz, at temperatures from 1.8–39° C (Fig. 1). The resistance at the two lowest frequencies tried was slightly higher than at 10 or 20 KHz, presumably because of polarization. Consequently, all subsequent experiments were done at 20 KHz. Agreement was adequate between the cell-volume fraction as measured by centrifugation of a give suspension (cytocrit method) and as calculated from Equation (1). Thus, the two suspensions shown in Figure 3 had 37.9 and 38.5% volume fractions as determined by the conductivity method, and 43.5% and 40.3% volume fractions as determined by cytocrit. Asami et al. (1976) have suggested that, since the cell-wall is permeable to the outer solution, the volume occupied by the wall should be subtracted from the total cell-volume. When this correction is made, the volume fractions occupied by cell material become 40.9% and 37.9% for the two suspensions. There is thus better agreement between the conductivity and cytocrit methods. It will be noticed that the results are not affected by the temperature at which the electrical resistance were measured.

Next, yeast cells were suspended in 4 M NaCl solutions, and the measurements repeated (Fig. 2). The same upward trend in resistance at the lower frequencies was noticed as with solutions of 40 mM NaCl. In Figure 3 the cell-volume fraction of the suspension shown is 24% (electrical resistance method) or 26.5% (cytocrit method), or 24.9% (cytocrit corrected for volume of cell-wall). Thus, the agreement between the electrical method and the cytocrit corrected for the volume of the cell-wall is very good. The volume fraction, as determined by the electrical resistance method, was unaffected by the temperature at which readings were made.

The results showed that it was possible to work at high salt concentrations with the apparatus available.

Table 4. Volume of cell suspension occupied by cells of *H. marismortuis*, as calculated from conductivity measurements of suspension and of supernatants at 20 kHz. The cell-volume fraction measured by centrifugation is also given. The resistance of each sample was measured not less than five times

Age of culture (h)	Temperature (°C)	Resistance, ohms		Cell volume fraction	
		Supernatant	Suspension	Conductivity (%)	Centrifugation (%)
24	20	3.78 ± 0.03	4.34 ± 0.01	8.9	22.4 ± 0.6
24	20	3.73 ± 0.05	4.31 ± 0.06	9.2	20.5 ± 0.6
48	20	3.94 ± 0.07	4.48 ± 0.12	8.3	17.1 ± 0.5
72	20	3.45 ± 0.14	4.15 ± 0.09	9.3	22.4 ± 0.6

Suspensions of *H. halobium* in medium with 4 M NaCl gave results showing that at 20 KHz the electrical behaviour of these cells is consistent with Model 1 i.e. there is a non-conducting layer situated virtually at the geometrical surface of the cell (see Table 3). Out of six different suspensions tested, only in one was there a significant discrepancy between the cell-volume fraction as determined by the electrical and cytocrit methods. One might have expected to obtain somewhat higher values from the cytocrit method, seeing that the cells are surrounded by a cell-envelope and that 6–8% of the cell-volume is situated outside the cell-membrane. However, the electrical behaviour of *H. halobium* is largely consistent with Model 1 i.e. the cells from cultures of up to 48 h of age are surrounded by a non-conducting membrane.

In contrast, in suspensions of Dead Sea Halobacterium in 3.5 M NaCl there was no agreement between the cell-volume fraction, as measured by cytocrit and as calculated from Fricke's equation (see Table 4). The discrepancy between the two sets of results is far too great to be ascribed to the presence of a conducting cell-envelope. It must be concluded that these cells do not fit the model which was applied to the yeast and *H. halobium* cells.

Discussion

The results of our measurements on the electrical conductivity of yeast and *H. halobium* cells are consistent with the hypothesis that these cells are surrounded at low frequencies by an electrically-insulating, ion-impermeable membrane. This was not the case for the Dead Sea Halobacterium; there was a large discrepancy between the cell-volume fraction as measured by cytocrit, and as measured by the electrical method. As already mentioned, similar discrepancies were noticed in *E. coli* and *Micrococcus lysodeikticus* and were found to be due to fixed charges in the cell-wall (Carstensen et al., 1967, 1968).

Let us see whether the same explanation could apply to the Dead Sea Halobacterium. For this we need to refer to Equation (5). In the first place, let us estimate k_3, the specific conductivity of the cell-envelope (the layer outside the cell-membrane). It

Table 5. Calculation of $\bar{k}_2$, effective conductivity of *H. marismortui* cell, from Equation (5) on assumption that cell has a conducting outer layer. Measured values of $\bar{k}_2$ are given as contrast with calculated values

Age of culture (h)	$\bar{k}_2$ calculated (Equation 5)	Measured
24	0.00660	0.143
24	0.00664	0.144
48	0.00632	0.139
72	0.00696	0.152

can be estimated that up to 20% of the cell-proteins are to be found in the cell-envelope, which occupies 5–10% of the total cell-volume. The proteins are thus three times as concentrated in the cell-envelope as in the bulk of the cell. The percentage of charge-bearing aminoacid residues in the cell-envelope proteins is the same as in the bulk proteins (Lanyi, 1974) and varies from 30–35%. Since the volume of packed cells/cell protein is of the order of 0.5 μl/100 μg, it can be calculated that there are about 0.7 moles of charged aminoacids residues per litre of cell-envelope material. This latter figure does not take the lipid charges of the membrane into account. However, c_3' has a minimum value of 2.1 M. Since $c_i = 3.5$ M, it can be calculated from Equation (7) that $c_3^+ + c_3^-$ cannot differ from c_i by more than 10%. It is not to be expected that u_3^+ or u_3^- be higher than the corresponding mobilities in the outside solution. Since conductivity is a function of ion concentration and mobility (Equation 6), and since these parameters have similar values in the cell-envelope and outside, it follows that the conductivity of the cell-envelope cannot be very different from that of the outside solution.

From Equation (5) and on assuming that $k_3 = k_1$, one can calculate $\bar{k}_2$, and compare the calculated and measured values (Table 5). This table shows that $\bar{k}_2$, as measured, is 20 times larger than $\bar{k}_2$ as calculated from Equation (5). It does not therefore seem likely that the conductivity of the Dead Sea Halobacterium can be caused by a high concentration of fixed charges in the outer layers of the cell. It is concluded that Model 2 is inconsistent with the electrical behaviour of *H. marismortui*.

Let us now consider Model 3 which is equivalent to a cell with conducting contents and conductivity k_2 surrounded by a permeable, conducting cell-membrane with conductivity k_3. The relevant Equations are (9) and (10): a, the cell-radius = 500 nm and is much larger than t, the thickness of the membrane, which is 5–10 nm.

Figure 4 is a numerical solution of k_3 as a function of k_2 for culture IV in Table 4. The other cultures give similar results. It can be seen that k_3 has two limiting values, the first at high k_2 (when $k_3 = \bar{k}_2 \cdot t/a$) and the second at low k_2. Where $k_2 = \bar{k}_2$ there is a rapid change in k_3 from one limiting value to the other. It can be argued from physical considerations that only a small region of the curve is applicable to *H. marismortui*. On *a priori* grounds, the penetration of ions through a typical cell-membrane is either by a "carrier" mechanism or through aqueous pores. Neutral "carriers" are unlikely to contribute to the electrical conductivity of the membrane and will be neglected in the coming discussion, and we will assume that ions

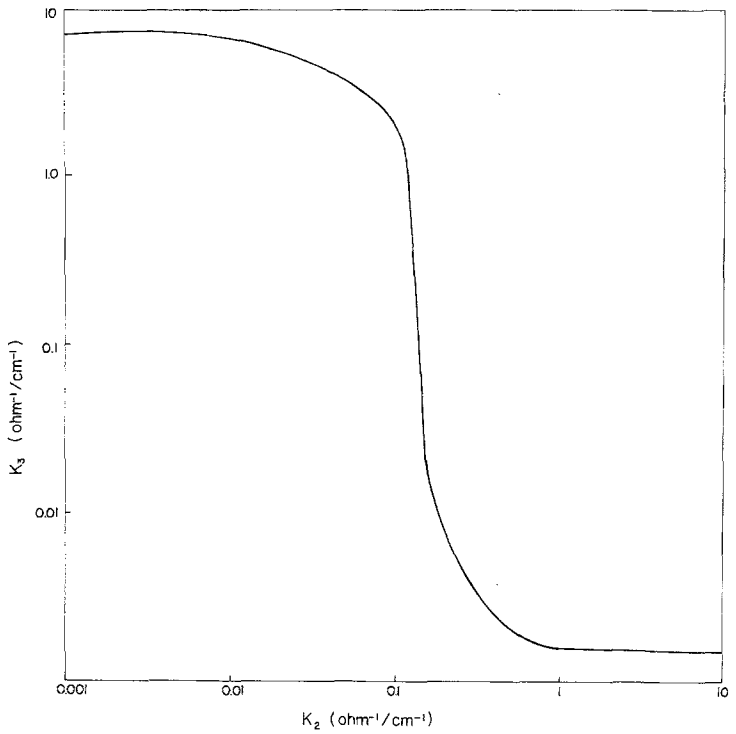

Fig. 4. k_2, conductivity of cell contents, using $\bar{k}_2$ otained from culture 4, Table 4 as a function of k_3, conductivity of cell-membrane, calculated from Equation (9)

cross the membranes through pores. An estimate of k_3 can be made on the assumption that the conductivity of the membrane is determined by the total volume of pores per volume of membrane and that the pores contain medium at the outside salt concentration. Were the total membrane volume to be occupied by medium, it would have a conductivity of $2 \times 10^5\ \Omega^{-1}$/cm. From Figure 4 the minimum value of k_3 compatible with the model is $1.5 \times 10^3\ \Omega^{-1}$/cm or 1% of the former value, which suggests that the area of aqueous pores would be 1% of the total membrane area. It is most unlikely that the pore area be any higher.

If a value of $1.5 \times 10^3\ \Omega^{-1}$/cm is accepted for k_3, then from Figure 4, k_2 must be $\gg 1.5 \times 10^{-1}\ \Omega^{-1}$/cm. k_2, the conductivity of the cell contents is determined principally by the major cell ions, Na^+, K^+, Cl^-. As these are present in molar amounts, the relative contribution of H^+ and OH^- can be assumed to be negligible. Were only Na^+ and Cl^- to be mobile, k_2 would equal $1.6 \times 10^{-1}\ \Omega^{-1}$/cm, which would mean that $k_3 > 4 \times 10^{-2}\ \Omega^{-1}$/cm. If k^+ were to be as mobile as in the bulk, $k_2 = 4 \times 10^{-1}$ and $k_3 = 2 \times 10^{-3}\ \Omega^{-1}$/cm. These latter values fit those regarded as likely in the preceding section and point to the conclusion that most of the ions inside the cells are mobile. The issue cannot finally be resolved by measurements in the low-frequency range; the conductivity of the cell contents can be determined with certainty only by measurements at higher frequencies where the membrane cannot exert any resistance.

In conclusion, *H. marismortui* has been shown to have unusual properties of electrical conductivity at a frequency of 20 KHz. The high conductivity which has been observed, is ascribed to the presence of pores in the membrane permitting ions to flow between the outside medium and the cell interior. The bulk of the membrane is presumably non-conducting in the usual way. In contrast, cells of yeast in 4 M NaCl behaved as insulated particles, as is consistent with their lack of permeability to sugars and ions (Ørskov, 1945). *H. halobium* behaved in the same way as yeast

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