BIOSYNTHESIS OF POLYUNSATURATED FATTY ACIDS FROM THE LINOLEIC ACID FAMILY IN CULTURED CELLS

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INTRODUCTION

HTC cells (designated HTC for hepatoma tissue culture) were derived from the ascites form of a rat-carried Morris hepatoma 7288 C (Thompson et al, 1966). Previous studies have revealed that cells of this kind are able to desaturate and elongate fatty acids. In this respect it was demonstrated that culture HTC cells preserved the ability to desaturate stearic to oleic acid (Δ9 desaturase), α-linolenic acid to octdeca-6,9,12,15-tetraenoic acid (Δ6 desaturase), and eicosa-8,11,14-trienoic acid to arachidonic acid (Δ5 desaturase) (Alaniz et al, 1975). They are also able to convert α-linolenic acid to higher homologs with 5 and 6 double bonds by desaturation and elongation reactions. These results also proved the existence of Δ4 desaturase activity (Alaniz et al, 1975). However, it was shown that the cell cultured in Swim's medium supplemented with serum possessed a very low capacity to convert labeled linoleic acid of the medium to arachidonic acid. Nevertheless, these tumor cells readily converted eicosa-8,11,14-trienoic acid to arachidonic acid (Alaniz et al, 1975; Gaspar et al, 1975). Therefore, the difficulty of these cells to synthesize arachidonic acid from linoleic acid may reside in a step previous to the Δ5 desaturation of eicosa-8, 11, 14-trienoic acid. This step could be a Δ6 desaturation of linoleic acid. The discrepancy between the biosynthesis of linoleic and α-linolenic acid series is difficult to explain considering that the same enzyme desaturates

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* The authors are members of the Carrera del Investigador Científico of the Consejo Nacional de Investigaciones Científicas y Técnicas.

N. G. Bazán et al. (eds.), *Function and Biosynthesis of Lipids*

linoleic and α-linolenic acids in Δ6 position (Brenner and Peluffo, 1966) (Brenner, 1971; Brenner, 1974) (Ninno et al, 1974).

For this reason it was important to investigate the routes of arachidonic acid synthesis from labeled linoleic acid and the possible incorporation of labeled acids in cell lipids.

MATERIAL AND METHODS

HTC 7288 C cells were maintained and grown at 37°C in confluent layers attached to glass on Swim's 77 medium supplemented with 10% calf serum (Thompson et al, 1966) (Alaniz et al, 1975). After 48 h, when the bottles contained approximately 5×10^6 cells, the medium was changed for 10 ml of S 77 medium without serum, to which 5 nmoles of $|1\text{-}^{14}C|$ linoleate (61 mC/mmole, 99 % radiochemical purity) per bottle was added, and the cells were maintained in this medium for 24 h. The acid was added as sodium salt bound to defatted albumin (Goodman, 1957) according to Spector et al (1965). After the end of the incubation period the attached cells were washed with 0.85 % NaCl, removed, and suspended in the same saline solution. An aliquot of the suspension was used to determine the amount of cellular protein by the method of Lowry et al (1951) and the rest was centrifuged. The lipids were extracted by the procedure of Folch et al (1957) and separated by thin layer chromatography (TLC). The solvent mixture used was chloroform-methanol-water 65:25:4 v/v/v. An aliquot of the corresponding fraction of the samples was counted in a Packard Tricarb Scintillation counter. Other aliquots were esterified. The distribution of the radioactivity between the fatty acids was determined by gas liquid radiochromatography (Alaniz et al, 1976). The labeled methyl esters were identified by equivalent chain length determination and comparison with authentic standards. The fatty acid composition of serum, HTC cells and lipid fractions of culture cells was analyzed by gas liquid chromatography. The specific radioactivities for linoleic and arachidonic acids were calculated with those data after measuring the radioactivity in an aliquot in which the mass distribution of the fatty acids had previously been determined by gas liquid chromatography in the presence of an internal standard of eicosaenoic acid.

RESULTS AND DISCUSSION

The fatty acid composition of serum and different lipid fractions of HTC cells is shown in Table 1. The pattern is similar to that of the serum used in the preparation of the medium, except for changes in oleic, linoleic and α-linolenic acids. Whereas the relative amount of oleic acid was higher in the cells than in the

Table 1. Fatty acid composition of serum and different lipid fractions of HTC cells.

Cells grown for 2 days with Swim's medium and calf serum were incubated with |1-^{14}C| linoleic acid for 24 h in a new Swim's medium without serum. Means of three bottles ± 1 SEM. Total fatty acids (TFA), phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), free acids (FA) and neutral lipids (NL) fractions.

Fatty acids	Serum	Lipid fractions				
	%	TFA %	PC %	PE %	FA %	NL %
14:0	1.3 ±0.1	1.9 ±0.2	3.6 ±0.2	0.2 ±0.03	1.9 ±0.4	3.3 ±0.1
16:0	27.8 ±1.2	25.0 ±1.5	46.2 ±1.3	12.7 ±1.4	23.6 ±4.2	26.9 ±2.3
16:1	3.7 ±0.4	4.5 ±0.6	3.3 ±0.1	2.1 ±0.3	4.9 ±0.6	3.9 ±1.3
18:0	21.9 ±1.1	17.6 ±0.3	6.8 ±0.4	27.0 ±1.9	22.1 ±3.5	10.9 ±0.6
18:1	22.6 ±1.5	41.8 ±2.2	36.1 ±0.05	46.7 ±1.0	35.9 ±3.7	45.8 ±2.8
18:2	15.8 ±0.5	5.9 ±0.3	2.8 ±1.4	4.9 ±1.1	9.5 ±1.9	7.9 ±0.8
18:3	4.4 ±0.2	tr.	tr.	tr.	tr.	tr.
20:4	2.5 ±0.4	3.3 ±0.2	1.0 ±0.1	6.5 ±0.5	2.1 ±0.8	1.3 ±0.03
Ratio 20:4/ 18:2	0.16	0.56	0.36	1.33	0.22	0.16

serum the percentages of linoleic and α-linolenic acid were lower. The low levels of linoleic and arachidonic acid in HTC cells could result from a difficulty of the cells to absorb linoleic acid readily, a very active catabolism of the acid, or an absence of low activity of any enzyme involved in the synthesis of polyunsaturated acids. The ready absorption of linoleic acid was proved by Gaspar

Table 2. Distribution of radioactivity in the lipids of HTC cells after incubation with |1-^{14}C| linoeic acid.

Results are the means of 3 bottles expressed as percentage of total radioactivity on the plate ± 1 SEM. Radioactivity on the rest of the plate makes up to 100 %. Experimental conditions as in Table 1.

Lipid fractions	Labeling distribution %
Phosphatidyl choline	33.0 ± 0.5
Phosphatidyl ethanolamine	22.8 ± 1.0
Free acids	14.5 ± 0.3
Neutral lipids	19.1 ± 0.9

et al* measuring the incorporation of labeled linoleic acid from the medium to the cell. The incorporation increased with time of incubation and the amount of acid in the medium. The same authors showed that the saturation curves of incorporation of linoleic, oleic and α-linolenic acids were similar. However, the plateau of the curve was lower for oleic and linoleic acids than for α-linolenic acid. Besides, linoleic acid is catabolized very little by the cells, since Gaspar et al* showed that labeled acid in the medium is preferentially incorporated rather than converted to CO_2.

The amount of arachidonic acid in all cell lipids is low. The highest proportion is found in the phosphatidyl ethanolamine fraction. Moreover, the arachidonic:linoleic acid ratio is the highest in the phosphatidyl ethanolamine fraction. The lowest ratios correspond to free and neutral lipid fractions. These results are similar to those reported previously (Wood, 1973; Wood and Falch, 1973). Therefore it is possible to admit that the fatty acid composition of HTC cells is regulated in an active way and the low content of arachidonic acid is not necessarily the only consequence of low linoleic acid uptake from the medium. Besides, the low content of linoleic and arachidonic acids is not apparently due to a preferential oxidation to CO_2.

The incorporation of labeled linoleic acid of the medium in

* Gaspar G., Alaniz M. J. T. de and Brenner R. R. Mol. and Cell Biochem. (sent for publication).

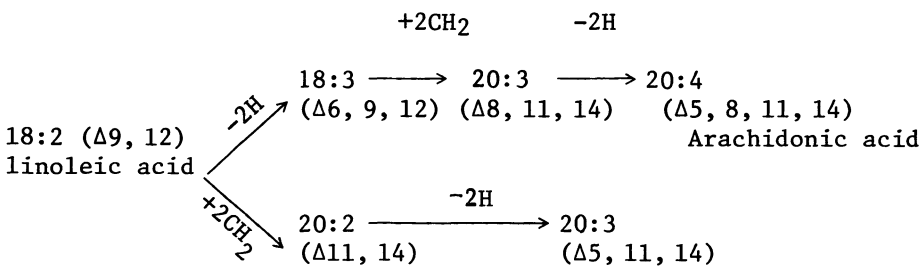
Table 3. Pattern of labeled fatty acids in HTC cells incubated in the presence of [1-^{14}C] linoleic acid

Results are the percentages of total measurable radioactivity. They are the means of three bottles ± 1 SEM. Experimental conditions and symbols as in Table 1.

Fatty acids	Lipid fractions % radioactivity distributions				
	TFA	PC	PE	FA	NL
18:2 (Δ9, 12)	84.3 ±1.7	79.0 ±4.3	83.9 ±0.9	100.0	91.0 ±0.6
20:2 (Δ11, 14)	5.8 ±0.6	9.1 ±2.2	4.8 ±0.7		9.0 ±0.6
20:3 (Δ5, 11, 14)	2.8 ±0.3	4.2 ±0.9	2.9 ±0.3		
20:3 (Δ8, 11, 14)	2.9 ±0.3	3.8 ±0.7	3.3 ±0.2		
20:4 (Δ5, 8, 11, 14)	4.2 ±0.8	3.9 ±0.9	5.1 ±0.3		

cell lipids is shown in Table 2. It shows that phospholipids incorporated the highest percentage of the radioactivity. This selective concentration of unsaturated acids in the phospholipids helped to investigate the products of linoleic acid conversion by analysis of the fatty acid composition of the labeled products. In Table 3 it is possible to recognize that the free acid fraction of the cell lipids contained only labeled linoleic acid. In neutral lipids the elongation product of linoleic acid: 20:2 (Δ11, 14) was also found. In phosphatidyl choline and phosphatidyl ethanolamine, not only were linoleic acid, 20:2 (Δ11, 14) and arachidonic acid incorporated, but also two 20:3 acids. These 20:3 acids were identified as 20:3 (Δ5, 11, 14) and 20:3 (Δ8, 11, 14) acids by comparison of the retention times with standards and with the data published by Ullman and Sprecher (1971).

The acids detected suggest the existence of two metabolic pathways in the conversion of linoleic acid to higher homologs. These pathways are equivalents to the routes used by HTC cells to desaturate and elongate α-linolenic acid (Alaniz et al, 1976). One of the routes is initiated by a Δ6 desaturation and the other by an



elongation. In the first route, through a Δ6 desaturation linoleic acid is converted to γ-linolenic acid which is rapidly elongated to 20:3 (8, 11, 14) and then desaturated in Δ5 to arachidonic acid. The elongation of linoleic acid in the second route leads to 20:2 (11, 14). This acid would not be desaturated to 20:3 (8, 11, 14) acid since it has been reported that a Δ8 desaturase is absent in liver (Alaniz et al, 1976; Sprecher et al 1975). However, 20:2 (11, 14) acid would be a substrate for the Δ5 desaturase and 20:3 (5, 11, 14) acid is so formed. This last pathway has been described in normal rat liver to occur in very specific conditions (Ullman and Sprecher, 1971).

The crescent importance of the elongation route that converts linoleic acid to 20:2 (Δ11, 14) and 20:3 (Δ5, 11, 14) acid and also converts α-linolenic acid to 20:3 (Δ11, 14, 17) and 20:4 (Δ5, 11, 14, 17) in HTC cells compared to normal tissue may be easily explained by a decrease of the activity of the Δ6 desaturase/elongating enzyme ratio in the transformed cells and, besides, by a relatively high Δ5 desaturation activity.

The Δ6 desaturase is a microsomal enzyme but the elongating enzymes may be found in microsomes and mitochondria. Therefore it is possible that the microsomes of HTC cells have been altered. It is suggestive that we have been unable as yet to obtain active microsomes from the cells in spite of using different mild procedures.

Experiments carried out by Dunbar and Bailey (1975) suggest that generally heteroploid or transformed cell lines lose Δ6 desaturase by dilation. HTC cells cannot be included in this generalization since they possess a Δ6 desaturase. However, Δ6 desaturation activity for linoleic acid is extremely low compared to α-linolenic acid.

Unsaturated acids are incorporated in a normal way in HTC cell phospholipids (Tables 1 and 2). Arachidonic acid would be preferentially incorporated in phosphatidyl choline and phosphatidyl ethanolamine. The measurement of the specific radioactivity of

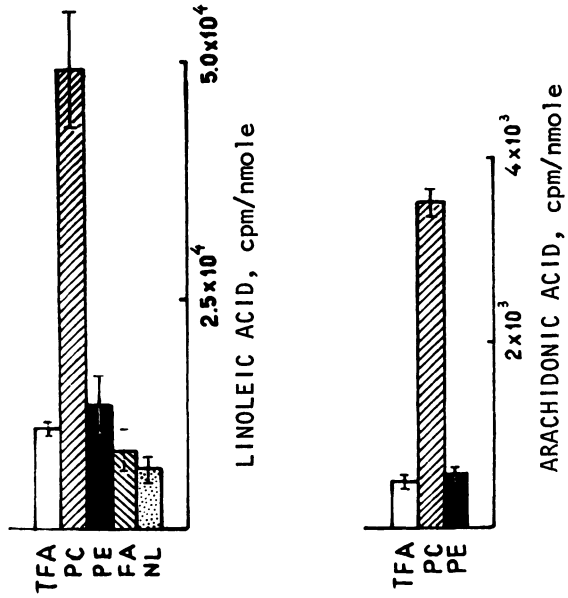


Fig. 1. Specific radioactivities of linoleic and arachidonic acids in different lipid fractions of HTC cells incubated in the presence of labeled linoleic acid.

Results are the means of three incubations. Vertical lines represent 1 SEM. Details of experimental conditions described in material and methods. Symbols as in Table 1.

linoleic and arachidonic acids in the different lipid fractions (Fig. 1) indicates that both acids present the highest values in phosphatidyl choline. However, the highest percentage of arachidonic acid was found in phosphatidyl ethanolamine (Table 1). Therefore it is reasonable to assume that labeled arachidonic acid is diluted in phosphatidyl ethanolamine by unlabeled endogenous acid. Both labeled acids would be incorporated in both phospholipids preferentially by an interchange reaction. Therefore, these results do not help to explain the specifically low desaturation activity for linoleic acid.

CONCLUSIONS

HTC cells incubated in Swim's 77 medium with 10 % calf serum contain small amounts of linoleic and arachidonic acids. Labeled linoleic acid of the medium is incorporated in phospholipids and neutral lipids. It is converted to higher homologs following two

routes. One route is 18:2 (Δ9, 12) ⟶ 18:3 (Δ6, 9, 12) ⟶ 20:3 (Δ8, 11, 14) ⟶ 20:4 (Δ5, 8, 11, 14). The other route is 18:2 (Δ9, 12) ⟶ 20:2 (Δ11, 14) ⟶ 20:3 (Δ5, 11, 14). These products are also incorporated in phosphatidyl choline and phosphatidyl ethanolamine. Linoleic acid Δ6 desaturation is very low compared to α-linolenic acid, whereas Δ5 desaturation of 20:3 (Δ8, 11, 14) to arachidonic acid is very high.

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