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Letters to the Editor

An Alternate Explanation for the Permeability Changes Induced by Electrical Impulses in Vesicular Membranes

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In a recent article, E. Neumann and K. Rosenheck (*J. Membrane Biol.* **10**:279, 1972) reported that catecholamines and ATP were released from vesicles upon the application of a pulsed electric field. They ruled out dielectric breakdown of the membrane as a possible cause for the release. It will be shown here that dielectric breakdown cannot be ruled out in their experiments.

If the vesicles are spherical and have a membrane whose resistivity is much larger than that of either the fluid inside the vesicle or the fluid outside the vesicle, the steady-state solution for the membrane potential caused by the electric field is

$$1.5 a E \cos \theta$$

where a is the radius of the vesicle and E is the magnitude of the externally applied field. θ is the angle between the radius drawn to a point on the membrane and the electric field. Since, as was stated in the article, the relaxation time is much faster than the decay time of the voltage pulse, the steady-state is established during the pulse. Using the values for a and E given in the article ($a = 1.2 \times 10^{-5}$ cm) ($E = 25$ kV/cm) we calculate a maximum potential of 450 mV superimposed on the intrinsic membrane potential. This voltage is large enough so that dielectric breakdown cannot be ruled out as a cause for the permeability changes.

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Potential Difference across Vesicular Membranes

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Consider a spherical vesicle having the physical dimensions a, b, d, and the conductivities λ_1, λ_2 as indicated in Fig. 1. The conductivity of the external medium is λ_3; the indices denote the three phases: vesicle core 1, membrane 2, and medium 3.

In an external homogeneous electric field, E, the contribution $\Delta\psi$ to the total membrane potential, due to conductivity differences may be formally determined by solving Laplace's equation in spherical coordinates: $\vec{\nabla}^2\psi(r, \theta) = 0$.

Among the boundary conditions for the interfaces at $r = a$ and $r = b$ are the continuity equations

$$\lambda_1 \left(\frac{d\psi_1}{dr}\right)_{r=a} = \lambda_2 \left(\frac{d\psi_2}{dr}\right)_{r=a},$$

$$\lambda_2 \left(\frac{d\psi_2}{dr}\right)_{r=b} = \lambda_3 \left(\frac{d\psi_3}{dr}\right)_{r=b}.$$

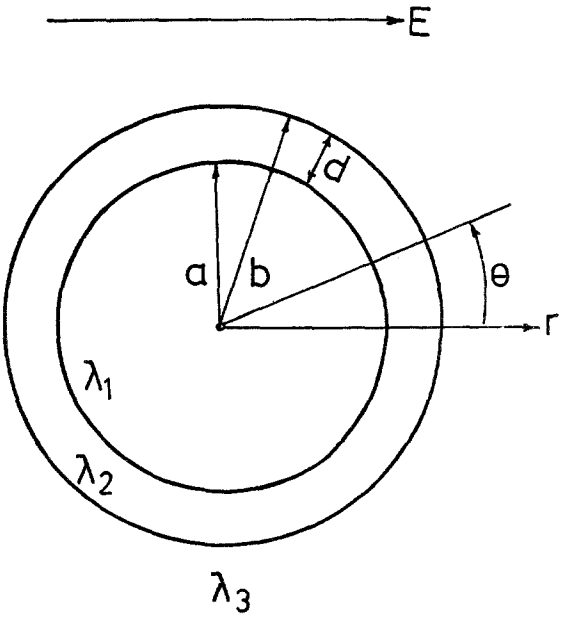

Fig. 1. Scheme of a diametral cross-section through a spherical vesicle; a and b are the inner and outer radii, respectively, of the membrane shell. E is the electric field vector and λ denotes the conductivity

In the case of the chromaffin granule the vesicle core contains electrolytes (A. D. Smith, 1968. *In*: The Interaction of Drugs and Subcellular Components in Animal Cells. P. N. Campbell, editor. p. 239. Churchill Ltd., London), partially as ionic associates. If the solution contains sufficient electrolyte, such that $\lambda_1 \simeq \lambda_3$ we may solve for $\Delta\psi$ in a simple way, using for example

$$\Delta\psi = (\psi_3)_{r=b} - (\psi_1)_{r=a}. \tag{1}$$

Following Maxwell's approach (J. C. Maxwell, 1904. A Treatise on Electricity and Magnetism, 3rd edition, Ch. 9. University Press, Oxford),

$$(\psi_1)_{r=a} = -\frac{9}{\lambda_1 \lambda_2} CE a \cos\theta \tag{2}$$

$$(\psi_3)_{r=b} = -E b \cos\theta - \frac{(\lambda_1 - \lambda_2)(2\lambda_2 - \lambda_1)}{(\lambda_1 \lambda_2)^2} (b^3 - a^3) CE b \cos\theta \tag{3}$$

with

$$C = \left[\frac{9}{\lambda_1 \lambda_2} + 2\left(\frac{1}{\lambda_2} - \frac{1}{\lambda_1}\right)^2 \left(1 - \left(\frac{a}{b}\right)^3\right)\right]^{-1}. \tag{4}$$

If the membrane thickness d is small compared to b, we may approximate the term $1 - (a/b)^3$ by $3\frac{d}{b}$. Thus, Eq. (4) becomes

$$C \simeq \frac{(\lambda/\lambda_1)^2}{9\lambda + 2(1-\lambda)^2 \frac{3d}{b}} \tag{5}$$

where we denote $\lambda = \frac{\lambda_2}{\lambda_1}$.

Inserting Eq. (5) into Eqs. (2) and (3) we obtain with Eq. (1) for the maximum value (i.e., for $\cos\theta = 1$):

$$\Delta\psi \simeq -\left\{\left(1 + \frac{1+\lambda-2\lambda^2)}{9\lambda + 2(1-\lambda)^2 \frac{3d}{b}}\right) b - \left(\frac{(1+\lambda-2\lambda^2)\left(1-\frac{2d}{b}\right)+9\lambda}{9\lambda + 2(1-\lambda)^2 \frac{3d}{b}}\right) a\right\} E. \tag{6}$$

Let us now consider the two extreme cases: $\lambda \ll 1$ and $\lambda \simeq 1$.

1) For $\lambda \ll 1$, and $\frac{2d}{b} \gg \lambda$, Eq. (6) is reduced to

$$\Delta\psi_{\max} \simeq -\left\{\left(1 + \frac{b}{6d}\right) b - \left(\frac{b-2d}{6d}\right) a\right\} E;$$

2) For $\lambda \simeq 1$,

$$\Delta\psi_{\min} \simeq -(b-a)E.$$

For the chromaffin granules where $b \simeq 12 \times 10^{-6}$ cm, $a \simeq 11 \times 10^{-6}$ cm, and $d \simeq 10^{-6}$ cm, and for $E = 25$ kV/cm we find the extreme values $\Delta\psi_{\max} \simeq$ 457 mV and $\Delta\psi_{\min} \simeq 25$ mV.

The actual value of $\Delta\psi$ lies between these two extreme values. At present there are, however, no data for the conductivities of the lipoprotein membrane and the core of the chromaffin granules. It is therefore not possible to give a reliable value for the contribution due to conductivity differences to the potential difference across the membranes of the chromaffin vesicles. Since there is spontaneous efflux, as well as uptake of catecholamines in the absence of an external field, the membrane must have a finite conductivity (Smith, *loc. cit.*). In our paper (*J. Membrane Biol.* **10**:279, 1972) we supported our conclusion that membrane damage was not the cause of the field-induced release, by the experimental fact that no protein is found in the vesicle supernatant after an impulse of the *initial* intensity of about 25 kV/cm (and exponentially decaying with time) has been applied. Release of internal soluble proteins would, however, be expected if the membrane structure is irreversibly destroyed in a dielectric breakdown. Although the possibility of local, transient and reversible "breakdown" causing the permeability change cannot be totally excluded, gross membrane damage is thus considered unlikely. Furthermore, recent field relaxation studies of chromaffin granules (K. Rosenheck & I. Pecht, *in preparation*) show that the overall distortion of the vesicle under the influence of the electric field is very small. This too suggests the absence of a dielectric breakdown with consequent membrane disruption in the presence of a transient field.

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