DIFFUSION POTENTIALS IN MODELS AND IN LIVING CELLS

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It is important to learn what fundamental properties of protoplasm are responsible for its electrical behavior. Progress in this field evidently depends on advances in physical chemistry and their use in biology.

Experiments on cells which are especially suitable for such studies show that they possess the properties of an aqueous system covered by a thin layer of non-aqueous material which is the chief seat of the electrical potentials. This material is present in exceedingly small amounts so that we can hardly hope to obtain enough for analysis. Failing this we may try to find models which act like the living cell. Much has been learned in this way.

A useful substance for this purpose is guaiacol which acts like certain protoplasmic surfaces in various ways, such as the following:[1]

1. It allows water to pass freely: it admits inorganic electrolytes and to a still greater extent certain "lipoid-soluble" substances.

2. It is more permeable to potassium salts than to sodium salts and more permeable to chlorides than to sulfates.

3. When it is shaken with 0.01 M NaCl and placed in a U-tube with aqueous 0.1 M NaCl on one side and aqueous 0.01 M NaCl on the other the dilute solution is electrically positive in the external circuit. This indicates that the mobility of Na^+ (*i.e.*, u_{Na}) is greater than that of Cl^- (*i.e.*, v_{Cl}). This applies also to KCl and to the guaiacolates of sodium and potassium (which will be called for convenience KG and NaG).

4. When aqueous 0.1 M KCl is placed on one side of guaiacol (previously shaken with 0.1 M NaCl) and aqueous 0.1 M NaCl is placed on the other the KCl is negative in the external circuit ("potassium effect"). This indicates that u_K is greater than u_{Na}. This applies also to KG and NaG.

The study of guaiacol has especial interest because the role of diffusion potentials can be determined with considerable precision since Shedlovsky and Uhlig,[2] with the aid of the moving boundary measurements in guaiacol made by Longsworth, have determined the mobilities in guaiacol of K^+, Na^+, and the guaiacol ion together with dissociation constants and activities.

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[1] *Cf.* Osterhout, W. J. V., Some models of protoplasmic surfaces, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1940, **8**, 51.

[2] Shedlovsky, T., and Uhlig, H. H., *J. Gen. Physiol.*, 1933–34, **17**, 549, 563.
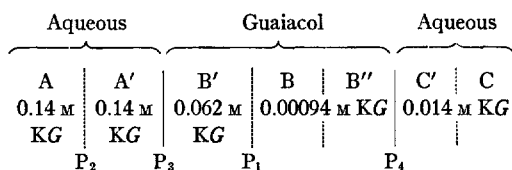
Using these data we can predict diffusion potentials in cells of the type

Calomel electrode	Aqueous KG concentrated	Guaiacol	Aqueous KG dilute	Calomel electrode
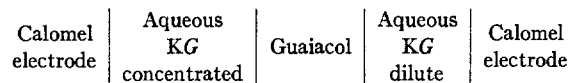
The calculations agree so well with the observed values that we may conclude that the latter are due to diffusion potentials.

This is important in its bearing on the study of bioelectric behavior. Since the equations for diffusion potentials can be used in dealing with guaiacol there is reason to suppose that they may also be employed for *Nitella* and for other cells whose behavior resembles that of guaiacol. Hence we may calculate relative mobilities of ions and partition coefficients in the non-aqueous layer which covers the surface of the protoplasm and determine the effects of metabolism and applied reagents on ionic mobilities and on partition coefficients. This provides a method of studying protoplasmic behavior which is decidedly promising.

As an example of the situation in models we may consider the following. Guaiacol shaken at 25°C. with aqueous 0.014 M KG until equilibrium was attained was placed in contact with aqueous 0.14 M KG on one side and with aqueous 0.014 M KG on the other. We may assume that the situation resembles that shown[3] in Scheme 1.

Aqueous		Guaiacol			Aqueous	
A	A'	B'	B	B''	C'	C
0.14 M KG	0.14 M KG	0.062 M KG	0.00094 M KG		0.014 M KG	
P₂	P₃	P₁		P₄		

SCHEME 1

Here A' and B' represent exceedingly thin layers on each side of the phase boundary. We make the usual assumption that they at once come into equilibrium with each other. This also applies to B'' and C'.

We may suppose that when the guaiacol is placed in contact with 0.14 M KG there is a movement of KG from A' to B' making the concentration of KG in B' approximately 0.062 M. The amount of KG moving in this way is very small and it is quickly replaced in A' because KG in the aqueous phase diffuses up to the boundary much faster than it diffuses in the guaiacol phase since the viscosity of the latter is about 7 times as great as that of the aqueous phase.[4]

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[3] Guaiacol in equilibrium with aqueous 0.14 M KG at 25°C. contains 0.062 M KG: in equilibrium with aqueous 0.014 M KG it contains 0.00094 M KG.

[4] If the partition coefficient of KG in guaiacol were unity and the viscosity of guaiacol about the same as that of the aqueous solution the concentration gradient of KG at A' would be about the same as in the adjacent region of A. Actually the

Hence any diffusion potential at P_2 may be neglected on account of the very small magnitude of the concentration gradient in that region.

The observed potential of the chain is therefore $P_1 + P_3 + P_4$ where P_3 and P_4 are phase boundary potentials. If we compute the value of P_1 and subtract it from the observed total value we can estimate the value of $P_3 + P_4$.

We may assume that at P_1 we have a diffusion potential between 0.062 M KG and 0.00094 M KG in the guaiacol phase. To compute this we may employ the usual equation[5]

$$P_1 = \frac{RT}{F} (2t_K - 1) \ln \frac{a_1}{a_2} \tag{1}$$

where a_1 and a_2 are the mean ionic activities of KG in guaiacol and t_K is the transference number of K^+ in guaiacol.

The values of t_K and t_{Na} were determined by Longsworth as 0.57 and 0.54 from moving boundary measurements in guaiacol.[2] According to Shedlovsky[6] the equivalent conductivity of KG in guaiacol at zero concentration, i.e. Λ_0 (KG), is 9.5 so that we have for the equivalent conductivity of G^- at zero concentration, i.e. $\lambda_G = 9.5(0.43) = 4.085$. For NaG we have $\Lambda_{0(NaG)} = 9.0$ and for $\lambda_G = 9.0$ (0.46) $= 4.14$. These values agree within the limits

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partition coefficient is 0.062 ÷ 0.14 = 0.44 so that KG does not pass as readily into the guaiacol as it would into an aqueous solution.

The situation can be illustrated by using a dye as the diffusing substance. For this purpose brilliant cresyl blue (National Aniline Company) was allowed to diffuse into 1 part of guaiacol plus 25 parts of chloroform. The partition coefficient of the dye depends on the pH of the aqueous solution: when this is about 5.8 the partition coefficient approaches 0.44.

We use a V-tube (not a U-tube) placing at the bottom the non-aqueous mixture of guaiacol plus chloroform. Above this we place in the left-hand arm an aqueous solution of buffer and in the right-hand arm brilliant cresyl blue dissolved in 0.01 M phosphate buffer solution at pH 5.8. Then diffusion takes place without producing a clear zone in the column of dye at the boundary (some dye enters the non-aqueous phase but this does not produce enough color to be visible). But if the partition coefficient is high (as when pure guaiacol is used as the non-aqueous mixture) the dye passes into the non-aqueous mixture faster than it can be brought up by diffusion and convection in the aqueous phase and in consequence a clear zone appears in the aqueous column of dye in the region of the phase boundary.

In Scheme 1 the movement of water across the boundary is neglected. If water tends to move from B' to A' at the start it tends to move back again as KG enters the guaiacol phase since guaiacol takes up more water when it contains KG: any movement of guaiacol may also be neglected since both aqueous phases are saturated with guaiacol.

[5] Cf. MacInnes, D. A., Principles of electrochemistry, New York, Reinhold Publishing Corporation, 1939, pp. 225, 232.

[6] Shedlovsky, T., J. Gen. Physiol., 1942–43, 26, 287.

of experimental error.[7] The average of these two values for λ_G is 4.11 which gives for λ_K 9.5 $-$ 4.11 $=$ 5.39 and for λ_{Na} 9.0 $-$ 4.11 $=$ 4.89. Hence we shall use for the transference number of K^+, i.e. $t_K = 5.39 \div 9.5 = 0.567$ and of Na, i.e. $t_{Na} = 4.89 \div 9.0 = 0.543$.

Equation (1) may also be written (for 25°C.)

$$P_1 = 59(2t_K - 1) \log \frac{C_1 \theta_1 \gamma_1}{C_2 \theta_2 \gamma_2} \tag{2}$$

where θ is the fraction dissociated, γ is the mean ionic activity coefficient, and the subscripts 1 and 2 refer to the two solutions.

We may also write

$$P_1 = 59 \frac{u_K - v_G}{u_K + v_G} \log \frac{C_1 \theta_1 \gamma_1}{C_2 \theta_2 \gamma_2} \tag{3}$$

where u_K and v_G are ionic mobilities, so[8] that $t_K = u_K \div (u_K + v_G)$ and $t_G = v_G \div (u_K + v_G)$: hence we may write

$$2t_K - 1 = \frac{2u_K}{u_K + v_G} - \frac{u_K + v_G}{u_K + v_G} = \frac{u_K - v_G}{u_K + v_G}$$

We also have

$$2t_K - 1 = \frac{\lambda_{(K)} - \lambda_{(G)}}{\lambda_{(K)} + \lambda_{(G)}}$$

In order to compute the values of θ and γ we determine the equivalent conductivity, Λ, at 25°C. (see Fig. 1). According to Shedlovsky[6] we may write

$$\theta = \frac{\Lambda F}{\Lambda_0}$$

where Λ_0 is the limiting equivalent conductivity at zero concentration (this is 9.5 for KG and 9.0 for NaG),[6] $F = 1 + z + (z^2 \div 2)$ and

$$z = \frac{\alpha \Lambda_0 + \beta}{\Lambda_0^{\frac{3}{2}}} \sqrt{C\Lambda}$$

where C is concentration in guaiacol. For guaiacol at 25°C. saturated with water Shedlovsky[2] gives the following values: $\alpha = 2.93$ and $\beta = 19.36$. Hence for KG we have $z = 1.611 \sqrt{C\Lambda}$ and for NaG we have $z = 1.694 \sqrt{C\Lambda}$.

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[7] MacInnes,[5] p. 332. In applying these results we assume that t_K is constant which is approximately correct since the nearer t_K is to 0.5 the less it changes as concentration changes. We also assume that γ is the same for both ions.

[8] MacInnes,[5] pp. 59, 60. For univalent electrolytes we have for the equivalent conductivity $\Lambda = \lambda^+ + \lambda^- = F a (u + v)$, where F is the Faraday, a is the fraction dissociated, λ^+ is the conductivity of the cation and λ^- of the anion.

For[9] 0.062 M KG at 25°C. Λ = 0.404 and θ = 0.0548; for 0.00094 M K$G \Lambda$ = 1.85 and θ = 0.208.

We may write according to Shedlovsky[6]

$$-\log \gamma = \frac{6.52\sqrt{C\theta}}{1.0 + 6.1\sqrt{C\theta}}$$

where γ is the mean ionic activity coefficient. We thus obtain for 0.062 M KG in guaiacol at 25°C. γ = 0.525 and for 0.00094 M γ = 0.824.
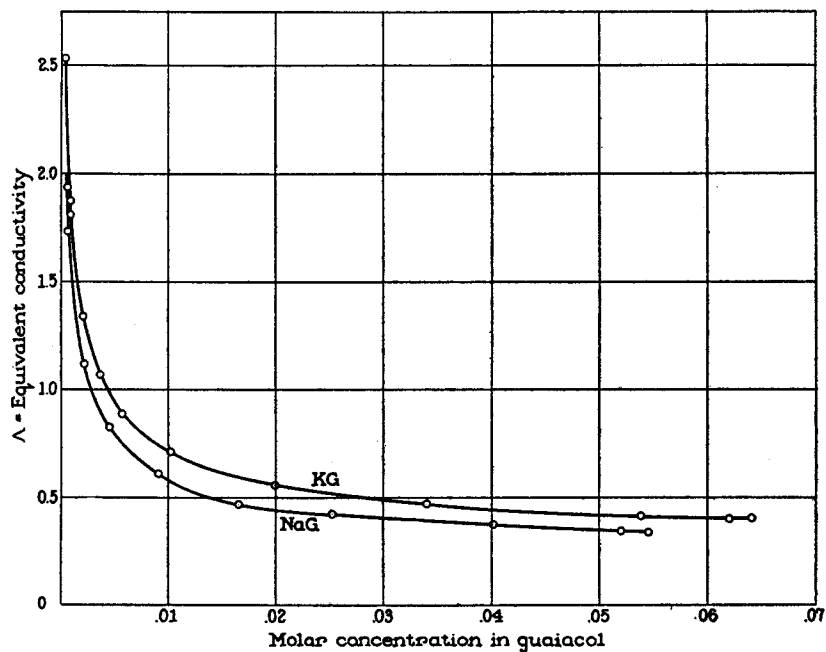

FIG. 1. Curves showing the equivalent conductivity of potassium guaiacolate, KG, and of sodium guaiacolate, NaG, at 25°C. in guaiacol saturated with water (at each concentration the guaiacol was shaken with the appropriate aqueous solution until equilibrium resulted).
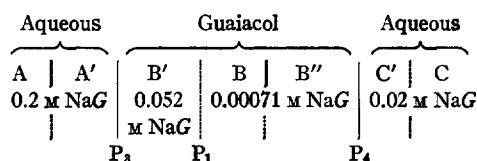
Inserting these values in equation (2) we have

$$P_1 = 59(2[0.567] - 1) \log \frac{0.062(0.0548)0.525}{0.00094(0.208)0.824}$$

$$= 59(0.134) \log \frac{0.00178}{0.000161}$$

$$= 59(0.134) \log 11.1$$

$$= 8.3 \text{ mv.}$$

The observed value is 8 ± 0.2 (12 observations).

[9] These values apply to guaiacol in equilibrium with aqueous 0.14 M and 0.014 M KG respectively.

It is of interest to note the difference between the concentration ratio 0.062 ÷ 0.00094 = 66 and the ionic activity ratio 0.00178 ÷ 0.000161 = 11.1. The latter does not differ much from the ratio of aqueous concentrations; *i.e.*, 0.14 ÷ 0.014 = 10. Hence if we did not know the activities in guaiacol we should not be greatly in error in taking the ratio of aqueous concentrations (see p. 299).

To measure the concentration effect of NaG the same method was used. The dilute aqueous solution was 0.02 m NaG which was shaken with the guaiacol, giving 0.00071 NaG in the guaiacol phase. At the other side was placed an aqueous solution of 0.20 m NaG, giving[10] 0.052 m NaG in B' as shown in Scheme 2.

Aqueous		Guaiacol			Aqueous	
A	A'	B'	B	B''	C'	C
0.2 m NaG		0.052	0.00071 m NaG		0.02 m NaG	
		m NaG				
	P₃		P₁		P₄	

SCHEME 2

In the guaiacol phase we have the following values: for 0.052 m NaG Λ = 0.346, θ = 0.0482, and γ = 0.562; for 0.00071 m NaG Λ = 1.93, θ = 0.228, and γ = 0.838.

Accordingly we have for the diffusion potential P_1

$$P_1 = 59(2[0.543] - 1) \log \frac{0.052(0.0482)0.562}{0.00071(0.228)0.838}$$

$$= 59(0.086) \log \frac{0.00141}{0.000136}$$

$$= 59(0.086) \log 10.4$$

$$= 5.2 \text{ mv.}$$

The observed value is 5 ± 0.1 mv. (8 observations).

It is interesting to note the difference between the ratio of concentrations 0.052 ÷ 0.00071 = 73 and that of the mean ionic activities 0.00141 ÷ 0.000136 = 10.4. The situation resembles that with KG where the corresponding values are 66 and 11.1 respectively. In this connection the following may be considered.

As long as the properties of the two phases remain unaltered the partition coefficient of the ionized portion of a solute is a constant when defined as

$$S_i = \frac{[C_i]}{[C_i']}$$

where $[C_i]$ refers to the non-aqueous and $[C_i']$ to the aqueous phase and the brackets denote activities. We have also $[C_i]^2 = K[C_u]$ where K is the dis-

[10] Guaiacol shaken with aqueous 0.2 m NaG at 25°C. contains 0.052 m NaG.

sociation constant and $[C_u]$ is the activity of the unionized portion in the non-aqueous phase. Hence we may write

$$S_i = \frac{\sqrt{K[C_u]}}{\sqrt{K'[C'_u]}}$$

The partition coefficient for the unionized portion is a constant when defined as

$$S_u = \frac{[C_u]}{[C'_u]}$$

Substituting this we obtain

$$S_i = \sqrt{\frac{KS_u}{K'}}$$

This treatment, due to Shedlovsky (personal communication), we may illustrate by the following in which hypothetical values are assumed in order to make a consistent scheme.*

Aqueous						S_u	Non-aqueous						S_i	S
$C' = C'_i + C'_u$	C'_i	γ'	$[C'_i]$	K'	$[C'_u]$		$[C_u]$	K	$[C_i]$	γ	C_i	$C = C_i + C_u$		
0.0012	0.0011	0.95	0.001	0.01	0.0001	4	0.0004	0.0001	0.0002	0.9	0.00022	0.00062	0.2	0.52
0.021	0.011	0.90	0.01	0.01	0.01	4	0.04	0.0001	0.002	0.68	0.003	0.043	0.2	2.0

* For a simpler scheme see Osterhout, W. J. V., *Biol. Rev.*, 1931, **6**, 400.

Here γ' is the ionic activity coefficient in the aqueous and γ that in the non-aqueous phase (it is assumed that $\gamma = 1$ for the unionized portion in both phases). S is the partition coefficient for the total concentration; *i.e.* for $(C_i + C_u) \div (C'_i + C'_u)$.

It is evident that S_u is constant at 4 and S_i is constant at 0.2. S increases from $0.00062 \div 0.0012 = 0.52$ at the lower concentration, to $0.043 \div 0.021 = 2.0$ at the higher because K' is greater than K so that the ratio of undissociated to dissociated is greater in the non-aqueous phase and hence the concentration in the non-aqueous phase increases faster than in the aqueous phase.

When $[C'_i]$ is multiplied by 10 we see that C'_i is multiplied approximately by 10, C' by 18, $[C_i]$ by 10 and C by 69. This recalls the situation in guaiacol, as described earlier (p. 298).

Guaiacol in contact with aqueous solutions of KG or NaG takes up more water when the concentration of these substances increases and as the plait point[11] is approached the two phases become more and more alike so that the

[11] *Cf.* Osterhout, W. J. V., and Murray, J. W., *J. Gen. Physiol.*, 1939-40, **23**, 365.

value of S_i approaches unity. Since the value of S_i in dilute solutions is much less than unity this taking up of water involves a rise in S_i. Hence the value of $[C_i]_1 \div [C_i]_2$ becomes progressively greater than that of $[C_i']_1 \div [C_i']_2$ (here the subscripts 1 and 2 refer to concentrated and dilute solutions respectively).

As previously stated (p. 295) the observed potentials are theoretically equal to $P_1 + P_3 + P_4$ and any excess of the observed values over the values calculated for P_1 might be regarded as due to $P_3 + P_4$; *i.e.*, to the phase boundary potentials. Since there is no excess, as shown in Table I, there is no reason to think that phase boundary potentials make any contribution to the observed values.

TABLE I

Concentration Effects of KG and NaG

Concentrations in aqueous phase	Concentrations in non-aqueous phase	Potential	
		Observed value	Calculated value of P_1 (diffusion potential)
0.14 M *vs.* 0.014 M KG	0.062 M *vs.* 0.00094 M KG	8	8.3
0.2 M *vs.* 0.02 M NaG	0.052 M *vs.* 0.00071 M NaG	5	5.2

EXPERIMENTAL

Guaiacol (Kahlbaum's c.p. crystallized or in some cases Eastman Kodak) was redistilled as described by Shedlovsky.[2] Owing to supercooling the guaiacol remained liquid at 25°C. at which temperature all the measurements were made.

The solutions of KG and NaG were prepared by shaking guaiacol with aqueous KOH or NaOH free from carbonates. The concentrations of KG and NaG in the guaiacol were determined by shaking the guaiacol with water and titrating the aqueous solution while in contact with the guaiacol, using methyl red as an indicator (the endpoint was pH 5.0). A correction was made for the guaiacol dissolved in the aqueous phase.

On standing in contact with air these solutions acquired a color which deepened with time and the p.d. measurements became less reliable. It was therefore necessary to use freshly made solutions (in some cases solutions stored under nitrogen and free from color were employed).

Conductivity measurements in guaiacol nearly saturated with water were made following in general the method of Shedlovsky[2] (but using a Washburn type cell with a cell constant of 0.03741). In addition a series of measurements was made on guaiacol solutions which had been shaken with aqueous solutions of KG or NaG until equilibrium was attained (these are designated as "saturated with water" (Fig. 1)).

The measurements of potential were made with a Compton electrometer (Cambridge Instrument Co.) used as a null instrument. The solutions were placed in a

grounded wire cage and the wires connecting with the electrometer were shielded microphone cable.

The choice of electrodes is important.[12] After experimenting with various kinds the choice fell upon the arrangement shown in Fig. 2. The guaiacol was contained in a breaker into which dipped 4 tubes filled with aqueous solution.[13] Thus in measuring the concentration effect of KG two tubes, A and B, were filled with a dilute aqueous solution of KG and the other two with a more concentrated aqueous solution
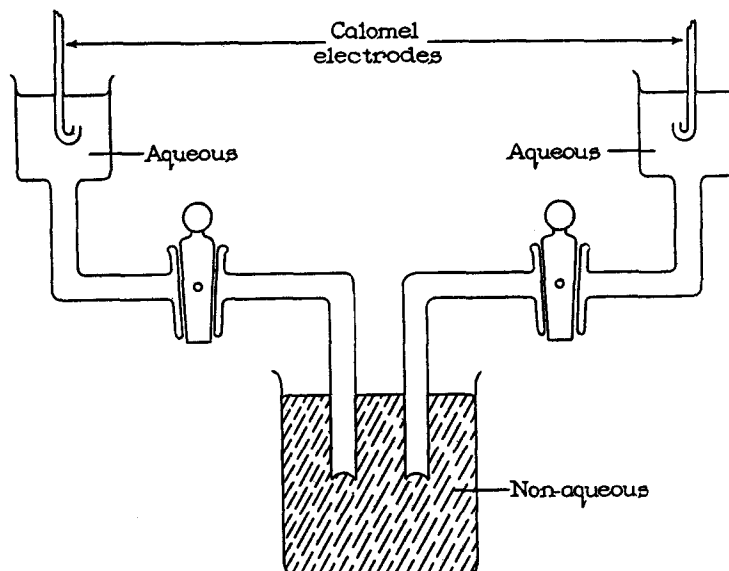

Fig. 2. To make liquid junctions between the guaiacol and aqueous solutions the latter are placed in tubes which dip into the guaiacol as shown. The path traversed by the electric current in the guaiacol has a large cross-section with no opportunity for short-circuiting by continuous aqueous films adhering to the glass. Four tubes are employed (only two are shown): they are connected in turn by means of calomel electrodes to a Compton electrometer.

of KG. All of these were allowed to dip simultaneously into the guaiacol and were connected in turn (with stopcocks closed) to the electrometer through calomel electrodes (filled with 3.5 M KCl). When the calomel electrodes were in proper condition the potential between A and B or between C and D did not exceed 1 or 2 mv. and for this a correction could be applied. Under these conditions a close agreement of the potentials between A and C, A and D, B and C, and B and D was regarded as indicating a satisfactory state of affairs.
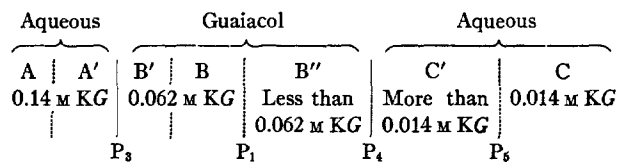
Under these conditions there was very little change of the values during the first hour (after this no readings were taken) except in the case of KG vs. NaG when the

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[12] Stopcocks in guaiacol should be avoided.

[13] The internal diameter of these tubes was 5 mm. No stopcock grease was used.

first reading was usually high, as explained later (p. 304). If a change occurred in measuring concentration effects the measurement was rejected.

Let us now turn to a different type of experiment in which the guaiacol is shaken at the start with the more concentrated aqueous solution. Here the diffusion potential P_5 in the dilute aqueous solution becomes important. We may picture the situation as in Scheme 3.

Aqueous		Guaiacol			Aqueous	
A	A'	B'	B	B"	C'	C
0.14 M KG		0.062 M KG		Less than 0.062 M KG	More than 0.014 M KG	0.014 M KG
	P_3		P_1		P_4	P_5

SCHEME 3

The maximum value possible for P_1 would exist if B contained 0.062 M KG and B", in equilibrium with C', contained 0.00094 M KG. This maximum value would be calculated as 8.3 mv. as described above (p. 297).

The maximum possible value for P_5 would result if C' contained 0.14 M KG, in equilibrium with 0.062 M KG in the guaiacol, and C contained 0.014 M KG. We should then have[14] for P_5 (assuming that concentrations in the aqueous solution are equal to activities)

$$P_5 = 59 \frac{\lambda_{(K)} - \lambda_{(G)}}{\lambda_{(K)} + \lambda_{(G)}} \log \frac{0.14}{0.014}$$

$$P_5 = 59 \frac{74 - 30}{74 + 30} \log \frac{0.14}{0.014}$$

$$= 25.0 \text{ mv.}$$

Here the dissociation in the aqueous phase is regarded as complete.

A measurement was made with the aqueous[15] solutions in direct contact (no guaiacol phase present). This gave 23 ± 0.4 mv. (8 observations).

Adding these maximum values we get $P_1 + P_5 = 8.3 + 23 = 31.3$ mv. This is, of course, larger than any value which could be realized in practice for the concentration of KG in C' would fall off rapidly since KG would diffuse away from C' into C much faster than it diffused up to C' in the guaiacol owing to the greater viscosity of the latter. The observed value is 19 ± 0.6 (12 observations).

As would be expected, we find that when we employ KCl in place of KG for the concentration effect we can neglect P_5 since its value is very small in all cases. Hence the observed value for the concentration effect of KCl is prac-

[14] The value of λ_K at 25°C. is taken as 74. That of λ_G is taken as 4.11 (7.2) = 30 since the viscosity of guaiacol is 7.2 times that of water (cf. Shedlovsky, T., and Uhlig, H. H., J. Gen. Physiol., 1933–34, 17, 549).

[15] The aqueous solutions were saturated with guaiacol.

tically the same whether we have a situation like that in Scheme 1 (p. 294) or like that in Scheme 3.

Similar considerations apply to NaG. The maximum value for P_1 would be 5.2 mv., as already noted (p. 298). For the maximum value of P_5 we have[16]
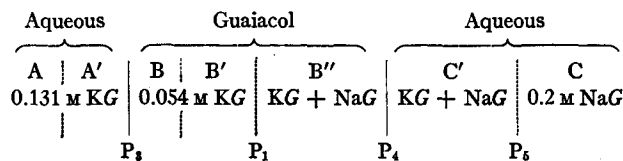
$$P_5 = 59 \frac{50 - 30}{50 + 30} \log \frac{0.2}{0.02}$$

$$= 14.8 \text{ mv.}$$

An actual test of the diffusion potential of 0.2 M NaG against 0.02 M NaG in water (no guaiacol phase present) gave 17 ± 0.1 mv. (4 observations).

Accordingly we have for the sum of the maximum values 5.2 + 17 = 22.2. The observed value is 15 ± 0.03 (4 observations) which is, as expected, much less.

Turning now to the effect[17] of KG vs. NaG (potassium effect) we find it advantageous for purposes of computation to set up a chain in which the concentrations of the two salts in the guaiacol phase are approximately equal. For this purpose guaiacol was shaken with 0.131 M KG and then placed in contact with 0.2 M aqueous NaG, as in Scheme 4.

Aqueous		Guaiacol			Aqueous	
A	A'	B	B'	B''	C'	C
0.131 M KG		0.054 M KG		KG + NaG	KG + NaG	0.2 M NaG
	P_3		P_1		P_4	P_5

SCHEME 4

The maximum value for P_1 would occur if B contained 0.054 M KG (with no NaG) and B" contained[18] 0.052 M NaG (with no KG). This value cannot be accurately computed. We may attempt an approximation by assuming that in the guaiacol concentrations are equal to activities and that the degree of dissociation is the same for KG and NaG.[19] If we neglect the difference in

[16] The value of λ_{Na} in water at 25°C. is taken as 50 and that of λ_G as 4.11 (7.2) = 30 since the viscosity of guaiacol is 7.2 times that of water.

[17] It may be noted that in the cases previously treated where different concentrations of the same salt were in contact it makes no difference theoretically whether the diffusion boundary is sharp or diffuse. This does not apply when different salts are in contact. Cf. MacInnes, D. A.,[5] chapter 8 and p. 224.

[18] Guaiacol shaken with aqueous 0.131 M KG at 25°C. contains 0.054 M KG: shaken with 0.2 M NaG it contains 0.052 M NaG.

[19] The degree of dissociation of KG and of NaG in guaiacol does not differ much (see footnote 6). The error involved in the assumption that concentrations in guaiacol are equal to activities is approximately the same for KG and NaG so that the resulting computation is not as far out as would otherwise be the case: it is lessened by the fact that only ratios are involved.
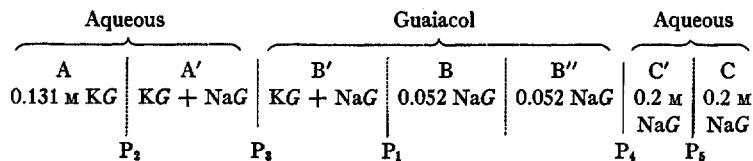
concentration between 0.054 M KG and 0.052 M NaG we may employ a modification of Henderson's equation[20]

$$P_1 = 59 \, \log \frac{\Lambda_{KG}}{\Lambda_{NaG}}$$

$$= 59 \, \log \frac{0.415}{0.346}$$

$$= 4.7 \text{ mv.}$$

Here Λ_{KG} is the equivalent conductivity of 0.054 M KG and Λ_{NaG} that of 0.052 M NaG (each shaken with the appropriate aqueous solution).

The maximum value of P_5 would occur if C' contained 0.131 M KG (with no NaG) and the adjacent layer of C contained 0.2 M NaG (with no KG). Experimental determination of this potential gives 2.5 $\pm$ 0.03 mv. (8 observations). For the total $(P_1 + P_5)$ we therefore have $4.7 + 2.5 = 7.2$. The observed value[21] is 8 $\pm$ 0.2 (12 observations).

If the guaiacol is shaken with 0.2 M NaG at the start we have the situation shown in Scheme 5. The maximum values of P_1 and P_2 are the same as for P_1 and P_5 in Scheme 3 so that $P_1 + P_2 = 7.2$. The observed value is 8 $\pm$ 0.1 (12 observations).

Aqueous			Guaiacol			Aqueous	
A	A′	B′	B	B″	C′	C
0.131 M KG	KG + NaG	KG + NaG	0.052 NaG	0.052 NaG	0.2 M NaG	0.2 M NaG
P_2	P_3	P_1			P_4	P_5

SCHEME 5

Attempts to measure the diffusion potential by bringing the two guaiacol solutions in contact did not give reproducible values. The difference in the specific gravity of the solutions was relatively small and a good deal of mixing occurred at the boundary.

We may sum up by saying that where we can calculate the P.D. most accurately (i.e. the concentration effect when the guaiacol has been previously shaken with the more dilute solution) it is clear that diffusion potentials account for the observed values (Table I, p. 300).

[20] Lewis, G. N., and Sargent, L. W., *J. Am. Chem. Soc.*, 1909, **31**, 363.   MacInnes,[5] p. 233.

[21] The first reading was usually a little higher but in the course of 10 minutes the readings showed a nearly constant value which is the one here reported. In the earlier stages of the work, before sufficient precautions were taken to avoid the use of colored solutions, higher and less reproducible values were obtained.

Experiments with living cells indicate[22] that diffusion potentials play the chief rôle in their electrical behavior. Here we use a different method of calculation. Since we do not know the mobilities or activities in the non-aqueous protoplasmic surface layer we cannot employ them to calculate potentials. We must reverse the process and calculate ionic mobilities from the observed potentials. It is of interest to see how closely we approximate the true values when we use this method with guaiacol.

To determine relative mobilities from the concentration effect we may put v_G equal to unity and designate it as $\bar{v}_G$. We then have for the concentration effect of KG (p. 297), putting $u_K \div v_G = \bar{u}_K$,

$$8 = 59 \frac{\bar{u}_K - 1}{\bar{u}_K + 1} \log \frac{a_1}{a_2}$$

With living cells there is a non-aqueous layer at the surface of the protoplasm and we may assume that the ratio of activities in this layer is the same as in the external aqueous solution (p. 306). Proceeding in the same way with guaiacol we may write (assuming that concentrations are equal to activities)

$$8 = 59 \frac{\bar{u}_K - 1}{\bar{u}_K + 1} \log \frac{0.14}{0.014}$$

whence $\bar{u}_K = 1.31$. This means that $u_K \div v_G = 1.31$. The actual value is the same as for $\lambda_K \div \lambda_G$, i.e. $5.39 \div 4.11 = 1.31$ (p. 296).

In the same way from the concentration effect of 0.02 vs. 0.20 M NaG which equals 5 (p. 298) we obtain $\bar{u}_{Na} = u_{Na} \div v_G = 1.19$. The actual value is $4.89 \div 4.11 = 1.19$ (p. 296).

In order to calculate t_K, the transference number of K^+, we have

$$t_K = \frac{\bar{u}_K}{\bar{u}_K + \bar{v}_G} = \frac{1.31}{1.31 + 1.0} = 0.567$$

The actual value of t_K as determined by Longsworth is 0.57 (or as worked out above 0.567 (p. 296)).

The corresponding values for NaG are

$$t_{Na} = \frac{\bar{u}_{Na}}{\bar{u}_{Na} - \bar{v}_G} = \frac{1.19}{1.19 + 1.0} = 0.543$$

[22] Osterhout, W. J. V., J. Gen. Physiol., 1929–30, 13, 715; 1939–40, 23, 53, 171. Osterhout, W. J. V., and Hill, S. E., J. Gen. Physiol., 1938–39, 22, 139. Hill, S. E., and Osterhout, W. J. V., J. Gen. Physiol., 1937–38, 21, 541; Proc. Nat. Acad. Sc., 1938, 24, 312. Damon, E. B., J. Gen. Physiol., 1932–33, 16, 375. Cowan, S. L., Proc. Roy. Soc. London, Series B, 1934, 115, 216. Erlanger, J., and Gasser, H. S., Electrical signs of nervous activity, The Eldridge Reeves Johnson Foundation for Medical Physics Lectures, Philadelphia, University of Pennsylvania Press, 1937, p. 134. Webb, D. A., and Young, J. Z., J. Physiol., 1940, 98, 299.

The actual value of t_{Na} as determined by Longsworth is 0.54 or as worked out above 0.543 (p. 296).

It is evident that the method of calculation used for living cells gives satisfactory approximations to the true values when applied to a non-aqueous substance like guaiacol.[23]

This result depends upon two factors.

1. The activity ratios. Designating the activity of the ionized portion of a solute as $[C_i]$ we may say that if the value of $[C_i]$ in the aqueous phase increases tenfold it will also increase tenfold in the non-aqueous surface layer of the protoplasm. This implies that the partition coefficient of $[C_i]$ is constant. As already stated (p. 298) this is true unless the non-aqueous phase changes its properties (e.g. by changing its content of water).

2. Diffusion potentials account for the observed P.D.'s. The present paper shows that this is true for guaiacol and previous papers indicate that it applies to the cells most carefully studied; i.e., to Nitella, Valonia, and Halicystis. For example in Nitella the changes in P.D. due to changes in the concentration of KCl closely approach the values predicted by the equation for diffusion potentials.[24, 25]

An extension of the method enables us to follow changes produced by reagents[26] or by metabolism[27] in mobilities and in partition coefficients.[28] For this purpose great accuracy is not needed since as a rule we are chiefly interested in qualitative results.

[23] It does not follow that it would be equally useful with all non-aqueous substances.

[24] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1937–38, **21**, 541. Also unpublished results with other salts. See also Blinks, L. R., The relation of bioelectric phenomena to ionic permeability and to metabolism in large plant cells, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1940, **8**, 208.

[25] In some cases at least an entering cation may combine with an organic anion X^- in the non-aqueous surface layer (Jacques, A. G., *J. Gen. Physiol.*, 1939–40, **23**, 41) but the anions in the external aqueous solution may also be important as shown by the change in P.D. when NO_3 is substituted for Cl (Blinks, L. R., The relation of bioelectric phenomena to ionic permeability and to metabolism in large plant cells, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1940, **8**, 204). The result would depend somewhat on the relative activity of X^-.

[26] Osterhout, W. J. V., *J. Gen. Physiol.*, 1938–39, **22**, 417; 1939–40, **23**, 171. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1938–39, **22**, 139; *Proc. Nat. Acad. Sc.*, 1938, **24**, 427.

[27] Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24**, 312. Osterhout, W. J. V., *J. Gen. Physiol.*, 1939–40, **23**, 429.

[28] The use of Henderson's equation in determining partition coefficients makes the results less accurate.

As examples we may mention studies on *Nitella* which show that the mobilities and partition coefficients are by no means the same in winter as in summer:[27] as a rule winter cells can be made to act like summer cells in these respects by leaching with distilled water which removes certain organic substances.

We also find that great changes are produced by reagents. For example, in *Nitella* certain mobilities and partition coefficients can be raised by guaiacol.[29] Guaiacol reverses the order of mobilities of K^+ and Na^+ in *Valonia*[30] so that instead of $u_K > v_{Cl} > u_{Na}$ we have $u_{Na} > v_{Cl} > u_K$ (thus showing that the surface is not a pore system).

Further studies in this field are very desirable.

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SUMMARY

The behavior of guaiacol resembles that of certain protoplasmic surfaces to such an extent that it can be advantageously used in models designed to imitate certain aspects of protoplasmic behavior. In these models the electrical potentials appear to consist of diffusion potentials and this may be true of certain living cells.

In dealing with models we determine ionic mobilities and use these to predict potentials.

In studying living cells we measure potentials and from these calculate ionic mobilities. The question arises, how far is this method justified. To test this we have treated guaiacol like a living cell, measuring potentials and from these estimating ionic mobilities.

The results justify the use of this method. This is of interest because the method is most useful in studying protoplasmic activity. In its extended form it enables us to follow changes in mobilities and in partition coefficients due to applied reagents and to metabolism.

[29] Osterhout, W. J. V., *J. Gen. Physiol.*, 1939–40, **23**, 171.

[30] Osterhout, W. J. V., *J. Gen. Physiol.*, 1936–37, **20**, 13.