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Effect of supplementation with exogenous fatty acid on the biological properties of a fatty acid requiring auxotroph of *Salmonella typhimurium*

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Abstract. The effects of changes in fatty acid composition of the cell membrane on different biological functions of *Salmonella typhimurium* have been studied with the help of a temperature sensitive fatty acid auxotroph which cannot synthesise unsaturated fatty acids at high temperature. On being shifted to nonpermissive temperature the cells continue growing for another one and half to two generations. The rates of protein and DNA syntheses run parallel to the growth rate but the rate of RNA synthesis is reduced. Further, there is a gradual reduction in the rate of transport of exogenous uridine and thymidine into the soluble pool. The transport process can be restored by supplementing the growth medium with *cis*-unsaturated fatty acids but not *trans*-unsaturated ones although the growth of the cells is resumed by supplementation with either *cis* or *trans*-unsaturated fatty acids. However, supplementation with *trans, trans*-unsaturated fatty acids leads to only partial recovery of the transport process. The rate of oxygen uptake is also affected in cells grown in the presence of the *trans*-unsaturated fatty acids, elaidic acid and palmitelaidic acid. Analysis of cells grown under different fatty acid supplementation indicate that fatty acid composition of the cell membrane, especially the ratio of unsaturated to saturated fatty acids varies with temperature shift and supplementation of the growth media with fatty acids.

Keywords. Transport; fatty acid auxotroph; *cis*- and *trans*-unsaturated fatty acids.

Introduction

The lipid composition of the cell membrane plays an important role in regulating the functions of the cells. The role of membrane lipid in various membrane associated biological functions of the cell has become discernible from the extensive studies carried out in different laboratories (*c. f.* Silbert, 1975; Cronan, 1978). Investigations have been carried out mostly with *Escherichia coli* and *Saccharomyces cerevisiae* and attempts have been made to alter the lipid composition of the

Abbreviations used: MM, Minimal medium; TLC, thin-layer chromatography; GLC, gas-liquid chromatography; PE, phosphatidyl-ethanolamine; PS, phosphatidyl serine; PG, phosphatidyl glycerol; PEGA, polyethylene glycol adipate; CL, cardiolipin.

cell by supplementation, genetic manipulation, alteration of the growth condition, etc. Effect of supplementation with *cis*- and *trans*-unsaturated fatty acids has not been studied. In the present investigation a conditional fatty acid auxotroph of *Salmonella typhimurium* which cannot synthesise unsaturated fatty acids at high temperature, say 37°C, has been used. The effects of supplementation of the medium with *cis*- and *trans* -unsaturated fatty acids on a variety of biological functions of the cell membrane have been studied. The primary object was to correlate the fluidity of the cell membrane with its functions by comparing the effects of *cis*- and *trans*-unsaturated fatty acids.

Working with the unsaturated fatty acid auxotrophs of *E. coli* several workers (Silbert and Vagelos, 1967; Silbert *et al.,* 1968, 1973) have demonstrated directly that the fatty acid composition of the membrane can be altered by supplying various fatty acids in the growth medium. Therefore it is most likely that similar alteration will take place in the fatty acid auxotroph of *S. typhimurium.* To alter the fluidity of the cell membrane the fatty acids used in the study varied in their chain length, degree of unsaturation and steric configuration. The systemic names and other details of the fatty acids used are provided in table 1.

Table 1. Fatty acids used.

Common name	Systematic name	No. of carbon atoms	Formula
Palmitic	n-Hexadecanoic	16	$CH_3(CH_2)_{14}COOH$
Palmitoleic	*cis*-Δ^9-Hexadecanoic	16	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$
Palmitelaidic	*trans*-Δ^9-Hexadecanoic	16	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$
Stearic	n-Octadecanoic	18	$CH_3(CH_2)_{16}COOH$
Oleic	*cis*-Δ^9-Octadecanoic	18	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
Elaidic	*trans*-Δ^9-Octadecanoic	18	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
Linoleic	*cis*-Δ^9-Δ^{12}-Octadecadienoic	18	$CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6$ COOH
Linolelaidic	*trans*-Δ^9-Δ^{12}-Octadecadienoic	18	$CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6$ COOH

Materials and Methods

Chemicals

[^{3}H]-L-Leucine (5200 mC/mmol), [^{3}H]-thymidine (6500 mC/mmol) and [14C] uridine (13·4 mC/mmol) were obtained from Bhabha Atomic Research Centre Bombay. 2,5-Diphenyloxazole (PPO) and 1,4 di 2-(4-methyl-5-phenyloxazolyl benzene (Dimethyl POPOP) were purchased from Amersham/Searle Corporation USA. The fatty acids used in the study were from Serdary Research Laboratories London, Ontario, Canada and obtained as generous gifts from Prof. B. D. Sanwa Department of Biochemistry, University of Western Ontario, Canada. Other chemicals were also of analytical grade.

Bacterial strain

S. typhimurium fabB2, a fatty acid biosynthetic mutant, which cannot grow at higher temperature (37°C or higher) unless supplemented with unsaturated fatty acid and is probably deficient in *β*-ketoacyl acyl carrier protein synthetase I (Hong and Ames, 1971) used in this study was a gift from Prof. B. Ames, Department of Biochemistry, University of California, Berkeley, California, USA. The strain LT2 was originally obtained from Prof. M. Levine, Department of Human Genetics, Ann Arbor, Michigan, USA.

Growth media

Minimal medium: S. typhimurium was normally grown in minimal medium (MM), the composition of which was as described by Chakravorty (1970). Glycerol (0·2%) was used as the carbon source.

Growth of fab B2 : When the auxotroph was grown in media supplemented with different fatty acids, 0·04% Brij-58 was added to the MM. At this concentration Brij-58 had no effect on the growth rate or the overall growth extent of the bacterium. Fatty acids were added at a concentration of 0·4 mM. Growth was measured by following the turbidity of the cell suspension at 610 nm. An absorbance of 0·1 is equivalent to $1{\cdot}3 \times 10^8$ cells per ml.

Measurement of oxygen uptake by the cells

Oxygen uptake measurement was based on the measurement of change in the quantity of dissolved oxygen in solution. The model 55 Oxygen Monitor of Yellow Spring Instrument Co., Yellow Springs, Ohio, USA was used for the purpose.

Isolation of lipids

Total lipid was extracted following the method of Bligh and Dyer (1959) using the solvent system chloroform: methanol (2:1 v/v), chloroform: methanol (1:2 v/v) and chloroform : methanol: water (1: 2:0·8 v/v/v). The amount of extractant used in each case was 5 ml/200 mg of dry cell. This procedure was repeated 3 times for complete extraction of lipids. 2,6-Ditertiary butyl -*p*-cresol (50 mg/1) was added in the solvents to protect the unsaturated fatty acids from aerial oxidation.

Thin-layer chromatography (*TLC*) *of lipids*

The individual phospholipids were characterized by comparing the R_f values with authentic standards and by spraying with specific reagents, *e.g.*, ninhydrin for phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS); and molybdenum blue for general phospholipid. Silica gel G, made by Centron India, was used for the separation of phospholipid.

Estimation of phospholipids

Total phospholipid and individual phospholipids were estimated colorimetrically (Ames and Dubin, 1960). Polar lipids chromatographing at the origin of the TLC. plates after separation of the neutral lipids with acetone were extracted thrice with

chloroform : methanol: water (65 : 25 :4 v/v/v). The extracts were combined, concentrated *in vacuo,* and then separated by preparative TLC (plate size 20 × 14 cm, thickness 0·25 mm) using the solvent system chloroform; methanol: water (1:2:0·8 v/v/v). Separated bands were scrapped off, extracted with the above solvent system and quantitated by phosphorous estimation.

Esterification of lipids

Total lipid was subjected to hydrolysis in the presence of 0·1 M methanolic sodium hydroxide at room temperature for 24 h. After hydrolysis, it was neutralised with 0·1 M acetic acid and fatty acid was extracted with diethyl ether and esterified with diazomethane. Formation of methyl ester was checked by TLC using n-hexane: diethyl ether: acetic acid (8 0:2 0:1 v/v/v) as the developing solvent and by spraying with 2', 7' dichlorofluorescein (0·2% in ethanol).

Gas-liquid chromatography (*GLC*)

Analytical gas Chromatograph (Pye Unicam series 104 Chromatograph) fitted with dual flame ionization detector was used. The fatty acid methyl esters were analysed on 10% polyethylene glycol adipate (PEGA) column (1·8 m × 2 mm), coated on a solid support (diatomite CAW) of 100-200 mesh. Nitrogen at a flow rate 40 ml/min was used as the carrier gas. The operating temperature was kept at 180°C. Peaks of the fatty acid methyl esters were identified by (i) comparing retention times with those of reference methyl esters and (ii) determining the carbon number of component esters (Ackman, 1963). Quantitation of the peaks was done by the triangular technique.

Incorporation of [^{14}C]*-uridine and* [^{3}H]*-thymidine into soluble pool and macromolecules*

Growing cells were labelled for 1 min with [^{14}C]-uridine (3·7 nmol, 5 × 10^5 counts/min/ml) or [^{3}H]-thymidine (4 nmol, 4·4 ×10^6 counts/min/ml) as described earlier (Rao *et al.,* 1972). The extent of incorporation into the soluble pool was calculated by substracting the amount incorporated into acid-precipitable fraction from the total incorporation.

Results

Effect of fatty acid on the growth of mutant

The conditional auxotroph fabB2 of 5. *typhimurium* can grow at nonpermissive temperature in MM if supplemented with unsaturated fatty acid. The generation times of the mutant in MM supplemented with different fatty acids were practically the same (table 2). The growth rate was slightly slower with *trans*-unsaturated fatty acids than with the corresponding *cis*-unsaturated ones except linolelaidic acid. The saturated fatty acids, however, failed to sustain the growth of the mutant at nonpermissive temperature. When shifted from 30°C (permissive temperature) to 37°C (nonpermissive temperature) the mutant grew for one and a half to two

Table 2. Generation time of fabB2 at 37°C in MM supplemented with different fatty acids.

Supplementation	Generation time (min)
Oleic	80
Elaidic	96
Palmitoleic	75
Palmitelaidic	90
Linoleic	85
Linolelaidic	92

The cells did not grow at 37'C without supplementation. The generation time of the cells at 30°C (without supplementation) is around 130 min.

generations and then lysed. When the cells were provided with elaidic acid they could only grow for 10-12 h after which they started lysing.

Effect of fatty acid supplementation on lipid composition

Total lipid as well as fatty acid composition of the cells grown under different experimental conditions were analysed to find out the respective changes. Results presented in table 3 indicate that phospholipid content is very low in cells grown in minimal media at 30°C. It varied a great deal in the cells grown at 37°C in the presence or absence of different fatty acids. However, there is no qualitative variation in phospholipid spectrum except for some quantitative differences. Phosphatidyl serine content of the cells grown in the absence of any supplementation either at 30°C or at 37°C for 3 h following a shift from 30°C is much higher than that of the cells grown at 37°C in the presence of fatty acids (table 3). The fact that cells grown at 30°C do not lyse but contain as much phosphatidyl serine suggests that phosphatidyl serine is not derived from the action of endogenous phospholipase released upon lysis of a fraction of cells. The PE constitutes the major component. The other components are CL, PG and PS. No other phospholipid could be detected. The ratio of acidic lipids (CL, PG and PS) *versus* Zwitterionic lipid (PE) varied a great deal.

The fatty acid profile (table 4) shows that the ratio of unsaturated fatty acid: saturated fatty acid in the cell membrane was more in *cis*-unsaturated fatty acid supplemented cells except in the case of linolelaidic acid, which is a *trans*, *trans*-unsaturated fatty acid and resembles *cis*-unsaturated fatty acids in physical properties. The ratio was very much reduced when the cells were grown in the presence of elaidic acid or at 37°C (for a few generations) which is the nonpermissive temperature for the synthesis of unsaturated fatty acids.

Rate of protein, RNA and DNA synthesis in mutant after temperature shift

The rate of protein synthesis after shift to nonpermissive temperature was found to be almost parallel to the growth rate upto 2 h, the period during which the cells

Table 3. Percentage of total lipid, total phospholipid and individual phospholipid of fabB2 mutant grown under different conditions.

	Growth condition							
	30°C	30°C and then shifted to 37°C for 3 h	Oleic acid at 37°C	Elaidic acid at 37°C	Palmito-leic acid at 37°C	Palmite-laidic acid at 37°C	Linoleic acid at 37°C	Linolela-idic acid at 37°C
Total lipid†	11·8	9·0	7·5	10·4	9·8	10·2	8·7	10·8
Phospholipid*	17·8	63·2	62·0	62·5	73·5	45·4	86·1	56·4
PE**	41·2	67·8	75·1	81·5	83·1	68·5	81·3	62·0
CL	11·2	4·7	15·3	6·2	13·0	17·6	10·2	22·6
PG	29·2	15·0	8·2	12·5	2·5	11·4	7·7	11·0
PS	12·4	12·5	1·4	0·2	1·4	2·5	0·8	4·4
Acyl-PG	—	—	—	—	—	—	—	—
$\frac{CL+PG+PS}{PE}$	1·12	0·47	0·33	0·23	0·20	0·45	0·23	0·61

† Percentage of dry weight of cell.
* Percentage of total lipid.
** Percentage of total phospholipid.
— Not detected.

Table 4. Total lipid fatty acid composition (wt. %) of fabB2 mutant grown under different conditions.

	Growth conditions							
Fatty acid components	30°C	30°C then transferred to 37°C for 3 h	Oleic acid at 37°C	Elaidic acid at 37°C	Palmito-leic acid at 37°C	Palmite-laidic acid at 37°C	Linoleic acid at 37°C	Linolelai-dic acid at 37°C
12:0	1·7	T	0·5	T	3·3	1·1	3·6	1·4
14:0	4·7	41·7	7·7	28·9	6·8	4·6	9·0	14·2
14:1	—	—	0·2	—	T	—	3·1	5·0
15:0	2·6	—	—	1·5	—	1·7	—	—
16:0	31·5	34·1	30·0	17·6	11·5	36·9	31·8	24·1
16:1	28·0	21·4	10·6	11·1	58·7	44·8	3·6	T
17:0	10·5	—	—	T	—	6·5	10·3	4·3
17:1	7·7	—	—	—	8·1	—	—	T
18:0	T	T	T	28·0	—	T	7·6	T
18:1	13·1	2·7	42·0	12·1	11·5	4·2	10·1	T
18:2	T	T	—	T	T	T	20·8	46·7
18:3	—	—	—	—	T	T	T	4·2
$\frac{\text{Unsaturated}}{\text{Saturated}}$ fatty acids	0·96	0·32	1·9	0·32	3·7	0·96	0·71	1·28

T, Trace; —, not detected.
The values represent average ;of 3 determinations.,

grew exponentially (figure 1A). After this the rate starts declining (figure 1A). The rate of RNA synthesis is slightly reduced immediately after the shift and continues at a reduced rate (figure 1B). However, the incorporation of exogenous uridine into free pool is drastically reduced from the time of shift to the higher temperature and there is negligible incorporation after 1 h. A similar effect on the transport of extracellular thymidine into intracellular pool was observed (figure 1C), although the rate of DNA synthesis increased exponentially along with the exponential growth of cells.

Effect of supplementation with cis or trans isomers of different fatty acids

It was of interest to study whether the rate of transport at 37°C can be recovered on addition of unsaturated fatty acids into the medium. The fatty acids were added 1 h after temperature shift and the rates of incorporation of uridine into RNA and intracellular pool were followed. Three sets of *cis* and *trans*-isomers of fatty acids used in the experiment were palmitoleic-palmitelaidic, oleic-elaidic, and linoleic-linolelaidic. As shown in figure 1A the rate of RNA synthesis decreased following temperature shift but on supplementation with palmitoleic or palmitelaidic acid the rate of RNA synthesis recovered (figure 2A) . The recovery was immediate in the case of palmitoleic acid supplementation whereas there was slight delay in the case of palmitelaidic acid. Such delay was consistent and not due to experimental errors. The effect of such supplementation on the rate of uridine incorporation into soluble pool, which reflects transport process, was however, somewhat different (figure 2B). Supplementation with palmitoleic acid resulted in the recovery of the rate of transport of uridine into cellular pool but palmitelaidic acid had no effect. Similar results were obtained with oleic and elaidic acid, the *cis* and *trans* isomers of mono-unsaturated fatty acid containing 18 carbon atoms (figure 2C and D) .

When the supplementation was made with the double *trans, trans*-unsaturated fatty acid, linolelaidic acid, its effect on uridine uptake is strikingly different from that caused by palmitelaidic and elaidic acid. The most interesting observation was, however, that in the presence of the double *trans* -unsaturated fatty acid, the rate of uridine transport starts recovering following the addition of linolelaidic acid (figure 2E and F) . The rate of transport is however, greater in the case of linoleic acid, *cis*-$\Delta^{9,}$ Δ^{12} -octadecadienoeic acid.

Relative rates of uridine incorporation

To confirm that the rate of uridine transport is regulated or influenced by the fatty acid composition of the membrane, further experiments were done with the cells grown in the presence of different fatty acids for a number of generations, the rationale behind the experiment being that if the cells are grown for several generations in medium containing a particular fatty acid their membrane lipids will contain predominantly that particular fatty acid. The two pairs of *cis*- and *trans*-unsaturated acids chosen for the experiment were oleic-elaidic and linoleic-linolelaidic. Cells were grown at 37°C for a number of generations in MM containing different fatty acids and were quickly washed free of adhering fatty acid in the medium by filtering and washing through Millipore filter with the same medium devoid of fatty acid. Cells were finally suspended in MM without any fatty

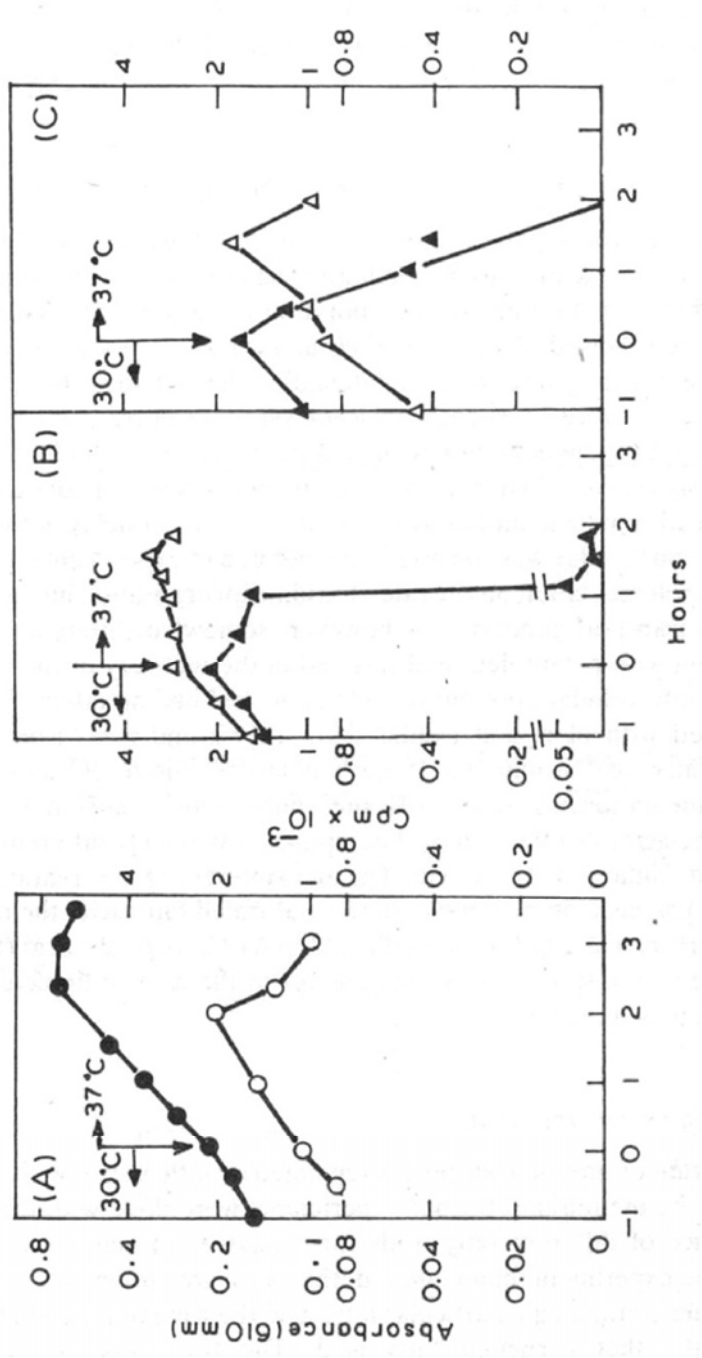

Figure 1. Synthesis of protein, RNA and DNA in the mutant fabB2 at nonpermissive temperature.

Cells ($2{\cdot}6\times10^8$/ml) growing exponentially at 30°C in MM were shifted to 37°C at 0 h and the rates of growth and protein synthesis (A), incorporation of [^{14}C]-uridine into RNA and intracellular pool; (B), incorporation of [^{3}H]-thymidine into DNA and intracellular pool; (C), were measured as described under 'materials and methods'. (●), Growth; (○), rate of protein synthesis; (△), rate of synthesis of RNA (B) and DNA (C); (▲), incorporation of uridine (B) and thymidine (C) into intracellular pool.

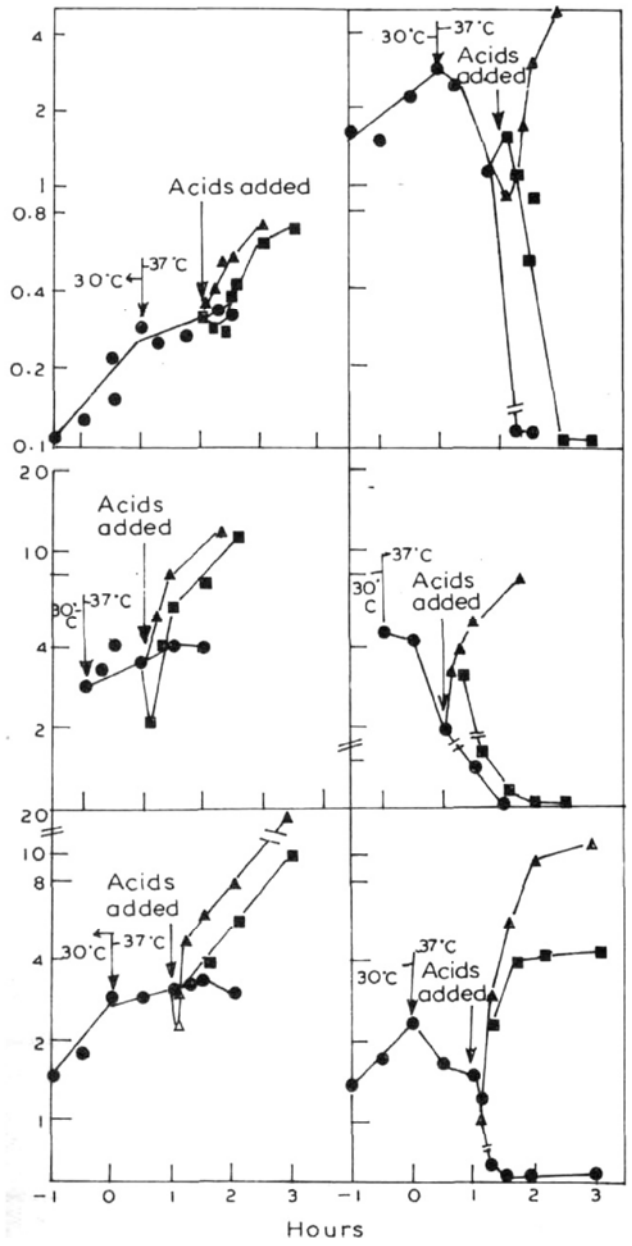

Figure 2. Effect of supplementation with *cis*- or *trans*-unsaturated fatty acids on the rates of incorporation of exogenous uridine into RNA and intracellular pool of fabB2 at nonpermissive temperature.

Cells ($2{\cdot}6 \times 10^8$/ml) growing exponentially in MM were shifted at 0 h from 30°C to 37°C as indicated in the figure. 1 h after the temperature shift they were divided into 3 batches. One batch was kept as unsupplemented control and to the remaining two batches two different fatty acids of the same chain length *cis*- or *trans-isomer* (0·4 mM) were added as follows: palmitoleic (A) and palmitelaidic acid (B); oleic (C) and elaidic acid (D) linoleic (E) and linolelaidic acid (F). The rates of incorporation of uridine into RNA and intracellular pool were estimated as described under 'materials and methods'.(●), Unsupplemented cells; (▲), supplemented with *cis*-unsaturated fatty acid; (■), supplemented with *trans*-unsaturated fatty acid.

acid. The incorporation of exogenous uridine into the RNA and soluble pool by such cells was then followed. The kinetics of incorporation presented in figure 3 agreed with the results presented in figure 2. The drastic reduction in the rate of incorporation of uridine into free pool was not the consequence of reduction in the rate of RNA synthesis (figure 3A) as cells treated with rifampicin (to block RNA synthesis) showed no reduction in the rate of incorporation of uridine into soluble pool (data not presented). There was no significant difference in the rates of uridine incorporation into RNA by linoleic and linolelaidic acid grown cells but there was difference both in the rates and extents of incorporation of uridine into free pool with this isomeric pair. These results confirmed our previous observation (figure 2 D and F) that the uridine transport could be recovered in the presence of linolelaidic acid, a *trans, trans*-unsaturated fatty acid.

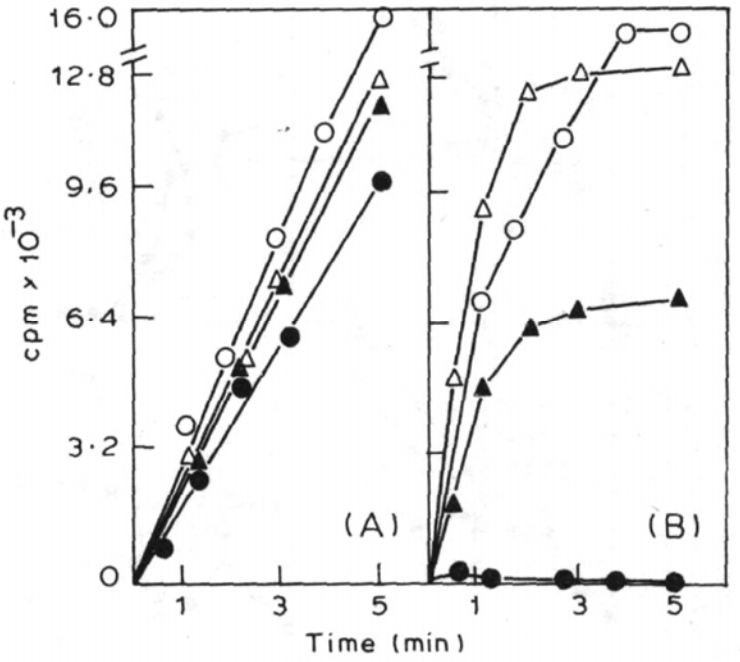

Figure 3. Incorporation of exogenous uridine into macromolecules (A) and soluble pool (B) of fabB2 grown in presence of *cis*- or *trans*-isomers of a mono- or di-unsaturated fatty acid.

The cells were grown for a number of generations and in media supplemented with different unsaturated fatty acid as indicated below. These exponentially growing cells were filtered through a Millipore filter, washed with media free of fatty acid (but containing Brij-58) and suspended in MM containing glycerol and no fatty acid at a density of $2{\cdot}6\times 10^8$ cells/ml. [^{14}CJ-uridine (20 nmol containing 6×10^5 cpm) was added per ml of cell suspension. At desired times 1 ml aliquots of cell suspension were pipetted out for measuring the incorporation of radio-activity into macromolecules (A) and soluble pool (B) as described under 'materials and methods'. (O), Oleic acid; (●), elaidic acid; (Δ), linoleic acid; (▲), linolelaidic acid.

Effect of fatty acid supplementation on the cellular respiration

As the respiratory enzymes are mostly membrane-bound the rate of respiration of the cells grown under supplementation with different fatty acids at 37°C was followed (table 5). The rate of respiration of the cells grown in the presence of elaidic acid is considerably reduced. The mutant grown in the presence of palmitelaidic acid also respired at a reduced rate, but there is little effect on the rate of respiration of the linoleic and linoelaidic acid supplemented cells. This is in agreement with the results presented in figure 2. Thus it is evident that the rate of respiration like uridine transport also depends on the fatty acid composition of the membrane.

Table 5. Oxygen uptake on glycerol by fabB2 grown in media supplemented with different fatty acids.

Cells grown with	μl O_2 uptake/5 min/ $2{\cdot}6\times10^8$ cells
Palmitoleic	8·0
Palmitelaidic	5·5
Oleic	9·5
Elaidic	4·5
Linoleic	8·0
Linolelaidic	7·5

The cells were grown at 37°C for several generations in MM containing different fatty acids as listed above. The exponentially growing cells were harvested at room temperature, washed and suspended in MM containing glycerol as the carbon source without any fatty acid supplementation. The rate of respiration of these cells in such unsupplemented media was followed at 37°C. The values were corrected for endogenous rate which was negligible.

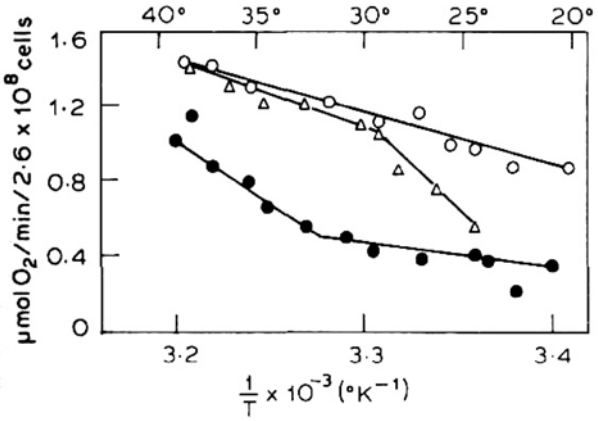

figure 4. Effect of temperature on the rate of oxygen uptake by cells grown in presence of oleic, elaidic and palmitelaidic acids.

The rate of respiration was measured at different temperatures and plotted against the reciprocal of temperature in Kelvin Unit. The remaining conditions are described in the text. (O), Oleic acid; (●), elaidic acid; (Δ), palmitelaidic acid.

To ascertain that the fatty acid supplementation in the medium not only changed the fatty acid composition of the membrane but also affected its fluidity, the effect of temperature on the rate of respiration was followed. For this purpose the cells grown on oleic or elaidic or palmitelaidic acid were used. The cells were grown at 37°C for several generations in MM containing any of the above mentioned fatty acids. The exponentially growing cells were harvested, washed and suspended in MM containing glycerol as the carbon source (without any fatty acid supplementation). The rate of respiration was followed at different temperatures. The results presented in figure 4 shows the transition in the rates of respiration in the case of elaidic and palmitelaidic acid supplemented cells, the transition temperature being 32°C and 29°C, respectively. The oleic acid grown cells do not show such transition within the temperature range of the experiment (20°C–40°C).

Discussion

That the fatty acid composition of the cell membrane can be influenced by environment such as temperature and nutrition is well documented in the case of fatty acid auxotroph of *E. coli* (Cronan, 1978; Silbert and Vagelos, 1967; Silbert *et al.,* 1968, 1973; Sinosky, 1971), Yeast (Stokes, 1971), *Neurospora* (Martin *et al.*, 1981; Aaronson *et al*., 1982) and some other organisms (Quinn, 1981). The results presented in this paper indicate that this is true in the case of the fatty acid auxotroph of *S. typhimurium.* Wild type *S. typhimurium* generally contains 90% phospholipid of the total lipid content (Ames, 1968). Variations to some extent have been observed in the fatty acid mutants of some organisms. However, 4 fold reduction in the phospholipid content of fabB2 mutant grown in MM at 30°C as compared to wild type (Ames, 1968) is rather significant. Decrease in the phospholipid content has been observed when *S. typhimurium* is grown in low phosphate medium (Cronan and Vagelos, 1972). A 10 fold decrease in phosphate resulted in 0·65 fold decrease in phospholipid content and a significant variation in the content of phospholipid. Results presented in table 3 indicate considerable variation in the phospholipid content of the cells grown under different conditions. The most striking feature of such alteration is the fact that the cells supplemented with *trans*-unsaturated fatty acid have a much lower ratio of unsaturated : saturated fatty acid. This suggests that the cells cannot either transport *trans*-unsaturated fatty acids into the cellular pool or incorporate into phospholipid as efficiently as *cis*-unsaturated one. Such distribution of saturated and unsaturated fatty acids must be affecting the fluidity of the membrane. The supporting evidence comes from the results presented in figure 4. The rate of respiration of cells grown in different fatty acids shows a characteristic transition temperature which is higher in elaidic acid grown cells than in oleic acid grown one. A more direct evidence involving measurement of the fluidity of the membrane vesicles prepared from cells grown under different conditions using electron spin labelled fatty acids or fluorescent probe is yet to be provided.

A number of parameters such as growth, RNA and DNA synthesis, transport of uridine and thymidine and respiration have been followed to study the biological functions of the cells. Of all the above parameters uridine transport has been studied in detail. On temperature shift for a period of 3 h, the unsaturated fatty

acid content of the cells decreased to 24%. *E. coli* auxotrophs starved for unsaturated fatty acids can grow well with 24% unsaturated fatty acid (Cronan and Gelman, 1973). However, fabB mutant of *S. typhimurium* lyses when the unsaturated fatty acid content is reduced to 24%. The type of unsaturated fatty acids present in the membrane of *E. coli* and *S. typhimurium* auxotrophs under the above conditions may be different and the physical properties of the membrane not only depend on the per cent of unsaturation but also on the type of unsaturation and chain length. Moreover, the threshold level of unsaturated fatty acids for *E. coli* and *S. typhimurium* may be slightly different. Such lysis has been reported previously for some unsaturated fatty acid auxotrophs of *E. coli* (Esfahani *et al.*, 1969, 1971) and the effect of temperature on cellular lysis indicated that lysis is due to decrease in the fluidity of the membrane. It is quite remarkable that under both these conditions *i.e.*, growth in the presence of elaidic acid and after temperature shift, the unsaturated: saturated fatty acids ratio is the same and very low, 0·32. It is expected that under such conditions membrane fluidity will be reduced considerably. The results clearly suggest that transport of uridine is very much influenced by the ratio of unsaturated and saturated fatty acids. *E. coli* cells starved for fatty acids become leaky before cellular lysis (Overath *et al.,* 1971; Nunn and Cronan, 1974). So it may be argued that lack of accumulation is due to leakage of accumulated metabolite out of the cell, although to minimise such a possibility pulse labelling experiment was carried out. The main objective of this type of experiments was to study the differential effect of supplementation with *cis* and *trans*-unsaturated fatty acids. Our results indicate that the net rate of transport increases on supplementation with *cis*-unsaturated fatty acids as compared with that of *trans*-unsaturated ones except in the case of linolelaidic acid (figure 2) which resembles *cis* fatty acids so far as the physical properties are concerned. In case, the drastic reduction in the rate of transport is due to the leakage in the membrane, it has to be assumed that supplementation with *cis*-fatty acids or linolelaidic acid repairs the leakage of the membrane. So the apparent leakage can be attributed to the ratio of unsaturated and saturated fatty acids. However, the experiments described in figure 3 have been carried out with cells growing exponentially in fatty acid supplemented media and the question of lysis of those cells does not arise and thus the possibility of leakage is minimum. Those cells also exhibit remarkable difference in the rate of transport of uridine depending on the nature of the fatty acid present in the growth media. These facts suggest that uridine transport across the cell membrane depends on physical properties of the membrane lipids which is influenced at least to some extent by the ratio of saturated and unsaturated fatty acids. Supplementation with linolelaidic acid allows the cells to recover uridine transport. The unsaturated and saturated fatty acids ratio of cellular lipid of cells grown in linolelaidic acid is 1·28, higher than elaidic acid grown cells. The ratio of unsaturated fatty acid and saturated fatty acid, however, provides a gross idea about the membrane fluidity as the chain length of the fatty acids also contributes to the state of membrane fluidity. For example, although the unsaturated and saturated fatty acids of cells grown in the presence of linoleic acid is 0·71, it can provide suitable environment (proper fluidity) for the functioning of the uridine transport system. On the other hand, palmitelaidic acid grown cells having unsaturated and saturated fatty acids ratio of 0·97 do not manifest the normal rate

of uridine transport or respiration. Esfahani *et al.* (1972) have studied the effects of phospholipids on succinic-ubiquinone reductase activity of *E. coli* membranes. They have demonstrated that the transition of Arrhenius plot of succinic ubiquinone reductase activity is dependent on the nature of the polar group of phospholipids. Overath *et al.* (1970) studied the transition temperature of a number of membrane associated functions and correlated the biological function with the physical state of the cell membrane. Our results not only indicate the importance of the physical state of the membrane but also suggest that the lipid composition surrounding the membrane proteins, besides the fluidity, is also involved in regulating membrane function. At 37°C cells grown in the presence of *cis*· unsaturated fatty acids do not exhibit the same extent of activity of different biological functions tested. Cardiolipin has been implicated to play an important role in mitochondrial respiration (Rattery *et al.,* 1975). The cytoplasmic petit mutants of yeast (Jakovcic *et al.,* 1971) and respiratory deficient (rd3) mutant of *Aspergillus niger* (Mandal *et al.*, 1978) which have poor respiratory capacity contain significantly decreased amount of cardiolipin. Under the present set of experiments cardiolipin content is significantly low in cells grown at non-permissive temperature and in the presence of elaidic acid. However, cardiolipin content of palmitelaidic acid grown cells is comparatively high, 17·6% as opposed to 4·7% and 6·2% in the earlier two cases. Hence it seems that the cardiolipin content alone cannot regulate the activity of respiratory enzymes and fluidity parameter of cells may also be involved with the respiratory activity of the membrane. Lipid profiles of the mutant of *S. typhimurium* grown under different conditions support the idea that membrane function is greatly influenced by membrane structure specially the lipid environment. Study of transport of a number of metabolites and the use of a variety of fatty acids may be useful for this purpose.

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