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# THE ROLE OF Δ-6- AND Δ-9-DESATURASE IN THE FATTY ACID METABOLISM OF HEPATOMAS WITH DIFFERENT GROWTH RATE.

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## Summary

The fatty acid composition of microsomal membranes from Morris hepatomas 9618A, slow growing, and 3924A, fast growing, confirm the higher content in oleic acid and the loss of PUFAs of the tumours with respect to controls. The specific activities of Δ-9-desaturase indicate alternative metabolic pathways for the increased production of oleic acid in the two hepatomas. The Δ-6-desaturase activity is much lower in tumours than in controls. However the loss of PUFAs found in tumours seems to be mostly due to a low content in linoleic acid.

## Introduction

The desaturase mechanism has been studied extensively in microorganisms, plants, and especially animals.

Fatty acids of dietary or endogenous origin may be extensively modified in the animals by combination of desaturation and chain elongation. These two processes can be used alternately to produce longer chain unsaturated fatty acids; the desaturation steps are slow and rate limiting, whereas the elongation steps are rapid. In animals, the double bonds can be introduced at the Δ-4, Δ-5, Δ-6 and Δ-9 position, with a cis configuration.

The introduction of the Δ-9 double bond by the microsomal fraction of liver requires stearic (18:0) or palmitic acid (16:0) as substrates, and generates respectively oleic acid (18:1 n-9) and palmitoleic acid (16:1 n-7), which can be further elongated to cis vaccenic acid (18:1 n-7). The introduction of the Δ-6 double bond requires linoleic (18:2 n-6) or α-linolenic acid (18:3 n-3) as substrates, and generates polyunsaturated fatty acids (PUFAs) of the n-6 and n-3 series, respectively.

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PUFAs are required for the normal structure of all cellular membranes; they are precursors of a whole series of second messengers generally known as the eicosanoids (1) and they are required for the normal transport of cholesterol around the body (2). Very recently it has been recognized that PUFAs have a whole range of other effects including interactions with almost all second messenger systems and modulation of the behaviour of most membrane bound proteins including receptors, ion channels and ATPases (3).

All intact cells contain large amount of PUFAs; yet there is substantial evidence that cancer cells are depleted in such fatty acids (1, 4-8), and their reduction is greater, the faster growing is the tumour (4, 6, 9, 10).

The reason for the reduced levels of PUFAs in many malignant tissues is not certain. The observed changes could be caused by reduced formation of these fatty acids from the parent linoleic and α-linolenic acids, or by an increased destruction or both.

Unexplained as well is the reason why concomitant with the decrease in PUFAs, a substantial increase in oleic acid has always been observed in Morris hepatoma microsomes (4, 6, 7).

Studies were then conducted to assess whether Δ-6- and Δ-9-desaturases are involved in this peculiar fatty acid metabolism of tumours.

## Materials and methods

[1-$^{14}$C] palmitic acid (59 mCi/mmol), [1-$^{14}$C] stearic acid (51 mCi/mmol), and [1-$^{14}$C] linoleic acid (53 mCi/mmol) were purchased from Amersham Life Science (Amersham, UK); NADH, coenzyme A (CoA), ATP, and 2',7'-dichlorofluorescein were obtained from Sigma Chemical Co. (St. Louis, MO). All unlabelled fatty acids were obtained from Nu-Check Prep. (Elysian, MN). Tris was obtained from Sigma Chemicals Co. (St. Louis, Mo), KCl from Merck (Darmstadt, Germany). All chemicals and solvents were of the highest analytical grade.

**Microsome preparation.** Morris hepatoma 9618A (slow growing) and 3924A (fast growing) were transplanted subcutaneously into both hind legs of inbred rats of the Buffalo and the ACI/T strains, respectively, grown and isolated as previously reported (11, 12). Total microsomes were obtained as a pellet from the postmitochondrial supernatant by centrifugation at 105,000 x g for 90 min. Total microsomes were washed free of sucrose twice in 0.15 M KCl at 105,000 x g for 30 min. and finally suspended in 50 mM Tris-HCl, 0.15 M KCl, pH 7.5.

**Desaturase assays.** The activity of the Δ-6-desaturase (D6D) enzyme was measured as previously described (13, 14). The reaction mixtures, containing 5 mM $MgCl_2$, 50 μM CoA, 2 mM ATP, 1mM NADH, 50 mM phosphate buffer (pH 7.4), 40 μM [1-$^{14}$C]linoleate and approx. 2.5 mg microsomal protein in a total volume of 1 ml, were incubated in a shaking water bath (37°C) for 20 min.

The activity of Δ-9-desaturase (D9D) enzyme was determined by measuring the conversion of [1-$^{14}$C] palmitic acid to [1-$^{14}$C] palmitoleic acid and of [1-$^{14}$C] stearic acid to [1-$^{14}$C] oleic acid. Reactions were started by adding 2.5 mg microsomal protein to tubes

containing 50 μM radiolabelled palmitic or stearic acid, 1.5 mM ATP, 1 mM NADH, 3 mM $MgCl_2$, 60 μM CoA, 50 mM phosphate buffer, as reported by De Antueno et al.. (15). Incubations were performed for 20 min in a shaking water bath (37°C).

The reactions were stopped by adding 4 ml of chloroform/methanol (1:1 v/v) and, after the addition of 2 ml of chloroform, lipids were extracted according to Folch et al. (16). Lipids were methylesterified with methanol/hydrochloric acid (5% by vol), as reported by Stoffel et al. (17). Fatty acid methyl esters were separated on thin layer chromatography plates coated with silica gel G, impregnated with 10% (w/v) $AgNO_3$, and identified by comparison with authentic standards. Plates were developed in hexane/diethyl ether (8:2 v/v), and spots made visible under ultraviolet light by spraying with 2',7'-dichlorofluorescein (0.2%, w/v in ethanol). The spots were scraped off into scintillation vials and immediately counted in 10 ml of liquid scintillation mixture (Instagel, Packard) using a Packard liquid scintillation spectrometer. Enzyme activity is expressed as pmol of the radioactive fatty acid converted into the product per min per mg microsomal protein.

Fatty acid analysis. Lipids were extracted according to Folch et al. (16) and methyl esterified with methanol/hydrochloric acid (5% by vol), as reported by Stoffel et al. (17). The fatty acid composition of microsomal total lipids was determined by gas chromatography (Carlo Erba mod. 4160) using a capillary column (SP 2340, 0.10-0.15 μM i.d. ) at a programmed temperature (160-210°C, with an 8°C/min gradient), as previously reported (18).

**Results and discussion**

In our previous studies on the lipid composition of Morris hepatomas microsomes, liver microsomes from rats of the ACI/T strain were used as control. In this studies we further used liver microsomes from the Buffalo strain as control for the hepatoma 9618A. The results, reported in Table I, show noticeable differences in the membrane fatty acid composition of the two control strains and confirm a significant reduction in PUFAs content in microsomes derived from the two hepatomas with respect to controls.

The unsaturation index is lower in tumour microsomes than in controls, and it is lowest in the fast growing 3924A hepatoma (table I). In microsomes from both slow growing 9618A and fast growing 3924A Morris hepatomas we find a significant reduction of the specific activity of D6D on linoleic acid (Table II). However linoleic acid level are not increased as they should (5), while arachidonic acid levels are always higher then those of the precursor, but for H3924A (Table I). Transformed and malignant cell lines have been reported to show loss of D6D or greatly reduced activity of the enzyme (19, 20). In the light of our results further studies are requested to clarify this point.

It is instead apparent that the level of 18:2 n-6 is low in both hepatomas. Since the rats have been fed the same diet, the low microsomal content in 18:2 n-6 can be due either to insufficient absorption or to destruction by peroxidation. Whichever the cause, the low content of linoleic acid in H9618A and H3924A microsomal fractions seems to be of paramount importance in causing the membranes to have a low level of PUFAs.

**Table I.** Total fatty acid composition (mol/100 mol) of control and tumor microsomes.

| Fatty acid | Buffalo (n=3) | H9618A (n=3) | ACI/T (n=3) | H3924A (n=3) |
|---|---|---|---|---|
| 16:0 | 17.24±2.17 | 26.02±0.48° | 23.21±0.76 | 28.57±1.65° |
| 16:1 n-7 | 2.05±0.81 | 9.66±0.49* | 1.48±0.20 | 4.10±0.35* |
| 17:0 | 0.77±0.08 | 0.59±0.15 | 0.83±0.05 | 3.38±0.46* |
| 18:0 | 26.67±0.58 | 8.49±0.37* | 18.12±0.68 | 15.57±0.15° |
| 18:1 n-9 | 8.05±0.71 | 26.48±0.09* | 10.20±0.14 | 26.56±1.95* |
| 18:2 n-6 | 12.60±0.68 | 8.37±0.19* | 18.71±0.40 | 8.59±1.26* |
| 20:4 n-6 | 23.05±1.83 | 13.96±0.60* | 19.23±0.65 | 8.04±0.50* |
| 20:5 n-3 | 0.55±0.05 | 0.47±0.05 | 0.41±0.23 | 0.54±0.33 |
| 22:4 n-6 | 1.10±0.21 | 1.44±0.40 | 0.89±0.14 | 2.28±0.22* |
| 22:5 n-6 | 0.21±0.11 | 0.27±0.16 | traces | traces |
| 22:5 n-3 | 1.04±0.05 | 1.32±0.07° | 1.86±0.13 | 0.60±0.36° |
| 22:6 n-3 | 6.67±0.56 | 2.81±0.05* | 4.07±0.25 | 1.33±0.30* |
| U.I. | 179.9±8.6 | 140.3±5.4° | 165.7±6.2 | 102.8±4.9* |

Total fatty acid composition (as methyl esters) was performed as reported in the Materials and Method section. U.I.: unsaturation index. The unsaturation index was calculated by taking the concentration of each fatty acid, multiplying it for the number of its double bonds, and adding up all the scores for all the fatty acids. The number of samples examined is reported in brackets. Data are means ± S.D. Statistical analysis was by the one way analysis of variance comparing each Morris hepatoma to the corresponding control: ° $p<0.01$, * $p<0.001$.

This lack of substrates is recognized to be one of the main reasons why tumour cells are resistant to lipid peroxidation (see ref. 10 for a review). The protection of cancer cells for lipid peroxidation can also be attributed to a high level of membrane antioxidants, particularly vit. E (21, 22). As well as being rich in vitamin E, malignant cells are often rich in oleic acid (4, 5, 23). In microsomes from Morris hepatomas we confirmed a very significant increase in the relative molar content of oleic acid (table I). It is not always appreciated that, for

**Table II.** Desaturase activities (pmol/min/mg microsomal protein) in control and tumor microsomes.

| | D9D on palmitic acid | D9D on stearic acid | D6D on linoleic acid |
|---|---|---|---|
| Buffalo | 95±5 (n=3) | 192±23 (n=8) | 511±21 (n=4) |
| H9618A | 96±5 (n=3) | 260±27* (n=8) | 112±4* (n=4) |
| ACI/T | 60±2 (n=4) | 87±18 (n=13) | 183±16 (n=6) |
| H3924A | 155±11* (n=4) | 53±19* (n=13) | 54±0.5* (n=4) |

Desaturating activities were measured as reported in the Method section. Each value represents the mean ± SD of the number of microsomal preparations reported in brackets. Statistical analysis was by the one way analysis of variance: *$p<0.001$ vs the corresponding control microsomes.

reasons which are not fully understood, oleic acid is an extremely effective antioxidant and is found in high concentrations in rapidly dividing tissues (23).

The activity of D9D on stearic acid (table II) in the microsomes of 9618A Morris hepatoma can explain the higher level of oleic acid found in this tumour; in fact D9D activity is significantly higher than in control microsomes. On the contrary, the activity of D9D on stearic acid is extremely low in microsomes from 3924A hepatoma. Otherwise, microsomes from 3924A hepatoma show a very high D9D activity on palmitic acid; the result of this increased activity could be increased levels of 16:1, which can be further elongated to 18:1. D9D activity on palmitic acid in 9618A microsomes is not significantly different from the activity found in microsomes from Buffalo rats.

The increase in 18:1 n-9 has been reported not only in cancer, but whenever a situation of n-6 and n-3 EFA depletion occurs (4, 5). Our previous observation that in the hepatomas membranes molecular order increases and fluidity decreases with respect to control, in a fashion which is inversely related to the growth rate and malignancy of the tumour (10), supports the point of view that the increased ratio 18:1/18:0 in tumours reflects

an inadequate attempt to compensate for the loss of PUFAs and therefore of fluidity, attempt in which H9618A is more successful than H3924A.

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