***cis*-Unsaturated fatty acids uncouple mitochondria and stimulate glycolysis in intact lymphocytes**

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In intact lymphocytes, linoleic acid acts as a typical mitochondrial uncoupler: it substantially decreases the mitochondrial membrane potential and the cellular ATP level, while stimulating oxygen consumption and glycolysis. Under these conditions the inhibition of cellular functions by linoleic acid cannot be attributed to its postulated effects on lipid domains in the plasma membrane.

cis-Unsaturated fatty acids have been found to affect a wide range of functions in different types of cells, whereas saturated and *trans*-unsaturated fatty acids have little or no equivalent effect. These results have been interpreted in terms of a model in which the different fatty acids interact with lipid domains of different composition within the plasma membrane (Karnovsky *et al.*, 1982). For example, cap formation on lymphocytes was inhibited by *cis*-unsaturated fatty acids; this was attributed to a perturbation of lipid domains, which resulted in the displacement of Ca^{2+} from a protein postulated to anchor cross-linked plasma-membrane receptors to the cytoskeleton (Klausner *et al.*, 1980). The relevance of this hypothesis to the molecular mechanism of receptor redistribution prompted us to investigate the phenomenon in detail. We concluded that the *cis*-unsaturated fatty acids acted as mitochondrial uncouplers in the intact lymphocytes, causing a reduction in cellular ATP levels. This provided a simpler and sufficient explanation of the inhibition of cap formation (Corps *et al.*, 1980). Hoover *et al.* (1982), however, have subsequently argued that our conclusion was based on artifacts in the measurement of cellular ATP levels and oxygen consumption.

Here we present independent experimental evidence that the *cis*-unsaturated fatty acid linoleic

acid substantially reduces the mitochondrial membrane potential in intact lymphocytes. In addition, we show that linoleic acid causes an increase in the rate of glycolysis, as revealed by an increased output of lactate from the lymphocytes. We also show that concentrations of linoleic acid that substantially uncouple mitochondria *in situ* do not affect our assay procedures for ATP and oxygen consumption; the artifacts observed by Hoover *et al.* (1982) presumably result from the particular experimental conditions used by those authors. These results confirm and extend our previous conclusion (Corps *et al.*, 1980) that any effects of *cis*-unsaturated fatty acids that might be attributed to interactions with lipid domains cannot be distinguished from the effects of these agents on energy conservation in lymphocytes.

Experimental

Mouse spleen lymphocytes were prepared as described previously (Corps *et al.*, 1980). Linoleic acid was prepared as a stock solution of 10 mg/ml in ethanol as described previously (Corps *et al.*, 1980; Klausner *et al.*, 1980; Hoover *et al.*, 1982), and the final concentration of ethanol was less than 1% (v/v) in all samples; the concentration of ethanol did not affect the cellular activities assayed or any of the assay procedures used.

The accumulation of [^{14}C]TPMP⁺ was determined essentially as described by Felber & Brand (1982). Cells (0.5 ml portions) were incubated for 30 min at 37°C with 3 μM -tetraphenylboron, 0.22 μCi of $^3\text{H}_2\text{O}$ (Amersham International; 25 mCi/g) and either 0.06 μCi of [^{14}C]inulin (sp. radioactivity 5 Ci/mol) or 0.02 μCi of [^{14}C]TPMP⁺

Abbreviations used: $\Delta\psi_m$, mitochondrial membrane potential; $\Delta\psi_p$, plasma-membrane potential; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazide; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; TPMP⁺, tetraphenylmethylphosphonium.

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(sp. radioactivity 10 Ci/mol). The cell suspension was centrifuged (30 s, 14000g), and the pellets were resuspended in 0.5 ml of distilled water or were disrupted with 1% (v/v) HClO_4 . After a second centrifugation, portions of the supernatants were counted for ^3H and ^{14}C nuclides in a Packard scintillation counter (Tri-Carb 300 C) with automatic d.p.m. correction. The inclusion of $^3\text{H}_2\text{O}$ and [^{14}C]inulin allows the determination of total and extracellular water volumes respectively, from which the accumulation of [^{14}C]TPMP $^+$ in the intracellular water volume can be calculated (Felber & Brand, 1982).

To observe the staining of lymphocyte mitochondria with Rhodamine 123, cells [$(3-4) \times 10^7/\text{ml}$] were incubated at 37°C with $1\text{ }\mu\text{g}$ of Rhodamine 123/ml and the appropriate concentration of linoleic acid, FCCP or ethanol. After 30 min the samples were diluted 1:1 with medium in order to decrease the background fluorescence, and the cells were then immediately examined under u.v. illumination in a Leitz Diavert microscope. Under these experimental conditions (i.e. $\leq 0.03\text{ }\mu\text{g}$ of Rhodamine 123 per 10^6 cells) the cellular ATP levels remained within 90–95% of those in control (untreated) samples; furthermore, the mitochondrial membrane potential of the cells treated with Rhodamine 123, measured by the accumulation of [^{14}C]TPMP $^+$, was not significantly different from that in control samples. It should be noted that higher concentrations of Rhodamine 123 can decrease lymphocyte ATP levels significantly by inhibiting mitochondrial electron flow at site 1 (T. Pozzan, unpublished work).

The assays of lymphocyte oxygen consumption and ATP content were performed essentially as described previously (Corps *et al.*, 1980). Oxygen consumption was measured at 37°C in a thermostatically controlled cuvette with a Clark-type

electrode. The measurement of lymphocyte ATP levels by the luciferin/luciferase assay was performed as described previously (Corps *et al.*, 1980), using either a DuPont 760 or a Packard 3100 Pico-lite Luminescence Biometer. Lymphocyte ATP levels were also determined by using the hexokinase/glucose-6-phosphate dehydrogenase method (Williamson & Corkey, 1969). For the measurement of lymphocyte lactate output, cell samples were centrifuged (3 s, 14000g) and portions of the supernatants were assayed by the lactate dehydrogenase method (Gutmann & Wahlefeld, 1974), with a Varian DMS 90 double-beam spectrophotometer; the reference cuvette contained samples of medium (with the appropriate additions of ethanol or linoleic acid in ethanol) that had been processed in the same way as the cell supernatants. In all experiments, control samples of ATP or lactate in the appropriate medium were treated with ethanol or linoleic acid in ethanol, and assayed in the same manner as the cell samples. Firefly lantern extract was obtained from Sigma; hexokinase, lactate dehydrogenase and glucose 6-phosphate dehydrogenase were from Boehringer; linoleic acid was from Koch-Light Co. All other chemicals were of analytical grade.

It should be noted that the present experiments differ from those described previously (Corps *et al.*, 1980; Klausner *et al.*, 1980; Hoover *et al.*, 1982) in that the data in the present paper were obtained at the physiological temperature of 37°C rather than at 22°C . We emphasize that, although the absolute rates of oxygen consumption and lactate output were higher at 37°C than at 22°C , there were no significant differences in the pattern of responses to linoleic acid at the different temperatures. Similarly, variations in the media used for different experiments (see the Figure and Table legends) did not significantly alter the effects of

Table 1. *Effect of linoleic acid and mitochondrial inhibitors on the accumulation of [^{14}C]TPMP $^+$ into lymphocytes*
The experimental protocols and calculations are described in the text. The cell density was 4.5×10^7 cells/ml. [^{14}C]TPMP $^+$ accumulation is expressed as the ratio $[\text{TPMP}^+]_{\text{in}}/[\text{TPMP}^+]_{\text{out}}$; [^{14}C]TPMP $^+$ binding corresponds to an accumulation ratio of 2.2. The addition of ethanol (1%, v/v, in all samples) did not affect the accumulation of [^{14}C]TPMP $^+$. Medium A: 140 mM-NaCl, 5 mM-KCl, 1 mM- CaCl_2 , 0.5 mM- MgSO_4 , 1 mM- Na_3PO_4 and 5.5 mM-glucose, buffered with 20 mM-Hepes to pH 7.4 at 37°C . Medium B: as medium A, except that [NaCl] and [KCl] were 20 mM and 125 mM respectively. Results are means $\pm$ S.D. from three experiments. Abbreviation used: ND, not determined.

Additions	[^{14}C]TPMP $^+$ accumulation ratio		$\Delta\psi_m$ (mV)		$\Delta\psi_p$ (mV)	
	Medium A	Medium B	Medium A	Medium B	Medium A	Medium B
Ethanol (1%, v/v)	53.1 ± 1.7	16.2 ± 4.0	-137	-126	—	—
Oligomycin (2 $\mu\text{g}/\text{ml}$)	44.8 ± 8.3	ND	-132	ND	—	—
FCCP (1 μM)	7.6 ± 0.2	4.3 ± 0.9	0*	0*	-45	-20
Linoleic acid (15 $\mu\text{g}/\text{ml}$)	12.5 ± 8.5	5.6 ± 1.0	-77	-67	—	—

* Assumed.

linoleic acid on lymphocyte ATP levels. The only experimental variable to have a significant effect was the cell density used, as we have noted previously (Corps *et al.*, 1980); as expected, when higher cell densities were employed, higher concentrations of linoleic acid were required in order to obtain a given effect on the parameters measured.

Results and discussion

Effects on mitochondrial membrane potential *in situ*

In Table 1 the effects of linoleic acid on the accumulation of [^{14}C]TPMP $^{+}$ into lymphocytes are compared with the effects of classical mitochondrial inhibitors, FCCP and oligomycin. The accumulation of [^{14}C]TPMP $^{+}$ into cells has been shown to be determined both by the plasma-membrane potential, $\Delta\psi_p$, and by the mitochondrial membrane potential, $\Delta\psi_m$ (Hoek *et al.*, 1980); the values of $\Delta\psi_p$ and $\Delta\psi_m$ can be calculated independently from the accumulation of [^{14}C]TPMP $^{+}$ by appropriate manipulations of the medium and/or the use of specific inhibitors (Hoek *et al.*, 1980; Felber & Brand, 1982). Thus, assuming that the mitochondrial uncoupler FCCP completely collapses $\Delta\psi_m$ (Hoek *et al.*, 1980; Felber & Brand, 1982), then the plasma-membrane potential $\Delta\psi_p$ can be calculated from the accumulation of [^{14}C]TPMP $^{+}$ in the presence of FCCP, by using the Nernst equation (1):

$$\Delta\psi_p = -2.3 \frac{RT}{F} \cdot \log \left(\frac{[\text{TPMP}^{+}]_{\text{free}}^{\text{FCCP}}}{[\text{TPMP}^{+}]_{\text{o}}^{\text{FCCP}}} \right) \quad (1)$$

$$[\text{TPMP}^{+}]_{\text{free}}^{\text{FCCP}} = [\text{TPMP}^{+}]_{\text{total}}^{\text{FCCP}} - [\text{TPMP}^{+}]_{\text{bound}}^{\text{FCCP}} \quad (2)$$

$$[\text{TPMP}^{+}]_{\text{bound}}^{\text{FCCP}} = 2.2 [\text{TPMP}^{+}]_{\text{o}}^{\text{FCCP}} \quad (3)$$

where $2.3RT/F$ has a value of 61.5 at 37°C, $[\text{TPMP}^{+}]_{\text{free}}^{\text{FCCP}}$ and $[\text{TPMP}^{+}]_{\text{o}}^{\text{FCCP}}$ are the internal and external concentrations of TPMP $^{+}$ respectively in the presence of FCCP, and 2.2 is the correction for the constant binding component of [^{14}C]TPMP $^{+}$ (Table 1; compare Felber & Brand, 1982). The mitochondrial membrane potential, $\Delta\psi_m$, can then be calculated according to eqn. (4):

$$\Delta\psi_m = -2.3 \frac{RT}{F} \cdot \log \left(\frac{[\text{TPMP}^{+}]_{\text{m free}}}{[\text{TPMP}^{+}]_{\text{cyt. free}}} \right) \quad (4)$$

where $[\text{TPMP}^{+}]_{\text{m free}}$ and $[\text{TPMP}^{+}]_{\text{cyt. free}}$ are the mitochondrial and cytoplasmic concentrations of free TPMP $^{+}$ respectively. If it is assumed that $[\text{TPMP}^{+}]_{\text{bound}}$ is always 2.2 $[\text{TPMP}^{+}]_{\text{o}}$, and taking into account that the mitochondrial volume represents 5% of the total cell volume:

$$[\text{TPMP}^{+}]_{\text{m total}} = ([\text{TPMP}^{+}]_{\text{total}} - [\text{TPMP}^{+}]_{\text{cyt. free}} - [\text{TPMP}^{+}]_{\text{bound}}) \times 20 \quad (5)$$

$[\text{TPMP}^{+}]_{\text{cyt. free}}$ can be calculated from $[\text{TPMP}^{+}]_{\text{o}}$ if it is assumed that $\Delta\psi_p$ is not affected by FCCP (see Felber & Brand, 1982). No attempts were made to correct for mitochondrial TPMP $^{+}$ binding. Thus $[\text{TPMP}^{+}]_{\text{m total}} = [\text{TPMP}^{+}]_{\text{m free}}$. The effects of reagents on $\Delta\psi_m$ may also be estimated by measuring the accumulation of [^{14}C]TPMP $^{+}$ in a high- K^{+} low- Na^{+} medium (see the legend to Table 1): under these conditions the value of $\Delta\psi_p$ is considerably reduced (Rink *et al.*, 1980; Felber & Brand, 1982) and the accumulation of [^{14}C]TPMP $^{+}$ is essentially determined by $\Delta\psi_m$ (Felber & Brand, 1982).

On the basis of the accumulation of [^{14}C]TPMP $^{+}$ the values calculated for $\Delta\psi_p$ and $\Delta\psi_m$ in control cells are -45mV and -137mV respectively (Table 1). This value for $\Delta\psi_p$ is similar to that previously reported by Felber & Brand (1982) and by Rink *et al.* (1980). Linoleic acid markedly reduces the accumulation of [^{14}C]TPMP $^{+}$ in both normal and high- K^{+} medium (Table 1), indicating that its predominant effect is to reduce $\Delta\psi_m$ rather than $\Delta\psi_p$ (see above). Thus, assuming that the plasma-membrane potential $\Delta\psi_p$ is not significantly affected, then in the presence of $15\text{ }\mu\text{g}$ of linoleic acid/ml $\Delta\psi_m$ is reduced from -137mV to -77mV in normal medium, and from -126mV to -67mV in high- K^{+} medium (i.e. about half of the effect of $1\text{ }\mu\text{M}$ -FCCP). In contrast, oligomycin has very little effect on the accumulation of [^{14}C]TPMP $^{+}$, as expected for an agent that inhibits

mitochondrial ATP synthesis without affecting $\Delta\psi_m$ (Nicholls, 1982). When an inhibitor of the respiratory chain (KCN) is added in addition to oligomycin, the accumulation of [^{14}C]TPMP $^{+}$ is similar to that obtained in the presence of FCCP or linoleic acid (results not shown), confirming that the normal cellular responses to mitochondrial inhibitors are obtained under the experimental

conditions used. We conclude that $\Delta\psi_m$ of lymphocyte mitochondria *in situ* is partially collapsed in the presence of linoleic acid.

Rhodamine 123, in common with other cationic dyes of the same group, is accumulated by isolated mitochondria according to the membrane poten-

tial, negative inside (Goldstein & Korczack, 1981), and it has been shown that the staining of mitochondria in intact cells by Rhodamine 123 can be used as a qualitative indicator of $\Delta\psi_m$ *in situ* (Johnson *et al.*, 1980; Goldstein & Korczack, 1981). In control cell samples (Fig. 1*a*) Rhodamine 123 appears to be distributed as a number of discrete bright dots, often polarized towards one end of the cell. If $\Delta\psi_m$ is collapsed, for example by using FCCP (Fig. 1*b*), the mitochondria can no longer be clearly distinguished from the rest of the cell, which is diffusely stained. Fig. 1(*c*) shows that linoleic acid has a similar effect to that of FCCP on the staining of lymphocyte mitochondria *in situ*, and in Table 2 the numbers of cells with discrete dots and diffuse staining are compared for control cell samples and for cells treated with FCCP or linoleic acid. More than 90% of the cells in control populations show the pattern of discrete dots (as in Fig. 1*a*), whereas most of the cells treated with linoleic acid or with FCCP are diffusely stained (as in Figs. 1*b* and 1*c*).

Metabolic effects of linoleic acid

The results obtained with [^{14}C]TPMP⁺ and Rhodamine 123 are consistent with an uncoupling action of linoleic acid on the mitochondria of intact lymphocytes. It would therefore be predicted that linoleic acid would increase mitochondrial oxygen consumption (Nicholls, 1982), and we have previously demonstrated this effect (Corps *et al.*, 1980). Hoover *et al.* (1982) have suggested that the increase in oxygen consumption that we detected after the addition of linoleic acid to lymphocytes was in fact an artifact that could be observed in the absence of cells. However, Fig. 2(*a*) shows that under our experimental conditions the addition of linoleic acid at 20 $\mu\text{g}/\text{ml}$ has only a minor effect on the oxygen-electrode trace in the absence of cells, compared with the substantial increase in the rate of oxygen consumption in the presence of cells (Fig. 2*b*). It should also be emphasized that the increase in oxygen consumption induced by linoleic acid is blocked by rotenone (Fig. 2*b*; see also Fig. 3 of Corps *et al.*, 1980), antimycin A or KCN (not shown), confirming that linoleic acid behaves as a classical mitochondrial uncoupler (Arslan *et al.*, 1981; Nicholls, 1982). In marked contrast, the oxygen-consumption data reported by Hoover *et al.* (1982) are not consistent with the normal response of isolated mitochondria or of cells to oligomycin. They showed that oligomycin increases oxygen consumption and stated that similar effects were obtained with FCCP. However, it is very well established that oligomycin is an inhibitor of mitochondrial ATPase, whereas FCCP is a classical uncoupler, and that these two agents therefore have opposite effects on oxygen consumption

Fig. 1. Effect of linoleic acid on the staining of lymphocyte mitochondria by Rhodamine 123

Lymphocytes (3×10^7 cells/ml in RPMI 1640 medium, buffered with 20 mM-Hepes at pH 7.4) were incubated at 37°C with: (a) 1% (v/v) ethanol; (b) 1 μM -FCCP; (c) 15 μg of linoleic acid/ml. Rhodamine 123 (1 $\mu\text{g}/\text{ml}$) was added after 5 min, and after 30 min the samples were diluted and examined as described in the Experimental section. Each cell was checked by through-focusing, which revealed that the spots seen in (a) were within the cell, and not on the surface. The photographs are representative of the staining patterns observed on the respective slides, i.e. few cells in (b) or (c) had discrete spots of fluorescence as seen in (a).

(Nicholls, 1982). Thus oligomycin decreases oxygen consumption and FCCP increases oxygen consumption (Nicholls, 1982), as we showed previously (Corps *et al.*, 1980; Arslan *et al.*, 1981). One of several possible explanations of the oxygen-

Table 2. Effect of linoleic acid on the staining of lymphocyte mitochondria by Rhodamine 123

The experimental protocol is described in the legend to Fig. 1. Ethanol, FCCP or linoleic acid was added 5 min before Rhodamine 123. At least 100 stained cells were counted per sample.

Additions	Proportion of cells with discrete dots (%)		Proportion of diffusely stained cells (%)	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Ethanol (1%)	96	94	4	6
FCCP (1 μ M)	17	7	83	93
Linoleic acid (15 μ g/ml)	16	3	84	97

Fig. 2. Effect of linoleic acid on lymphocyte oxygen consumption

(a) Medium only; (b) lymphocytes (6×10^7 cells/ml) were incubated at 37°C in Earle's Balanced Salts Solution without Ca^{2+} . The arrows ($\downarrow$) indicate the following additions: oligo., 1 μg of oligomycin/ml; Lin.A, 20 μg of linoleic acid/ml; rot., 2 μM -rotenone. The addition of the volume of ethanol equivalent to that added with the linoleic acid had no effect on the measured rate of oxygen consumption in either the absence or the presence of cells.

electrode artifacts observed by Hoover *et al.* (1982) might be the low cell concentration used by those authors, which necessitates the amplification of the oxygen-electrode output. We have used a high cell concentration [$(50\text{--}80) \times 10^6$ cells/ml], which allows any spurious electrode effects to be clearly distinguished from genuine changes in the rate of cellular oxygen consumption (Fig. 2).

An alternative explanation for the increase in oxygen consumption is that linoleic acid might be acting as a preferential substrate. This is unlikely, however, since linoleic acid, in common with other uncoupling agents (Pozzan *et al.*, 1980), decreases lymphocyte ATP levels (Fig. 3a, and Corps *et al.*,

1980). Although confirming our experimental observations, Hoover *et al.* (1982) suggested that the effects were due to the interference of linoleic acid with the ATP assay. Under our experimental conditions, however, the addition of up to 100 μg of linoleic acid/ml has no effect on the luminescence intensities obtained in the assay of standard solutions of ATP (Fig. 3b); this concentration of linoleic acid is considerably greater than those that decrease lymphocyte ATP levels ($<10 \mu\text{g}/\text{ml}$; Fig. 3a). In many variations of the luciferin/luciferase assay protocol we have detected effects of linoleic acid on the assay of ATP standard samples only when the luciferin/luciferase mixture was diluted at least 10-fold and the concentration of linoleic acid was increased above 100 $\mu\text{g}/\text{ml}$. The effect of linoleic acid on lymphocyte ATP levels was confirmed by using the hexokinase/glucose-6-phosphate dehydrogenase method; the results obtained with the luciferin/luciferase assay and the hexo-kinase/glucose-6-phosphate dehydrogenase method differed by less than 10% in all samples measured.

The output of lactate from lymphocytes is an indicator of the rate of glycolysis under various conditions (Roos & Loos, 1973; Hume *et al.*, 1978), and is increased by mitochondrial inhibitors (Roos & Loos, 1973). In mouse lymphocytes, we find that the mitochondrial inhibitors sodium azide and FCCP each give a concentration-dependent increase in the output of lactate over the concentration ranges of the inhibitors that reduce cellular ATP levels. The maximal stimulation of lactate output was from 3–4 fmol/h per cell in control cells to 9–12 fmol/h per cell in those cells where the ATP levels were reduced by more than 60% (with 5 mM-azide or 300 nM-FCCP; results obtained in six independent experiments). In Fig. 3(a) we show that linoleic acid stimulates the glycolytic output of lactate from lymphocytes at concentrations that do not affect the assay of standard solutions of lactate (Fig. 3c). It is possible that mechanisms other than the inhibition of mitochondrial ATP production are involved in the stimulation of glycolysis by linoleic acid. However, it may be noted that the

Fig. 3. Effect of linoleic acid on lymphocyte ATP levels and lactate output

(a) Lymphocytes (2×10^7 /ml in Earle's Balanced Salts Solution without Ca^{2+} , buffered with 10 mM-Hepes at pH 7.3) were incubated for 30 min at room temperature with the indicated concentration of linoleic acid. They were then centrifuged (3s, 14000g) and resuspended at 10^7 cells/ml in fresh medium at 37°C. Samples were removed for ATP assay after 15 min, and the total lactate output was assayed over 60 min. (b) ATP standard solutions (2 μM, 5 μM and 10 μM) were prepared by dilution of a 10 mM stock solution in the medium described above. Linoleic acid was added and the samples were processed in the same manner as the cell samples. (c) Lactate standard solutions were prepared in the medium described above. Linoleic acid was added and the samples were processed in the same manner as the cell samples. The indicated concentrations of lactate were those present in the final assay mixture, from which the calculated values of A_{340} are 0.061, 0.122 and 0.184 respectively, given ϵ_{340} (NADH) = $6.22 \times 10^3 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$. In all samples (cell suspensions and standard solutions) the concentration of ethanol was 1% (v/v), which had no effect on the results obtained. Similar results

maximal stimulation of lactate output with linoleic acid (Fig. 3a) is similar to that obtained in response to the classical mitochondrial inhibitors.

In conclusion, we have shown that linoleic acid acts on intact lymphocytes in a manner characteristic of a classical mitochondrial uncoupler. In addition, linoleic acid stimulates glycolysis in lymphocytes, but this is not sufficient to prevent the cellular ATP levels from falling sharply. The precise mechanism of uncoupling by linoleic acid remains to be determined. Linoleic acid could either have a direct uncoupling effect on the mitochondria, or an indirect effect, for example by increasing calcium cycling in the mitochondria. Whatever the mechanism of the uncoupling effect, however, the resultant reduction in cellular ATP levels is sufficient to have wide-ranging effects on lymphocyte functions: it inhibits cap formation (Corps *et al.*, 1980; Pozzan *et al.*, 1980), blocks the ligand-induced increase in cytoplasmic Ca^{2+} levels (Pozzan *et al.*, 1982), and inhibits mitogenic stimulation (compare the results of Roos & Loos, 1973). Thus the hypothesis that the effects of *cis*-unsaturated fatty acids on cellular functions are due to perturbation of lipid domains in the lymphocyte plasma membrane cannot be accepted until this mechanism can be distinguished from the effects of ATP depletion.

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