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FREQUENCY DEPENDENCE OF THE FROG SKIN IMPEDANCE

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SUMMARY

At frequencies between 20 Hz and 1 kHz the impedance locus of the isolated frog skin is circular; below 20 Hz the resistive component of the impedance is frequently greater than would be expected from extrapolation of the high-frequency locus. At frequencies greater than twice the highest frequency at which there are deviations from the circular locus the variation of impedance Z with angular frequency ω is closely described by the equation $Z = r_1 + r_0/[1+(j\omega\tau)^{1-\alpha}]$, where j is $\sqrt{-1}$, r_1 and r_0 are resistances, τ is a time constant and α a constant in the range 0.02–0.14.

INTRODUCTION

The frequency dependence of the electrical impedance Z of a tissue is conveniently represented by the impedance locus, a graph of negative reactive component $-X$ against resistive component R, with frequency as implicit parameter. The impedance locus of a cell membrane is frequently a circular arc with its centre below the R axis [1]. This implies that the complex impedance is described by the equation

$$Z = R + jX = r_1 + r_0/[1 + j^{1-\alpha} f(\omega)], \tag{1}$$

where j is $\sqrt{-1}$, r_1 and r_0 are constants with the dimensions of resistance and α is a constant in the range 0–1; $f(\omega)$ is a function of angular frequency ω which increases monotonically with ω and for which $f(0) = 0$ [1, 2]. For the few cell membranes which have been studied in detail, impedance is often described by the more restrictive equation,

$$Z = r_1 + r_0/[1 + (j\omega\tau)^{1-\alpha}], \tag{2}$$

where τ is a time constant. This equation predicts in addition the way in which the frequencies are distributed around the circular arc. Equation 2 is formally identical to the empirical Cole–Cole equation [3] for the variation of permittivity with frequency.

If $\alpha = 0$, Equation 2 gives the impedance of a circuit consisting of a resistance r_1 in series with a parallel arrangement of a resistance r_0 and a capacitance C, where $\tau = r_0 C$. Values of α greater than zero imply that the behaviour of the parallel r–C element is not ideal, but deviates in a rather specific way. In preparations consisting of a single cell membrane the value of α is small and it seems clear that a circuit of essentially the above description, with the membrane behaving as a leaky parallel-plate capacitor, is responsible for conformity with Equation 2 [1].

Brown and Kastella [2] concluded that the frog skin impedance obeys Equation 2 but the one graph they present shows considerable deviations between experimental values and those predicted by Equation 2. It was therefore of interest to investigate the point more closely.

METHODS

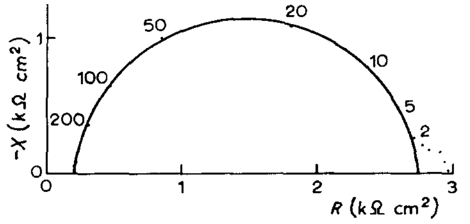
Impedance was measured by the method described previously [4]. An area (7.1 cm^2) of frog (*Rana temporaria*) skin was bathed in Ringer solution in a double-chamber apparatus; the steady potential difference across the skin was greater than 50mV in all cases. Sine-wave currents from an oscillator (Hewlett-Packard 3310A) were passed through the skin; the current electrodes were discs of platinum coated with platinum black and had a negligible polarisation impedance. The voltage across the skin was measured via salt bridges and calomel half-cells. Voltage and current were displayed on an oscilloscope operating in the X–Y mode and were measured directly from the oscilloscope screen. The peak-to-peak voltage across the skin was less than 40 mV; no non-linearities were apparent at this voltage amplitudes, in agreement with previous findings [5, 6]. Impedance was measured over the range 0.05 Hz to 2 kHz, taking three frequencies per decade. Values of resistive and reactive components of the impedance were calculated and graphs of the impedance locus were drawn. At the lowest frequencies the resistive component could be measured with a precision rather better than 1 %; the same absolute precision applied to the reactive component at all frequencies and to the resistive component at higher frequencies. This precision is relevant only if the skin impedance was steady during the period of time over which measurements were made (about 25 min). Impedance at the lowest frequency did not change by more than 6 % over this period. Measurements over the range 1–1000 Hz, which were used in further analysis, took about 10 min, and the slow drift in impedance was presumably correspondingly smaller during this time. The frequency scale of the oscillator was calibrated by counting pulses from the 'sync' output over appropriate periods of time.

RESULTS AND DISCUSSION

An example of an impedance locus is shown in Fig. 1. The locus is close to a semicircle over the range 2–2000 Hz and it therefore appears that Equation 1 is obeyed in this range. The graph shows deviations from the semicircle at 1 Hz and below and so Equation 1 cannot be obeyed at these frequencies. Similar deviations can be seen in loci described previously [4].

Conformity with Equations 1 and 2

Ignoring for the moment the low-frequency deviation we may ask, do the points which lie on a circular arc obey Equation 2? The problem can be dealt with by investigating variation of the ratio u/v (Fig. 2) with frequency [1]. Equation 2 predicts that $u/v = 1/(\omega\tau)^{1-\alpha}$. However, the experimentally-determined ratio is subject to larger errors at very low and very high frequencies than at intermediate



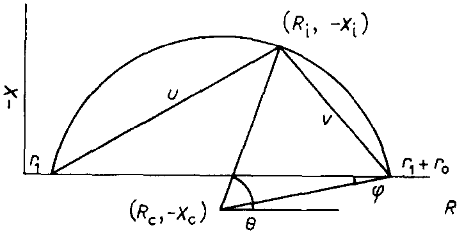


Fig. 1. An example of an impedance locus of frog skin. The negative reactive component $-X$ is plotted against the resistive component R for frequencies in the range 0.05–2000 Hz, taking frequencies in a 1–2–5 sequence. Numbers on the graph are frequencies in Hz. The semicircle was fitted to the points in the frequency range 5–1000 Hz. Points at frequencies of 1 Hz and below were considered to lie outside the semicircle.

Fig. 2. Idealised impedance locus, showing the meaning of parameters used in the text. The centre of the circular locus is at $(R_c, -X_c)$; $(R_i, -X_i)$ is an experimental point.

values, presenting difficulties in analysis. A better way of dealing with the question is to investigate variation of the angle θ (Fig. 2) with frequency; this angle is expected to be approximately homoscedastic. Moving the origin to the centre of the circular locus and transforming to polar coordinates shows that Equation 2 implies that

$$\tan\theta = \frac{2(\omega\tau)^{1-\alpha}\cos\varphi + \tan\varphi[1+2(\omega\tau)^{1-\alpha}\sin\varphi + (\omega\tau)^{2(1-\alpha)}]}{1-(\omega\tau)^{2(1-\alpha)}} \tag{3}$$

where φ is defined in Fig. 2 and $\alpha = \varphi/90$, if φ is measured in degrees. For an experimental point $(R_i, -X_i)$ the value of θ is given by

$$\tan\theta = (X_c - X_i)/(R_i - R_c) \tag{4}$$

where $(R_c, -X_c)$ is the position of the centre of the semicircle. Theoretical and experimental values of θ can then be compared.

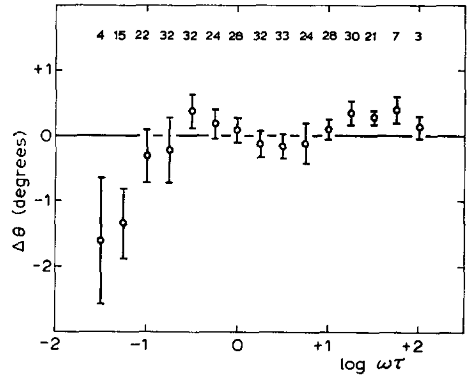
In order to work out either theoretical or experimental values of θ it is necessary to know the position of the centre of the semicircle. It was possible to determine the position roughly by eye from the experimental points but a better estimate was obtained with the aid of a computer program. This program found by iteration a position of the centre at which there was a minimum in the sum of squares of the distances of the experimental points from the semicircle; that is, a minimum in $\Sigma\{[(X_c-X_i)^2+(R_i-R_c)^2]^{\frac{1}{2}}-\rho\}^2$, where ρ is the mean distance of the points from the centre, $\Sigma[(X_c-X_i)^2+(R_i-R_c)^2]^{\frac{1}{2}}/n$, n being the number of points. Finding the position of the centre makes no assumptions about the way in which θ depends on frequency.

Knowledge of the position of the centre of the semicircle and the value of its radius fixes the values of α and φ. For a given value of τ, theoretical values of θ at the appropriate frequencies can then be worked out. For each skin the value of τ was found which gave the best fit to the experimental points; this was again carried out with the aid of a computer program, which found by iteration the value of τ for which $\Sigma(\theta_c-\theta_0)^2$ was a minimum, θ_c and θ_0 being respectively the calculated and experimental values of θ. Individual values $\Delta\theta$ of $\theta_c-\theta_0$ were then tabulated as a function of frequency.

Differences between calculated and observed values of θ may have two sources; a random component due to experimental error, variation of impedance with time, etc., and a component due to non-conformity with Equation 2. If some other relationship described the variation of impedance with frequency it might be expected that $\Delta\theta$ would show a systematic dependence upon frequency; any such dependence would be obscured by the random component. Some idea of the size of the random component and its possible dependence upon frequency can be gained by investigating the variability of $\Delta\theta$ within small ranges of frequency, from skin to skin. The value of τ varied widely, from 1.6 to 17 ms; in order to compare the values of $\Delta\theta$ between skins it seemed appropriate to group the values in equally-spaced ranges of log $\omega\tau$. This is equivalent to grouping the values in ranges of log f/f_0, where f is the measuring frequency in Hz and f_0 is the frequency at which $-X$ is a maximum; $f_0 = 1/2\,\pi\tau$. Four groups per decade change in $\omega\tau$ were taken. The standard deviation of $\Delta\theta$ was rather less than 1° over the whole range, about what would be expected for the stated precision of the experiment.

The observed average values of $\Delta\theta$ for 39 skins are shown in Fig. 3, together with 95 % confidence limits for the means, calculated from the t-distribution. Points at low frequencies which appeared to lie outside the semicircle were excluded from the analysis. At frequencies above 1000 Hz both theory and experiment gave values of θ close to $180°-\varphi$, so that there was little scope for the existence of a difference between θ_c and θ_0. Similarly below 1 Hz (in the absence of a low-frequency deviation) both values of θ were close to φ. All points outside the frequency range 1–1000 Hz were therefore excluded. Fig. 3 shows a clear discrepancy between theory and experiment at low frequencies ($\log \omega\tau < -1$), corresponding to frequencies less than $f_0/10$.

If not only the points lying outside the semicircle were excluded, but also those at the next higher frequency, the discrepancy vanished (Fig. 4). There remained a



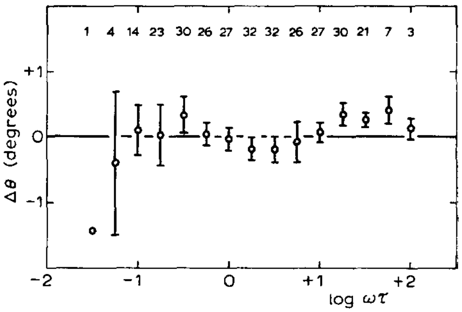


Fig. 3. Means of differences $\Delta\theta$ between calculated and observed values of θ, as a function of log $\omega\tau$, where ω is the angular frequency and τ the time constant of each skin. The mean values and 95 % confidence limits for the mean are shown, together with the numbers of values in each range of log $\omega\tau$. Values of impedance in the range 1–1000 Hz were used in the analysis but points at low frequencies which appeared to lie outside the extrapolation of the semicircular locus were excluded.

Fig. 4. As Fig. 3, but excluding not only points outside the semicircle but also those at the next higher frequency.

small oscillation in $\Delta\theta$ with maxima at values of $\log \omega\tau$ of about -0.5 and 1.5, and a minimum at about 0.5; the discrepancy appears to be antisymmetric about $\log \omega\tau = 0$. However the discrepancy is small, no more than 0.4°, and is well within the accuracy of the experiment; Equation 3 is a good description of the data used in Fig. 4.

As well as investigating variation of θ with $\log \omega\tau$, variation of the distance of each point from the centre of the semicircle was examined in a similar way. There was no evidence for a systematic frequency dependence of the difference between individual and mean values of this distance, for the data used in either Fig. 3 or Fig. 4. The standard deviation of the distances of the experimental points from the centre was less than 1 % of the mean distance in all but three cases. This confirms that Equation 1 applies to both sets of data.

In about one-half of the skins (21 out of 39) only points at frequencies less than $0.1 f_0$ had to be excluded from the analysis shown in Fig. 4, and in all but seven, only points at less than $0.2 f_0$. In the frequency range $0.1 f_0 - 10 f_0$, therefore, Equation 2 is a tolerably good description of the variation of impedance with frequency for most skins.

While an impedance described approximately by the equation $Z = r_1 + r_0/(1 + j\omega\tau)$ might be expected for a cell membrane, it is not clear why the real behaviour should deviate in the way described by Equation 2 [1]. It seems doubtful whether variations in the permittivity of the membrane dielectric with frequency can account for the applicability of Equation 2 [8]. A distribution of time constants, rather than a single value, can give rise to a non-zero value of α; it is possible to see how such a distribution could arise in a complex tissue such as the frog skin but less clear for the single cell membrane of the squid giant axon, where Hodgkin et al. [9] found $\alpha \approx 0.1$ and Curtis and Cole [10] found a mean value of 0.16 (although more recent work [11] has found that capacitance is independent of frequency, i.e. $\alpha = 0$).

Equation 2 was used by FitzHugh and Cole [8] to predict the time-course of the response of voltage across a membrane to passage of a step of current. The fact that the frog skin impedance obeys Equation 2 means that this predicted voltage response also applies to the skin. Measurements of frog skin capacitance have been made by carrying out a detailed analysis of the voltage response to a current step [12, 13; Smith, P.G., unpublished]; with a knowledge of the exact form of the response it becomes possible to assess the accuracy of this method of analysis.

Values of the parameters

Mean values and standard deviations of the parameters, calculated from the analysis of 39 skins shown in Fig. 4, were as follows: r_1 (after subtraction of the resistance of the solution between the voltage electrodes and the skin on the two sides), $23 \pm 20\ \Omega\text{cm}^2$; r_0, $2850 \pm 1320\ \Omega\text{cm}^2$; τ, 7.1 ± 4.2 ms; and α, 0.087 ± 0.027. If it is assumed that a capacitance in parallel with r_0 is responsible for the time constant τ then the value of this capacitance is $2.39 \pm 0.49\ \mu\text{F/cm}^2$.

The value of α found here is not markedly different from the values found in the early work on the squid giant axon [9, 10]. This suggests that inhomogeneities resulting from complexity of structure in the frog skin play a minor role in determining the value of α. The skin capacitance, when Ringer solution bathes both sides of the skin, appears to be determined by the cell membranes facing the outer surface [4]. At least part of the difference between the value of the frog skin capacitance and the "typical" cell membrane value of 1 μF/cm^2 [1] can be accounted for by undulations in the outer cell membranes [4]. The values for capacitance and the parameters r_0 and α reported here are not substantially different from those published previously [2, 4, 12, 13].

The resistance r_1 represents the part of the total skin resistance which is not in parallel with a membrane capacitance; that is, the resistances of the corium and cornified layer, and the epithelial cytoplasm. The small value of r_1, in comparison with that of r_0, indicates that these structures present little hindrance to ion movement through the skin.

The low-frequency deviation

The problem of the origin of the low-frequency deviation remains. Its presence was previously ascribed to the capacitance of the inward-facing membrane of the skin [4]. Another possibility [7] is that the deviation is the result of a time-dependent redistribution of ions in a membrane caused by the changing voltage across the membrane. This latter explanation appears to rely upon a frequency-dependent non-linearity in the current–voltage relation of the skin; a small non-linearity may have gone undetected but any large effect would have been noticed. This explanation can only apply, therefore, in experiments where the low-frequency deviation was small, and the finding (Smith, P. G., unpublished) of a deviation in experiments in which there were much smaller voltages across the skin suggests that even here it may not be the true explanation. However, in favour of it is the occasional occurrence of a small inductive, rather than capacitative, reactance at very low frequencies; this finding can easily be explained [7] on the basis of a redistribution of ions in a membrane but is not predicted by any combination of membrane capacitances.

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