oxidation via the tricarboxylic acid cycle. The metabolism of \([^{14}C]\)ethanolamine to fatty acids via acetate has been shown to occur in mammals (Sprinson & Coulon, 1954; Sprinson & Weliky, 1969), but not previously in a bacterium. The decreased incorporation of \([^{14}C]\)ethanolamine that occurs when an alternative N source is present is probably related to the repression by \((NH_4)_2SO_4\) of the phospholase (deaminating) involved in ethanolamine catabolism to acetaldehyde (Jones et al., 1973). L-Asparaginase is found at high activity in \(E.\) \(carotovora\) (Cammack et al., 1972) but other N. sources may be deaminated less readily. Whereas N sources seem to regulate the metabolism of ethanolamine to acetaldehyde, the effect of acetate and other acetogenic C sources is likely to be the diluting-out of \([^{14}C]\)acetyl-CoA, derived from acetaldehyde, with non-radioactive material. Quantitative results suggest that the route from \([^{14}C]\)ethanolamine to the phosphatidyl base may not involve acetyl-CoA but may be more direct.

Experiments with a wide variety of bacteria showed that all those capable of using ethanolamine as an N source for growth (for which vitamin B\(_{12}\) was sometimes necessary) incorporated \([^{14}C]\)ethanolamine into cell substance, but to a variable extent. Bacteria incapable of catabolizing ethanolamine, under a variety of circumstances, appear to be incapable of utilizing it biosynthetically.

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The Role of Unsaturated Fatty Acids in Mitochondrial Membrane Functions

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The proportion of unsaturated fatty acids in the membrane lipids of \(ole-1\), a fatty acid desaturase mutant of \(Saccharomyces cerevisiae\), is manipulated within the range 6–85\% of total fatty acids by growing the organism in media containing defined supplements of Tween 80 (Proudlock et al., 1971). Mitochondria whose membrane fatty acids are less than 20\% unsaturated lose the ability to couple phosphorylation to oxidation both \(in vivo\) and \(in vitro\) (Haslam et al., 1971). The loss of coupling is reversed by the incorporation of unsaturated fatty acids \(in vivo\), and recoupling is unaffected by inhibitors of protein synthesis, indicating that the phenomenon is purely a lipid lesion. The energy-linked ejection of protons by mitochondria is also lost as a result of fatty acid depletion, and the passive permeability of the mitochondria to protons increases (Haslam et al., 1973c). Since chemical uncouplers also cause the two latter effects, the enhanced proton permeability induced by changed lipid composition of the membrane is an adequate explanation of the loss of coupled phosphorylation.

The mode of entry of protons into the unsaturated fatty acid-depleted organelles was investigated. One possible mechanism of proton entry is via an aberrant proton-
translocating ATPase.* The activity of the mitochondrial ATPase in unsaturated fatty acid-depleted mitochondria is normal at 28°C, and is inhibited by oligomycin and the protein ATPase inhibitor from ox heart mitochondria. However, Arrhenius plots of the temperature-dependence of the enzyme revealed profound effects on the kinetics at lower temperatures. There are discontinuities in the Arrhenius plots of mitochondrial ATPase for organelles containing the whole range of fatty acid compositions, but depletion of unsaturated fatty acids causes a marked shift in the transition temperature to higher values (Haslam et al., 1973a). This confirms that lipid composition has a profound effect on the kinetics of membrane-bound enzymes, and is consistent with the hypothesis that transitions in Arrhenius plots indicate a phase separation in the membrane lipids in the vicinity of the enzyme.

Fig. 1 shows that Arrhenius plots of the passive permeability of mitochondria to protons are entirely different to those of the ATPase. Fully supplemented mitochondria are highly impermeable to protons over the whole range of temperature 4–34°C. The Arrhenius activation energy of only 9 kJ/mol suggests a diffusion-limited process. In contrast, the proton permeability of unsaturated fatty acid-depleted mitochondria is very high, particularly at higher temperatures, and the Arrhenius activation energy is 38 kJ/mol, which could indicate a chemically mediated process. However, Arrhenius plots for proton permeability do not show discontinuities and are unaffected by oligomycin, indicating that the additional proton entry is probably not catalysed by the ATPase.

The effects of unsaturated fatty acid depletion on mitochondrial macromolecular synthesis have been studied on Linnane’s laboratory by using anaerobically grown cells and the ole-1 mutant. Depletion of unsaturated fatty acids in both systems causes an

*Abbreviation: ATPase, adenosine triphosphatase.

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**Fig. 1. Effects of unsaturated fatty acid depletion on the passive entry of protons into mitochondria**

The half-times of passive proton entry into mitochondria were determined as described by Haslam et al. (1973c). ●, 83% of fatty acids unsaturated, Arrhenius activation energy = 9 kJ/mol; ■, 20% of fatty acids unsaturated, Arrhenius activation energy = 38 kJ/mol.
Table 1. Parameters of ATP uptake by mitochondria containing high and low concentrations of unsaturated fatty acids

The rates and extents of atractylate-sensitive ATP uptake by arsenate-depleted mitochondria were determined as described by Haslam et al. (1973b). Other additions, where indicated, were: 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole, 1 μM; valinomycin, 0.3 μg/ml, plus KCl (10 mM); nigericin, 0.3 μg/ml.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>With high fatty acid content</th>
<th>With low fatty acid content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsaturated content of fatty acids (%)</td>
<td>85</td>
<td>20</td>
</tr>
<tr>
<td>Inhibition by atractylate (%)*</td>
<td>75</td>
<td>58</td>
</tr>
<tr>
<td>$V_{\text{max}}$ (pmol/s per mg of protein)†</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>Rate of ATP uptake (pmol/s per mg of protein)†</td>
<td>6.1</td>
<td>1.4</td>
</tr>
<tr>
<td>$+ 4,5,6,7$-Tetrachloro-2-trifluoromethyl-benzimidazole</td>
<td>11.7</td>
<td>1.2</td>
</tr>
<tr>
<td>$+ \text{Valinomycin, KCl, nigericin}$</td>
<td>8.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Binding sites (pmol/mg of protein)</td>
<td>450</td>
<td>50</td>
</tr>
</tbody>
</table>

* Inhibition of the uptake of ATP (1 μM) by atractylate (100 μM).
† Rates of uptake of ATP (1 μM).

inhibition of mitochondrial protein synthesis and a diminution in the amount of mitochondrial ribosomal RNA (Watson et al., 1971; Marzuki et al., 1975). Moreover, mitochondrial DNA replication is inhibited, leading to the induction of the cytoplasmic petite mutation (Marzuki et al., 1974). Induction of cytoplasmic petite mutants has also been observed when intramitochondrial ATP production and the transport of cytoplasmic ATP into mitochondria are inhibited simultaneously (Subik et al., 1972). Depletion of unsaturated fatty acids will prevent endogenous production of ATP owing to the uncoupling of oxidative phosphorylation. If the mitochondrial ATP transporter is also inhibited by the changes in membrane lipid composition, this would cause a fall in mitochondrial ATP concentrations and account for the inhibition of macromolecular synthesis. The effects of changed fatty acid composition on the properties of the adenine nucleotide transporter are shown in Table 1. Depletion of unsaturated fatty acids greatly diminishes both the rate and extent of ATP uptake by mitochondria, and also lowers the sensitivity to atractylate. Similar results are obtained with freshly isolated mitochondria, in which uptake into the endogenous pool of mitochondrial adenine nucleotides predominates, and with arsenate-depleted organelles, in which the major part of ATP uptake is binding to membrane sites. Preloading of fresh or arsenate-depleted mitochondria with ATP, as described by Groot et al. (1975), does not affect the results. The rate of uptake of ATP by supplemented mitochondria is enhanced by the addition of chemical uncouplers or by valinomycin plus nigericin, indicating that ATP entry is opposed by the membrane potential, whereas in the depleted organelles uncouplers have no effect on ATP uptake, as the mitochondria are already uncoupled, owing to the changes in lipid composition. Thus unsaturated fatty acid depletion inhibits the adenine nucleotide transporter independently of its uncoupling action, and the combination of these two distinct effects probably causes indirectly an inhibition of all intramitochondrial processes that require ATP.

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High-Performance Liquid Chromatography of Ubiquinones, Ficaprenols and Dolichols

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High-performance liquid chromatography offers several advantages over other conventional chromatographic systems. These include speed, direct quantitative assay of solutes, ease of recovery of solutes and the absence of a requirement of volatility of the solute. Separation of the isoprenologues of families of polyisoprenoid compounds requires reversed-phase partition chromatography, and in this area the availability of stable chemically bonded stationary phases for use in high-performance liquid chromatography is an added advantage. We are reporting here some preliminary observations relating to the application of this technique to the analytical and preparative separation of the components of families of the biologically important polyisoprenoid compounds the ubiquinones, the ficaprenols (Stone et al., 1967) and the pig liver dolichols (Hemming, 1970). Conventional reversed-phase partition t.l.c. was used to check the purity of the recovered solutes (Dunphy et al., 1966).

The chromatographic system used was a Waters Associates model ALC 100 liquid chromatograph fitted with a refractive-index detector, a u.v. monitor (254 nm) and an M6000 pumping system. Of several bonded reversed-phase stationery phases tried, \( \mu \)-Bondapak C\(_18\)/Porasil (<10 \( \mu \)m; Waters Associates) was the most successful, and results mentioned here were obtained with a pre-packed column (30 cm \( \times \) 5 mm internal diam.) at room temperature. Ubiquinones were detected and assayed with the u.v. monitor and the polyisoprenoid alcohols with the refractive-index detector.

The separation of a synthetic mixture of ubiquinones-7, -8, -9 and -10 was very successful. However, with a single solvent adequate separation of ubiquinone-7 and ubiquinone-8 resulted in long retention times for ubiquinone-10. For this reason a linear gradient of solvent composition from methanol–water (9:1, v/v) to dry methanol at 4 ml/min \( (t_0 = 0.75 \text{ min}) \) over 1 h was found convenient. With approx. 25 \( \mu \)g of each component, ubiquinones-7, -8, -9 and -10 had retention times \( (t_k) \) of 8, 12.5, 19.5 and 24 min with band-widths \( (t_w) \) of 1.5, 2.0, 2.5 and 2.5 min respectively. Reversed-phase t.l.c. confirmed the complete separation of each component. This procedure appeared capable of satisfactory operation in the 10 ng–1 mg range. The upper limit and speed of the separation could probably be increased by further modification of the parameters. The method revealed the presence of several unsuspected minor contaminants.

The separation of the components of a natural mixture of ficaprenols-10, -11 and -12 was less good than that of the ubiquinones, but was still complete when assessed by reversed-phase t.l.c. The most satisfactory result was achieved with methanol–water \( (1:24, v/v) \) as solvent at 2 ml/min \( (t_0 = 1.5 \text{ min}) \). A load of 3 mg of ficaprenol mixture gave for ficaprenols-10, -11, and -12 \( t_k \) values of 14, 23 and 37.8 min with \( t_w \) values of 1.5, 2.4.