EFFECT OF "FASTING" AND DIFFERENT CONCENTRATIONS OF GLUCOSE ON α-LINOLENIC ACID METABOLISM IN HTC CELLS. CORRELATION WITH THE ULTRASTRUCTURAL STUDY

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Summary

HTC cells are able to convert α-linolenic acid into higher homologs by desaturating and elongating reactions. When the cells were cultured in a Krebs Ringer bicarbonate solution ("fasted cells") a decrease in both biosynthetic reactions took place. "Refeeding" the cells with Swim's 77 medium without glucose enhanced the biosynthesis of polyunsaturated fatty acids from α-linolenic acid family, but when glucose was added to the medium, α-linolenic acid was preferably elongated rather than converted into eicose-pentaenoic acid.

The ultrastructural study revealed HTC cells with a simple cytoplasmic organization, showing little evidence of their origin from hepatocytes. The cells cultured in a complete medium appeared well preserved and were similar to those "fasted" for 12 hours and "refed" for another 12 hours using Swim's 77 medium without serum. The amount of glucose in the medium plays a role in preserving the cell structure. This effect does not occur if glucose is added in the absence of aminoacids and vitamins.

Introduction

It has been established that lipids containing particularly polyunsaturated fatty acids of the α-linolenic series, are involved intimately in cell structure and function, especially at the membrane level. Besides, it is known that there exist important differences in lipid metabolism[1] between normal and neoplastic tissues.

We have previously demonstrated that a hepatoma tissue culture (HTC) cell line, derived from a Morris minimal deviation hepatoma, was able to desaturate and elongate α-linolenic acid[2]. In this cell type, it was possible to show that α-linolenic acid was converted into higher homologs by a desaturating route that synthetized mainly 18:4 (Δ6, 9, 12, 15) 20:4 (Δ8, 11, 14, 17) and 20:5 (Δ5, 8, 11, 14, 17) acids and by an elongating route that produced 20:3 (Δ11, 14, 17) and 20:4 (Δ5, 11, 14, 17) acids. We could also demonstrate the effect of different carbon sources upon the metabolism of α-linolenic acid in HTC cells. "Fasting" decreased both biosynthetic routes, whereas glucose reactivated only the elongating pathway. Aminoacids produced an increase in the conversion of α-linolenic acid into eicose-5, 8, 11, 14, 17-pentaenoic acid[2]. These results are independent of hormonal influence. The effect of glucose and "fasting" upon the desaturation reaction was similar to that obtained in *in vivo* experiments. PELUFFO et al.[3] have shown that a glucose diet decreased the desaturation of linolenic acid into α-linolenic acid in nonfasted animals. GOMEZ DUMM et al.[4] have assumed that 96 hr of fasting cause a decrease of linolenic acid desaturation in rats.

The purpose of the present paper was to study the effect of "fasting" and different

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Table 1
Effect of "fasting" and different concentrations of glucose on the conversion of 1[^{14}C]α-linolenic acid into higher homologs, in HTC cells[a].

Fatty acids	Label distribution % (b)					
	Complete medium	24 hr "fasted"	Cells "refed" with S-77 medium			"refed with glucose
			Without glucose	Plus glucose 0.3 g ‰	Plus glucose 1.0 g ‰	
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
18:3 (Δ9, 12, 15)	58.6±1.9	90.4±2.3	73.3±1.0	87.1±2.0	88.9±2.3	86.0±1.6
20:3 (Δ11, 14, 17)	5.9±0.4	0.3±0.02	3.6±0.7	2.5±0.3	2.8±0.3	6.2±0.5
20:4 (Δ5, 11, 14, 17)	4.9±0.7	1.1±0.3	7.8±1.3	2.0±0.05	1.8±0.1	4.2±0.1
20:4 (Δ8, 11, 14, 17)	5.8±0.5	1.2±0.4	4.1±0.3	3.4±0.8	2.9±0.3	1.9±0.05
20:5 (Δ5, 8, 11, 14, 17)	21.3±1.1	7.0±1.6	8.6±1.6	2.8±0.3	1.4±0.05	1.7±0.04

(a) 5 million cells per bottle incubated 24 hr with 2.5 nmol 1[^{14}C]α-linolenic acid in Swim's 77 medium.
(b) Percentages represent the mean 1 SEM of 3 determinations. Experimental conditions for groups 1 to 6 are described in "material and methods".

concentration of glucose upon the metabolism of α-linolenic acid in HTC cells, trying to correlate the biochemical results with the ultrastructural findings.

Material and Methods

Radiochemicals:
1-[^{14}C]α-linolenic acid (47.1 mC/mmol, 99% radiochemically pure) was obtained from The Radiochemical Centre, Amersham, England.

Culture conditions:
The cell line used came from an ascites tumor which had been derived from a solid hepatoma (#7288 c)[5]. These cells were maintained and grown at 37 °C in confluent layer attached to glass on Swim's 77 medium, supplemented with 10% calf serum[5] using conventional sterile conditions as described previously[6].

Assay procedure
The cells were detached from the glass surface with the aid of 0.25% trypsin solution. They were suspended in culture medium and counted in a hemocytometer. Aliquots (2.5×10^6 cells per bottle) were cultured in 70 cm^2 flasks with 20 ml Swim's 77 medium supplemented with 10% calf serum as described before[6]. Some groups of cells were used after 48 hr, when the bottles contained approximately 5×10^6 cells (Group 1). In other bottles the medium was changed for 10 ml of Krebs Ringer bicarbonate solution and the cells were maintained under these conditions for 12 hr. After this period, 1[^{14}C]α-linolenic acid (0.5 nmol per million of cells) was added into the medium and the incubation continued for another 12 hr (group 2). These cells are called "fasted" cells. Simultaneously, with the addition of the labeled acid some groups of cells were "refed" with Swim's 77 medium without serum and without glucose (group 3), and other groups were "refed" with the same medium and different concentrations of glucose: 0.3 g ‰ (group 4) and 1 g ‰ (group 5). Other cells were "refed" for 12 hr only with glucose (1 g ‰) in Krebs Ringer bicarbonate solution (group 6). For biochemical study, the labeled acid was added, during the last 12 hr of incubation.

The sodium salt of the labeled acid bound to defatted albumin[7] according to SPECTOR et al.[8] in a ratio of 2 moles fatty acid per mole of albumin was used in all the experiments. After 12 hr of incubation with 1-[^{14}C]α-linolenic acid the attached cells were washed twice with 0.85% Na Cl, removed from the container with a rubber policeman, collected into tubes and centrifuged at 2000 × g for 5 min. The saline solution was then decanted and the cells were saponified at 85 °C with 2 ml of 10% K OH in ethanol for 45 min. The fatty acids were extracted from the acidified solution with light petroleum (b.p. 30–40 C) and esterified with methanolic H Cl 3 N for 3 hr at 60 °C. The distribution of radioactivity between the fatty acids was determined by gas liquid

radiochromatography in an apparatus equipped with a Packard proportional counter[9]. The samples were analyzed using a column packed with 15% diethylene glycol succinate on chromosorb W (80–100 mesh) at 180 °C. The acids were identified by the procedure already described[10].

Ultrastructural study:
The cells were removed from the bottles and centrifuged at 1000 × g for 5 min. The pellets were then fixed in MILLONIG[11] fixative for 1.5 hr, dehydrated in increasing concentration of ethanol and embedded in Araldite[12]. Sections were made with glass knives and an LKB Ultratome III, mounted on copper grids and stained with uranium-lead. They were examined with a Siemens Elmiskop I electron microscope at 60 kV.

Results

Ultrastructural study:
At low magnification, all the groups showed different proportion of intact and necrotic or disrupted cells. The intact cells (Fig. 1) were very well preserved and appeared free or related to each other by means of irregular microvilli, and less frequently, through junctional complexes. The nuclei were variable in shape and contained dense large nucleoli. Organelles were not abundant and generally appeared grouped over a cytoplasmic area. The rest of the cytoplasm showed a large amount of free ribosomes. Glycogen, lipid droplets and pinocytotic vesicles were not abundant.

Cells from group 1 and 5 presented similar features. Both groups had a low proportion of necrotic cells. Mitochondria showed normal appearance (Fig. 2). The presence of some dilated mitochondrial cristae was not a common finding. The endoplasmic reticulum was mainly of the rough type (Fig. 5) and appeared as flattened sacs or vesicles, scattered over the cytoplasmic matrix. The Golgi complex was not very well developed and some lysosomes of the autophagic vacuole type were occasionally seen. Several cells possessed a clearly defined annulate lamellae (Fig. 2, inset), frequently seen in some tumoral and embryonic cells.

There were no remarkable differences between groups 2, 3, 4 and 6. A high proportion of necrotic cells was common in these groups. Many intact cells showed definite irregular outline of nuclei with small clear round areas in the peripheral chromatin (Fig. 3). Most of the mitochondria showed a considerable dilatation of the cristae, which were diminished in number, and an increase in the density of the matrix (Fig. 4). The rough endoplasmic reticulum was frequently formed by dilated vesicles (Fig. 6). Variable kinds of lysosomes were usually seen (Fig. 4). The rest of the organelles did not show any important difference with respect to groups 1 and 5.

Biochemical study
As it is shown in Table 1, "fasting" produces a decrease in the conversion of α-linolenic acid into higher homologs (group 2) compared with cells maintained in complete medium (group 1). The "refeeding" with Swim's 77 medium without glucose (group 3) produces an increase in eicose-11, 14, 17-trienoic acid levels. This increase is not so high as the one observed with complete medium. Besides, an enhancement of the transformation of α-linolenic acid into eicose-5, 11, 14, 17 and 8, 11, 14, 17-tetraenoic acids and eicose-5, 8, 11, 14, 17-pentaenoic acid also occurs.

When glucose is added to Swim's 77 medium (group 5), even at a low concentration (group 4), a decrease in the conversion of α-linolenic acid into higher homologs takes place. This decrease is especially marked for eicose-5, 11, 14, 17-tetraenoic and eicose-5, 8, 11, 14, 17-pentaenoic acids. The decrease is slightly higher when the medium contains glucose at a concentration of 1 g ‰ (group 5).

Discussion

The ultrastructure of transplantable Morris hepatomas had been thoroughly studied by HRUBAN et al.[13,14] It reveals many differences attributed to several variables, like growth rate, genetic and nutritional factors. In general, fast-growing hepatomas do not retain many of the features of the hepatocyte fine structure[15]. This is the case with this study, which reveals cells with a simple cytoplasmic organization that shows little evidence of its origin from hepatic cells. As far as we know, this is the first study

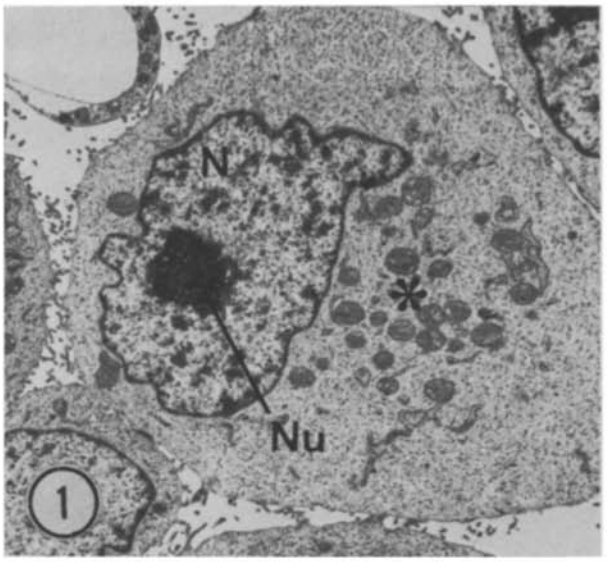

Fig. 1. A low magnification view of cells from group 5. The nucleus (N) looks rather irregular and contains a dense nucleolus (Nu). Most of the organelles (asterisk) are grouped in an area near the nucleus. Numerous microvilli are seen on the surface of the cells (×2.500).

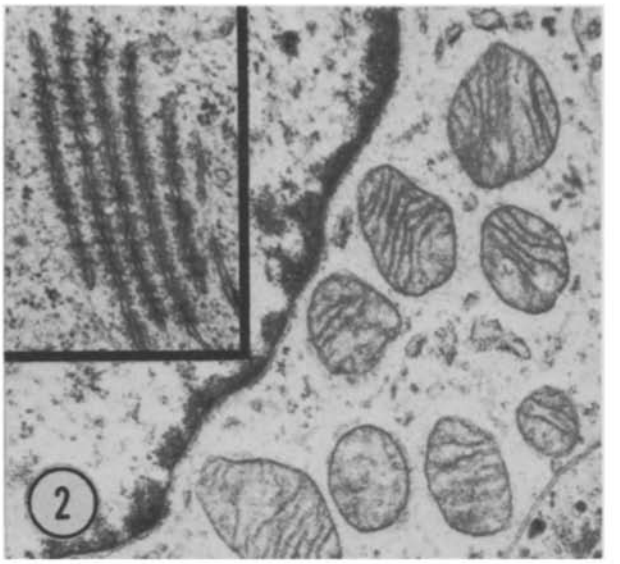

Fig. 2. A cell from group 5. Mitochondria showing normal appearance. (×18.000). *Inset*: annulate lamellae in a cell from the same group (×25.000).

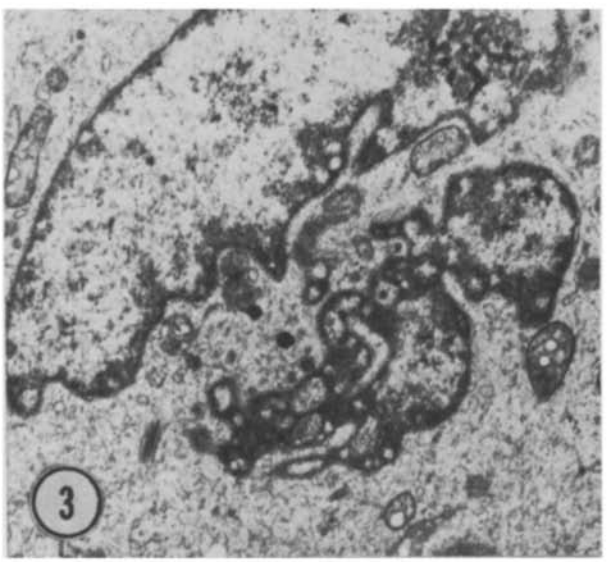

Fig. 3. Group 2. Irregularly shaped nucleus. The chromatin shows clear round areas in the periphery (×4.000).

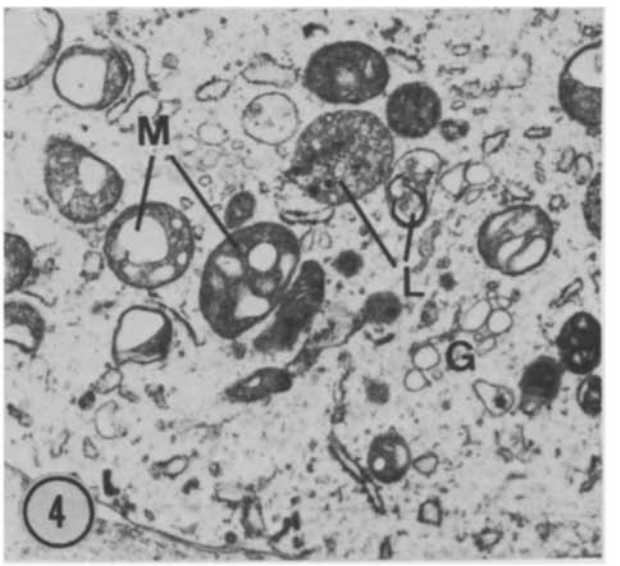

Fig. 4. Group 4. Some mitochondria (M) with dense matrix and conspicuous dilatation of the cristae, lysosomes (L) and a small Golgi complex (G) can be seen in this figure (×18.000).

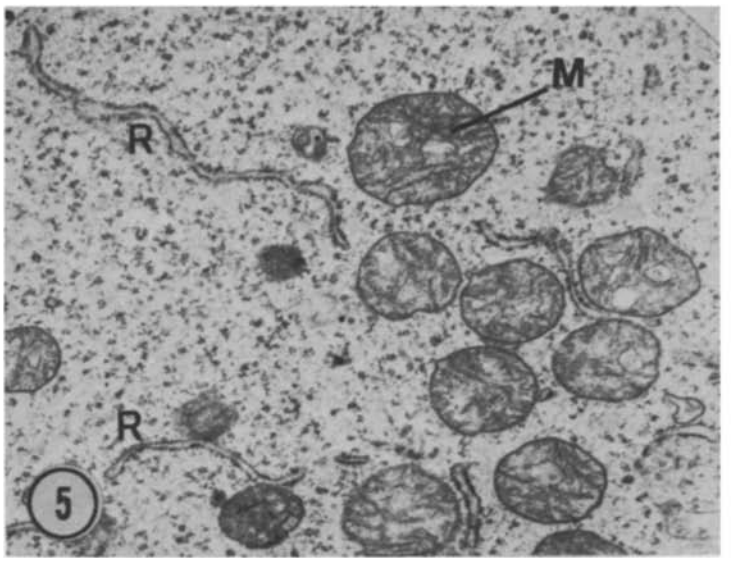

Fig. 5. Group 1. Rough endoplasmic reticulum profiles (R) and some mitochondria (M) are seen in a portion of cytoplasm, where numerous free ribosomes occupy areas without organelles. Only a few mitochondrial cristae appear dilated (×18.000).

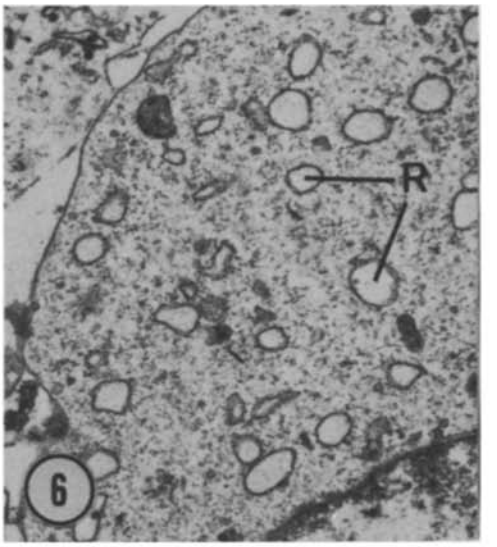

Fig. 6. Dilated rough endoplasmic reticulum (R) in the cytoplasm of a cell from group 6 (×15.000).

regarding to the ulstrastructure of cells from a hepatoma under present culture conditions.

From morphologic observations, it could be concluded that most of the cells cultured for 24 hr in a medium composed of 1g ‰ of glucose, 10% calf serum and sufficient amounts of aminoacids, vitamins and minerals (group 1) display a fine structure that is well preserved after a routine fixation. Only few cells are disrupted or necrotic. The general appearance of the nucleus and organelles would indicate that under this experimental procedure which is similar to that used in other related works[2], the cells do not show any important abnormal changes due to the environmental conditions. Thus, this study would provide useful morphologic evidence for further biochemical experiments.

Comparing groups 1 and 5, it could be assumed that 12 hr of "fasting" did not greatly modify the fine structure of the cultured cells when medium containing 1 g ‰ of glucose was added for another 12 hr period, even in the absence of 10% calf serum.

The cells, under various experimental conditions employed for groups 2, 3, 4 and 6 were rather similar. However, they showed some important differences compared to groups 1 and 5. It seems that the amount of glucose in the medium plays an important role in preserving the appearance of cell structures, like the nucleus, mitochondria and endoplasmic reticulum under the present culture procedure. Moreover, glucose would also modify the degree of cell necrosis, under the same conditions. However, when 1 g ‰ of glucose is added, in the absence of aminoacids and vitamins in the medium (group 6), this effect did not occur.

The decrease in the conversion of α-linolenic acid into higher homologs observed in cultured cells after "fasting" for 24 hr, is similar to that shown in a previous work[2]. In spite of the fact that glucose seems to be an important factor in preventing the cell ultrastructure, the metabolism of α-linolenic acids would indicate that glucose causes a decrease in the conversion of α-linolenic acid especially into 20:5 (Δ5, 8, 11, 14, 17). These results are in accord with those obtained earlier[2] where it was reported that "refeeding" with glucose, as the only carbon source, would restablish the levels of eicose-11, 14, 17-trienoic acid-which is the elongated product of α-linolenic acid-but it would not restablish the levels of eicose-5, 8, 11, 14, 17-pentaenoic acid.

The results observed after "refeeding" with a medium lacking glucose, but containing sufficient amounts of aminoacids, vitamins and

minerals (group 3) fit well with the observation that aminoacids produce an enhancement in 20:5 (Δ5, 8, 11, 14, 17) acid when used as the only carbon source after "fasting"[2]. This is also consistent with data obtained in experiments in vivo in which fasting depress Δ6 desaturation[4,17] and elongation[18] of fatty acids.

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