

MICROELECTRODE STUDIES ON THE MEMBRANE PROPERTIES OF ISOLATED MITOCHONDRIA, II. ABSENCE OF A METABOLIC DEPENDENCE*

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Abstract.—Mitochondrial membrane potentials and resistances have been determined under various metabolic conditions by the use of piezoelectric-driven microelectrodes. The mean state 3 potentials (about +19 mv) are higher than the potentials in state 4 (about +9 mv). DNP in uncoupling concentrations or KCN have no significant effect on membrane resistance or potential. Previous experiments have shown the potential to be sensitive to mitochondrial swelling and have suggested that it depends predominantly on anionic distribution.

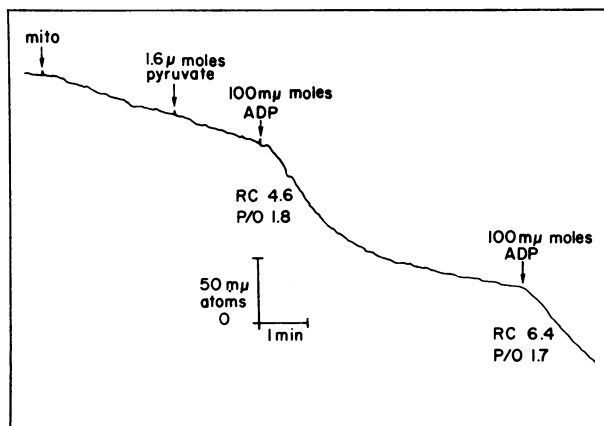
Introduction.—In a previous paper¹ we have discussed the measurement of mitochondrial membrane potentials and resistances by means of piezoelectric-driven microelectrodes in *Drosophila* mitochondria 3–4 μ in diameter. The magnitude and properties of the potential and resistance in state 4 led to the conclusion that the measured potential was across the mitochondrial semipermeable membrane and that this potential (approximately +9 mv, positive inside) could not play a significant role in oxidative phosphorylation.

In subsequent experiments the membrane properties have been studied under the following conditions:² (a) state 4 (pyruvate, P_i), (b) state 3 (pyruvate, ADP, P_i), (c) state 1 (P_i), (d) state 4, 3, 1 plus KCN, (e) state 4 plus 2,4-dinitrophenol.

Materials and Methods—All methods for the isolation of the mitochondria, analytical techniques, and the measurement of the electrical properties have been previously described^{1, 3}

Results.—Respiratory control of the mitochondrial preparation was determined polarographically. A typical record is shown in Figure 1. For a total of 33 determinations the mean respiratory control rate was 4.3 (range 3.0–7.2). The

FIG. 1—Oxygen consumption in *Drosophila* mitochondria. Mitochondria were incubated under state 4 conditions of Fig. 2, except without methyl cellulose. Equivalent records were obtained with methyl cellulose present. Additions, as indicated; final cuvette vol, 1.6 ml; reaction mixture contained 0.06 mg mitochondrial protein.



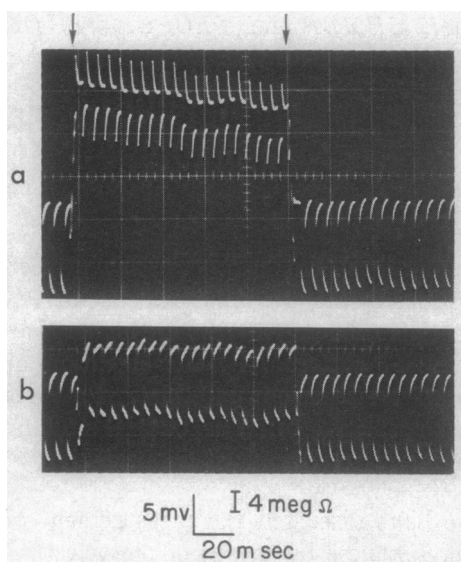


FIG. 2.—Relatively stable potential and resistance changes as determined during state 3 or state 4 respiration. (a) State 3 determination carried out in a medium containing 0.25 *M* sucrose, 1 mM pyruvate, 1 mM EDTA, 1 mM K_2HPO_4 , 50 mM KCl, 10 mM Tris-Cl, pH 7.4, 1% BSA, methyl cellulose (approximately 30 gm/l), and 2 mM NaADP (Grade I, Sigma Chemical Co., St. Louis, Missouri). (b) State 4 determination in the same medium as (a) but without NaADP. The arrows indicate the insertion and withdrawal of the electrode. The scales are as indicated. The bar represents the change in square pulse height equivalent to a 4 meg Ω change in input resistance.

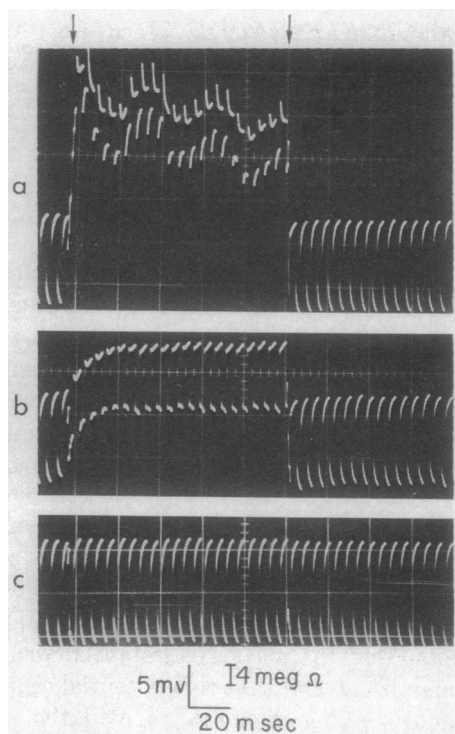


FIG. 3.—Records illustrating commonly observed oscillations and slow transients during mitochondrial impalements. (a) State 3 determination. (b) State 4 determination. Conditions are the same as those of Fig. 2. (c) A control where the electrode is advanced by the 100-volt driving pulse without impalement. The arrows indicate insertion and withdrawal of the electrode.

P/O with pyruvate as substrate was 2.2 (range 1.5–2.9). Methyl cellulose (added to immobilize the mitochondria) had no effect on the respiratory control. It was necessary to use 1 per cent bovine serum albumin in both the isolation and incubation medium to maintain respiratory control in these preparations.

Records of the oscilloscope traces illustrating relatively stable potential and resistance changes recorded during impalement of mitochondria in state 3 or state 4 respiration are shown in Figure 2. The more common records obtained are shown in Figure 3. Frequently the potential and resistance changes showed oscillations or slow transients. Table 1 summarizes the results of three experiments comprising 60 impalements with preparations in state 4 or state 3. A comparison of the mean values for state 3 and state 4 shows an increase in state 3 of approximately +10 mv in membrane potential and 1.1 $\Omega \cdot cm^2$ in membrane resistance. However, subsequent experiments indicate that these changes are not linked to metabolism.

Data showing the effect of the respiratory inhibitor KCN on the electrical

parameters of the mitochondria in various conditions are given in Table 2. The results indicate that the potential (state 4) is altered only slightly and not in a consistent way by the presence of 2 mM KCN. Also, removal of pyruvate, the substrate, is without effect on the potential. It should be noted, however, that respiration takes place without pyruvate (see Fig. 1), presumably by oxidation of endogenous substrate. Increase in membrane potential in the presence of NaADP is still observed, despite the presence of KCN.

Table 3 summarizes two experiments illustrating measurements made in the presence of the uncoupler 2,4-dinitrophenol (DNP). There is no apparent effect on either membrane potential or resistance, a finding in disagreement with

TABLE 1. Summary of state 3 and state 4 values for membrane potential (E) and membrane resistance (R).

Expt. number	E		R		$\frac{E\text{-state 3}}{E\text{-state 4}}$
	+mv state 4	+mv state 3	$\Omega \cdot \text{cm}^2$ state 4	$\Omega \cdot \text{cm}^2$ state 3	
1	6.0 \pm 0.6 (7)	11.6 \pm 0.6 (7)	2.7 \pm 0.2 (6)	3.4 \pm 0.1 (6)	1.9
2	9.2 \pm 0.8 (12)	22.1 \pm 2.6 (9)	2.1 \pm 0.2 (11)	3.2 \pm 0.3 (9)	2.4
3	10.5 \pm 1.1 (13)	22.0 \pm 1.7 (12)	2.3 \pm 0.3 (11)	4.0 \pm 0.3 (12)	2.1
Avg	8.6 mv	18.6 mv	2.4 $\Omega \cdot \text{cm}^2$	3.5 $\Omega \cdot \text{cm}^2$	2.1

The conditions for state 3 and state 4 are the same as those in Fig. 2. The values for potential are determined from the displacement of the oscilloscope trace from the baseline. Changes in electrode resistance are determined from the difference in square pulse height when the electrode is within the membrane as opposed to when it is withdrawn (see Fig. 2). The value for R is calculated for an average particle diameter of 3.5 μ , and the mitochondrion is assumed to be spherical. The deviations shown are the standard errors. The numbers in parentheses are the number of determinations.

TABLE 2. Membrane potential (E) and resistance (R) measurements under various conditions.

Expt. I	Conditions	E	R
		+mv	$\Omega \cdot \text{cm}^2$
Expt. I	(a) None (state 4)	10.9 \pm 2.1 (8)	1.5 \pm 0.3 (8)
	(b) + 2 mM KCN	6.3 \pm 1.2 (9)	1.1 \pm 0.2 (9)
	(c) 2 mM KCN	6.0 \pm 1.4 (10)	1.0 \pm 0.2 (10)
	No substrate		
Expt. II	(d) + 2 mM KCN	22.0 \pm 3.0 (4)	2.3 \pm 0.3 (4)
	+ 2 mM NaADP		
	(a) None (state 4)	3.4 \pm 0.5 (7)	0.5 \pm 0.1 (7)
	(b) + 2 mM KCN	7.3 \pm 2.3 (8)	1.1 \pm 0.3 (8)
Expt. II	(c) + 2 mM KCN	5.9 \pm 1.2 (8)	1.0 \pm 0.2 (8)
	No substrate		
	(d) + 2 mM KCN	18.3 \pm 5.1 (6)	2.8 \pm 0.6 (6)
	+ 2 mM NaADP		

Conditions for state 4 are the same as those of Fig. 2. Presentation of the data is the same as in Table 1.

TABLE 3. The effect of 2,4-dinitrophenol on membrane potential (E) and resistance (R).

Expt. I	Conditions	E	R
		+mv	$\Omega \cdot \text{cm}^2$
Expt. I	None (state 4)	9.4 \pm 1.7 (7)	1.8 \pm 0.3 (7)
	+ 1×10^{-4} DNP	9.8 \pm 1.4 (5)	2.4 \pm 0.3 (5)
Expt. II	None (state 4)	13.1 \pm 1.5 (9)	2.3 \pm 0.2 (9)
	+ 1×10^{-4} DNP	16.0 \pm 1.4 (10)	2.3 \pm 0.2 (10)

Conditions for state 4 are the same as those of Fig. 2. The data are presented as in Table 1.

similar work on artificial membrane systems.⁴ That dinitrophenol was effective as an uncoupler was demonstrated in parallel polarographic experiments. Addition of 1×10^{-4} M DNP resulted in a threefold increase in respiration (not shown).

Discussion.—In our previous paper, factors bearing on the evaluation of these potential and resistance measurements have been presented.¹ With these reservations in mind, the data presented offer no support for the coupling of mitochondrial oxidative-phosphorylation to a membrane potential. In the state 3 or phosphorylating condition the potentials never exceed +35 mv, with mean values of about +20 mv. In state 4 the mean membrane potential is approximately +9 mv. The sign of the potential is the opposite of that predicted by the chemiosmotic mechanism, and the magnitude is well below that required for a sole active role in phosphorylation (200–300 mv).⁵ Furthermore, DNP has been shown to uncouple these mitochondrial preparations. However, there is no significant alteration of membrane resistance or potential, in contrast to the prediction of the chemiosmotic mechanism.⁵ Experiments of Chance and Mela^{6, 7} and Addanki⁸ have also found no significant effect of DNP on the pH gradient across the mitochondrial membrane in state 4. This would suggest that no change in membrane permeability to protons was induced by DNP. However, it should be noted that these experiments give no information on the magnitude of cationic or anionic gradients or whether they were affected by DNP. Tedeschi and Horn⁹ have shown that DNP results in no significant change in permeability of rat liver mitochondria to certain penetrants, suggesting little change in membrane resistance, as observed in our experiments. In contrast, large increases in permeability are induced by DNP in artificial membrane preparations.⁴ Our data would suggest that the effect of DNP as an uncoupler does not reside in its ability to alter the resistance and therefore the permeability of the membrane. However, effects on the permeability of some specific component cannot be excluded.

With respect to the ineffectiveness of KCN in altering the electrical parameters, it is interesting to note that Van Dam and Tsou¹⁰ have shown that a considerable accumulation of anions can take place in the absence of metabolism. The anions appear to accumulate as the consequence of the presence of immobile internal positive charges.⁵ This may explain the lack of effect of KCN on the membrane potential in our experiments. It has been previously suggested¹ that the measured potential reflects the distribution of anions.

The increase in potential in the presence of NaADP (state 3 conditions) is not metabolically dependent, since KCN does not interfere with the effect. A possible explanation is that Na⁺ possesses a greater mobility in the membrane than does ADP.¹¹ This increased mobility of Na⁺ would lend the interior a more positive charge.

Note added in proof: In more recent experiments we have estimated the distribution of C¹⁴-labeled anions. Both the sign and the magnitude of the measured potential agree closely with potentials calculated from the anion distribution by the use of the Nernst equation.

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