Mitochondrial Membrane Potentials Measured with Microelectrodes: Probable Ionic Basis

Abstract. *The membrane potentials of isolated* Drosophila *mitochondria have been measured under a variety of conditions by microelectrodes driven by a piezoelectric device. The results support the interpretation that the potentials are the result of a distribution of ions imposed by a Donnan effect.*

Membrane potentials and resistance have been measured in isolated *Drosophila* mitochondria 3 to 4 μ in diameter (*1*). The measurements were made on mitochondria immobilized in a medium of high viscosity and impaled by means of piezoelectric-driven microelectrodes. The state-4 potential of over 70 impalements with 8 separate preparations was measured as 9.8 ± 2.5 mv (standard deviation; inside positive). In these same experiments the membrane resistance was 2.2 ± 0.5 ohm cm^2. The value for membrane resistance is comparable to that observed in the human erythrocyte (approximately 3 to 10 ohm cm^2) (*2*). It is also of the same order of magnitude as the resistances measured in several other systems, such as glial cells in tissue culture [3 to 10 ohm cm^2 (*3*)] rat liver cells *in situ* [37 ohm cm^2 (*4*)], and *Drosophila* gland cell nuclei [1 ohm cm^2 (*5*)]. In state 3, the mitochondrial membrane potential is higher (about 19 mv), but this increase is unaffected by the metabolic inhibitor KCN (*1*). The measured potential is sensitive to changes in external osmotic pressure at constant ionic strength (*1*) and to certain changes in external ionic composition. These observations, in conjunction with the observed transients in resistance and potential during impalement, indicate that the measured potential is across the mitochondrial semipermeable membrane.

The magnitude of the membrane potential required for a role in oxidative-phosphorylation has been estimated to be between -210 and -270 mv [inside negative (*6*)]. This conclusion is based on the questionable assumption (*7*) that the measured K^+ distribution in rat liver mitochondria treated with valinomycin represents an equilibrium distribution.

The possibility of a membrane potential sustained by K^+ has also been pointed out (*8*). However, in our experiments, the membrane potential remains unaffected by varying the K^+ in the external medium while keeping constant the osmotic pressure. For example, the potential in state 4 at 10 m*M* KCl was 8.4 ± 2.7 mv and 9.0 ± 2.2 mv in two independent experiments. At 50 m*M* KCl, the potential was 9.2 ± 2.2 and 10.4 ± 4.0 mv, respectively. The magnitude, sign, and insensitivity of the potentials to the presence of 2,4-dinitrophenol or KCN do not support the concept that a membrane potential plays a significant role in phosphorylation (*1*).

The possible basis for the membrane potential is of considerable interest. Harris and Pressman (*9*) suggested that, in isolated rat liver mitochondria, the membrane potential is positive inside and is approximately 23 to 37 mv. This prediction is based on the measured C^{14}-distribution between the mitochondria and the medium of C^{14}-labeled univalent, divalent, and trivalent anions (*9*) and the use of the Nernst equation. Similar anionic distributions have been presented (*10*), and in some works the anionic distribution was only slightly affected by metabolic inhibitors. The anionic distribution was considered to be imposed by the presence of a positive fixed charge. There is considerable evidence that a significant portion of mitochondrial protein is cationic (*11*) and therefore capable of supporting such a distribution independently of metabolism. Such a hypothesis (which essentially postulates a Donnan distribution) predicts the sign of the potential reported in our work and also its order of magnitude. For these reasons we explored distributions of added C^{14}-labeled anions in mitochondria isolated from *Drosophila* and also determined the membrane potentials (Table 1).

Use of the Nernst equation (Eq. 1), together with the measured distribution of anions, allows calculation of the membrane potential

$$E = (2.3/nF)\, RT \log r \qquad (1)$$

where E represents the membrane potential, F the Faraday constant, R the gas constant, T the temperature (°K), n the valency of the anion, and r the ratio of concentrations (inside to outside; presumably the Donnan ratio). The membrane potential predicted from the distribution of each anion tested is shown in Table 1 along with the measured potential.

These results show that the values predicted by the distribution of the an-

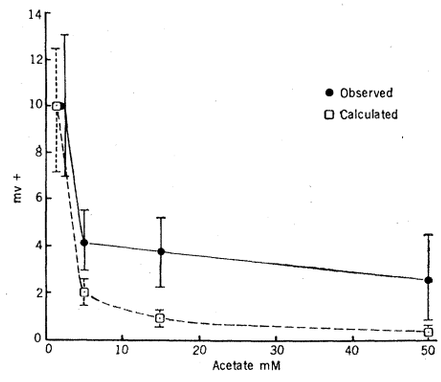

Fig. 1. Observed and calculated mitochondrial membrane potentials at increasing external tris-acetate concentration. The method for measurement of potential has been described (*1*). The calculated potentials are derived from Eqs. 1 and 2, assuming the fixed species z_pP imposing a Donnan distribution remains constant and that A_0 is represented by acetate. The deviations on the observed curve are the standard errors. Each point on the observed curve represents eight or more individual mitochondrial impalements. The deviations of the calculated curve are derived for maximum or minimum values of z_pP. The incubation medium is the same as in Table 1 with tris-acetate added at the concentrations indicated.

Table 1. The distribution of various C^{14}-labeled anions in *Drosophila* mitochondria and a comparison of the membrane potential calculated from this distribution to that directly observed with microelectrodes. The method is similar to that of Harris and Van Dam (*10*) except that osmotically inactive water is determined by the use of C^{14}-carboxydextran (molecular weight, 60,000 to 90,000). The method of measurement of membrane potential has been described (*1*). The values for internal anion concentration represent the mean ±S.D. of eight or more samples. The calculated potentials are determined from Eq. 1. The incubation medium contained 0.25*M* sucrose, 1 m*M* ethylenediaminetetraacetate, 10 m*M* KCl, 1 m*M* KH_2PO_4, 0.01*M* tris-Cl *p*H 7.4, 1 percent bovine serum albumin, and the concentration of C^{14}-labeled anion or KCN indicated. In the experiments in which the potential was measured, 3 percent methyl cellulose was added to immobilize the mitochondria.

C^{14}-Anion	Concentration		Membrane potential	
	External (m*M*)	Internal (m*M*)	Calculated (+mv)	Observed (+mv)
Acetate (monovalent)	1.0	1.4 ± 0.8	8.6 ± 11.1	9.8 ± 3.1
Succinate (divalent)	1.0	2.2 ± 0.8	9.9 ± 4.1	8.8 ± 2.2
Citrate (trivalent)	5.0	12.3 ± 1.0	7.6 ± 1.0	9.8 ± 1.2
Citrate $+10^{-3}M$ KCN	5.0	10.8 ± 1.1	6.5 ± 1.0	8.8 ± 1.2

ions agree very closely with the experimental values. Also, the agreement is good even though the ratio r varies from anion to anion because of the increasing valency as predicted by Eq. 1. The measured potential as well as the citrate distribution is unaffected by KCN.

It is possible to test further the applicability of Eq. 1 and the accompanying assumption that the anionic distribution is imposed by a Donnan effect brought about by a positive internal fixed charge. The Donnan ratio r of Eq. 2 (*12*) for the univalent ion would be a function of the positive charges (z_pP) per concentration of fixed or impermeable molecules (P) and the anion

$$r = \frac{z_pP}{2A_o} + \left[\left(\frac{z_pP}{2A_o}\right)^2 + 1\right]^{1/2} \quad (2)$$

present in the outside medium (A_o). An increase in A_o would lead to a drop in r and consequently (see Eq. 1) to a drop in the measured potential. Alternatively, a decrease in z_pP brought about by osmotic swelling of the mitochondria should also decrease r.

With the mean state-4 potential of +9.8 mv, Eq. 1 leads to the calculation of an r of 1.5 for the case of a monovalent anion. With an external anion concentration of 1 mM (either pyruvate or acetate at 1 mM) the z_pP of Eq. 2 corresponds to approximately 0.8 mM.

Figure 1 illustrates an experiment in which the external tris(hydroxymethyl)-aminomethane (tris) acetate concentration is varied from 1 mM to 50 mM. The values calculated for the membrane potential at different acetate concentrations (with Eqs. 1 and 2 at z_pP = 0.8 mM) are shown by the lower dashed line. The upper line represents the observed curve where the potentials were measured directly with the microelectrodes. Although the two curves are not superimposable, the observed potentials are not too distant from the predicted values. Acetate causes little or no swelling under these conditions as determined in two independent experiments by the photometric technique of Tedeschi and Harris (*13*). The maximum difference between the control and the experimental values was negligible throughout the range used.

When osmotic swelling is induced (table 2 in *1*) by suspending the mitochondria in a hypotonic medium (109 milliosmolal), the potential drops by an average of 6.7 mv from the control potential (in a 450 milliosmolal solution). Since the amount of z_pP is presumably fixed, a fourfold increase in volume would reduce the concentration to one-fourth of its original value. It is possible to calculate from Eqs. 1 and 2 that the potential should drop approximately 7.5 mv, in agreement with the measured value.

The results support the hypothesis that the measured potential is across the mitochondrial semipermeable membrane. The properties of the potential suggest that it is the result of the distribution of anions imposed by a Donnan effect.

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Ecdysone Analog: Conversion to Alpha Ecdysone and 20-Hydroxyecdysone by an Insect

Abstract. *The tritium-labeled synthetic ecdysone analog Δ^7-5β-cholestene-2β,3β,14α-triol-6-one terminated diapause when injected into diapausing tobacco hornworm pupae and was converted into tritium-labeled α-ecdysone and tritium-labeled 20-hydroxyecdysone. About half of the crystalline α-ecdysone and 20-hydroxyecdysone isolated from the tobacco hornworms 7½ to 8½ days after injection was derived from endogenous steroid precursors and half from the transformation of the synthetic ecdysone analog.*

Certain synthetic ecdysone analogs exhibit molting-hormone activity in ligated abdomens of *Diptera* (*1*) and severely inhibit larval growth and ovarian maturation in several species of insects (*2*, *3*). In addition to these effects, injection of the synthetic ecdysone analog Δ^7-5β-cholestene-2β,3β,-14α-triol-6-one (**1**, triol) terminated pupal diapause in the tobacco hornworm *Manduca sexta* (Johannson). In association with this physiological phenomenon we report our results on the conversion of the [^{3}H]triol to [^{3}H]α-ecdysone (**2**) and [^{3}H]20-hydroxyecdysone (**3**) in this insect.

The 1α[^{3}H]triol was prepared from 1α[^{3}H]cholesterol according to procedures used for the synthesis of the unlabeled compound (*4*); [observable specific activity, 1757 disintegrations per minute (dpm)/μg (*5*)]. Male, diapausing, tobacco hornworm pupae (669) collected from the field were injected in the ventral intersegmental membrane between the fifth and sixth abdominal segments with a microsyringe. The [^{3}H]triol was administered at 10 μg per gram of body weight (1 μg/μl, in 60 percent acetone solution). Initially we observed that doses of 1 to 10 μg per gram of body weight terminated diapause in the treated organisms and that the externally visible character of eye pigmentation (*6*) served as a valid and reliable indicator for ascertaining termination of diapause or spontaneous development, or both, in the intact organism. With eye pigmentation as the criterion, we harvested 621 insects (1.99 kg, 3.5×10^7 dpm) 7½ to 8½ days after injection. To determine the metabolic fate of the triol, we extracted the hornworms as described (*7*). The crude extractive (2.0 g, 1.9×10^7 dpm) was first fractionated on silicic acid (*8*) (Table 1) because this column permitted the separation of the unmetabolized triol from the ecdysones and more polar metab-