Membrane potential of mitochondria in intact lymphocytes during early mitogenic stimulation

Martin D. BRAND and Suzanne M. FELBER

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.

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1. The mitochondrial membrane potential ($\Delta\psi_m$) in intact lymphocytes was calculated by measuring the distribution of radiolabelled methyltriphenylphosphonium cation. The value obtained was 120 mV. 2. The pH gradient across the mitochondrial membrane *in situ* (ΔpH_m) was estimated to be 73 mV (1.2 pH units). Thus the electrochemical gradient of protons was about 190 mV. 3. Addition of the mitogen concanavalin A did not alter $\Delta\psi_m$, showing that, if movement of Ca^{2+} across the inner membrane of lymphocyte mitochondria occurs when concanavalin A is added, it is accompanied by charge-compensating ion movements.

Various techniques have recently become available for measuring $\Delta\psi_m$ in intact cells. Uptake of radioactive lipophilic cations such as TPP^+ or TPMP^+ has been used in neuroblastoma cells (Deutsch *et al.*, 1979), hepatocytes (Hoek *et al.*, 1980), synaptosomes (Scott & Nicholls, 1980; Scott *et al.*, 1980; Deutsch *et al.*, 1981) and adipocytes (Davis *et al.*, 1981; Davis & Martin, 1982a,b; Vallano & Sonenberg, 1982). Qualitative estimates have also been made by using safranin in Ehrlich-ascites-tumour cells (Åkerman, 1979) and hepatocytes (Åkerman & Järvisalo, 1980) and with fluorescent probes in several cell types (Johnson *et al.*, 1981; Goldstein & Karczack, 1981; Darzynkiewicz *et al.*, 1981; Levenson *et al.*, 1982). Quantitative assessment of $\Delta\psi_m$ in lymphocytes has not been reported.

Mitogenic stimulation of lymphocytes is accompanied within 5 min by an increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ (Tsien *et al.*, 1982a,b). Lymphocyte mitochondria are competent to take up Ca^{2+} at low concentrations (Dippenaar & Brand, 1982), and uptake of

Ca^{2+} partially depolarizes isolated liver mitochondria (Mitchell & Moyle, 1969). The decrease in $\Delta\psi_m$ in isolated fat-cells treated with insulin has been tentatively attributed to uptake of Ca^{2+} by the mitochondria (Davis *et al.*, 1981). These observations prompted us to examine $\Delta\psi_m$ in lymphocytes treated with the mitogen concanavalin A to see whether alterations in $\Delta\psi_m$ occur, perhaps indicating mitochondrial Ca^{2+} movements during mitogenic stimulation. In the present paper we show that $\Delta\psi_m$ in lymphocytes is about 120 mV and estimate $\Delta\bar{\mu}\text{H}_m^+$ to be about 190 mV. Addition of concanavalin A does not alter $\Delta\psi_m$.

Experimental

Cells and mitochondria

Pig lymphocytes were prepared from lymph nodes dissected from the mesentery of a freshly slaughtered pig as described by Felber & Brand (1982a). Mouse spleen cells were prepared from 6–8 week-old Balb/c mice by an identical method. Pig lymphocyte mitochondria were isolated as described by Dippenaar & Brand (1982).

Assays

Protein was measured by a biuret method (Gornall *et al.*, 1949). Glutamate dehydrogenase (EC 1.4.1.2) was assayed by the method of Schmidt (1974) and citrate synthase (EC 4.1.3.7) by the method of Bergmeyer *et al.* (1974).

Cell pellets for electron microscopy were fixed in 4% glutaraldehyde in 0.1 M-sodium cacodylate buffer, stained with uranyl acetate and Reynolds

Abbreviations used: TPMP^+ , methyltriphenylphosphonium; TPP^+ , tetraphenylphosphonium; $\Delta\psi_p$, electrical potential across the plasma membrane (positive outside, given a positive sign throughout the present paper); $\Delta\psi_m$, electrical potential across the mitochondrial inner membrane; ΔpH_p , pH difference across the plasma membrane (acid outside); ΔpH_m , pH difference across the mitochondrial inner membrane (acid outside); $\Delta\bar{\mu}\text{H}_m^+$, electrochemical gradient of H^+ across the mitochondrial membrane; FCCP, carbonyl cyanide *p*-tri-fluoromethoxyphenylhydrazone; DMO, 5,5-dimethyl-oxazolidine-2,4-dione; $[\text{Ca}^{2+}]_{\text{cyt}}$, free Ca^{2+} concentration in cell cytoplasm.

Table 1. *Specific activities of mitochondrial marker enzymes in intact pig lymphocytes and isolated pig lymphocyte mitochondria*

Assays are described in the Experimental section. Results are expressed as means $\pm$ S.E.M. of experiments on the numbers of different cell preparations shown in parentheses.

	Glutamate dehydrogenase sp. activity (m-units/mg of protein)	Citrate synthase sp. activity (m-units/mg of protein)	Volume (μ l/mg of protein)
Intact lymphocytes	5.0 $\pm$ 0.2 (9)	128 $\pm$ 15 (7)	3.3 $\pm$ 0.4 (3)
Isolated lymphocyte mitochondria	23.3 $\pm$ 0.9 (5)	530 $\pm$ 65 (6)	0.7 $\pm$ 0.1 (4)

lead stain and embedded in Araldite before cutting of silver sections.

Cell and mitochondrial volumes

The procedures described in Felber & Brand (1982a) were used to determine intracellular and extracellular volumes of pelleted cells, using [3 H]-inulin and 3 H $_2$ O, and intramitochondrial and extramitochondrial volumes of pelleted isolated mitochondria by using [U- 14 C]sucrose and 3 H $_2$ O.

Radiolabelled-ion uptake

The methods described in Felber & Brand (1982a) were followed, using [3 H]inulin as extracellular marker and [U- 14 C]sucrose as extramitochondrial marker.

Materials

86 RbCl, [3 H]inulin and 3 H $_2$ O were from Amersham International, Amersham, Bucks., U.K. [3 H]TPMP $^+$ bromide was from New England Nuclear Chemicals, Dreieich, Germany. RPMI 1640 culture medium was from Flow Laboratories, Irvine, Scotland, U.K. FCCP was from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K. Concanavalin A was from Miles Laboratories, Stoke Poges, Slough, Berks., U.K. [3 H]TPP $^+$ was a gift from Dr. H. R. Kaback. All other reagents were of analytical grade.

Results

Measurement of $\Delta\psi_m$

The uptake of a lipophilic cation such as TPMP $^+$ by cells is a function of both $\Delta\psi_p$ and the membrane potential of any internal organelle. Only mitochondria, however, have sufficient membrane potential and volume to give measurable extra cation uptake. The relationship between $\Delta\psi_m$ and TPMP $^+$ distribution at 37°C is:

$$\Delta\psi_m \text{ (mV)} = 61.5 \log \left\{ \frac{V_c \cdot a_m}{V_m \cdot a_e} \left(\frac{[\text{TPMP}^+]_e \cdot [\text{TPMP}^+]_{\text{tot}}}{[\text{TPMP}^+]_c \cdot [\text{TPMP}^+]_e \cdot a_e} - 1 \right) \right\} \quad (1)$$

where V = volume, a = TPMP $^+$ activity coefficient and subscripts c, m, e and tot = cytoplasmic + nuclear, mitochondrial, extracellular

Table 2. *Mitochondrial matrix volume as a percentage of intracellular volume in pig lymphocytes*

For the enzymes, % mitochondrial volume/cell volume = $100 \times \{[\text{sp. activity (cell)}]/(\text{cell volume})\} / \{[\text{sp. activity (mitochondria)}]/(\text{mitochondrial volume})\}$, from the data of Table 1. The electron-microscopy data were obtained by the method of Weibel (1969) and corrected by subtracting 40% for the intermembrane space (Loud, 1968); 33 electron micrographs of 46 cell profiles were used.

Method	Calculated mitochondrial volume (% of intracellular volume)
Enzymes	
Glutamate dehydrogenase	4.6
Citrate synthase	5.2
Volume ratios	4.7
Electron microscopy	4.7
Mean	4.8

and total taken up respectively (see Scott & Nicholls, 1980). Thus to calculate $\Delta\psi_m$ in lymphocytes we need to know (i) the proportion of the cell volume occupied by mitochondria, (ii) the activity coefficient of TPMP $^+$ in each compartment and (iii) the ratio of concentrations of TPMP $^+$ in the whole cell and in the cytoplasm relative to the medium.

(i) *Determination of the fraction of lymphocyte volume occupied by mitochondria.* We used two independent approaches to estimate this. The first was to measure the specific activities of two exclusively mitochondrial enzymes, glutamate dehydrogenase and citrate synthase, in both intact cells and isolated mitochondria (Table 1). From the measured cell and mitochondrial volumes we calculated enzyme activities per unit volume, and, from the ratio of these, the proportion of the cell occupied

by the mitochondria (Table 2). This calculation assumes that the mitochondria do not change

volume when isolated, which will lead to some inaccuracy. If we also assume that the mitochondrial protein concentration is similar to that of whole cells, we can calculate mitochondrial volume/cell volume by direct comparison of mitochondrial and cell volumes per mg of protein (Table 2). The second approach was to measure mitochondrial volumes from electron micrographs of whole lymphocytes by using the procedures of Weibel (1969).

Table 2 shows that all these estimates of mitochondrial volume in lymphocytes were similar: mitochondria make up about 5% of the volume of a lymphocyte. A value of 4.8% was used in all the calculations reported in the present paper, corresponding to $V_c/V_m = 19.8$ in eqn. (1).

(ii) *Determination of TPMP⁺ activity coefficients a_e , a_c and a_m .* Strictly, we calculate not activity coefficients, but correction factors. We assume the activity coefficient of TPMP⁺ in the medium (a_e) to be 1. We have previously measured the activity coefficient in the cytoplasm (a_c) by comparing $[TPMP^+]_c/[TPMP^+]_e$ with $[Rb^+]_c/[Rb^+]_e$ in the presence of valinomycin to abolish $\Delta\psi_m$ and allow equilibration of $^{86}Rb^+$ across the plasma membrane. We found a value of 1 for a_c , but with a small potential-independent binding term equivalent to 1.8-fold accumulation of TPMP⁺ (Felber & Brand, 1982a). All values for TPMP⁺ accumulation reported here are corrected for this binding component.

The corrections for binding and activity in mitochondria (a_m) were assayed by setting different values of $\Delta\psi_m$ in isolated lymphocyte mitochondria using valinomycin and different extramitochondrial $[K^+]$, and comparing $[^3H]TPMP^+$ and $^{86}Rb^+$ accumulation ratios (Fig. 1). From these data we took a value for a_m of 0.25 (by extrapolation) to $[TPMP^+]_m/[TPMP^+]_e = 360$, which is the value we find for $[TPMP^+]_m/[TPMP^+]_e$ in intact cells by using the data of Table 3.

(iii) *Determination of TPMP⁺ accumulation ratios and $\Delta\psi_m$ and $\Delta\psi_p$.* We have already shown that the ratio $[TPMP^+]_c/[TPMP^+]_e$ can be measured in the presence of uncouplers such as FCCP to abolish TPMP⁺ uptake into mitochondria (Felber & Brand, 1982a). FCCP does not itself alter $\Delta\psi_p$ (measured qualitatively with fluorescent probes) and therefore does not affect the distribution of TPMP⁺ in equilibrium with $\Delta\psi_p$. $[TPMP^+]_c/[TPMP^+]_e$ and $[TPMP^+]_{tot}/[TPMP^+]_e$ in eqn. (1) are therefore the accumulation ratios for TPMP⁺ in the presence and absence of FCCP respectively. Fig. 2 shows that the accumulation ratio for TPMP⁺ was about 100 in control cells, similar to our previous reports (Felber & Brand, 1982a,b). Other experiments gave values of 200 or more [see Felber & Brand (1983a) and Table 3]. Fig. 2 also

Fig. 1. Comparison of $[^3H]TPMP^+$ and $^{86}Rb^+$ uptake in isolated lymphocyte mitochondria

Lymphocyte mitochondria (3 mg of protein/ml) were suspended in medium containing 250 mM-sucrose, 10 mM-Tes (2-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino)ethanesulphonate) (lithium salt), 1 mM-lithium EGTA, 2 mM-lithium succinate, 0.5 mg of valinomycin/ml, pH 7.2, with either 0.02 μ Ci of $^{86}Rb^+$ /ml and 0.1 μ Ci of $[^3H]TPMP^+$ /ml (500 nM) or 1.5 μ Ci of 3H_2O /ml and 0.13 μ Ci of $[U-^{14}C]$ sucrose/ml. After 2 min the suspension was centrifuged in an Eppendorf bench centrifuge and accumulation ratios were measured as described for intact cells in the Experimental section. Data are means of two experiments carried out in duplicate.

Fig. 2. Measurement of TPMP⁺ accumulation in pig lymphocytes

Cells (3×10^7 /ml) were incubated in RPMI medium containing 3 μ M-tetraphenylboron, 30 nM- $[^3H]TPMP^+$ (0.01 μ Ci/ml) and 10 mM-Hepes at pH 7.3. Where indicated 10 μ M-FCCP was added to half the cells. Samples were centrifuged and accumulation ratios were calculated as described in the Experimental section. The results are from one representative experiment carried out in duplicate.

Table 3. TPMP⁺ and DMO accumulation ratios and derived values of $\Delta\psi$ and ΔpH in pig lymphocytes

[³H]TPMP⁺ accumulation ratios were measured at 20 min as described in Fig. 2 and the Experimental section. FCCP (5 μ M) was present for $\Delta\psi_p$ determinations. [¹⁴C]DMO accumulation ratios were measured after 5 min as described in Fig. 4(b) and the Experimental section. $n = 45$ for TPMP⁺ experiments; $n = 3$ for DMO experiments. Values are means $\pm$ S.E.M. Values for P were calculated by using Wilcoxon's signed rank test. Abbreviation used: N.S., not significant.

	Control	+ Concanavalin A (5 μ g/ml)	P for control versus concanavalin A
TPMP ⁺ accumulation ratio	181.2 $\pm$ 11.5	162.2 $\pm$ 7.3	<0.01
TPMP ⁺ (FCCP) accumulation ratio	9.15 $\pm$ 0.27	8.52 $\pm$ 0.21	<0.01
$\Delta\psi_p$ (mV)	59.1 $\pm$ 0.8	57.2 $\pm$ 0.7	<0.01
$\Delta\psi_m$ (mV)	120.2 $\pm$ 1.0	119.5 $\pm$ 0.9	N.S.
DMO accumulation ratio	0.86	0.95	
ΔpH_m (mV)*	73	79	
$\Delta\mu H_m^+$ (mV)	193	199	

* Calculated by the method of Davis *et al.* (1982), assuming a cytoplasmic pH of 7.0, i.e. $\Delta pH_p = 0.3$ pH unit with or without concanavalin A (Rink *et al.*, 1982).

shows that addition of FCCP brought the distribution of TPMP⁺ to equilibrium with $\Delta\psi_p$ within 5–10 min at a value of about 9 (i.e. $\Delta\psi_p$ of 59 mV), in agreement with our earlier values with FCCP present throughout the experiment (Felber & Brand, 1982a,b, 1983a).

Table 3 reports the mean values from a number of experiments for TPMP⁺ accumulation with and without FCCP, and the values for $\Delta\psi_m$ and $\Delta\psi_p$ derived from them with eqn. (1). $\Delta\psi_p$ was 59 ± 1 mV; $\Delta\psi_m$ was 120 ± 1 mV.

Estimation of ΔpH_m and $\Delta\mu H_m^+$

Although our main interest was in $\Delta\psi_m$, we made a rough estimate of ΔpH_m by measuring uptake of the weak acid DMO and taking published values for ΔpH_p (see Table 3). The value obtained was 73 mV. Thus $\Delta\mu H_m^+$ was about 190 mV in these cells (Table 3).

Effect of concanavalin A on $\Delta\psi_m$ and ΔpH_m

To see whether mitogenic stimulation of lymphocytes changes the components of $\Delta\mu H_m^+$, as might be expected if the mitochondria take up a large amount of Ca²⁺ after stimulation (see Davis *et al.*, 1981) we repeated our measurements on lymphocytes with concanavalin A present. Table 3 shows the small concanavalin A-dependent depolarization of $\Delta\psi_p$, which we have previously reported (Felber & Brand, 1983a). However, there was no detectable change in $\Delta\psi_m$. The decrease in accumulation of TPMP⁺ caused by concanavalin A was accounted for entirely by the drop in $\Delta\psi_p$.

The crude estimate of ΔpH_m using DMO showed either an increase from 73 mV to 79 mV, assuming no change in ΔpH_p , or no change in ΔpH_m , assuming a slight alkalization (0.05 pH unit) of the cytoplasm in the presence of concana-

Fig. 3. $\Delta\psi_m$ and $\Delta\psi_p$ in pig lymphocytes: time course of concanavalin A effects

Conditions were as in Fig. 2, except that 25 mM-Hepes was used. For each time point the isotopes (and 5 μ M-FCCP where $\Delta\psi_p$ was measured) were added only 20 min before samples were taken for assay. Where indicated 5 μ g of concanavalin A/ml (conA) was present from the start. Results are means for two experiments carried out in duplicate.

valin A. Our experiments have not measured ΔpH_p , and so do not distinguish between these possibilities. Note that these values are inaccurate because of the small amount of uptake they represent and because we have carried out no corrections for binding.

Time course of concanavalin A effects

Fig. 3 shows the effect of concanavalin A on $\Delta\psi_p$ and $\Delta\psi_m$ over 200 min. The decrease in $\Delta\psi_p$ that we have previously reported over the first 30 min of stimulation (Felber & Brand, 1983a) was maintained or even increased over the first few hours with concanavalin A, presumably because of a

Fig. 4. Effect of concanavalin A on DMO accumulation by pig lymphocytes

(a) Conditions were as for Fig. 2, except that 25 mM-Hepes, pH 7.3, was present and the isotopes used were $0.5 \mu\text{Ci}$ of $[^{14}\text{C}]\text{DMO}/\text{ml}$ ($4.6 \mu\text{M}$) and $1.0 \mu\text{Ci}$ of $[^3\text{H}]\text{inulin}/\text{ml}$ ($56 \mu\text{M}$). A correction for extracellular medium in the pellet was calculated for each sample. (b) Conditions were as for Fig. 3, except that the isotopes used were $0.2 \mu\text{Ci}$ of $[^{14}\text{C}]\text{DMO}/\text{ml}$ ($1.8 \mu\text{M}$) and $0.5 \mu\text{Ci}$ of $[^3\text{H}]\text{inulin}/\text{ml}$ ($53 \mu\text{M}$). A correction for extracellular medium in the pellet was calculated for each sample. The pH of the medium was 7.3, and did not change during the experiment. Results show a representative experiment. Symbols: $\circ$, + concanavalin A; $\bullet$, control.

maintained increase in P_{Na} (electrogenic permeability coefficient for Na^+) (Felber & Brand, 1983b). There was no increase in $^{86}\text{Rb}^+$ accumulation rates over this period (results not shown). $\Delta\psi_{\text{m}}$ remained similar with and without concanavalin A during the experiment.

Fig. 4 shows corresponding experiments with DMO. The small increase in DMO accumulation was maintained for at least 2 h. Thus the effects of concanavalin A on $\Delta\psi_{\text{p}}$, and on $\Delta\text{pH}_{\text{m}}$ or $\Delta\text{pH}_{\text{p}}$, are persistent.

Effect of concanavalin A on $\Delta\psi_{\text{p}}$ and $\Delta\psi_{\text{m}}$ in mouse spleen cells

Kiefer *et al.* (1980) and Kiefer & Schulze (1982) have reported that mitogens cause a large depolarization of several cell types, including mouse spleen cells, monitored with TPP^+ . However, as we have pointed out (Felber & Brand, 1982a), the corrections they applied are inappropriate, and do not allow them to distinguish between depolarization of $\Delta\psi_{\text{p}}$, and $\Delta\psi_{\text{m}}$, or both, nor do they allow calculation of true values of $\Delta\psi_{\text{p}}$.

Fig. 5. Effect of concanavalin A on TPP^+ accumulation by mouse spleen lymphocytes

(a) Cells $[(1-3) \times 10^7/\text{ml}]$ were incubated at 37°C in RPMI medium + 10 mM-Hepes, pH 7.3, in the presence of 5% foetal-calf serum. $\bullet$, Control; $\circ$, + $2 \mu\text{g}$ of concanavalin A/ml. For each time point $0.06 \mu\text{Ci}$ of $[^3\text{H}]\text{TPP}^+/\text{ml}$ ($25 \mu\text{M}$) (and $5 \mu\text{M}$ -FCCP where indicated) were added 20 min before samples were taken for assay as described in the Experimental section. The results are means of two experiments. (b) Cells were incubated as described above. $\bullet$, Control; $\circ$, + $2 \mu\text{g}$ of concanavalin A/ml. For each time point $0.06 \mu\text{Ci}$ of $[^3\text{H}]\text{TPP}^+/\text{ml}$ was added at either $25 \mu\text{M}$ or $0.5 \mu\text{M}$, 20 min before samples were removed for assay. The results are from one representative experiment. No correction was made for any TPP^+ binding to cell components.

We have therefore applied our methods to mouse spleen cells.

Fig. 5 shows the uptake of TPP^+ into mouse spleen cells in the presence of foetal-calf serum essentially as described by Kiefer *et al.* (1980). Concanavalin A consistently decreased TPP^+ accumulation. To distinguish between effects on $\Delta\psi_{\text{p}}$ and $\Delta\psi_{\text{m}}$ we added FCCP. Control experiments using bis-oxonol to monitor $\Delta\psi_{\text{p}}$ (results not shown) revealed that FCCP did not itself alter $\Delta\psi_{\text{p}}$ in these cells, and that the Ca^{2+} -dependent K^+

Fig. 6. Effect of concanavalin A on TPMP⁺ accumulation by mouse spleen lymphocytes

Cells $[(1-3) \times 10^7/\text{ml}]$ were incubated at 37°C in RPMI + 10 mM-Hepes, pH 7.3. ●, Control; ○, +2 μg of concanavalin A/ml. For each time point, 3 μM -tetraphenylboron, 0.1 μCi of [³H]TPMP⁺ (50 nM) (and 50 μM -FCCP where indicated) were added 20 min before samples were removed for assay as described in the Experimental section. The results are from one representative experiment. No correction for TPMP⁺ binding was made.

channel was fully open as it is in pig lymphocytes (Felber & Brand, 1983a). In the presence of FCCP concanavalin A still lowered TPP⁺ accumulation, showing that $\Delta\psi_p$ was the main site of concanavalin A action. The depolarization was about 3–5 mV, much less than that reported by Kiefer *et al.* (1980). If we estimate $\Delta\psi_p$ by subtracting TPP⁺ accumulation, as those authors have, then our results would indicate (incorrectly) a much larger depolarization. The contribution of $\Delta\psi_m$ to TPP⁺ uptake under the conditions of Kiefer *et al.* (1980) does not seem to be large. Fig. 5(b) shows that lowering the concentration of TPP⁺ 50-fold gave much greater TPP⁺ accumulation, suggesting that the higher TPP⁺ concentrations caused damage to mitochondria with a decrease of $\Delta\psi_m$ in these cells.

Fig. 6 shows the uptake of TPMP⁺ by mouse spleen cells under conditions similar to those we used for pig lymphocytes. It is clear that, although $\Delta\psi_m$ was not stable, the results are very similar to those seen in pig lymphocytes (Felber & Brand, 1982a, 1983a; the present work). We conclude that the apparent decreases in $\Delta\psi_p$ in mouse spleen cells reported by Kiefer *et al.* (1980) are genuinely decreases in $\Delta\psi_p$, but that the corrections applied by those authors led them to overestimate greatly the size of the depolarization of $\Delta\psi_p$ caused by concanavalin A. The depolarization seen in mouse spleen cells is small, and is very similar to that in pig lymphocytes.

Discussion

Estimates of $\Delta\psi_m$ in intact cells made by using uptake of radiolabelled cations such as TPMP⁺ vary between 75 and 180 mV. The more reliable values, with low TPMP⁺ concentrations, are above 150 mV (see the introduction for references). Our $\Delta\psi_m$ value for lymphocytes of 120 mV is therefore a little lower than expected. Our estimate of $\Delta\bar{\mu}H_m^+$ is about 190 mV, which should be compared with values for isolated liver mitochondria of 170–230 mV in State 4 (e.g. Nicholls, 1974; Rottenberg, 1975). That lymphocyte mitochondria are not uncoupled, but are between State 3 and State 4 in our cells, is shown by the 2–4-fold stimulation of the whole-cell rate of oxygen consumption by 10 μM -FCCP. However, the cells are turning ATP over quite rapidly, since inhibitors of ATP synthesis (oligomycin at 40 $\mu\text{g}/\text{ml}$ and bongkrekic acid at 20 μM) halve basal respiration (M. D. Brand & S. M. Felber, unpublished work).

Although we have attempted to control for major effects, there may be systematic errors that make our $\Delta\psi_m$ values a little inaccurate. However, the technique is very sensitive to small changes in $\Delta\psi_m$, and so we can say with some confidence that concanavalin A does not alter $\Delta\psi_m$ in lymphocytes. This is in contrast with the situation in isolated fat-cells, where insulin and α -adrenergic agonists decrease $\Delta\psi_m$ (Davis *et al.*, 1981; Davis & Martin, 1982b). It may be that this effect in fat-cells is caused by net entry of Ca^{2+} from cytoplasm to mitochondria, although other explanations are possible.

In lymphocytes there is good evidence that concanavalin A raises $[\text{Ca}^{2+}]_{\text{cyt}}$ (Tsien *et al.*, 1982a,b). Since isolated lymphocyte mitochondria transport Ca^{2+} in the same way as those from other tissues such as liver (Dippenaar & Brand, 1982), we would expect this rise in $[\text{Ca}^{2+}]_{\text{cyt}}$ to be followed by entry of Ca^{2+} into the mitochondria, as proposed in other tissues (see, e.g., Denton & McCormack, 1980). We have some evidence that this is in fact what happens, as judged by concanavalin A activation of lymphocyte pyruvate dehydrogenase (Baumgarten *et al.*, 1983).

Isolated liver mitochondria show a depolarization of $\Delta\psi_m$ when they take up Ca^{2+} (Mitchell & Moyle, 1969). From the present work it is clear that any Ca^{2+} influx or efflux into or out of lymphocyte mitochondria is not accompanied by depolarization or hyperpolarization of $\Delta\psi_m$ in intact cells. One possibility is that lymphocyte mitochondria do not take up or release Ca^{2+} on concanavalin A stimulation (but see Mikkelsen & Schmidt-Ullrich, 1980; Baumgarten *et al.*, 1983). More probably, compensating $\Delta p H_m$ -linked uptake of anions such as phosphate or bicarbonate occurs, followed by

increased respiration, leading to no significant change in $\Delta\psi_m$.

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References

- Åkerman, K. E. O. (1979) *Biochim. Biophys. Acta* **546**, 341–347
- Åkerman, K. E. O. & Järvisalo, J. O. (1980) *Biochem. J.* **191**, 183–190
- Baumgarten, E., Brand, M. D. & Pozzan, T. (1983) *Biochem. J.* **216**, 359–367
- Bergmeyer, H. U., Gawehn, K. & Grassl, M. (1974) in *Methods in Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 443–444, Academic Press, London
- Darzynkiewicz, Z., Staiano-Coico, L. & Melamed, M. R. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2383–2387
- Davis, R. J. & Martin, B. R. (1982a) *Biochem. J.* **206**, 611–618
- Davis, R. J. & Martin, B. R. (1982b) *Biochem. J.* **206**, 619–626
- Davis, R. J., Brand, M. D. & Martin, B. R. (1981) *Biochem. J.* **196**, 133–147
- Denton, R. M. & McCormack, J. G. (1980) *FEBS Lett.* **119**, 1–8
- Deutsch, C., Erecińska, M., Werrlein, R. & Silver, I. A. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2175–2179
- Deutsch, C., Drown, C., Rafalowska, U. & Silver, I. A. (1981) *J. Neurochem.* **36**, 2063–2072
- Dippenaar, N. G. & Brand, M. D. (1982) *Biochem. J.* **202**, 731–737
- Felber, S. M. & Brand, M. D. (1982a) *Biochem. J.* **204**, 577–585
- Felber, S. M. & Brand, M. D. (1982b) *FEBS Lett.* **150**, 122–124
- Felber, S. M. & Brand, M. D. (1983a) *Biochem. J.* **210**, 885–891
- Felber, S. M. & Brand, M. D. (1983b) *Biochem. J.* **210**, 893–897
- Goldstein, S. & Korczack, L. B. (1981) *J. Cell Biol.* **91**, 392–398
- Gornall, A. G., Bardawill, C. J. & David, M. M. (1949) *J. Biol. Chem.* **177**, 751–766
- Hoek, J. B., Nicholls, D. G. & Williamson, J. R. (1980) *J. Biol. Chem.* **255**, 1458–1464
- Johnson, L. V., Walsh, M. L., Bockus, B. J. & Chen, L. B. (1981) *J. Cell Biol.* **88**, 526–535
- Kiefer, H. & Schulze, R. (1982) *Biosci. Rep.* **2**, 583–588
- Kiefer, H., Blume, A. J. & Kaback, H. R. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2200–2204
- Levenson, R., Macara, I. G., Smith, R. L., Cantley, L. & Housman, D. (1982) *Cell* **28**, 855–863
- Loud, A. V. (1968) *J. Cell Biol.* **37**, 27–46
- Mikkelsen, R. B. & Schmidt-Ullrich, R. (1980) *J. Biol. Chem.* **255**, 5177–5183
- Mitchell, P. & Moyle, J. (1969) *Eur. J. Biochem.* **7**, 471–484
- Nicholls, D. G. (1974) *Eur. J. Biochem.* **50**, 305–315
- Rink, T. J., Tsien, R. Y. & Pozzan, T. (1982) *J. Cell Biol.* **95**, 189–196
- Rottenberg, H. (1975) *J. Bioenerg.* **7**, 61–74
- Schmidt, E. (1974) in *Methods in Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 650–656, Academic Press, London
- Scott, I. D. & Nicholls, D. G. (1980) *Biochem. J.* **186**, 21–33
- Scott, I. D., Åkerman, K. E. O. & Nicholls, D. G. (1980) *Biochem. J.* **192**, 873–880
- Tsien, R. Y., Pozzan, T. & Rink, T. J. (1982a) *Nature (London)* **295**, 68–71
- Tsien, R. Y., Pozzan, T. & Rink, T. J. (1982b) *J. Cell Biol.* **94**, 325–334
- Vallano, M. L. & Sonenberg, M. (1982) *J. Membr. Biol.* **68**, 57–66
- Weibel, E. R. (1969) *Int. Rev. Cytol.* **26**, 235–302