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Biosynthetic Labelling of Membrane Lipids of Eukaryotic Cells in Tissue Culture by a Novel Type of Fluorescent Fatty Acids

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Summary: ω-Anthryl labelled fatty acids with hydrocarbon chains of different lengths (C_8, C_{11}, C_{15}) and different degrees of unsaturation have been incorporated into the membrane lipids of three different cell lines in tissue culture by addition of these ^{3}H-labelled precursor fatty acids to the growth medium. The cell lines were baby hamster kidney cells (BHK 21), Chang liver cells and the RN6 cell line derived from a chemically induced Schwannoma tumor cell clone. Cell growth was normal. The quantitative analysis on the basis of radioactivity determinations demonstrated that the fluorescent-labelled fatty acids were introduced into the neutral lipid fraction (triglycerides, diglycerides, and cholesterol esters, all present in small amounts), but mainly into the phospholipid classes phosphatidylcholine, -ethanolamine and -serine, and to a lesser extent, as *N*-acyl component of sphingolipids (sphingomyelins, ceramides, mono- and diglycosylceramides).

Cell fractionation studies indicated that the membranes of all subcellular particles were labelled with the fluorescent probes in their lipid moieties. These ω-anthryl fatty acids are the first type of fluorescent lipid precursors which can be incorporated biosynthetically in vivo into membrane lipids of eukaryotic cells. This effective incorporation of the bulky fluorescent anthryl group in the terminal position of fatty acids of different chain lengths into the complex membrane lipids of the cell gives proof of 1) their uninhibited membrane transport, 2) their activation by the acyl-CoA synthetase and 3) their substrate properties for the *O*-acyl and *N*-acyl transferases in phospho- and sphingolipid biosynthesis.

Key words: Novel fluorescent fatty acids, eukaryotic cells in culture, biosynthetic labelling, fluorescent complex membrane lipids, distribution of fluorescent fatty acids.

Enzymes:
Acyl-CoA synthetase, acid:CoA ligase (AMP-forming) (EC 6.2.1.3);
Glycerophosphate acyltransferase, acyl-CoA:*sn*-glycerol-3-phosphate *O*-acyltransferase (EC 2.3.1.15);
Sphingosine acyltransferase, acyl-CoA-sphingosine *N*-acyltransferase (EC 2.3.1.?);
Phospholipase A_2, phosphatide 2-acylhydrolase (EC 3.1.1.4).

Abbreviations:
PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; SPM, sphingomyelin; CE, cholesterolester; TG, triglyceride; DG, diglyceride; CMH, monoglycosylceramide; CDH, diglycosylceramide.
Example for the shorthand nomenclature: 11-A-11: $2^{6,9}$ = 11-(9-anthryl)-6*c*,9*c*-undecadienoic acid.

Biosynthetische Markierung von Membran-Lipiden in eukaryoten Zellen in Gewebe-Kultur durch eine neue Klasse fluoreszenzmarkierter Fettsäuren

Zusammenfassung: ω-Anthrylfettsäuren mit verschiedenen Kohlenwasserstoff-Kettenlängen (C_8, C_{11}, C_{15}) und unterschiedlichem Grad der Ungesättigtheit wurden durch Zugabe der freien Säure zum Nährmedium in die Membranlipide von drei verschiedenen Zellstämmen in der Gewebekultur, nämlich BHK 21, Chang-Leber und einem Schwann-Zell-Tumorklon (RN6) inkorporiert. Die quantitativen Analysen auf der Basis der Radioaktivitätsbestimmung zeigen, daß diese fluoreszenzmarkierten Fettsäuren in die Fraktion der Neutrallipide (Triglyceride, Diglyceride und Cholesterinester), die alle nur in geringen Mengen vorliegen, und in die Phospholipide (Phosphatidylcholin, -äthanolamin und -serin) eingebaut werden. In letzteren acylieren sie vorwiegend die 2-Position des Glycerophosphatskeletts. Ebenfalls werden sie zur *N*-Acylierung in der Biosynthese der Sphingolipide (Sphingomyelin, Ceramid, Mono- und Diglykosylceramid), wenn auch verglichen mit den Phospholipiden in wesentlich geringerem Maße, herangezogen.

Vorliegende Zellfraktionierungsuntersuchungen beweisen ferner, daß die Membranen aller subzellulären Partikeln mit dieser Fluoreszenzsonde in den Membranlipiden markiert sind. Es handelt sich bei diesen ω-Anthrylfettsäuren um die ersten fluoreszenzmarkierten Lipidvorstufen, die für die Biosynthese in vivo von Membranlipiden eukaryoter Zellen Verwendung finden können. Der effektive Einbau dieser mit der terminal lokalisierten raumfüllenden fluoreszierenden Anthrylgruppe substituierten Fettsäuren in die komplexen Membranlipide der Zelle setzt ihren ungehinderten Membrantransport, ihre Aktivierung durch die Acyl-CoA-Synthetase sowie die Substrateigenschaften für die *O*-Acyltransferasen in der Phospho- und Sphingolipidbiosynthese voraus.

The study of lipid-lipid and lipid-protein interactions in artificial membranes (liposomes, lipoproteins) and simple and more complex natural membranes requires a wide variety of physical and chemical techniques and methods to elucidate the structural architecture and the functions of the hydrophilic and hydrophobic moieties of different lipid classes in these macromolecular arrangements. Four techniques are used in this laboratory to get insight into the details of the arrangement of the lipids and their interactions on a molecular level: radioisotopic labelling[1,2], ^{13}C and ^{31}P nuclear magnetic resonance spectroscopy[3-7], and lipid labelling with photosensitive groups and determination of the nearest neighbour in the macromolecular structures under study after photosensitization and fluorescence spectroscopy[9,10]. The drawback of fluorescence spectroscopy so far has been the inability to incorporate fluorescent probes into distinct positions of the lipid phase, since the probes hitherto used are freely mobile fluorescent molecules and structurally unsuitable for biosynthetic incorporation in membrane structures. We therefore developed a novel type of fluorescent labelled fatty acids (ω-anthryl fatty acids). Their properties were tested after incorporation by chemical synthesis into phospholipids and cholesterol esters[9]. The potency of these fluorescent probes was demonstrated by their ability to indicate changes in the mobility of the fluorescent groups by temperature-dependent fluorescence intensity and fluorescence polarization measurements as shown by phase transitions and the influence of membrane proteins on these properties[10].

One of the most important properties of physical and chemical probes is their efficient biosynthetic incorporation into the structures under study. This has been clearly demonstrated for ^{13}C-labelled[5,6] fatty acids and fatty acids with photosensitizable groups[8].

This paper reports the results of studies which prove that the novel type of fluorescent-labelled ω-anthryl fatty acids can be incorporated biosynthetically into the complex membrane lipids of eukaryotic cells in tissue culture. ω-Anthryl fatty acids added to the growth medium of three cell clones in tissue culture – BHK 21, Chang liver and RN6 cells[11] – were incorporated efficiently into neutral, phospho- and sphingolipids of the membranes of these cells. Quantitative

data are reported on the yield of the incorporation, since the radioactive labelling of the precursor fluorescent fatty acids facilitated these analyses.

Material and Methods

[6,7-$^3H_2$]8-(9-Anthryl)-6*c*-octenoic (specif. radioactiv. 50 μCi/μmol), [6,7,9,10-$^3H_4$]11-(9-anthryl)-6*c*,9*c*-undecadienoic (specif. radioactiv. 58 μCi/μmol) and [10,11,13,14-$^3H_4$]15-(9-anthryl)-10*c*,13*c*-pentadecadienoic acids (specif. radioactiv. 32 μCi/μmol) were synthesized in this laboratory and their fluorescence properties described[9,10].

BHK 21 cells, Chang liver and RN6 cells were each grown in five Roux flasks (250 cm^3) of Dulbecco medium[12] supplemented with 10% tryptose broth, 2% fetal calf serum and desthiobiotin (5 μg/100 m*l*) for 6 - 10 h. After seeding the cells, the fluorescent labelled fatty acids were added as ethanolic solutions. The medium was 5×10^{-6}M in fatty acid. Cells were grown to confluency under an atmosphere of 5% CO_2/95% air at 37 °C. Confluency was reached after 48 h for BHK 21 and Chang liver cells and 36 - 48 h for RN6 cells. The medium was decanted, the monolayer washed with phosphate-buffered saline and cells were harvested by scraping them from the glass surface with a rubber policeman. They were pelleted by centrifugation at $2000 \times g$ for 20 min, resuspended and washed twice with medium and phosphate-buffered saline and again pelleted. The cells were suspended in 5 m*l* water and sonicated for brief periods. 10-μ*l* portions were used for radioactivity determination. The suspension was then lyophilized. Lipids were extracted by refluxing for 10 min with chloroform/methanol (2:1 v/v) and then stirring with chloroform/methanol 1:2 for 20 min. The combined lipid extracts were taken to dryness, dissolved in chloroform and samples were withdrawn for determining the total radioactivity and for thin-layer chromatography. The lipid extract was separated into the fractions of neutral lipids, glycosphingolipids and phospholipids by standard procedures[13] using silicic acid columns (5 g). Neutral lipids obtained in the chloroform eluant (100 m*l*), glycosphingolipids in the acetone/methanol fraction (100 m*l*) and phospholipids in the methanol fraction (100 m*l*) were further analyzed for the distribution of radioactivity and fluorescence by one- or two-dimensional thin-layer chromatography. The following solvent systems were used for analytical and preparative thin-layer chromatography: neutral lipids, light petroleum 30 - 60 °C/ether/acetic acid 70:30:1 (I); neutral glycosphingolipids, chloroform/methanol/water 100:42:6 (II); phospholipids one-dimensional, chloroform/methanol/acetic acid/water 50:30:8:4 (III); two-dimensional[14], 1) chloroform/methanol/ammonia (10%) 60:30:6 (IV), and 2) chloroform/methanol/acetone/acetic acid/water 75:15:30:15:7.5 (V). Radioactive bands were isolated and phosphorus determined according to a modification of the Bartlett procedure[15,16]. Phospholipase A_2 hydrolysis (*Crotalus adamanteus*) was carried out as described before[16].

Radio thin-layer scans were performed with a Berthold scanner, model LB 2723 equipped for two-dimensional radioscanning. Fluorescent bands were photographed under UV light illumination.

Results

Three lines of eukaryotic cells were grown on a medium supplemented with three ω-anthryl fatty acids of different chain lengths. These rather divergent cell lines were: BHK 21 cells, Chang liver cells and the RN6 Schwannoma cell clone. The fluorescent fatty acids were tritium labelled at their *cis*-double bonds: 8-(9-anthryl)-6*c*-octenoic, 11-(9-anthryl)-6*c*,9*c*-undecadienoic and 15-(9-anthryl)-10*c*,13*c*-pentadienoic acid.

BHK 21 cells: 3 μmol of the aforementioned ω-anthryl fatty acids were added to the growth medium of semi-confluent BHK 21 cells. The monolayers in the flasks growing on the two shorter chain probes showed confluency after 48 h, whereas cells growing with 15-(9-anthryl)-10*c*,13*c*-pentadienoic acid were not grown to complete confluency during this period of time. The lipid isolation and the separation by column and thin-layer chromatography was carried out following standard procedures. Fig. 1 presents a two-dimensional thin-layer analysis of the total lipid extract of BHK 21 cells grown on 11-(9-anthryl)-6*c*,9*c*-undecadienoic acid. Table 1 summarizes the yield of recovered ^3H-labelled fluorescent precursors and their incorporation into lipid fractions.

The lipid fractions were further separated by thin-layer chromatography in order to determine the distribution of the anthryl labelled fatty acids in the lipid classes (Fig. 2a - c). Furthermore the specific activities of the phospholipid classes were determined (Table 2).

Phosphatidylcholine from the three experiments was purified and subjected to phospholipase A_2 (*Crotalus adamanteus*) treatment. The fatty acid released enzymatically and the lysolecithin

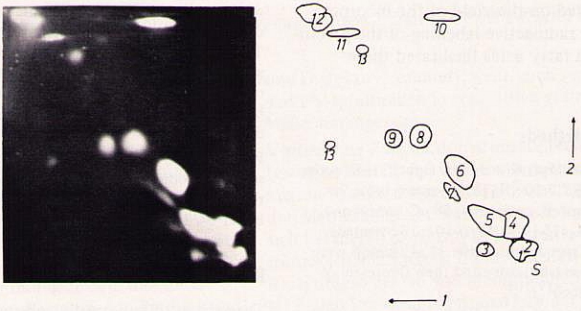


Fig. 1. Two-dimensional separation of total lipid extract of BHK 21 cells grown on a medium supplemented with 11-(9-anthryl)-6*c*, 9*c*-undecadienoic acid.

Visualization: left, fluorescence; right, charring with sulfuric acid. First dimension, solvent system IV second dimension, solvent system V. 1 = sphingomyelin, 2 = hematoside, 3 = triglycosylceramide, 4 = phosphatidylserine, 5 = phosphatidylcholine, 6 = phosphatidylethanolamine, 7 = diglycosylceramide, 8 = cardiolipin, 9 = monoglycosylceramide, 10 = fatty acid, 11 = ceramide, 12 = triglyceride and cholesterol esters, 13, not identified.

formed were separated by preparative thin-layer chromatography and the distribution of the fluorescent labelled precursor within the phospholipid molecule calculated from the radioactivity present in these two fractions.

The short, medium and long chain substituted ω-anthryl fatty acids are transferred by the acyl-CoA transferase preferentially to the 2-position of the glycerol backbone. 82 - 84% of each anthryl fatty acid was found in the 2-position of the respective phosphatidylcholines. Since the fluorescent fatty acids are radioactively labelled, it is possible to determine the number of ω-anthryl fatty acids incorporated per phospholipid molecule from the specific radioactivity of the phospholipid and the specific radioactivity of the respective precursor fatty acid. The following Table 3 summarizes the number of fluorescent fatty acids per 100 phospholipid molecules.

Chang liver cells: Monolayers of Chang liver cells were incubated under the conditions described for BHK 21 cells with the same fluorescent fatty acid precursors. The analysis for their quantitative distribution in phospholipids, glycosphingolipids and neutral lipids followed the same route outlined before. Fig. 3 represents the two-dimen-

Table 1. Uptake and distribution of fluorescent labelled fatty acids in BHK 21 cells and their lipid classes.

8-A-8:1^6 = 8-(9-anthryl)-6*c*-octenoic acid
11-A-11:$2^{6,9}$ = 11-(9-anthryl)-6*c*,9*c*-undecadienoic acid
15-A-15:$2^{10,13}$ = 15-(9-anthryl)-10*c*,13*c*-pentadecadienoic acid

Lipid extract	8-A-8:1^6	11-A-11:$2^{6,9}$	15-A-15:$2^{10,13}$
Radioact. [dpm]	6.9×10^7	1.8×10^8	7.3×10^7
-% of administered acid	6.9	15	12
Lipid fraction	% of total radioactivity		
Neutral lipids	6.5	57.3	37.6
Glycosphingolipids	17.9	8.3	3.9
Phospholipids	75.6	34.4	58.5

Table 2. Distribution of ω-anthryl fatty acids in phospholipids of BHK 21 cells and specific radioactivities of phospholipids.

Phospholipid class	8-A-8:1^6		11-A-11:$2^{6,9}$		15-A-15:$2^{0,13}$	
	[%]	Spec. act. [dpm/μmol]	[%]	Spec. act. [dpm/μmol]	[%]	Spec. act. [dpm/μmol]
SPM	2.3	5.8×10^6	7.2	9.9×10^6	8.2	1.0×10^7
PC	51.0	5.0×10^6	70.3	6.3×10^6	70	1.8×10^7
PS/PI	6.4	–	8.2	–	12.4	–
PE	40.3	8.9×10^6	14.0	7.7×10^6	9.4	4.5×10^6

sional radioscan (left) and fluorescence of the total lipid extract of Chang liver cells grown on the medium supplemented with 15-(9-anthryl)-10, 13-pentadecadienoic acid. Table 4 summarizes the incorporation into the three lipid fractions: neutral lipids, glycosphingolipids and phospholipids, and also includes specific radioactivities of the main phospholipids and a calculation of the number of fluorescent fatty acids per 100 molecules of phospholipid molecules.

Table 3. Number of ω-anthryl fatty acid molecules incorporated per 100 phospholipid molecules.

Phospholipid	8-A-8:1^6	11-A-11:$2^{6,9}$	15-A-15:$2^{10,13}$
SPM	5	8	8
PC	5	7	20
PE	9	6	14

Table 4. Incorporation and distribution of ω-anthryl fatty acids in Chang liver lipids.
a) Lipid fractions and b) phospholipid classes (% of total radioactivity in phospholipids) with spec. activities.
FA/100 PL = number of ω-anthryl fatty-acid incorporated into 100 phospholipid molecules.

a)	8-A-8:1^6	11-A-11:$2^{6,9}$	15-A-15:$2^{10,13}$
Total lipid extract % of administered radioactivity	20	8.5	7
	% of incorporated radioactivity		
Neutral lipids	9	52	49
Glycosphingolipids	3	6	5
Phospholipids	88	41	46

b)	8-A-8:1^6			11-A-11:$2^{6,9}$			15-A-15:$2^{10,13}$		
Phospholipids	[%]	10^{-6} x Spec. act. [dpm/μmol]	FA / 100 PL	[%]	10^{-6} x Spec. act. [dpm/μmol]	FA / 100 PL	[%]	10^{-6} x Spec. act. [dpm/μmol]	FA / 100 PL
SPM	8.4	4.5	4	9.9	3.1	3	6.0	7.4	6
PC	45.8	8.2	7	70.0	12	9	79.1	9.2	22
PS	8.1	4.2	4	4.1	3.6	3	4.6	16	13
PE	37.7	5.8	5	22	3.1	2	10.3	4.3	10

Analysis of the positional distribution of the three acids in purified phosphatidylcholine of Chang liver was carried out by phospholipase A_2 hydrolysis and determination of the radioactivity in free fatty acid and lysolecithin formed.

Again the striking preference of the ω-anthryl fatty acyl transfer to the 2-position became apparent: 8-A-8 : 1^6, 82%; 11-A-11 : $2^{6,9}$, 79%; and 15-A-15 : $2^{10,13}$, 62%.

RN6 cells (Schwannoma cell clone): RN6 cells are derived from a chemically induced mouse Schwann cell tumor[11]. This cell clone was incubated with the aforementioned three ω-anthryl fatty acids of different alkyl chain length in order to prove the applicability of our fluorescent probes, not only for the incorporation into complex lipids of epithelial and parenchymal derived cells, but also nervous tissue. Indeed, the ω-anthryl fatty acids were incorporated into the lipids of RN6 cells without any visible changes in the morphology and viability of these cells under the conditions of the incubation also used for BHK 21 and Chang liver cells.* The incorporation (% of radioactive fatty acid added to the medium) increased with increasing chain length: 8-(9-anthryl)-6*c*-octenoic acid, 8%; 11-(9-anthryl)-6*c*, 9*c*-undecadienoic acid, 13.5%; and 15-(9-anthryl)-10*c*,13*c*-pentadecadienoic acid, 33%. Fig. 5 shows the distribution of fluorescent labelled fatty acids in phospholipids of RN6 cells. Silicic acid chromatography separated the neutral, glycosphingo- and phospholipids.

* The viability of the cells was tested by further passing them. Their doubling time was the same as for unlabelled cells (12 - 14 h).

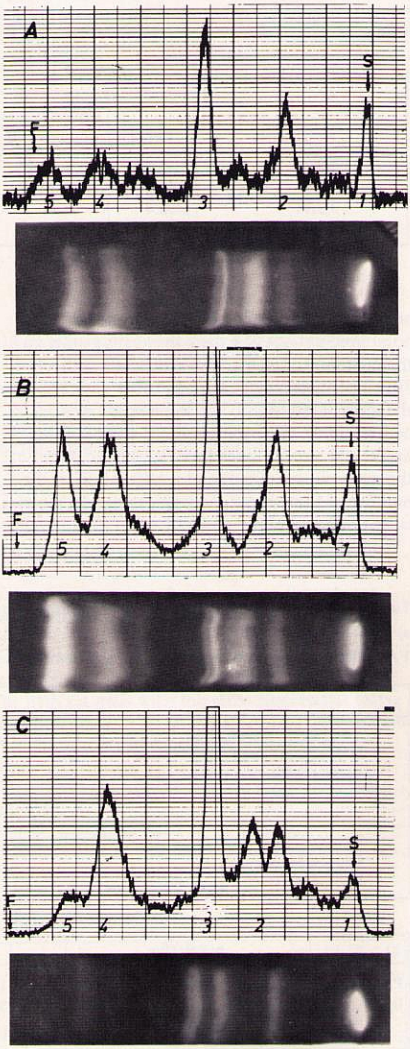
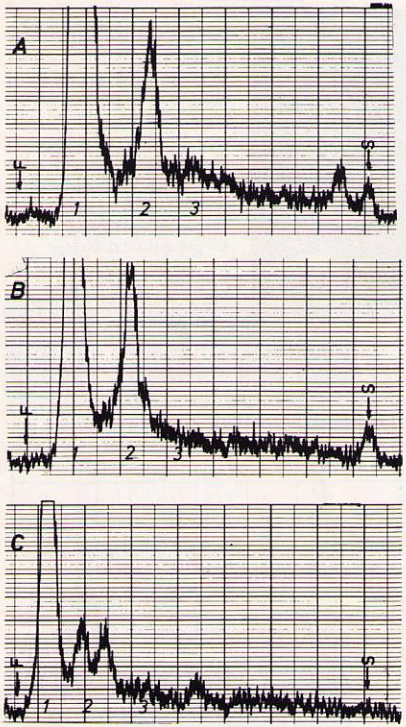
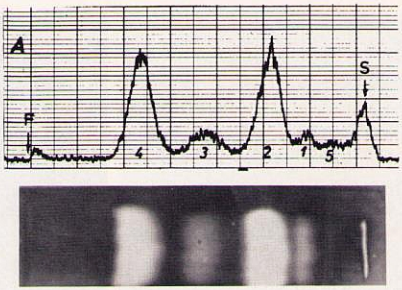
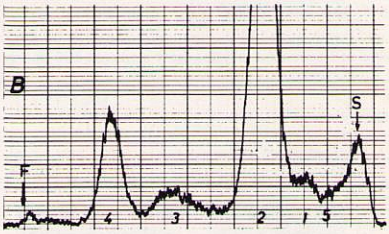


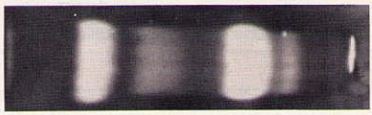
Fig. 2a - c. Distribution of fluorescent labelled fatty acids in a) neutral lipids, b) glycosphingolipids, c) phospholipids of BHK 21 cells.
A, 8-(9-anthryl)-6*c*-octenoic acid; B, 11-(9-anthryl)-6*c*,9*c*-undecadienoic acid; C, 15-(9-anthryl)-10*c*,13*c*-pentadecadienoic acid.

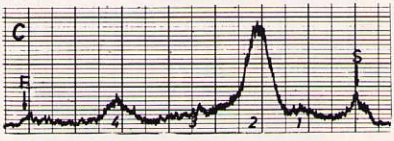
a) Neutral lipids of BHK 21 cells. Solvent system I. 1 = phospholipids, 2 = diglycerides (1,2- and 1,3-diglycerides), 3 = fatty acids, 4 = triglycerides, 5 = cholesterol esters.

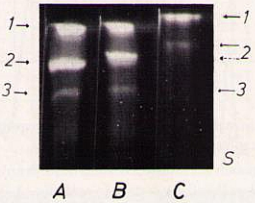












b) Glycosphingolipids. Solvent system II. 1 = ceramide, fatty acid, 2 = monoglycosyl ceramide hexoside, 3 = diglycosylceramide.

c) Phospholipids. Solvent system III. 1 = sphingomyelin, 2 = phosphatidylcholine, 3 = phosphatidylserine, 4 = phosphatidylethanolamine, 5 = lysophosphatidylcholine. Unidentified radioactivity at start.

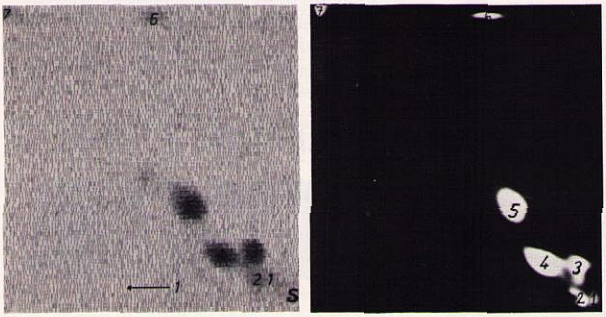


Fig. 3. Two-dimensional radioscans and fluorescence of total lipids isolated from Chang liver cells grown on 15-(9-anthryl)-10*c*,13*c*-pentadecadienoic acid. First dimension, solvent system IV. Second dimension, solvent system V. 1 = hematoside, 2 = sphingomyelin, 3 = phosphatidylserine, 4 = phosphatidylcholine, 5 = phosphatidylethanolamine, 6 = fatty acid, 7 = neutral lipids.

Table 5. Incorporation and distribution of ω-anthryl fatty acids in RN6 cells.
a) Lipid fractions and b) phospholipid classes (% of total radioactivity in phospholipids), specific radioactivities and number of fluorescent labelled fatty acids per 100 phospholipid molecules (FA/100 PL).

a)	8-A-8:1^6	11-A-11:$2^{6,9}$	15-A-15:$2^{10,13}$
Total lipid extract % of administered radioactivity	8	13.5	33
	% of incorporated radioactivity		
Neutral lipids	12	74.2	42.4
Glycosphingolipids	8.7	7.8	6.4
Phospholipids	79.3	18.0	51.2

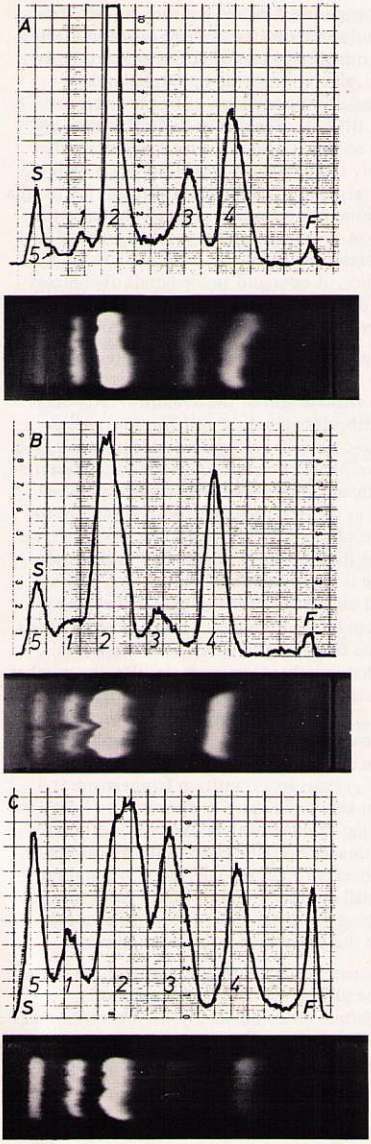
b)	8-A-8:1^6			11-A-11:$2^{6,9}$			15-A-15:$2^{10,13}$		
Phospholipids	[%]	10^{-6} × Spec. radioact. [dpm/μmol]	FA / 100 PL	[%]	10^{-6} × Spec. radioact. [dpm/μmol]	FA / 100 PL	[%]	10^{-6} × Spec. radioact. [dpm/μmol]	FA / 100 PL
SPM	5.8	1.9	2	7.8	6.0	5.5	0.8	0.62	1
PC	43.4	3.1	3	69.9	8.6	8	69.8	11	16
PS/PI	10.3	4.1	4	2.6	1.5	1.5	2.3	2.4	3.5
PE	40.5	4.4	4	19.7	3.1	2.5	27.1	6.0	8.5

Table 5 summarizes the analytical data regarding distribution of radioactive ω-anthryl fatty acids in a) neutral, glycosphingo- and phospholipid fractions and b) specific radioactivities and number of respective ω-anthryl fatty acids incorporated per 100 phospholipid molecules.

The fluorescent fatty acids with short (8 C-atoms) and medium (11 C-atoms) alkene chains were

Fig. 4. Radioscan and fluorescence of phospholipid fraction of Chang liver cells. ▷

Cells were grown on Dulbecco's medium supplemented with A, 8-(9-anthryl)-6*c*-octenoic acid; B, 11-(9-anthryl)-6*c*,9*c*-undecadienoic acid; C, 15-(9-anthryl)-10*c*,13*c*-pentadecadienoic acid. Solvent system III. 1 = sphingomyelin, 2 = phosphatidylcholine, 3 = phosphatidylserine, 4 = phosphatidylethanolamine, 5 = front activity (unidentified).



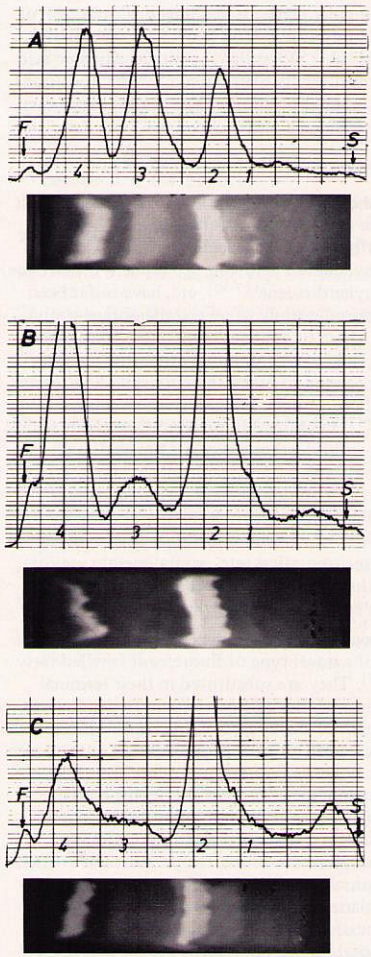


Fig. 5. Radioscan and fluorescence of phospholipid fraction of RN6 cells grown on Dulbecco's medium supplemented with A, 8-(9-anthryl)-6*c*-octenoic acid; B, 11-(9-anthryl)-6*c*,9*c*-undecadienoic acid; C, 15-(9-anthryl)-10*c*,13*c*-pentadecadienoic acid.

Solvent system III. 1 = sphingomyelin, 2 = phosphatidylcholine, 3 = phosphatidylserine, 4 = phosphatidylethanolamine.

preferentially incorporated into the 2-position of phosphatidylcholine (66 and 73%, respectively), whereas the 15-(9-anthryl)-pentadecadienoic acid was almost evenly distributed (53% in 2-position) in the 1- and 2-position of this phospholipid class.

Discussion

A number of polycyclic aromatic compounds with suitable fluorescent properties such as 8-anilino-1-naphthalenesulfonic acid[18], *N*-phenyl-1-naphthylamine), perylene, 1,6-diphenylhexatriene, 2-methylanthracene[17-30], etc. have so far been applied in the study of several membrane properties. These fluorescent compounds have either hydrophilic or hydrophobic properties and are therefore used to probe the corresponding regions of artificial or natural membranes. Waggoner and Stryer[31] developed fluorescent long chain acids [12-(9-anthrylcarbonyloxy)stearic acid] and aminosulfonates (octadecylnaphthylaminesulfonate) of amphiphilic nature to mimic membrane lipid properties. All these probes lack properties which would facilitate their biosynthetic incorporation into natural membranes or allow the exact determination of their localization in macromolecular structures under study.

Our recent chemical studies led to the development of a novel type of fluorescent labelled fatty acids[9]. They are substituted in their terminal position with the anthryl residue. Depending on the length of the hydrocarbon chain of the acyl residues substituting the 9-position of anthracene they may act as fluorescent "rulers" in a bilayer or in membranes. We have also incorporated these acids chemically into phosphatidylcholine molecules and described their usefullness to monitor phase-transition temperatures of artificial and natural membranes by fluorescence intensity and polarization measurements[10]. After the unsuccessful attempts (unpublished results) to incorporate these fluorescent probes biosynthetically into the fatty acid auxotrophic *E. coli* mutant (K1060) we made the striking observation that the anaerobic *Bacteroides melaninogenicus* or *B. thetaiotaomikron* do incorporate these fluorescent probes (unpublished results). This bacterial strain produces almost exclusively branched iso-fatty acids and β-hydroxy fatty acids[32].

In this paper we describe the very efficient uptake and incorporation of three ω-anthryl fatty acids with alkyl chains of different length into neutral, glycosphingo- and phospholipids of three eukaryotic cell lines of rather divergent origin, BHK 21, Chang liver and a Schwann cell tumor cell line (RN6). The results indicate that not only the uptake of these fatty acids with the bulky terminal anthryl residue through the plasma membrane is functioning properly, but also the acyl-CoA-synthetase and acyl-CoA-*O*- and *N*-acyltransferases use these acids as substrates with the formation of ester and amide bonds of complex lipids. The incorporation of the acids does not interfere with the viability of the cells.

The analytical data given here indicate that the degree of uptake and incorporation is dependent on the chain length of the ω-anthryl fatty acids: 8-(9-anthryl)-octenoic < 11-(9-anthryl)-undecadienoic < 15-(9-anthryl)-pentadecadienoic acid. If we compare phosphatidylcholine and phosphatidylethanolamine, the two main phospholipid classes in the three cell lines, with respect to the amount of ω-anthryl fatty acid incorporated, we observe that the biosynthesis of phosphatidylcholine is regulated in such a way that only a limited number of short chain ω-anthryl fatty acids can be incorporated. This number increases with the chain length. On the other hand, in phosphatidylethanolamine molecules, the number of fluorescent fatty acids decreases with chain length. Previous studies[10] have shown that the perturbation of lipid bilayers by phosphatidylcholine containing a short chain ω-anthryl fatty acid (C_8) leads to a transition temperature (t_t) interval below the sharp t_t measured by light scattering. It might well be that the location of the anthracene residue close to the glycerolphosphate backbone allows only the incorporation of a small number of anthryl fatty acids without disturbing the membrane function. The regulation of this phenomenon is not understood.

Preliminary cell fractionation studies have shown that the plasma membrane, mitochondria, endoplasmic reticulum and lysosomal membranes are labelled*. Since the plasma membrane is heavily labelled biosynthetically, the usefulness

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of these fluorescent fatty acids for the study of many membrane properties such as fluidity, transport, fusion etc. becomes apparent.

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