Influence of chain length of pyrene fatty acids on their uptake and metabolism by Epstein–Barr-virus-transformed lymphoid cell lines from a patient with multisystemic lipid storage myopathy and from control subjects

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The uptake and intracellular metabolism of 4-(1-pyrene)butanoic acid (P4), 10-(1-pyrene)decanoic acid (P10) and 12-(1-pyrene)dodecanoic acid (P12) were investigated in cultured lymphoid cell lines from normal individuals and from a patient with multisystemic lipid storage myopathy (MLSM). The cellular uptake was shown to be dependent on the fatty-acid chain length, but no significant difference in the uptake of pyrene fatty acids was observed between MLSM and control lymphoid cells. After incubation for 1 h the distribution of fluorescent fatty acids taken up by the lymphoid cell lines also differed with the chain length, most of the fluorescence being associated with phospholipid and triacylglycerols. In contrast with P10 and P12, P4 was not incorporated into neutral lipids. When the cells were incubated for 24 h with the pyrene fatty acids, the amount of fluorescent lipids synthesized by the cells was proportional to the fatty acid concentration in the culture medium. After a 24 h incubation in the presence of P10 or P12, at any concentration, the fluorescent triacylglycerol content of MLSM cells was 2–5-fold higher than that of control cells. Concentrations of pyrene fatty acids higher than 40 μM seemed to be more toxic for mutant cells than for control cells. This cytotoxicity was dependent on the fluorescent-fatty-acid chain length (P12 > P10 > P4). Pulse–chase experiments permitted one to demonstrate the defect in the degradation of endogenously biosynthesized triacylglycerols in MLSM cells (residual activity was around 10–25% of controls on the basis of half-lives and initial rates of P10- or P12-labelled-triacylglycerol catabolism); MLSM lymphoid cells exhibited a mild phenotypic expression of the lipid storage (less severe than that observed in fibroblasts). P4 was not utilized in the synthesis of triacylglycerols, and thus did not accumulate in MLSM cells: this suggests that natural short-chain fatty acids might induce a lesser lipid storage in this disease.

INTRODUCTION

Multisystemic lipid storage myopathy (MLSM) is a rare inborn error of metabolism generally characterized by the association of muscular weakness, ichthyosis, multisystemic triacylglycerol storage, Jordans' anomaly (fat vacuoles in leucocytes) without deficiency in carnitine or carnitine acyltransferase (Jordans, 1953; Chanarin *et al.*, 1975; Angelini *et al.*, 1978; Angelini, 1984). When grown in a medium supplemented with 10% fetal calf serum cultured skin fibroblasts from MLSM patients contained numerous cytoplasmic lipid vacuoles and accumulated 8–10 times more triacylglycerols than controls (Chanarin *et al.*, 1975; Radom *et al.*, 1988*a*). When radioactive fatty acids were used, MLSM cultured skin fibroblasts stored more radiolabelled triacylglycerols than did controls (Radom *et al.*, 1987*a*; Di Donato *et al.*, 1988).

Cultured human skin fibroblasts have been used as cellular model of MLSM (Chanarin *et al.*, 1975; Radom *et al.*, 1987*a*,*b*, 1988*a*; Di Donato *et al.*, 1988), but this experimental model has several disadvantages: limited lifespan, aging in culture and modification of enzyme activities during senescence (Sun *et al.*, 1975). In contrast, Epstein–Barr-virus-transformed lymphoid cell lines (LCL) have several advantages over fibroblasts: unlimited lifespan, short doubling time and growth in suspension, permitting the production of large quantities of cellular material (Pope *et al.*, 1968; Glade & Beratis, 1976; Povey, 1976; Salvayre *et al.*, 1983). In a previous paper the validity of this new experimental model was demonstrated: LCL from a MLSM patient showed a marked accumulation of triacylglycerols (Radom *et al.*, 1988*b*).

Elsewhere, the use of pyrene derivatives of fatty acids and phospholipids to measure lateral diffusion, lipid exchange between different vesicles, translocation, phase-separation phenomena and lipid–protein interaction has been widely reported (Galla & Hartman, 1980). Pyrene lipids have been used as substrates for lipolytic enzymes, e.g. sphingomyelinase and lipases (Nègre *et al.*, 1986, 1988*b*; Levade *et al.*, 1986; Salvayre *et al.*, 1986; Klar *et al.*, 1988). Previous studies have demonstrated that 12-(1-pyrene)dodecanoic acid (P12) was taken up by cultured cells and incorporated in the cellular neutral lipids and phospholipids (Morand *et al.*, 1982, 1984; Gatt *et al.*, 1988). Measurement of the incorporation and of the intracellular metabolism of this intensely fluorescent fatty acid have been used to differentiate normal and lipidotic cells (Levade & Gatt, 1987; Radom *et al.*, 1987*b*).

In this paper we report the uptake and the utilization of pyrene fatty acids with various chain lengths by LCL from normal subjects and from a patient affected with MLSM, as well as the

Abbreviations used: MLSM, multisystemic lipid storage myopathy; P4, 4-(1-pyrene)butanoic acid; P10, 10-(1-pyrene)decanoic acid; P12, 12-(1-pyrene)dodecanoic acid; LCL, lymphoid cell lines.

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effect of high pyrene-fatty-acid concentrations on the cell viability.

MATERIALS AND METHODS

Chemicals

4-(1-Pyrene)butanoic acid (P4), 10-(1-pyrene)decanoic acid (P10), and P12 were purchased from Molecular Probes (Eugene, OR, U.S.A.), bovine fatty-acid-free albumin from Sigma (St. Louis, MO, U.S.A.), silica-gel G thin layers from Merck (Darmstadt, Germany), RPMI 1640, fetal-calf serum, glutamine, streptomycin and penicillin from Seromed (Strasbourg, France), Ultroser HY from IBF (Villeneuve-la-Garenne, France), phospholipase A_2 from pig pancreas (Boehringer-Mannheim, Germany) and the other reagents and organic solvents from Merck or Prolabo (Paris, France).

Cell culture

LCL were established by Epstein–Barr-virus transformation (B95/8 strain) of blood B-lymphocytes (Pope *et al.*, 1968; Salvayre *et al.*, 1981) from normal individuals (lines Be, C_{49} and Lau) and from a woman affected with MLSM (line Bo). LCL were grown at 37 °C in CO_2/air (1:19) in RPMI 1640 medium supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), glutamine (2 mM) and 10% inactivated fetal-calf serum. Cell viability was assessed by the Trypan Blue dye-exclusion test and by [^{3}H]thymidine incorporation.

Incubation of LCL with fluorescent fatty acids

LCL were usually grown in RPMI 1640 containing 2% Ultroser HY (a serum substitute) for 2 days before studies were initiated. The pyrene fatty acid was solubilized in dimethyl sulphoxide (final concn. 0.05%), added at the indicated concentration to the incubation medium (RPMI 1640 containing 2% Ultroser HY) and preincubated at 37 °C for 30 min. At the beginning of the pulse, the medium containing pyrene fatty acid was mixed with the cell suspension in tissue-culture flasks (final vol. 3 ml/flask). The final concentration of pyrene fatty acid in the medium was 33 nmol/ml and the initial concentration of cells was 1×10^6.ml. Under these conditions, pyrene fatty acid and dimethyl sulphoxide had no adverse effect on cell viability (as determined by the Trypan Blue-dye-exclusion test). At the end of the incubation period with the fluorescent fatty acid derivative, lymphoid cells were sedimented by centrifugation, washed once with phosphate-buffered saline (8 mM-sodium phosphate/0.14 M-NaCl, pH 7.4) supplemented with 10 μM-BSA (essentially fatty-acid-free) and twice with the phosphate-buffered saline. For chase experiments, after incubation for 24 h with the fluorescent fatty acid, the cells were pelleted by centrifugation (time 0 of the chase) and chased for the indicated periods in a medium containing 10% (v/v) fetal-calf serum.

Lipid extraction and analysis

The cell pellets were suspended in 0.8 ml of distilled water and sonicated (2 cycles of 15 s; Soniprep 150 sonicator). The lipids of the cell suspensions were extracted with chloroform/methanol (2:1, v/v) (Folch *et al.*, 1957). The fluorescent lipid classes were separated by on silica-gel G plates, using the following solvent systems: light petroleum (b.p. 35–60 °C)/diethyl ether/acetic acid (80:20:1, by vol.), for the separation of the neutral lipids and chloroform/methanol/water (50:21:3, by vol.) for the separation of the phospholipids; then lipids were scraped off and their relative fluorescence intensity was determined as previously described (Radom *et al.*, 1987*b*).

In order to determine the position of the pyrene fatty acid in the phospholipid molecules, the total phospholipid fractions were hydrolysed with phospholipase A_2 (Okuyama & Nojima, 1965). The lysophospholipids obtained were analysed for their pyrene fatty acid content as described above.

The critical micellar concentration of pyrene fatty acids in phosphate-buffered saline was evaluated as reported by Galla *et al.* (1979). Proteins were determined by the method of Lowry *et al.* (1951), with BSA as standard. Statistical analysis was done using the Student *t* test (Schwartz, 1981).

RESULTS

Kinetics of uptake and incorporation of fluorescent fatty acids

The time course of the fatty acid uptake by LCL was investigated by using three fluorescent fatty acids at 37 °C in the absence of fetal-calf serum (Fig. 1). The rate of incorporation of fatty acid in LCL was related to the acyl chain length: the initial rates evaluated during the first minutes of the pulse (under the conditions used, the linearity of the uptake did not hold beyond 1–2 min) were 0.04 and 0.04 nmol/min per mg for P4, 1.67 and 1.84 nmol/min per mg for P10, and 3.75 and 3.93 nmol/min per mg for P12 in MLSM and control cells respectively, these results indicating no significant difference between control and MLSM cells. After a 1 h pulse, the amount of fluorescent fatty acid taken up by the cells was about 1, 10 and 20 nmol/mg of cell protein for P4, P10 and P12 respectively.

The distribution of fluorescent fatty acids in the various lipid classes of lymphoid cells also differed with the fatty acid chain length (Fig. 2). Most of the P10 and P12 was incorporated into phospholipids and triacylglycerols, whereas P4 was only incorporated into phospholipids. At any time during the pulse, the fluorescent phospholipid levels were not significantly different between control and MLSM cells. The incorporation of fluorescent fatty acids into triacylglycerols was obviously dependent on the chain length: P10 and P12 were incorporated in triacylglycerols, whereas P4 was not. When evaluated in the first minutes of the pulse, the initial rates of fluorescent triacylglycerol biosynthesis were 0.12 and 0.11 nmol/min per mg of cell protein with P10, and 0.75 and 0.70 nmol/min per mg with P12, in control and MLSM cells respectively; after a 1 h pulse, the values were 3.2 and 3.1 with P10 and 8.2 and 7.5 nmol/mg in MLSM and control cells respectively.

Effect of the concentration of pyrene fatty acids on their uptake and metabolism

The incorporation of pyrene fatty acids into lymphoid cells after 24 h incubation was dependent on their chain length and concentration in the culture medium (Fig. 3). The amounts of P10 and P12 incorporated into MLSM cells were 1.2–1.5-fold greater than in controls, whereas with P4 no difference was observed between MLSM and normal cells (Fig. 3*a*).

The analysis of fluorescent phospholipids revealed several differences in the metabolic utilization of pyrene fatty acids. When P10 (33 μM) was used, the fluorescence in phospholipids was distributed as follows: phosphatidylcholine, 55 ± 4%; phosphatidylethanolamine, 40 ± 2%; phosphatidylinositol and/or phosphatidylserine, 5 ± 1%. Similar results were obtained with P12. When P4 was used, phosphatidylcholine represented more than 73% of the fluorescent lipids (results not shown). To determine the location of the pyrene fatty acids on the glycerol backbone of the phospholipids, phospholipase A_2 was allowed to react with the total phospholipid extracts until there was complete hydrolysis of the phospholipids; the quantitative analysis of the products showed that P10 and P12 were mainly incorporated into the *sn*-1 position, whereas P4 was only incorporated into the *sn*-2 position (Table 1).

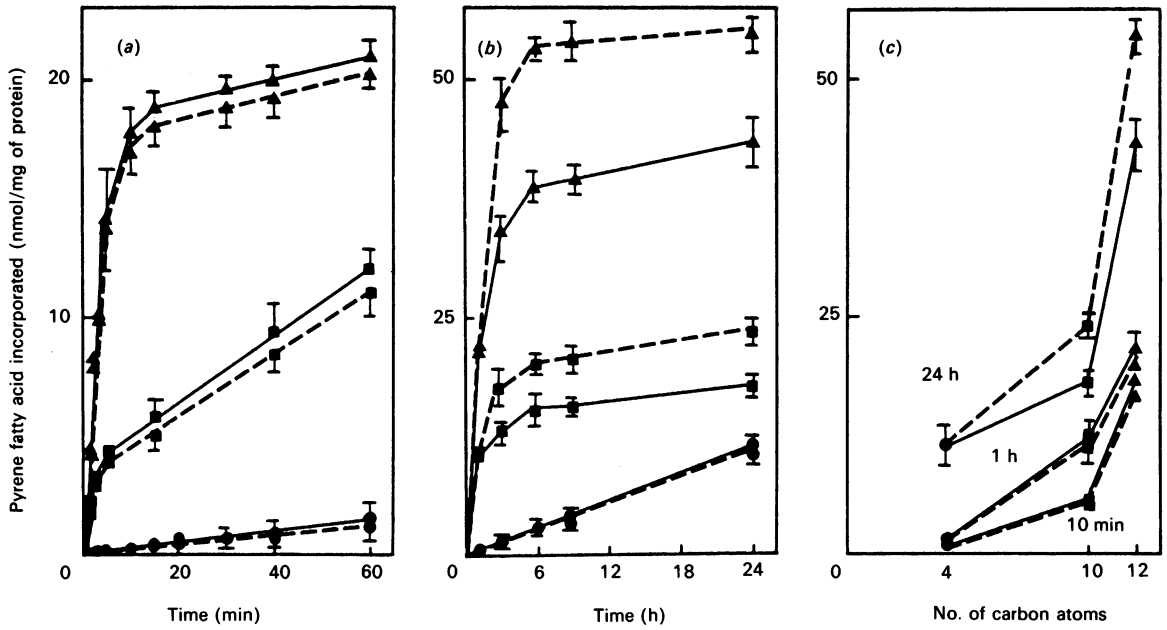

Fig. 1. Kinetics of uptake of P4 (●), P10 (■) and P12 (▲) by lymphoid cells from MLSM (---) and controls (——), during short (*a*) and longer (*b*) pulse (*c* shows uptake of fatty acids as a function of fatty acid carbon-atom number)

Pyrene fatty acid (33 nmol/ml) was dispersed in warmed culture medium (RPMI 1640 containing 2% Ultroser) and added to the cells. Incubation was stopped after various times by cooling the Petri dishes on ice (for time 0 the cells were immediately put on ice after the addition of the fluorescent fatty acids). The cells were washed once with phosphate-buffered saline containing 10 μmol of BSA (fatty-acid-free)/l and twice with phosphate-buffered saline without albumin. Fluorescent cellular lipids were extracted and quantified as described in the Materials and methods section. Each point represents the mean for four separate experiments.

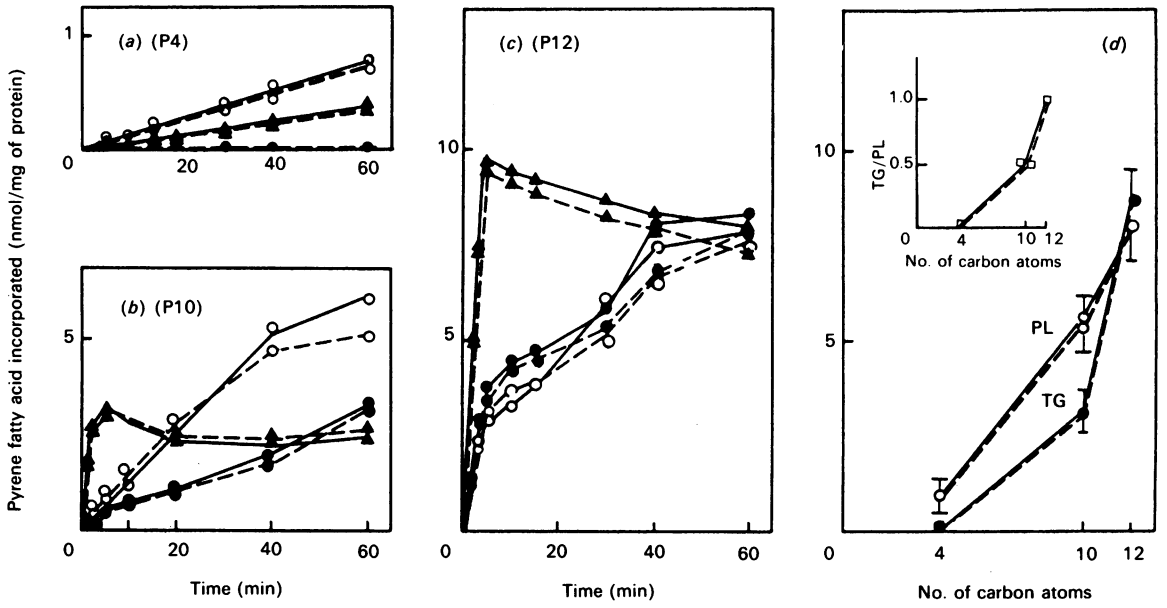

Fig. 2. Distribution of fluorescent fatty acids (P4, P10 and P12 in *a*, *b* and *c* respectively) into the cellular lipids from MLSM (-----) and control (——) cells [*d* shows incorporation, after a 60 min pulse, of pyrene fatty acids into phospholipids (PL) and into triacylglycerols (TG) as a function of carbon atom number of fluorescent fatty acids; the insets show TG/PL ratios]

These data were obtained from the analysis of the fluorescent lipids of the Fig. 1(*a*) experiments. Lipids were extracted from the cells, separated and quantified as described in the Materials and methods section. ▲, Non-esterified fatty acids; ●, triacylglycerols; ○, polar lipids.

The incorporation of the various fluorescent fatty acids into cellular neutral lipids was markedly different. When P4 was used, no fluorescent triacylglycerol or cholesteryl ester was detected. When P10 and P12 were used, fluorescent triacylglycerols and non-esterified fatty acids were present, as well as minor amounts of cholesteryl esters. At the end of the 24 h pulse period, an accumulation of fluorescent triacylglycerols was observed in MLSM cells, accounting for the difference in the fluorescence of total lipid extracts between the two cell lines (Figs. 3*b* and 3*c*). The triacylglycerol storage in MLSM cells was already evident at low concentrations of P10 or P12. At high concentrations of P10 and P12 (i.e. at 80 μM), the incorporation of fluorescent fatty acid

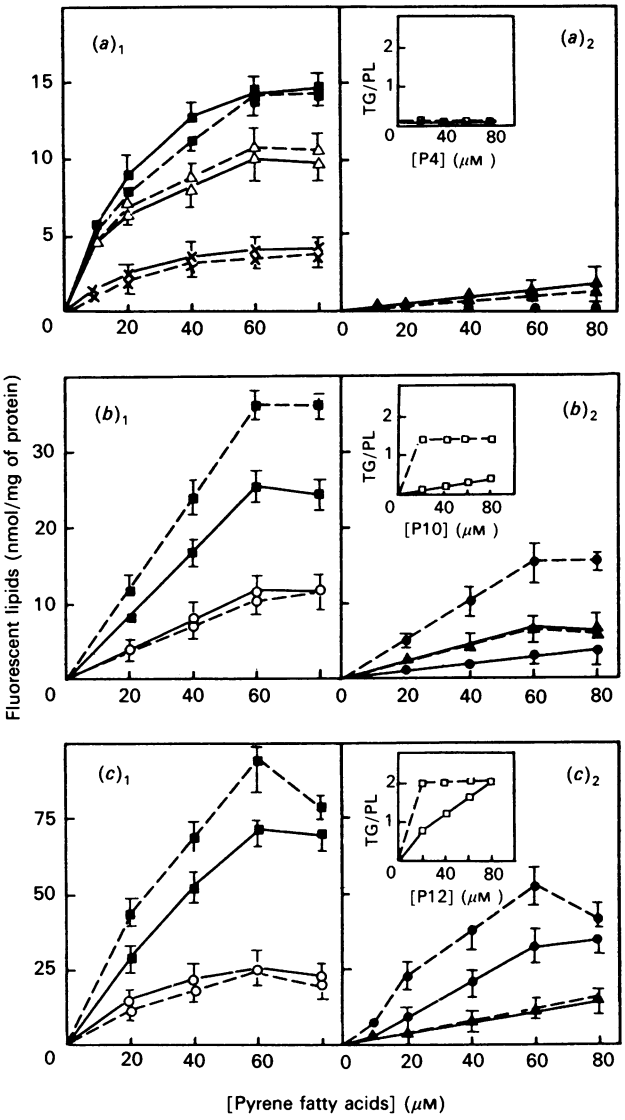

Fig. 3. Effect of the concentration of pyrene fatty acid in the culture medium on its incorporation into lipids of MLSM (----) and normal (——) LCL

LCL were incubated at 37 °C for 24 h in RPMI 1640 supplemented with 2% Ultroser and various pyrene-fatty-acid concentrations. The cells were washed, the lipids were extracted, separated, and their fluorescence intensity was determined as described in the Materials and methods section. (*a*) P4; (*b*) P10; (*c*) P12; the insets show triacylglycerol (TG)/phospholipid (PL) ratios; $(a)_1$, $(b)_1$, $(c)_1$ ■, total lipids; ○, phospholipids; △, phosphatidylcholine; ×, phosphatidylethanolamine; $(a)_2$, $(b)_2$, $(c)_2$ ▲, non-esterified fatty acids; ●, triacylglycerols.

into total lipids and particularly into triacylglycerols reached a plateau, or even decreased. This result prompted us to check the cytotoxicity of increasing doses of fluorescent fatty acids.

Effect of pyrene-fatty-acid chain length on cell viability

Incubation of cells for periods up to 24 h in the presence of relatively low fluorescent-fatty-acid concentrations (10–40 μM) had no adverse effect on cell viability, as assessed by Trypan Blue dye exclusion (Table 2); similar results were obtained by measuring [^{3}H]thymidine incorporation (results not shown). By contrast, concentrations of fluorescent fatty acid greater than 40 μM appeared to be cytotoxic. The cytotoxicity of fluorescent fatty acids was dependent on the fatty acid chain length (cytotoxicity of P12 > P10 > P4). The control cells were less sensitive than the MLSM lymphoid cells, which accumulated larger amounts of triacylglycerols.

Table 1. Position of the pyrene fatty acid (PFA) incorporated into glycerophospholipids of LCL

Control cells were incubated for 24 h with pyrene fatty acid (33 nmol/ml). The analysis of fluorescent lipids was performed as described in the Materials and methods section. Values (means ± S.D. for three separate experiments) are expressed as percentages, in each position (*sn*-1 or *sn*-2), of the total fluorescent fatty acid of the analysed lipid.

		Percentage of lipid at:	
PFA	Position ...	*sn*-1	*sn*-2
P4		0	100
P10		63±4	37±2
P12		61±3	39±2

Table 2. Effect of the concentrations of pyrene fatty acids (PFA) on the cell viability of normal and MLSM LCL after a 24 h pulse period

The number of living cells is expressed as a percentage of total cells. Results are from four experiments. *Denotes values for the MLSM patient's cells that are statistically different from that of controls ($P < 0.01$).

		Cell viability (%)					
Concn.	PFA ...	P4		P10		P12	
(μM)	Cells ...	Controls	MLSM	Controls	MLSM	Controls	MLSM
0		96±2	93±4	96±2	93±4	96±2	93±4
10		95±2	93±4	93±2	93±4	94±2	93±4
40		95±2	93±3	91±3	92±2	91±3	93±4
60		92±1	80±1*	83±1	68±2*	73±2	50±2*
80		84±1	73±1*	74±1	36±5*	50±2	24±1*

Degradation of cellular fluorescent lipids

The time course of the catabolism of the endogenously biosynthesized fluorescent lipids was monitored by incubating the cells for 15–20 days in full medium (Fig. 4). When cells pulsed with P4 were chased for 19 days, the fluorescence decreased rapidly, similarly to control and MLSM cell lines (Fig. 4*a*). For cells pulsed with P10, the level of P10 lipids decreased with time in both cell types, but more slowly in MLSM cells (Fig. 4*b*). At time 0 of the chase the content of P10 triacylglycerols was 4 times higher in MLSM cells than in controls. During the chase period P10 triacylglycerols decreased, but a clear difference in the apparent half-lives of P10 triacylglycerols between the two cell lines was observed (11±2 and 1±0.3 days for MLSM and control cells respectively). The ratio of triacylglycerols to phospholipids increased with time in MLSM cells, whereas it decreased in controls (Fig. 4b_2 inset). Very similar results were obtained when P12 was used, the half-lives of P12 triacylglycerols being 12±3 days and 2±0.5 days in MLSM and control cells respectively. Short chase experiments allowed one to calculate the initial rates of triacylglycerol degradation: for P10, 1.8±0.4 and 0.4±0.1 nmol/day per mg of cell protein and, for P12, 7±1 and 1.4±0.4 nmol/day per mg of cell protein in control and MLSM cells respectively.

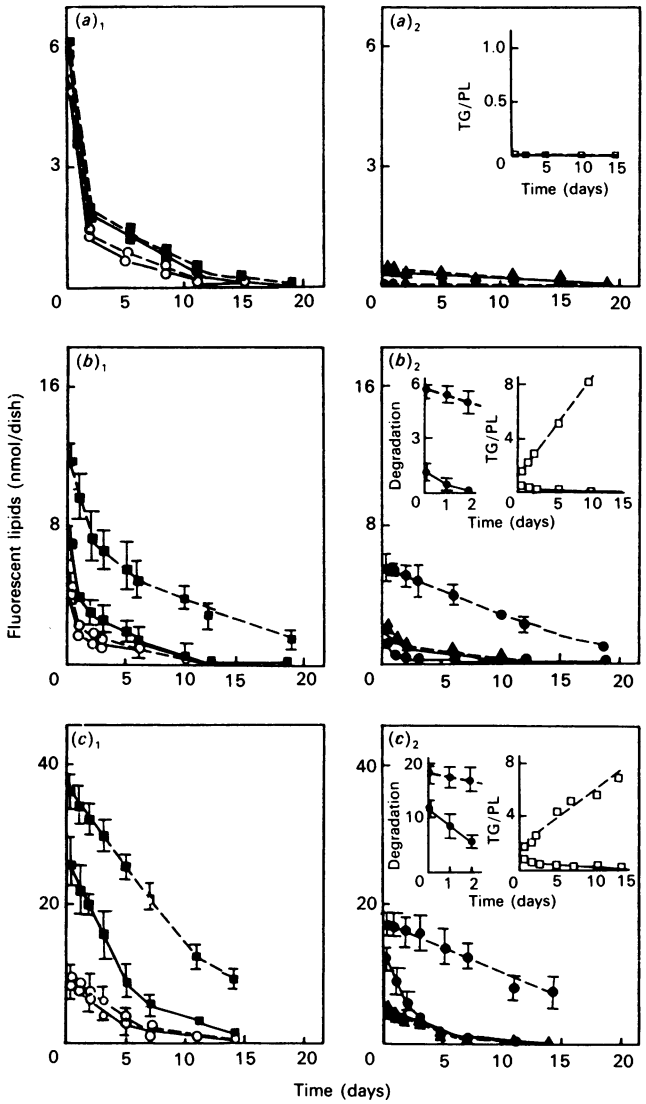

Fig. 4. Degradation of the fluorescent lipids in MLSM (----) and normal (——) LCL

The cells were incubated at 37 °C for 24 h (pulse period) in RPMI 1640 supplemented with 2% Ultroser HY and pyrene fatty acid (33 nmol/ml). Then the medium was removed and replaced by fresh medium supplemented with 10% fetal-calf serum, and cells were incubated at 37 °C (chase period). At the indicated times, cells were washed, lipids were extracted, separated, and fluorimetrically quantified as described in the Materials and methods section. The amount of fluorescent lipid is expressed as nmol of pyrene fatty acid per dish (at time 0 of the chase; protein concentrations were 0.45 and 0.5 mg/dish for MLSM and control cells respectively). (*a*) P4; (*b*) P10; (*c*) P12; the insets on the left in $(b)_2$ and $(c)_2$ show degradation (nmol/dish) of the fluorescent triacylglycerols during the first 2 days of the chase (expanded initial part of the chase); the insets on the right in $(b)_2$ and $(c)_2$ and the only inset in $(a)_2$ show triacylglycerol (TG)/phospholipid (PL) ratios. The symbols are defined in Fig. 3.

During the chase, the decrease in the cell-associated fluorescence was accompanied by an increase in the fluorescence in the culture medium (Table 3). The fluorescence recovered in the culture medium during the chase experiments was due to non-esterified fluorescent fatty acid. When MLSM and control cells were pulsed with P4, almost all the intracellular fluorescence was recovered in the culture medium after 10 days' chase. In contrast, for cells pulsed with P10 and P12, the release of the fluorescence into the culture medium was slower in MLSM than in normal cells.

Table 3. Release into the culture medium of pyrene fatty acid (PFA) by control and MLSM lymphoid cells during the chase period

Under the experimental conditions reported in Fig. 4, the lipids from the culture medium were extracted and analysed as described in the Materials and methods section. The amount of fluorescent lipid recovered in the medium is expressed as a percentage of pyrene fatty acid incorporated in cellular lipids at the end of the pulse period. Results are means ± S.D. for four experiments. *Denotes values for MLSM cells that are statistically different from that of controls ($P < 0.01$).

		Fluorescent lipid recovered (%)					
	PFA ...	P4		P10		P12	
Chase (days)	Cells ...	Controls	MLSM	Controls	MLSM	Controls	MLSM
5		79±2	79±1	80±3	54±5*	65±5	25±5*
8		89±3	86±3	86±3	71±2*	83±4	56±6*
12		92±4	91±4	99±1	81±2*	93±2	68±5*
15		98±3	95±3	99±1	85±3*	99±2	75±3*

DISCUSSION

Pyrene fatty acids and their derivatives have been previously used as fluorescent probes to study lipid metabolism in living cells (Morand *et al.*, 1984; Levade & Gatt, 1987; Radom *et al.*, 1987*b*). In the present paper the comparative study of the uptake of three pyrene fatty acids with various chain lengths and of their metabolic utilization by LCL from MLSM and controls allows one to demonstrate that: (i) the uptake of fluorescent fatty acids by lymphoblasts, their subsequent incorporation into phospholipids and triacylglycerols and their cytotoxic effect are strongly dependent on their chain length; (ii) there is a defect in the catabolism of the endogenously biosynthesized triacylglycerols in the lymphoid cells from MLSM.

When administered to the cells at relatively low concentration (33 μM), without any adverse effect on the cell viability, the pyrene fatty acids were taken up by the lymphoid cells with a clear selectivity for the longer-chain fatty acid derivatives. The difference of uptake could be related to the value of their critical micellar concentration (1300 μM, 80 μM and 1 μM, for P4, P10 and P12 respectively) and to the relative solubility of the pyrene derivatives in the membrane lipid phase and in the aqueous phase. Our data well agree with those reported by Morand *et al.* (1982) on the preferential uptake of P12 compared with P10 in HL60 cells, and with the results obtained using natural fatty acids with various chain lengths in adult rat heart myocytes (De Grella & Light, 1980*a*,*b*). In agreement with Pownall & Smith (1989), the short-acyl-chain derivative, P4, was less efficiently taken up and utilized by cells than the longer-chain pyrene fatty acids, P10 and P12.

The fact that the metabolic utilization of fluorescent fatty acids is dependent on the acyl chain length could be explained in at least three possible ways: (i) the amount of non-esterified fatty acid associated with the cells (increasing from P4 to P12), (ii) the rate of acyl-CoA formation, which is also acyl-chain-length-dependent (Bremer & Norum, 1982), and (iii) the acyl-chain-specificity of the acyltransferases (Coleman & Bell, 1976). All the pyrene fatty acids tested were incorporated into phospholipids, but only P10 and P12 were incorporated into triacylglycerols. Their incorporation into triacylglycerols prevailed when the cells received high influx of fluorescent fatty acids, in agreement with the results observed with natural fatty acids (Mackenzie *et al.*, 1966; Schneeberger *et al.*, 1971). The lack of P4 triacylglycerols

is not due to a defect of P4-CoA synthesis, since P4 phospholipids (P4 phosphatidylcholine and P4 phosphatidylethanolamine) were synthesized. This lack could be explained in two ways: (i) the diacylglycerol acyltransferase cannot utilize P4-containing substrates (P4 diacylglycerols or P4-CoA), in agreement with previous results reporting that this enzyme does not use butyryl-CoA (Coleman & Bell, 1976), but utilizes only acyl-CoA with an acyl chain longer than 12 carbon atoms (Hosaka *et al.*, 1977); (ii) the low concentration of intracellular P4, since triacylglycerol synthesis is stimulated by a high intracellular influx of fatty acids, as discussed above (Schneeberger *et al.*, 1971).

The metabolic defect of MLSM cells was investigated in short-time-pulse and pulse–chase experiments. The short pulse permitted evaluation of initial rates of fatty acid uptake. Our values are consistent with those found by Morand *et al.* (1982) for HL60 cells and by Samuel *et al.* (1976) for cardiac cells. These short-time experiments investigated the first phase of P10 and P12 uptake, which is thought to represent the association with, and the insertion of the fluorescent fatty acid into, the cell plasma membrane (Nahas *et al.*, 1987). The lack of significant difference between MLSM and control cells permits us to conclude that the metabolic defect of MLSM does not result from an abnormal (increased) transport of fatty acids. The triacylglycerol levels during a short time pulse are probably representative of the biosynthetic rate of cellular triacylglycerols; thus the similarity of the values observed in control and MLSM cells permits the conclusion that an excess of triacylglycerol biosynthesis is not the primary anomaly of MLSM. The kinetics of P10 or P12 triacylglycerol accumulation in MLSM lymphoblasts (the accumulation occurs only after 2 or 3 h incubation with the fluorescent fatty acids and becomes evident after 24 h) suggest that the triacylglycerol storage in MLSM cells results from a defect in a metabolic step active after biosynthesis, e.g. an anomaly in the cellular transport of triacylglycerols or a block in their degradation. This conclusion is further supported by the chase experiments, which showed a considerably delayed catabolism of P10 or P12 triacylglycerols in MLSM cells (the residual activity in mutant cells being around 10–15% of controls, on the basis of the half-lives of triacylglycerols). Consistent with these findings were the lower rates of release of fluorescent fatty acid into the culture medium by MLSM cells compared with control cells. Our results on the metabolic defect in MLSM lymphoid cells are in good agreement with previous results obtained in cultured skin fibroblasts (Radom *et al.*, 1987*a*,*b*; Di Donato *et al.*, 1988). However, the degradation of triacylglycerols was not as completely blocked in lymphoid cells as in fibroblasts, suggesting some residual catabolic activity in the lymphoblasts from the MLSM patient (about 20–25% of controls on the basis of the initial catabolic rates). This mild phenotypic expression of the disease in lymphoid cells is in good agreement with the lack of triacylglycerol storage in circulating blood lymphocytes (Chanarin *et al.*, 1975).

The block of endogenously biosynthesized triacylglycerols seems to be the primary metabolic defect of MLSM, but the molecular basis of this catabolic block is still unknown, since assays *in vitro* did not show any deficiency in lipase activities of cultured fibroblasts (Di Donato *et al.*, 1988) or lymphoid cells (Radom *et al.*, 1988*b*, 1989). Several hypotheses can be proposed, including the deficiency of a lipase activator molecule or the inaccessibility of triacylglycerols to the lipase.

The cytotoxicity induced by high concentrations of pyrene fatty acids increased with the chain length of the pyrene fatty acids, with the total fluorescence associated with the cells and/or with the cellular content of non-fluorescent triacylglycerols. The mechanism of the cytotoxicity of fluorescent fatty acids could involve a photochemical effect in cells containing high levels of pyrene lipids (Fibach & Gatt, 1987). The significantly higher sensitivity of MLSM cells (to the toxic effect of fluorescent fatty acids) is probably related to their higher lipid content. The cytotoxicity could result from either a direct effect of fluorescent lipids or an indirect effect of cytoplasmic vacuoles filled with natural triacylglycerols, which could amplify the emission intensity of fluorescent lipids, as has demonstrated in Wolman cells (Nègre *et al.*, 1988*a*).

Epstein–Barr-virus-transformed LCL seem to be a valid experimental model system of MLSM that exhibits a mild phenotypic expression of the lipid storage [less severe than that observed in fibroblasts by Radom *et al.* (1987*b*)]. Thus those LCL may constitute a good model system complementing that with cultured fibroblasts, with which to gain an insight into normal intracellular neutral lipid metabolism and the pathogeny of MLSM. A comparison of the metabolic utilization of pyrene fatty acids with different acyl chain lengths showed that P4 was not utilized in the synthesis of triacylglycerols and thus did not accumulate in MLSM cells. These data suggest that natural short-chain fatty acids could induce diminished triacylglycerol storage in this disease, in spite of their possible chain-length elongation.

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