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Incorporation and metabolic conversion of saturated and unsaturated fatty acids in SK-Hep$_1$ human hepatoma cells in culture

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Abstract

We report here a study of the incorporation and metabolism of various long chain fatty acids in SK-Hep-1 cultured hepatoma cells. Medium supplementation with radiolabelled palmitic, stearic, linoleic, α-linolenic and eicosa-8,11,14-trienoic acids (1 μM, 24 H) resulted in an active uptake of each of these precursors by the cultures. Subsequent analysis of the cellular lipids indicated that they exhibit almost all the enzymic activities of polyunsaturated fatty acid metabolism that are characteristic of normal hepatic cells. With respect to the desaturation capacities of this cell line, although α-linolenic acid reacted more extensively than did linoleic acid and the conversion of 8,11,14-eicosatrienoic acid by the △ 5 specific enzyme was more avid than had been previously seen in normal rat or human liver: the saturated fatty acids constituted relatively poor substrates, being preferentially chain-elongated rather than (mono) desaturated at the △ 9 position. Analysis of the fatty acid profiles of total cellular lipids and of various lipid subclasses, however, revealed a relative paucity of essential fatty acids when compared with the abundance of endogenous monoenoic acids (particularly oleic). Of the total cellular fatty acids, 58% were present in the form of phospholipids; with 33% of the remaining 42% (i.e., the neutral lipids) being associated with triacylglycerol fraction. Within the total lipids, phosphatidyl-choline and phosphatidyl-ethanolamine were the major sites for the incorporation of all metabolic products derived from the incubated radiolabelled 16- and 18-carbon fatty acid precursors, whereas the phosphatidyl-inositol fraction was the predominat recipient of nascent arachidonic acid when the eicosatrienoate was the substrate. The express purpose of this investigation was to characterize the biochemical routes involved in the anabolism of various essential fatty acids in the human hepatocyte, through the use of cultured human hepatoma cells as an experimental model system. In view of the similarities between certain aspects of the polyunsaturated fatty acid metabolism of these cells and the corresponding properties of other mammalian hepatic or liver-derived tissues, the data presented here would thus constitute a significant beginning alone those lines. Moreover, considering the extreme difficulty in obtaining for such investigation relevant tissue samples from normal human sources, we regard these results – and the availability for use of this particular human hepatoma cell line – as important new developments in the effort to characterize a useful experimental model both for gaining immediate information and for designing future experiments. (Mol Cell Biochem **117:** 107–118, 1992)

Key words: desaturases, elongases, fatty acid composition, phospholipids, neutral lipids, human hepatoma, SK-Hep$_1$ cultured cells

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Introduction

The specialized functions of differentiated mammalian cells require the utilization of certain fatty acids. For this purpose, specific fatty acids are selectively incorporated into the different classes of membrane phospholipids and a few polyunsaturated fatty acids are released for the utilization in the synthesis of their oxygenated derivatives [1–4]. Excellent reviews on the biochemical and physiologic roles of polyunsaturated fatty acids in humans are available [5–9]. Although much information within this field has been gained from studies on isolated cultured cells derived from a variety of animals [4, 10–17] and humans [4, 18–20], no relevant experiments have been carried out with human liver-derived cells. In view of the difficulties encountered in obtaining human hepatocytes under physiological conditions [21–25] along with inter-species differences in lipid metabolism [3, 26], hepatocyte cell lines derived from human tumors have been used to represent an alternative model for studies on human hepatic lipid metabolism. For this reason, the present investigation was undertaken to determine polyunsaturated fatty acid uptake and metabolism in the human hepatoma line SK-Hep-1. The advantage of this system was the ability to investigate the enzymic activities involved in fatty acid biosynthesis (elongases and/or desaturases) in a chemically defined medium, thus avoiding the complex background of hormones, metabolites, transport proteins and other biological factors always present in the intact mammalian organism. In an effort to elucidate the biochemical routes of polyunsaturated fatty acid biosynthesis in the human species in particular, we supplemented the normal growth medium of the SK-Hep-1 cells with a chemically defined (i.e., serum-free) one containing a given fatty acid of interest and then examined the incorporation of this precursor into various lipid classes along with its concomitant anabolism to higher polyenoic acid derivatives.

Materials and methods

Chemicals

The following radioactive fatty acids were used: [1-^{14}C]linoleic (52.6 mCi/mmol, 98% pure), [1-^{14}C]α-linolenic (51.0 mCi/mmol, 98% pure) and [1-^{14}C]eicosa-8,11,14-trienoic (54.9 mCi/mmol, 99% pure) from Amersham Corp., Arlington, IL and [1-^{14}C]palmitic acid (58.7 mCi/mmol, 99% pure) and [1-^{14}C]stearic acid (58.9 mCi/mmol, 99% pure) from New England Nuclear Corp., Boston, MA. Unlabeled palmitic, stearic, linoleic and α-linolenic, eicosa-8,11,14-trienoic and eicosa-11-monoenoic acids were provided by Nu-Chek Prep, Elysian, MN. All acids were stored in benzene under an atmosphere of nitrogen at −20°C. Concentrations and purities were confirmed by both liquid-scintillation counting and gas-liquid chromatography (GLC) of fatty acid methyl esters (FAME) prepared in the presence of internal standards. Neutral lipids and phospholipids used as standards for thin-layer chromatography (TLC) were obtained from Serdary Research Lab. Inc., London, Ontario, Canada. FAME mixtures for GLC identifications and defatted bovine albumin were from Sigma Chemical Co., St. Louis, MO. All chromatographic solvents were RPE grade and obtained from Carlo Erba, Milan, Italy. Other chemicals used were reagent grade and provided by local commercial sources.

Cell culture

The human cell line SK-Hep-1 was originally isolated from a malignant adenocarcinoma of liver in 1971 [27, 28]. The cells were grown in surface culture in 70 cm^2-flasks at 37°C in 20 ml of Swim's 77 (S-77) medium, supplemented with 10% (v/v) calf serum [29], by means of conventional sterile conditions as described previously [30]. Fatty acid composition of serum-supplemented Swim's S-77 medium was (in percentages): 1.1 lauric, 16.3 palmitic, 3.8 palmitoleic, 15.4 stearic, 19.9 oleic, 22.7 linoleic, 1.1 gamma-linolenic, 10.5 alpha-linolenic, 0.5 eicosatrienoic (n-9), 1.6 eicosatrienoic (n-6), 3.1 docosapentaenoic (n-3) and 2.9 docosahexaenoic (n-3). The remainder consisted of small amounts of 22-C fatty acids from n-3 and n-6 series. When the cells were in the logarithmic phase of growth (48 h after seeding, the culture medium was replaced by Minimum Essential Medium Zinc-option (IMEM-Zo) [31] minus linoleic acid and containing N-2-hydroxyethylpiperazine-N-2-ethanosulphonic acid (HEPES) (5 mM). The cells were maintained in this medium for 24 h. Then, the IMEM-Zo medium was replaced by the same medium supplemented with different fatty acids at a concentra-

tion of 1.0 μM (0.5 μCi/flask). The cells were then incubated under these conditions for another 24 h. A control group of flasks was treated in parallel but not given any fatty acid supplements. The acids were added as their sodium salt bound to defatted albumin according to Spector *et al.* [32] in a ratio of 6 nmol of fatty acid to 1 nmol of the protein. Control cells were supplemented with defatted albumin at the same concentration. At the end of each experiment, the attached cells were washed three times with 5 ml of ice-cold physiologic saline solution, detached from the growing surface mechanically through the use of a rubber-tipped spatula, and pelleted at 500 × g for 10 min. The sedimented cells were removed and washed twice by centrifuging as before, but for 15 min in 15 ml of the saline solution. The final pellet was resuspended in 5 ml of the same solution. An aliquot of this suspension was used to determine cell viability [33] and the amount of cellular protein [34]. The rest was centrifuged under the latter conditions; the supernatant was discarded; and the pellet was processed for lipid analysis.

Cellular lipid extraction and separation

Lipids were extracted from the pellets using the method of Folch *et al.* [35]. The organic phases, containing more than 97% of the total radioactivity, were further separated by two chromatographic procedures. In the first system, the neutral lipids were partitioned by TLC on 0.25 mm thick silica gel G-60 plates by means of a solvent system containing hexane/diethylether/acetic acid (80:20:1, v:v:v) [36]. Under these conditions the phospholipid fraction remained at the origin. Individual neutral lipid species were identified by comparison with a standard mixture containing (Rf values in parenthesis) monoacylglycerides (MAG) (0.10), diacylglycerides (DAG) (0.20), cholesterol (CHO) (0.31), free fatty acids (NEFA) (0.40), triacylglycerides (TAG) (0.72), fatty acid methyl esters (FAME) (0.81), and cholesterol esters (CHOE) (0.96), which species were applied to each plate. Phospholipids were separated by means of a second TLC system [37] involving two successive partitionings in the same direction. The first mobile phase was chloroform/methanol/ammonium hydroxide/water (70 : 25 : 3.5 : 1.5, v : v : v : v), whereas the second was chloroform/methanol/acetic acid/water (80 : 10 : 2 : 0.75, v : v : v : v). The different subclasses of phospholipids were identified by means of the following standard mixture run in parallel with the samples (Rf values in parenthesis): lysophosphatidyl-choline (LPL) (< 0.15); phosphatidyl-serine (PS) (0.19); phosphatidyl-inositol (PI (0.25); sphingomyelin (SM) (0.30); phosphatidyl-choline (PC) (0.39); phosphatidyl-ethanolamine (PE) (0.55); cardiolipin (CL) (0.69) and phosphatidyl-glycerol (PG) (0.71).

Lipid analysis

After development, plates were dried under nitrogen. Lanes containing the experimental samples were covered with a glass plate while the markers were briefly exposed to a spray of methanolic iodine (1.5%, w/v). Areas of interest were scraped off the plates and the isolated lipids recovered by elution with chloroform/methanol (1:1, v/v). After drying, the samples were transesterified by heating in 2.5 ml 4N HCL/methanol at 68°C for 3 h under nitrogen. The resulting FAME were extracted thrice with 2 ml of hexane. The methanol phase was analyzed for phosphorus content according to Chen *et al.* [38], while the hexane phase was evaporated and the residue prepared for GLC analysis by dissolution in 20–30 μl of hexane containing 1 μg/tube of eicosa-11-monoenoic acid as an internal standard. The distribution of radioactivity among the FAME was analyzed in an Acromat GC-100 apparatus (Redhill, Surrey, England) with a flame-ionization detector by means of a 6-foot glass column packed with 10% (w/w) SP-2330 on 100-200 mesh chromosorb WAW-DMCS (Supelco Inc., Bellefonte, PA). After a 4-min initial hold, the oven was programmed from 140 to 220°C at 1.5°C/min. The distribution of [^{14}C] radioactivity among the eluted FAME was determined with a Packard 894 Gas-Flow Proportional Counter (Downers Grove, I11) interfaced to the chromatograph through a nominal 10:1 splitter (total sample:fatty acid). A linear dual-pen recorder (LKB-2210 Two Channel Recorder, Bromma, Sweden) was used to obtain simultaneous radioactivity and mass tracings for each chromatographic separation. The FAME were quantified according to their peak areas and individual FAME were calculated as the percentages of total FAME identified in a given sample. Mass-composition analyses of FAME derived from all samples were made on a GLC Hewlett-Packard 5840-A apparatus (Avondale, PA) equipped with a 5840-A terminal computer integrator system. The column was packed as described above and the temperature of the oven programmed from 140 to 220°C at 3°C/min after a 1-min initial hold. The FAME

were identified by comparison of their relative retention times with authentic standards and the mass distribution was calculated electronically by quantification of peak areas. In some experiments, TLC plates were scanned for radioactivity (radio-TLC) using a TLC-Proportional Radioactivity Scanner, Berthold LB-2832, Wildbad, FRG, equipped with a Hewlett-Packard 3396-A Data Station. Regions of interest were scraped off the plates and isolated lipids recovered for radioactivity measurement in a Wallac 1214 Rackbeta Liquid Scintillation Counter (Pharmacia, Turku, Finland) (97% efficienty for ^{14}C) interfaced to an Olivetti M-240 Computer System. No differences were found between quantification of radioactivity by TLC-scanning or liquid-scintillation counting. Data are reported as the mean and standard error calculated from 3–5 independent analyses.

Results and discussion

The fatty acid profiles of total lipids and different lipidic subclasses from SK Hep-1 hepatoma cells are presented in Table 1. As can be seen, the fatty acid composition of this human-hepatoma line is similar to that frequently observed in tumor tissues; being characterized by an only modest level of the essential fatty acids, arachidonic and linoleic, and a relative abundance of monoenoic acids, in particular oleic [4, 10, 14, 39–43]. The analysis of the fatty acid composition of various lipid fractions isolated from SK-Hep-1 cells after 48 h of incubation in IMEM-Zo medium, revealed that 58.2% of the fatty acids were present in phospholipids, with the remaining 41.8% being found in the neutral lipids. Within this latter percentage, 32.5% were located in the triacylglycerol fraction. The determination of individual FAME derived from phospholipids (Table 1) showed that palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) [n-6] were the predominant fatty acids. In addi-

Table 1. Per-cent distribution of fatty acids within total lipids and lipidic subclasses of SK-Hep-1 human hepatoma cells.

Fatty acids	Total lipids[a]	Lipidic subclasses[b]									
		Phospholipids							Neutral lipids		
		PC	PE	PI	PS	LPL	SM	PG + CL	TAG	CHOE	NEFA
14:0	0.5	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
16:0	17.4	3.8	0.7	0.7	1.2	0.5	1.0	0.6	8.6	1.0	0.3
16:1	3.2	0.8	0.3	0.2	0.0	0.1	0.0	0.1	1.3	0.2	0.1
18:0	22.8	4.1	2.6	3.1	2.8	1.3	0.6	0.5	6.2	0.8	0.8
18:1	30.3	7.0	5.1	2.4	1.2	0.6	0.5	0.4	10.3	1.4	1.4
18:2(n-6)	8.2	1.9	1.7	0.4	0.2	0.1	0.1	0.1	2.9	0.3	0.5
18:3(n-3) + (n-6)	0.5	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0
20:2	0.6	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0
20:3(n-9)	1.1	0.2	0.2	0.1	0.2	0.0	0.1	0.0	0.3	0.0	0.0
20:3(n-6)	1.5	0.2	0.5	0.2	0.2	0.0	0.0	0.0	0.2	0.1	0.1
22:0 + 20:4(n-6)	4.8	0.5	1.1	0.9	0.6	0.2	0.2	0.1	0.8	0.1	0.3
20:5(n-3)	1.7	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.2	0.5	0.1
22:2(n-6)	0.7	0.1	0.6	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
22:4(n-6)	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:5(n-6)	0.5	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1
22:5(n-3)	1.9	0.2	0.6	0.1	0.2	0.1	0.3	0.0	0.3	0.1	0.0
22:6(n-3)	4.0	0.4	0.6	0.5	0.2	0.2	0.2	0.1	1.2	0.8	0.1
Fraction%		19.5	14.6	8.9	7.1	3.3	2.9	1.9	32.5	5.5	3.8
Total%	100				58.2%					41.8%	

[a] Results are expressed as the percentage distribution of the fatty acids found in total cellular lipids (mean of three independent analyses with variations not greater than 8% of the mean for any component shown).

[b] Per-cent contribution of each lipid subclass within the total lipids. For the key to the abbreviations used, c.f. *Materials and methods*. Percentages less than 0.1% are expressed as '0.0'. Analyses of the samples were performed as described in *Materials and methods*.

tion, there was a significant decrease in arachidonate content compared to the values shown by different authors for normal liver [39]. In all neutral lipid fractions, 18:1 was the major fatty acid. Considerable amounts of saturated fatty acids (16:0 and 18:0) were present in the triacylglycerol fraction. Either traces or non-detectable amounts of polyunsaturated fatty acids derived from arachidonate or eicosapentaenoate were found in all the fractions studied, except for 22:6 (n-3), it representing 4.0% of the total fatty acids and being mainly located in PE, PI and PC. The highest monoenoic/saturated fatty acid ratio was observed in PS (4.59) and the lowest ratio in PE (0.86), while the 20:4 (n-6)/18:2 (n-6) ratio was both particularly high in PS (2.76) and PI (2.06) and specially low in PC (0.28) and TAG (0.27) (Table 1).

Figure 1 shows the distribution of the major phospholipid classes derived from SK-Hep-1 cells expressed as phosphate content per mg of cellular protein. PC was the major phospholipid, attaining $15 \times (10)^{-2}$ μ moles of P per mg cellular protein, followed by PE. The amount of PI and PS was considerably lower, while the contribution of all other phospholipids together was only about 15%. Our results (Table 1 and Fig. 1) indicate clearly that SK-Hep-1 cells contain phospholipids and neutral lipids in a proportion similar to that observed in normal liver [39, 42, 43]. Previous studies on the distribution of lipid in the hepatomas of experimental animals have shown that the composition of such tumors varies widely, differing not only from that of normal liver tissue but also among the various liver carcinomas [39]. These discrepancies in the relative amounts of the major lipid groups demonstrate the marked and wide-ranging aberrant effect of malignancy upon lipid metabolism. The analyses of these differences thus give no consistent pattern with which to classify the hepatomas on the basis of their lipid composition. Furthermore, the mechanism underlying the production or accumulation of large amounts of neutral lipids in certain hepatomas is still unknown. There could possibly be, for example, an effect of malignancy on lipoprotein synthesis that diminishes the secretion of TAG from hepatoma cells [39].

After 24 h of culture in presence of 1 μM additions of [1-^{14}C]16:0, [1-^{14}C]18:0, [1-^{14}C]18:2 (n-6), [1-^{14}C]18:3 (n-3) or [1-^{14}C]20:3 (n-6), all labelled fatty acids were taken up by the cells (Fig. 2). The maximum incorporation of label (61%) was observed in linoleic acid, followed by α-linolenic acid. Stearic and eicosatrienoic acids were incorporated to nearly equal extents, where-

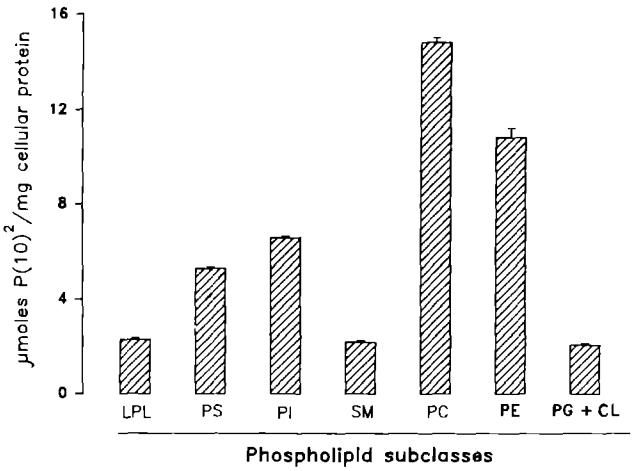

Fig. 1. Distribution of phosphate content in the phospholipid subclasses of SK-Hep-1 hepatoma cells. Results are expressed as the mean of four determinations ± 1 SEM. For technical details see *Materials and methods.*

as palmitic acid showed the lowest incorporation (40%). These results showed that SK-Hep-1 cells exhibit an ample capacity to assimilate either saturated or polyenoic fatty acids from the culture medium under standard experimental conditions, even though the essential fatty acids linoleic and α-linolenic were incorporated 22% more extensively than were the saturated acids. The fatty acids incorporated into the tumor cells were subsequently actively metabolized. Palmitic acid (Fig. 3A) was desaturated to palmitoleic acid, a result that is compatible with the existence of a Δ 9 desaturase enzyme. Quite probably, 16:0 was also elongated to 18:0 and this acid, in turn, was further desaturated at that same Δ 9 position to oleic acid; though, we obviously can not rule out the possibility of the contribution of a direct elongation of palmitoleic acid to this end result. Such an assumption would possibly explain the relatively high ratio of 18:1/18:0 found in the total lipid fraction as compared to that of 16:1/16:0. Consistent with our observations here on the monoenoate metabolism of the SK-Hep-1 cells, certain authors have proposed that the monoenoic/saturated fatty acid ratio could prove to be a useful marker reflecting both growth rate and malignancy in different human neoplasms, especially hepatomas [44–47]. By contrast, when [1-^{14}C] stearic acid was employed as the exogenous saturated fatty acid available, this precursor was scarcely converted to oleic acid at all (Fig. 3B). The small amount of [1-^{14}C]16:0 found in the radiochromatogram, may be considered to have arisen from the β-oxidation of labeled stearic acid followed by *de novo*

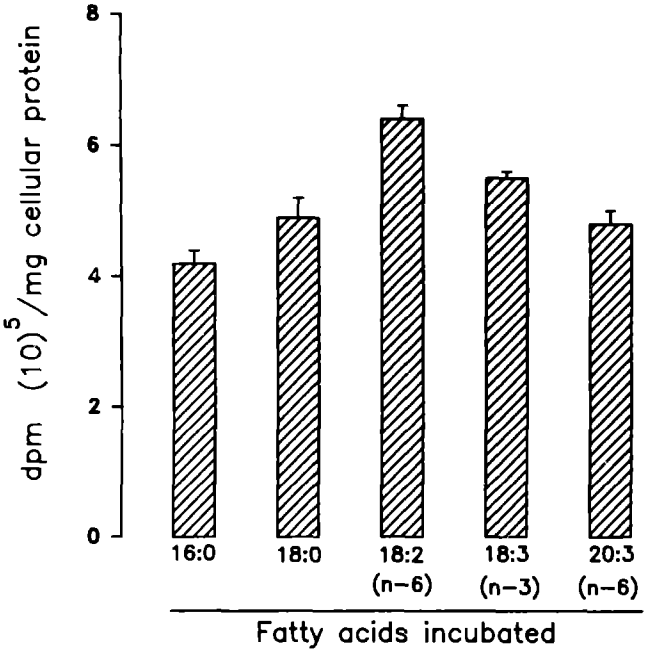

Fig. 2. Uptake of radioactivity in SK-Hep-1 hepatoma cells incubated in the presence of various [1-^{14}C]fatty acids. Results are expressed as dpm per mg of cellular protein (mean of four determinations ± 1 SEM). All fatty acids were added at 1 μM concentration (0.5 μCi/bottle) as sodium salt bound to defated albumin for 24 h. For experimental details see *Materials and methods*.

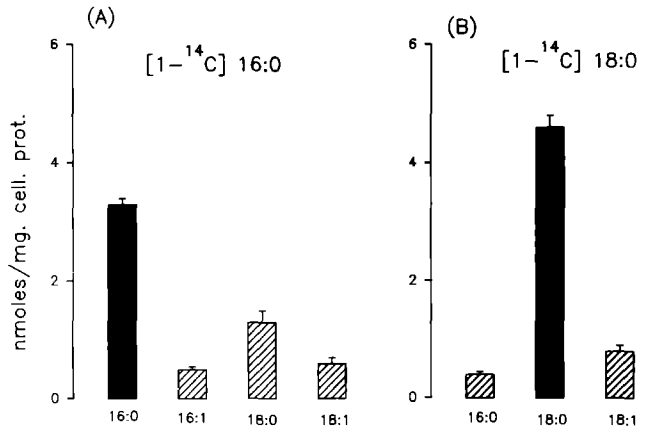

Fig. 3. Metabolic products obtained after incubation of SK-Hep-1 hepatoma cells with [1-^{14}C]palmitic (A) or [1-^{14}C]stearic (B) fatty acids. Results are expressed as nmol of each fatty acid per mg of cellular protein (mean of four determinations ± 1 SEM). The substrates were added as sodium salt bound to delipidated albumin at a final concentration of 1 μM (0.5 μCi/bottle) and incubated with the cells for 24 h. Radiochromatographic analyses of total cellular lipids were performed as indicated in *Materials and methods*.

synthesis. Comparisons between the results shown in Fig. 3A and B would seem to indicate that, despite the fact that this kind of tumor cell exhibits △ 9 desaturase activity, saturated fatty acids are primarily elongated rather than monodesaturated. The explanation as to how SK-Hep-1 cells exhibit high levels of monoenoic acids simultaneously with a relatively low △ 9 desaturation capacity awaits further clarification. One possibility, however, is that the cultured cells take up oleic and palmitoleic acids selectively from the serum lipids with a resulting partial inhibition of the endogenous synthesis of these monounsaturates. A second conceivable explanation could involve a selective inhibition of the catabolism of monoenoic fatty acids. Other studies carried out in human cells have demonstrated that external lipids can alter the relative contribution of preformed (i.e., endogenously synthesized) fatty acids within the total tissue lipid composition [48]. Moreover, Oshima *et al.* [49] have already demonstrated that, when different cell strains were cultured in serum-free medium, (i.e. in the absence of exogenous fatty acids), an increase in the content of endogenously synthesized monounsaturated fatty acids was seen to occur; presumably as a compensation for the lack of available polyunsaturates. For this reason, it is possible that the SK-Hep-1 line, as with other cells in culture, may adjust their monoenoic fatty acid metabolism in accordance with the supply of external lipids.

With respect to the function of polyenoates, arachidonic acid is known to be one of the main structural fatty acids of phospholipids and a prostanoid precursor as well [1–4, 18]; moreover, this polyenoate in particular plays an important role in membrane anatomy and physiology [50, 51]. Since we initially found that SK-Hep-1 cells contained somewhat depressed levels of arachidonic acid (Table 1), we supplemented their incubation medium with [1-^{14}C]eicosatrienoic acid and in this way demonstrated that these tumor-derived cells can actively transform that labelled substrate into arachidonic acid (Fig. 4). These results accordingly indicate clearly that SK-Hep-1 cells have an ample △ 5 desaturating capacity. The fact that this kind of cultured cell possesses a functional △ 5 desaturase that is directly involved in arachidonate biosynthesis is of extreme interest since the issue as to whether or not adult human liver expresses this activity is still controversial: for example, whereas Chambaz *et al.* [52] reported the presence of a △ 5 desaturase in three human fetal livers, Blond *et al.* [53] found only a very low activity of this enzyme in microsomes from four adult livers. Thus, although the latter results suggest that △ 5 desaturase activity remains unexpressed in adult hepatic tissue, the

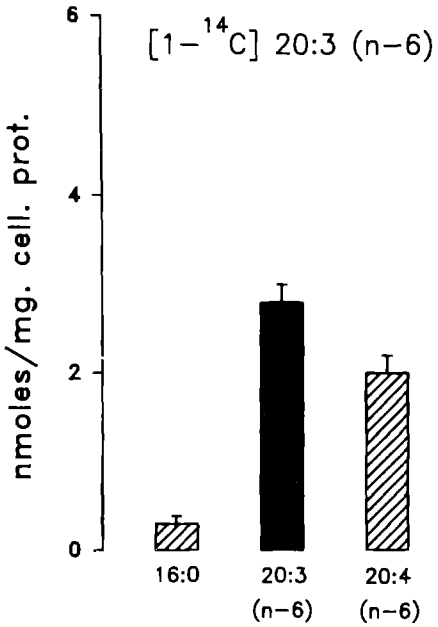

Fig. 4. Metabolic products obtained after incubation of SK-Hep-1 hepatoma cells with [1-^{14}C]eicosa-8,11,14-trienoic acid. Results are expressed as nmol of each fatty acid per mg of cellular protein (mean of four determinations ± 1 SEM). The substrate was added as the sodium salt bound to delipidated albumin at a final concentration of 1 μM and incubated with the cells for 24 h. Radiochromatographic analyses of total cellular lipids were performed as indicated in *Materials and methods.*

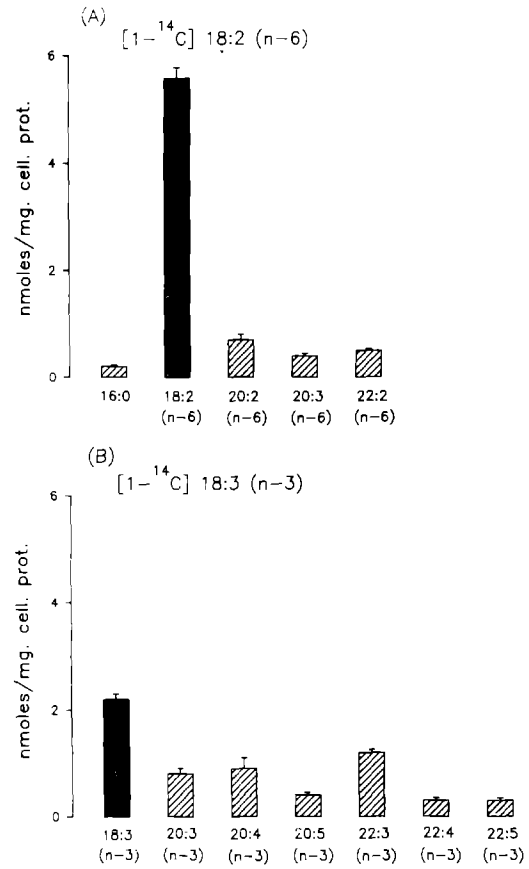

Fig. 5. Metabolic products obtained after incubation of SK-Hep-1 hepatoma cultured cells with [1-^{14}C]linoleic (A) or [1-^{14}C]linolenic (B) fatty acids. Results are expressed as nmol of each fatty acid per mg of cellular protein (mean of four determinations ± 1 SEM). Each substrate was added as the sodium salt bound to delipidated albumin at a final concentration of 1 μM (0.5 μCi/bottle) and incubated with the cells for 24 h. Radiochromatographic analyses of total cellular lipids were performed as indicated in *Materials and methods.*

findings from our experiments with SK-Hep-1 cells indicating a highly active △ 5 desaturase in human hepatoma tissue would likely argue to the contrary. Accordingly, we would reason that, despite the formal possibility of there occurring an ectopic genetic expression at this locus in these malignant, aneuploid cells, it would rather appear more probable that they are instead simply 'breeding true' to the phenotype of their normal counterparts *in vivo*. The significance of our findings, however, can obviously not be fully evaluated until similar data are available from normal hepatocytes. In support of this point of view, we would cite the following examples from the literature indicating – albeit indirectly – that human-liver tissue *in vivo* may well convert 8,11,4-eicosatrienoic acid to arachidonate: Earlier studies by Nakazawa and Goto [54] provided evidence for an inverse correlation between the abundance of arachidonate in the phospholipid fractions of hepatomas and their metastatic invasiveness of other tissues. More recently, Chapkin *et al.* [55] demonstrated that it is the △ 5 desaturase activity of tumor cells that regulates their eicosanoid metabolism. They further suggested that the metastatic capacity of malignant cells might vary in proportion to the magnitude of their endogenous prostaglandin (PG) ratio PG_2/PG_1; which quantity, in turn, is an inverse function of the △ 5 desaturation of eicosa-8,11,14-trienoic acid to arachidonate. Conclusions such as these, tangential to the issue in question here, if proved correct through future studies, would *ipso facto* infer the presence of high △ 5 desaturase levels in the normal (i.e. non-malignant) human hepatocyte. The following results from our laboratory, though somewhat inconsistent with this tumorigenicity model, nevertheless might serve to explain the aforementioned findings with the SK-Hep-1 cells: using another hepatoma line, HTC derived from a (chemically induced) transplantable (Morris) tumor in the rat – we observed a △ 5 desaturase activity that was even higher than that present in normal rat liver; since

such a high △ 5 desaturation capacity in those hepatoma cells is consistent with our present findings with the SK-Hep-1 cells, we were reinforced in our conclusion that the metabolic block in arachidonic acid biosynthesis from linoleate as the precursor must reside in some prior anabolic step. Thus, in order to investigate this possibility, we conducted experiments to measure directly the metabolic fate of [1-^{14}C]linoleic acid: Accordingly, the characterization of the FAME derived from this essential fatty acid precursor revealed that, despite the ample incorporation of this exogenous substrate into cellular lipids (Fig. 2), no detectable arachidonate was formed (Fig. 5A). Though the linoleate chain-elongation products, eicosa-11,14-dienoate and docosa-13-16-dienoate, plus the desaturated derivative, docosa-5,11,14-trienoate, were nonetheless present.

Despite, however, these observations and conclusions with respect to linoleate, we observed a different pattern of anabolic conversion with α-linolenate as a metabolic precursor: here the conversion to the elongated-plus-desaturated product 20:4(8,11,14,17) occurred far more readily, followed by the further anabolism to higher-chain analogues (Fig. 5B). Thus, we would interpret such results as indicating that these cells possess a △ 6 desaturase capacity for α-linolenic acid despite their much diminished ability to perform the analogous reaction with linoleate. Although this discrepancy with respect to the substrate permissivity of the △ 6 desaturase activity in SK-Hep-1 cells might *prima facie* appear a contradiction since a △ 6 desaturase should desaturate both linoleic and α-linolenic acid at the 6,7 position [30, 56–59], these observations may nevertheless be explained in either of two conceivable ways: There may exist two distinct hepatic enzymes in mammals, including man; one for each of the fatty acid substrates, with the hepatoma cells having undergone some genetic or epigenetic change such that the gene encoding the linoleate-specific △ 6 desaturase is impaired in either its function or expression. In this regard, that the mammalian △ 6 desaturase may be a heterogeneous enzyme has recieved some support in the literature [60, 61]. Alternatively, if there does, in fact, exist only a single enzyme and corresponding structural gene, that unique genetic locus in the SK-Hep-1 cells may have become altered in its coding sequence in such a way that its gene product, the △ 6 desaturase, now recognizes linoleate as a poor substrate. Indeed, as evidenced in human-liver microsomes [62] and cell-culture lines [4] – including diploid fibroblasts [63] and endothelial cells [48] – as well as in cultured rat hepatoma cells [12, 14, 30, 60, 64], the putative 'wild-type' △ 6 desaturase itself exhibits a marked preference for α-linolenic acid over linoleate as a substrate so that, given an even further diminution in the conversion of the latter, such as is found here, the level of formation of higher metabolic products becomes prohibitively low for the detection of significant quantities of arachidonate. Consistent with either of these two notions, Dunbar and Bailey have suggested that heteroploid transformed cell lines tend to lose △ 6

Table 2. Distribution of the radioactivity within the lipidic subclasses of SK-Hep-1 hepatoma cells after incubation in the presence of various [^{14}C]fatty acids.

	[1-^{14}C] Fatty acid incubated				
	16:0	18:0	18:2(n-6)	18:3(n-3)	20:3(n-6)
PS	0.7 ± 0.3	3.7 ± 0.5	7.5 ± 0.9	1.5 ± 0.1	0.0
PI	6.9 ± 0.4	12.6 ± 1.6	11.9 ± 0.7	4.3 ± 0.8	38.0 ± 2.0
SM	14.6 ± 0.5	1.3 ± 0.5	0.0	1.1 ± 0.4	0.0
PC	48.6 ± 0.9	44.2 ± 0.7	57.3 ± 2.6	48.6 ± 1.6	29.0 ± 1.9
PE	10.5 ± 0.3	22.4 ± 0.6	16.1 ± 1.4	35.8 ± 1.1	29.1 ± 1.3
PG + CL	7.6 ± 0.4	2.7 ± 0.4	0.0	1.1 ± 0.3	0.0
NEFA	1.1 ± 0.4	4.4 ± 0.4	6.5 ± 0.7	4.8 ± 0.7	1.3 ± 0.5
CHOE	0.7 ± 0.2	5.2 ± 0.9	0.5 ± 0.1	2.7 ± 0.2	1.5 ± 0.3
TAG	9.3 ± 1.4	3.5 ± 0.6	0.0	0.0	0.9 ± 0.1
[^{14}C]Phospholipids/ [^{14}C]Neutral lipids	8.0 ± 0.9	6.6 ± 0.3	13.3 ± 1.0	12.3 ± 0.6	26.0 ± 1.1

Results are expressed as the per-cent distribution of the total radioactivity incorporated into the cells (mean of four independent determinations ± 1 SEM). The cells were incubated in the presence of 1 μM [^{14}C]fatty acid for 24 h and then processed as indicated in the *Materials and methods*. The radioactivity recovered in cellular lipids varied from 95 to 99% of the [^{14}C] added to the medium. '0' indicates a negligible amount (< 0.1%). In all the experiments, traces of radioactivity were detected in the lysophospholipid fraction.

desaturase activity through genetic deletion [65], the most extreme of all mutational possibilities; thus, in a similar fashion, there may occur more conservative modifications of genetic structure at this locus as well.

Table 2 shows the per-cent distribution of radioactivity found in the major cellular lipid subclasses after incubation with different [^{14}C]fatty acids. When [^{14}C]eicosa-8,11,14-trienoic acid (20:3[n-6]) was added to the incubation medium, the radioactivity was found primarily in the PI fraction (ca. 40%) and secondarily in PC and PE (ca. 30%). Other [^{14}C]fatty acids and their metabolites were mainly incorporated into PC (ca. 50%) and to a minor extent into PE and PI. Both the PC and the PE fractions were, however, the major sites of radioactivity derived from α-linolenic acid. As one means of evaluating these data analytically, we calculated for each group the ratio of the total radioactivity found in the phospholipid classes (Table 2, rows 1–6) to that detected in the neutral-lipid species (Table 2, rows 7–9) and present these figures in row 10 of the table. Consistent with what might be expected from the known requirement of polyunsaturates within the phospholipids of cellular membranes for the maintenance of normal fluidity within their lipid phases, this ratio proved to be 2- to 3-fold higher with the three polyunsaturated precursors incubated (Table 2, columns 3–5) than with the two saturated acids examined (Table 2, columns 1 and 2).

Table 3. Per-cent distribution of radioactive fatty acids incorporated in total lipids and lipidic subclasses of SK-Hep-1 hepatoma cells.

Fatty acid incubated	Metabolites	[^{14}C] in total FAME[a]	Lipid subclasses[b]								
			PS	PI	SM	PC	PE	PG + GL	NEFA	CHOE	TAG
[^{14}C] 16:0	16:0	70.0	3.9	5.7	7.3	46.1	16.0	3.3	6.9	0.7	10.1
	16:1	3.7	0.0	0.0	0.0	0.0	27.0	0.1	0.1	13.3	59.5
	18:0	20.5	1.5	4.8	2.9	53.7	23.4	3.9	0.0	1.5	8.3
	18:1	5.8	4.0	10.0	4.0	6.0	63.5	0.4	0.1	4.0	8.0
[^{14}C] 18:0	16:0	1.5	0.3	33.0	0.0	40.0	26.6	0.0	0.0	0.0	0.1
	18:0	81.9	4.9	7.1	1.7	51.2	14.4	3.5	5.8	6.8	4.6
	18:1	16.6	0.1	24.6	5.9	18.8	50.0	0.2	0.0	0.2	0.2
[^{14}C] 18:2 (n-6)	16:0	0.5	0.5	77.7	0.1	0.2	0.2	0.5	0.1	2.7	18.0
	18:2	91.9	7.6	12.2	6.0	55.0	17.1	0.0	0.0	1.0	1.1
	20:2	6.6	6.1	9.0	0.1	78.0	3.0	0.4	1.0	1.2	1.2
	20:3	0.5	0.2	0.3	0.1	60.0	38.4	0.2	0.1	0.4	0.3
	22:2	0.5	0.5	0.2	0.8	15.0	78.5	0.5	0.0	1.5	3.0
[^{14}C] 18:3 (n-3)	18:3	47.5	0.1	1.9	2.1	53.6	28.6	1.1	6.3	3.4	2.9
	20:3	12.3	5.1	20.0	4.1	20.7	25.2	0.1	0.1	14.4	10.3
	20:4*	14.2	2.3	22.4	7.5	24.7	31.0	0.2	0.1	7.9	3.9
	20:5	5.4	0.1	8.0	0.0	30.6	50.0	0.2	0.2	4.4	6.5
	22:3	15.5	0.1	0.5	0.4	26.7	71.3	0.3	0.1	0.4	0.2
	22:4	2.5	0.0	0.0	0.0	52.0	48.0	0.0	0.0	0.0	0.0
	22:5	2.6	0.0	0.0	0.0	1.5	98.0	0.5	0.0	0.0	0.0
[^{14}C] 20:3 (n-6)	16:0	1.0	0.4	28.0	0.1	20.0	1.5	0.0	7.3	2.7	40.0
	20:3	64.3	0.5	35.0	0.7	28.0	31.0	1.0	1.6	1.6	0.6
	20:4	34.7	0.4	43.0	0.1	29.9	24.0	0.3	0.6	1.4	0.3

[a] Results are expressed as the percentage distribution of the total radioactivity added to the incubation media (means of two independent analyses, with variation among replicates not greater than 10%).

[b] Per-cent contribution of each lipid subclasses to the radioactivity found in total lipids. For the key to the abbreviations used, c.f. *Materials and methods.*

Percentages less than 0.1% are shown as '0.0'.

* Corresponds to the sum of 20:4△(8,11,14,17) + 20:4△(5,11,14,17) fatty acids.

The cells were incubated for 24 h in the presence of the indicated fatty acid (1 μM) complexed to delipidated albumin and then processed as indicated in *Materials and methods.*

The per-cent distribution of [^{14}C]fatty acids taken up by SK-Hep-1 cells as well as the metabolic products derived from these acids and incorporated into both total lipids and the major lipid subclasses is presented in Table 3. [^{14}C]Palmitic acid was preferentially incorporated into phospholipids although significant amounts were also found in neutral lipids. Only a minor fraction of the total cellular radioactivity was present in the NEFA, with the major fatty acid representative among these being the incubated precursor itself since virtually none of its metabolic derivatives were present in significant amounts in this fraction. This pattern also held true for the other [^{14}C] fatty acids incubated; with sole exception of 18:2(n-6), where the major labelled NEFA species was the elongation product 20:2. Within the total FAME, significant amounts of 16:0 were converted to 16:1, elongated and further desaturated to 18:1 (Table 3, column 2; Fig. 3A). These metabolic products were preferentially incorporated as 18:0 in PC and as 18:1 in PE (Table 3). A similar distribution of precursor and monodesaturated product between PC and PE was observed when the cells were incubated in the presence of [^{14}C]stearic acid, though in the former instance the TAG fraction constituted a major site for the incorporation of palmitoleic acid. When the hepatoma cells were incubated with [^{14}C]linoleic acid, the precursor was mainly incorporated into PC unaltered. Modest amounts of 18:2 were, however, elongated to 20:2 and 22:2 and desaturated to 20:3. Among these products, the two 20-carbon species were located in that same fraction, whereas the major site for the incorporation of 22:2 was the PE subclass (Table 3). By contrast, as indicated earlier, no labelled arachidonate was detected (Fig. 5A). When SK-Hep-1 cells were incubated in the presence of [^{14}C]α-linolenic acid, the substrate was elongated to the 20:3 and 22:3 and desaturated to the 20:4 and 20:5 fatty acids (Fig. 5B). The distribution of these acids among lipid subclasses was somewhat complex. Both the PC and the PE fractions actively incorporated all metabolic products from α-linolenic acid. Polyunsaturated fatty acids of 20 and 22 carbons either were present in a greater proportion in PE or were distributed similarly between PC and PE. In this regard, docosatetraenoic acid was the exceptional member of the (n-3) family in that it was found almost entirely (98%) in PC. Within the PI fraction, linolenic acid was poorly incorporated itself, but its 20:3 and 20:4 products comprised the predominant radiolabelled species. This finding was not observed when the cells were incubated in the presence of linoleic or saturated fatty acids, where the labelled precursor in question contributed significantly to the PI fraction. With respect to the distribution of [^{14}C]fatty acids derived from labelled eicosatrienoic acid, although the PI fraction was the major site for the incorporation of this substrate and its metabolic product, arachidonic acid; considerable amounts of 20:3 and 20:4 were also found in the PC and PE subclasses. The parallel pattern of incorporation of 20:3 and 20:4 into specific phospholipid pools suggests that both fatty acids may be bound at the usual β-position in these lipid moieties. The facile conversion of 20:3 to 20:4 was previously observed in other human hepatoma cells [60] and in HTC cells [14]. In an experimental situation this metabolic characteristic could be employed to effect a deliberate enrichment of those specific phospholipid subclasses with arachidonic acid by incubating the SK-Hep-1 cells with 20:3 instead of 20:4. Small amounts of eicosatrienoic acid were converted into palmitic acid by β-oxidation and *de-novo* synthesis (Table 3; Fig. 4), with this saturated fatty acid being found mainly in TAG fraction (Table 3). By contrast, the principal loci of incorporation of the labelled palmitate derived from other radioactive precursors in this same manner among the various phospholipid subclasses were: for [^{14}C]18:0, PI plus PC plus PE and, for [1-^{14}C]18:2, PI nearly exclusively; the carbon from 18:3 was, however, apparently not recycled in such a fashion in these cells (Table 3). From the results presented in this table, we conclude that there are clearly marked differences among the various polyunsaturated fatty acid precursors with respect to their pattern of incorporation into the different phospholipid subclasses

In conclusion, the observations documented here argue cogently that SK-Hep-1 human hepatoma cells constitute a useful cell-culture model for the study of polyunsaturated fatty acid metabolism under different experimental conditions since the presence in these cells of all but one of the relevant elongases and desaturases is unquestionable. These findings and the model system we have hereby characterized are of considerable significance, especially in view of the difficulties in obtaining samples of human liver for investigation. The complete veracity of these findings can, however, only be achieved when similar studies are eventually performed directly on normal human hepatocytes.

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