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MEDIUM CHAIN LENGTH FATTY ACIDS STIMULATE TRIACYLGLYCEROL SYNTHESIS IN TISSUE CULTURE CELLS

BEATE HORNUNG, EBERHARD AMTMANN and GERHARD SAUER*
German Cancer Research Center, Institute for Virus Research, W-6900 Heidelberg, Federal Republic of Germany

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Abstract—In the presence of undecanoic acid (C11) or lauric acid (C12) the synthesis of triacylglycerols was stimulated up to 10-fold both in tumor cell lines and in normal cell lines. Monocarboxylic acids of shorter or longer chain length either had no effect at all or were less effective. The increased triacylglycerol production was demonstrated, on the one hand, by the incorporation of radiolabeled glycerol into triacylglycerols and, on the other, by the incorporation of radiolabeled monocarboxylic acids, the incorporation of all (1-^{14}C)-labeled monocarboxylic acids (C6, C12, C16, C18) regardless of their chain length, being preferentially enhanced by C11 and C12. C12 stimulated the *de novo* synthesis of triacylglycerols to such a degree that a 7-fold increase in the total amount of triacylglycerols per cell was observed during the first 10 hr of incubation. After removal of C12 from the tissue culture medium levels of triacylglycerols reach initial values again within 6 hr, indicating that the stimulatory effect of C12 is dependent on its continued presence. This led to the speculation that medium chain length monocarboxylic acids might be involved in the control of triacylglycerol synthesis.

Despite many efforts, chemotherapy has met with only limited success in the experimental treatment of human carcinoma xenotransplants during recent years. A complete regression of such tumors in athymic mice can be considered a rare event and for the time being we, generally, have to be content with temporary regression of certain tumor models, mainly after combination therapy with various cytostatic agents.

We have developed a novel approach by which it is possible to achieve antitumor effects on human carcinoma xenotransplants that are known to be largely resistant to chemotherapy, such as squamous non-small cell lung carcinomas and colorectal carcinomas [1]. The principle of the (systemic) treatment is based on a single infusion of recombinant human tumor necrosis factor (rhTNF†) with D609 and lauric acid (C12). The D609/C12 combination was shown to enhance the antitumor activity of rhTNF *in vivo* and *in vitro*, while C12 or D609 alone had no such enhancing effect [1, 2]. In the absence of rhTNF, D609 also displayed selective antitumor effects *in vitro* when applied at the same time as monocarboxylic acids with chain lengths between 10 and 14 carbon atoms [3, 4]. The chain lengths of the monocarboxylic acids proved to be crucial, as those with shorter or longer chains had no effect. So far, the role of the medium chain length monocarboxylic acids in this combination therapy is not fully understood. However, there is experimental evidence indicating a correlation between energy depletion in cells and the antitumor activity of rhTNF, D609 and C12 [2]. Therefore, we examined the effect of C12 on lipid metabolism and its possible correlation with the energy supply of the cell. Mammalian adipose tissue, like most tissues, is able to synthesize only monocarboxylic acids longer than C14 [5]. If C12 is present in high concentrations in plasma, however, e.g. in coconut oil (50% C12)-fed animals, it is incorporated into the triacylglycerols of body fat [6]. The medium chain length triacylglycerols are hydrolysed and cleared rapidly in the liver and are therefore a more readily available lipid fuel than the conventional long chain length triacylglycerols [7]. On the basis of tissue culture experiments, we observed that C12 was not only incorporated into triacylglycerols, but also stimulated the *de novo* synthesis of triacylglycerols. Monocarboxylic acids of shorter and longer chain length either had no effect at all or were less effective. Apparently, there is a correlation between the stimulation of triacylgylcerol synthesis and the enhancement of the antitumor activity of D609 in the presence of medium chain length monocarboxylic acids. In addition to its antitumor activity D609 is capable of inhibiting the growth of various taxonomically unrelated DNA- and RNA-viruses [8, 9]. In this case, too, in the presence of monocarboxylic acids with 10–14 C atoms the antiviral activity is enhanced [10]. Here, we report that medium chain length monocarboxylic acids are likely to be involved in the control of triacylglycerol synthesis.

* Corresponding author: Prof. Dr G. Sauer, German Cancer Research Center, Institute for Virus Research, W-6900 Heidelberg, F.R.G. Tel. (49) 06221-424604; FAX (49) 06221-49665.

† Abbreviations: D609, tricyclodecan-9-yl-xanthogenate; rhTNF, recombinant tumor necrosis factor; HeLa, human cervix carcinoma cells; HEL, human embryonic lung; HTB72, human melanoma; MEF-K1, mouse embryonic fibroblast; CV-1, African green monkey kidney cells; L929, mouse fibroblast; SW707, human colon rectum carcinoma; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; PG, phosphatidylglycerol; DG, diacylglycerol; TG, triacylglycerol.

MATERIALS AND METHODS

Chemicals. [1-^{3}H]Glycerol (5 Ci/mmol), [1-^{14}C]-lauric acid (56.6 mCi/mmol), [1-^{14}C]palmitic acid (55.3 mCi/mmol) and [1-^{14}C]stearic acid (55.3 mCi/mmol) were purchased from Amersham/Buchler (Braunschweig, F.R.G.), sodium salt of [1-^{14}C]-octanoic acid (58 mCi/mmol) from NEN-du Pont (Dreieich, F.R.G.). Monocarboxylic acids were obtained from Merck (Darmstadt, F.R.G.). They were dissolved in 80% acetone and 20% H_2O as 1% stock solutions.

Cell lines. HeLa (human cervix carcinoma cells), HEL (human embryonic lung), HTB72 (human melanoma), MEF-K1 (mouse embryonic fibroblast, transformed by BPV), CV-1 (African green monkey kidney cells), SW707 (human colon rectum carcinoma) and L929 (mouse fibroblast) were used for the experiments.

Cell culture. Cells were seeded in Eagle's Basal Medium with 2.2 g/L $NaHCO_3$, 10% fetal calf serum, 1% streptomycin and 1% penicillin at an initial density of 1×10^6 cells/petri dish. Incubation took place in a 5% CO_2/95% air atmosphere at 37°. Prior to treatment with monocarboxylic acids the cells were grown to confluence unless indicated otherwise in the figure legends. After treatment the cell number was determined after trypsination of parallel cultures in a hematocytometer.

Extraction of lipids. Cells were washed twice with cold phosphate-balanced isotonic salt solution, scraped from the dishes and collected by centrifugation. Total lipids were extracted by the method of Bligh and Dyer [11]. The lipids in the combined organic phases were dried *in vacuo* and resuspended in 20 μL $CHCl_3/CH_3OH$ (1:2, v/v).

TLC of total lipids. A 5-μL aliquot of the lipid extract was analysed by TLC on silica gel plates (60, F254, Merck). $CHCl_3/CH_3OH$/25% NH_3 (65:35:4, v/v/v) (= solvent 1) or petrolether/diethylether/acetic acid (40:60:1, v/v/v) (= solvent 2) was used as developing solvent. Five microliters of a mixture of known pure phospholipids (PE, PI, PS, PG and PC), 5 μL of diacylglycerols [1,2 dipalmitoyl-*sn*-glycerol and 1,3 dipalmitoyl-*sn*-glycerol) and 5 μL of each triacylglycerol (1,2 distearoyl-3-palmitoylglycerol, 1,2 dioleoyl-3-stearoylglycerol and 1,2 dilauroyl-3-myristoyl-glycerol) were chromatographed as references. Lipids were visualized by iodination prior to detection and quantitation by radiodensitometry with a Berthold Linear Analyser. The counting efficiency of the radiodensitometer was determined with the aid of defined amounts of ^{14}C or ^{3}H. This factor was used for the calculation of molar amounts of incorporated tracers.

Hydrolysis of triacylglycerols and analysis of fatty acids. The silica gel spots identified as triacylglycerols were scraped from the plates, extracted with 1 mL 20% acetone/H_2O followed by 1 mL 80% acetone/H_2O. The combined supernatants were dried *in vacuo*, resuspended in 100 μL 1 M KOH, incubated for 15 min at 100° and acidified with acetic acid. The fatty acids produced were eluted with hexane, dried *in vacuo*, dissolved in 5 μL acetone and analysed by TLC on silica gel plates (RP-18, F254, Merck).

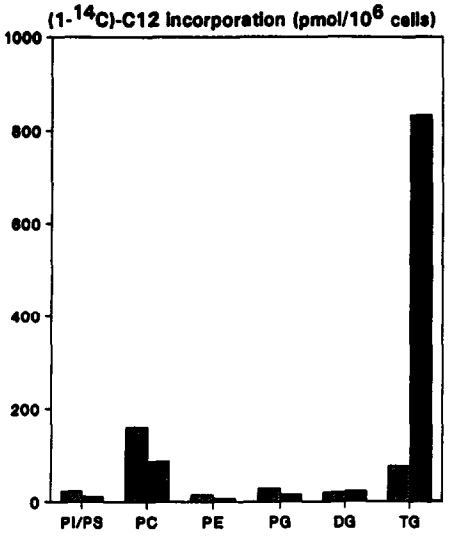

Fig. 1. Incorporation of [1-^{14}C]lauric acid into total cell lipids. HeLa cells were incubated with 1.76 μM [1-^{14}C]C12 in the presence or absence of 200 μM C12 for 6 hr. The total lipids were extracted and aliquots were separated by TLC in solvent 1 or solvent 2. ^{14}C Label in the individual spots was quantitated by radiodensitometry and molar concentrations were calculated. Values are means from three cultures; SD was smaller than 15%. Shaded bars, untreated; filled bars, treated with C12.

Acetonitrile/acetic acid/H_2O (35:5:10, by vol.) (= solvent 3) was used as developing solvent, and 5 μL each of ^{14}C-labeled C8, C12 and C18 were chromatographed as references.

Enzymatic quantitation of triacylglycerols. An enzymatic assay (GPO-Trinder; Sigma Chemical Co. Munich, F.R.G.; Catalogue No. 339) was used for the quantitative determination of total triacylglycerols. After extraction of total lipids the dried lipid extract was resuspended in 10 μL isopropanol and mixed with GPO-Trinder enzyme solution. Triacylglycerols were hydrolysed by lipase to glycerol and free fatty acids. The glycerol produced was measured by coupled enzyme reaction catalysed by glycerol kinase, glycerol phosphate oxidase and peroxidase.

RESULTS

As a first approach to its biochemical function, the metabolic fate of C12 in the cell was examined. HeLa cells were treated with 1.76 μM ^{14}C-labeled C12. To each of three cultures 200 μM unlabeled C12 was added and 6 hr later the lipids were extracted from the treated cells and separated by TLC in solvent 1. To distinguish between di- and triacylglycerols an aliquot was also separated by TLC in solvent 2. The incorporation of ^{14}C into the cell lipids was determined by radiodensitometry (Fig. 1). In the presence of 200 μM C12 the ^{14}C label was preferentially found in the triacylglycerol fraction. The incorporation of [1-^{14}C]C12 into this fraction was increased by 8-fold as compared with the untreated control. In contrast, the amount of

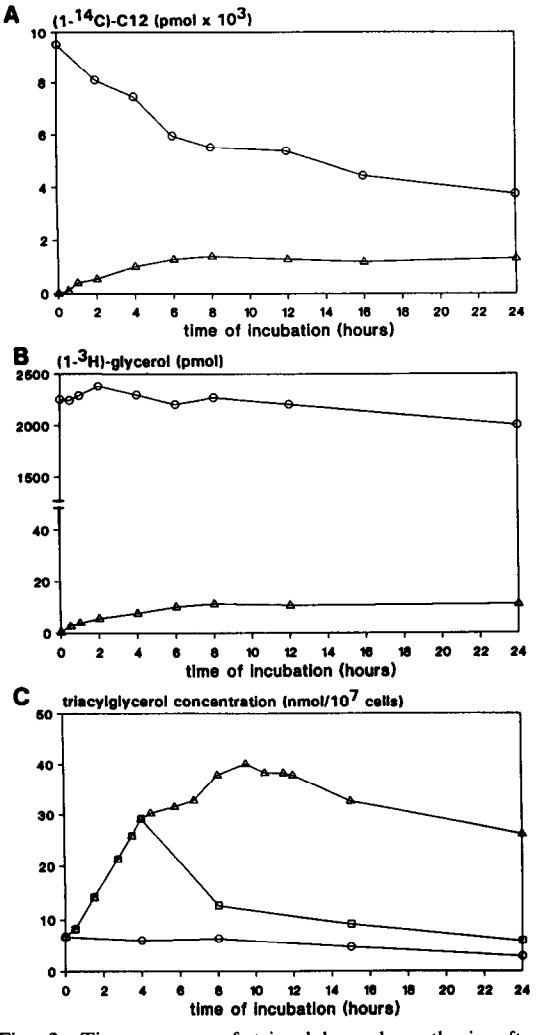

Fig. 2. Time course of triacylglycerol synthesis after treatment with C12. HeLa cells were grown to subconfluence and incubated in the presence or absence of 200 μM C12 and 2.2 μM [1-^{14}C]C12 or 0.1 μM [1-^{3}H]glycerol. After 4 hr of C12 treatment nine cultures were refed with C12-free tissue culture medium. At the indicated time points the total lipids were extracted from three cultures and the amount of triacylglycerols was quantitated by the enzymatic method. ^{14}C-Labeled lipids were separated by TLC in solvent 2 and ^{3}H-labeled lipids in solvent 1. The radioactivity in the triacylglycerol fraction was quantitated by radiodensitometry and the molar concentrations were calculated from the obtained data. (A) [1-^{14}C]C12 in medium (circles), ^{14}C label in triacylglycerol fraction (triangles). (B) [1-^{3}H]-Glycerol in medium (circles), ^{3}H label in triacylglycerol fraction (triangles). (C) Triacylglycerol content in untreated cultures (circles), C12-treated (triangles), treated with C12 for 4 hr (squares). Values are means from three cultures; SD was smaller than 10%.

radioactivity in the various phospholipids and in the diacylglycerols was not increased after treatment with C12.

The incorporation of ^{14}C into triacylglycerols was found to be linear over the first 8 hr (triangles, Fig. 2A). Thereafter, it reached a plateau despite the availability of sufficient amounts of [1-^{14}C]C12 in the tissue culture medium (circles, Fig. 2A). In fact, after 8 hr approximately 65% of the initial input was still present. Essentially similar results were found when the incorporation of [1-^{3}H]glycerol in triacylglycerols was studied in the presence of C12 (Fig. 2B). Again, during the first 8 hr the label was incorporated in a linear manner, followed by a plateau. The [1-^{3}H]glycerol in the tissue culture medium remained almost constant throughout the entire experiment (90% of the initial concentration was left after 24 hr of incubation). Only 2% of the radiolabeled glycerol was transported through the cell membrane and incorporated into triacylglycerols. However, the increase in the total amount of triacylglycerol per cell after incubation with 200 μM C12 and [1-^{14}C]C12 or [1-^{3}H]glycerol was similar (triangles, Fig. 2C) as the cells obviously use their endogenous glycerol for triacylglycerol synthesis. In order to determine the actual amount of triacylglycerol in the cell after incubation with C12 the enzymatic GPO-Trinder assay system was employed and a continuous increase over the first 10 hr was detected (triangles, Fig. 2C) resulting in a 7-fold increase over the untreated controls (circles, Fig. 2C). When C12 was removed from the tissue culture medium after a 4-hr treatment, the amount of triacylglycerol decreased to the initial value within 6 hr (squares, Fig. 2C).

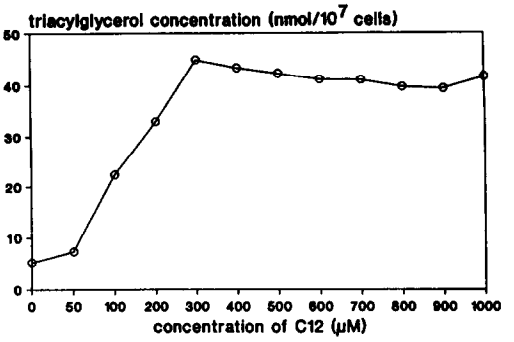

Fig. 3. Dose-response correlation of triacylglycerol synthesis in the presence of C12. HeLa cells were grown to subconfluence and treated with increasing concentrations of C12. The total lipids were extracted 4 hr later and the amount of triacylglycerol was quantitated by the enzymatic method. Values are means from three cultures; SD was smaller than 10%.

Total triacylglycerol increased in a dose-dependent manner after treatment with 50–300 μM C12 (Fig. 3). In the presence of 300 μM C12 a 7-fold increase in total triacylglycerol per cell was seen after as short a time as 8 hr of treatment (data not shown).

To identify a putative *de novo* synthesis of triacylglycerol, HeLa cells were labeled with [1-^{3}H]-glycerol and simultaneously treated with 200 μM C12. Other HeLa cell cultures were prelabeled with [1-^{3}H]glycerol for 16 hr, followed by treatment with 200 μM C12. In the case of simultaneous labeling and treatment only, an increase of ^{3}H incorporation

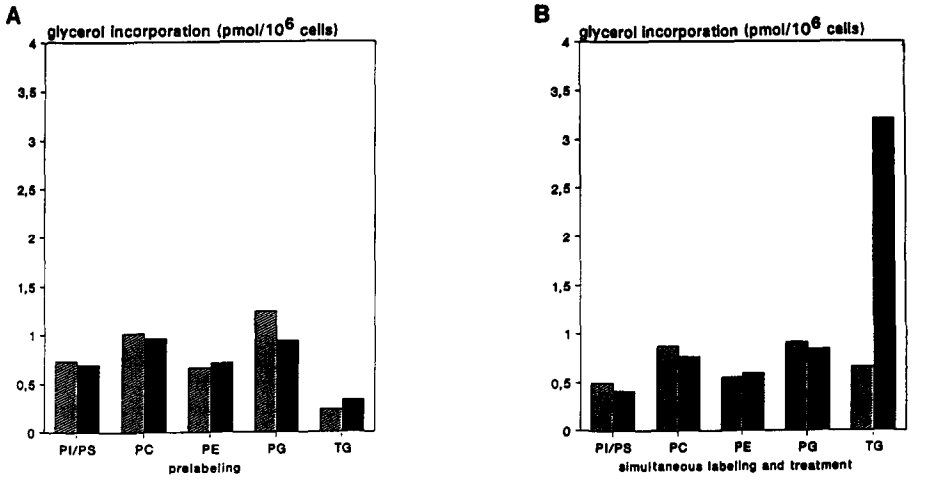

Fig. 4. Incorporation of [1-^{3}H]glycerol into total cell lipids. (A) HeLa cells were prelabeled with 0.05 μM [1-^{3}H]glycerol for 16 hr, washed and incubated in the presence or absence of 200 μM C12 for 6 hr. (B) HeLa cells were grown to confluence and incubated with 0.05 μM [1-^{3}H]glycerol in the presence or absence of 200 μM C12 for 6 hr. The total lipids were extracted and separated by TLC in solvent 1. ^{3}H Label in the individual spots was quantitated by radiodensitometry and molar concentrations were calculated. Values are means from three cultures; SD was smaller than 15%. Shaded bars, untreated; filled bars, treated with C12.

into the triacylglycerol fraction was observed in the presence of C12 (Fig. 4). This leads to the conclusion that the observed stimulatory effect is due to *de novo* synthesis of triacylglycerol from glycerol and fatty acids.

Next, the effect of the chain length of fatty acids on the stimulation of triacylglycerols was studied. HeLa cells were treated with 1.76–1.90 μM (1-^{14}C)-labeled fatty acid (C8, C12, C16, C18) and 200 μM unlabeled C12 or equimolar amounts of fatty acids with between 6 and 18 C atoms. Total lipids were extracted and separated by TLC and the incorporation of ^{14}C into triacylglycerol fraction was determined by radiodensitometry. It can be observed (Fig. 5) that the synthesis of triacylglycerols was stimulated particularly by medium chain length fatty acids (C11 and C12). Both shorter and longer fatty acids were less effective or entirely ineffective. Surprisingly, the incorporation of all labeled fatty acids that were present in the tissue culture medium (^{14}C-labeled C8, ^{14}C-labeled C12, ^{14}C-labeled C16, ^{14}C-labeled C18) was stimulated by C11 and C12. Essentially, the same results were obtained during three runs of the experiments. In agreement with the above data, the total amount of triacylglycerols was found to be stimulated by medium chain length fatty acids (C11–C14) by a factor of between 5 and 10, while in the presence of shorter and longer chain length fatty acids (C8 or C18) the triacylglycerol content was either unaffected or, at most, duplicated (data not shown).

The newly synthesized triacylglycerols were eluted from the silica gel plates and hydrolysed with KOH. The liberated fatty acids were separated by TLC parallel with ^{14}C-labeled C8, C12 and C18 as markers (Fig. 6). In the presence of ^{14}C-labeled C12, all labeled fatty acids that were incorporated into triacylglycerols during C12 stimulation could be recovered as unprocessed C12. Similarly, ^{14}C-labeled C8 and to some extent ^{14}C-labeled C8 was found unaltered in triacylglycerols after C12 treatment (data not shown).

To determine whether the observed stimulatory effect of medium chain length fatty acids on triacylglycerol synthesis was specific to HeLa cells or whether it could also be observed in cell lines of different origin, tumor cells (HeLa, L929, HTB72, SW707, MEF-K1) and non-transformed cell lines (HEL, CV-1) were studied with regard to a putative stimulation of triacylglycerol synthesis by medium chain length fatty acids. The cells were treated with ^{14}C-labeled C16 and unlabeled C8, C11, C12 and C15. The incorporation of ^{14}C into the triacylglycerol fraction was determined after a 6-hr treatment. Preferential stimulation of triacylglycerol synthesis by C11 and C12 can be observed as a general feature in all these cell lines (Fig. 7).

DISCUSSION

It has already been reported that fatty acids are important effectors that stimulate to different extents triacylglycerol synthesis [12–15]. Nevertheless, some such as eicosapentaenoic acid may even display inhibiting activities [16].

Here, we describe an increased synthesis of triacylglycerols in the presence of C11 and C12 in various normal and transformed cell lines. Generally, triacylglycerols are synthesized from fatty acids and glycerol. The effect of C12 on the incorporation of radiolabeled fatty acids and on the incorporation of radiolabeled glycerol into the cell lipids was examined. After treatment with lauric acid, radiolabeled C12 was preferentially incorporated into

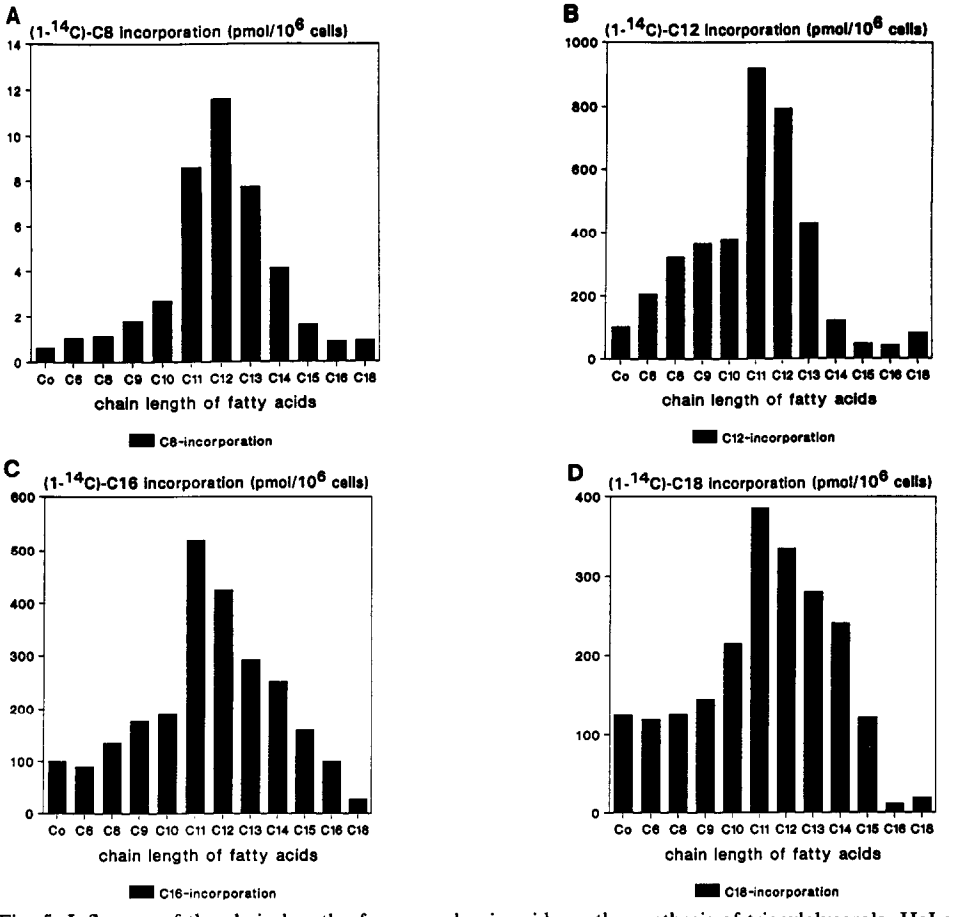

Fig. 5. Influence of the chain length of monocarbonic acids on the synthesis of triacylglycerols. HeLa cells were incubated with (A) 1.9 μM [1-^{14}C]C8, (B) 1.76 μM [1-^{14}C]C12, (C) 1.81 μM [1-^{14}C]C16 or (D) 1.81 μM [1-^{14}C]C18. Simultaneously, the cells were treated with 200 μM C12 or with equimolar concentrations of various monocarboxylic acids, as indicated. Five hours later the total lipids were extracted and separated by TLC in solvent 2. ^{14}C Label in the triacylglycerol fraction was quantitated by radiodensitometry and molar concentrations were calculated.

the triacylglycerol fraction while the incorporation of radiolabeled C12 into the phospholipid and diacylglycerol fraction was not influenced. After an 8-hr treatment with C12 there was no further incorporation of [1-^{14}C]C12 into the triacylglycerol fraction despite the availability of sufficient [1-^{14}C]-C12 in the tissue culture medium, indicating that the synthetic activity apparently had reached its optimum.

Upon labeling of the cells with [1-^{3}H]glycerol during treatment with C12, an increase of ^{3}H label in the triacylglycerol pool was observed. In contrast, when the cells were prelabeled with [1-^{3}H]glycerol, the treatment with C12 failed to increase the ^{3}H label in the triacylglycerol fraction. This indicates that *de novo* synthesis of triacylglycerols from glycerol and fatty acids was taking place rather than replacement of fatty acids in triacylglycerols or the processing of phospholipids.

An enhanced *de novo* synthesis of triacylglycerols would presumably result in an increase in total triacylglycerol. Accordingly, we found that the amount of triacylglycerols per cell was enhanced by C12 in a dose-dependent manner during the first 10 hr of incubation. In the presence of 200 μM C12 a 7-fold increase in the total amount of triacylglycerol was induced. After removal of C12 the level of triacylglycerol per cell returned to the initial value, implicating a decomposition.

In the experiments where ^{14}C-labeled C12 was employed to monitor the C12-induced stimulation of triacylglycerol synthesis the concentration of C12 in the medium of treated cultures was in an approximately 100-fold excess of the concentration in the controls. In view of the 8-fold increase of [1-^{14}C]C12 incorporation (Fig. 1) one might expect a final 800-fold greater amount of triacylglycerols in the treated cells. This, however, disregards the (unknown) concentration of fatty acids that is present *a priori* in the batches of fetal calf serum that were added to the medium. Thus, only a stimulation of the amount of triacylglycerols by 7-fold was actually detected (triangles, Fig. 2C).

Regarding the *de novo* synthesis of triacylglycerols,

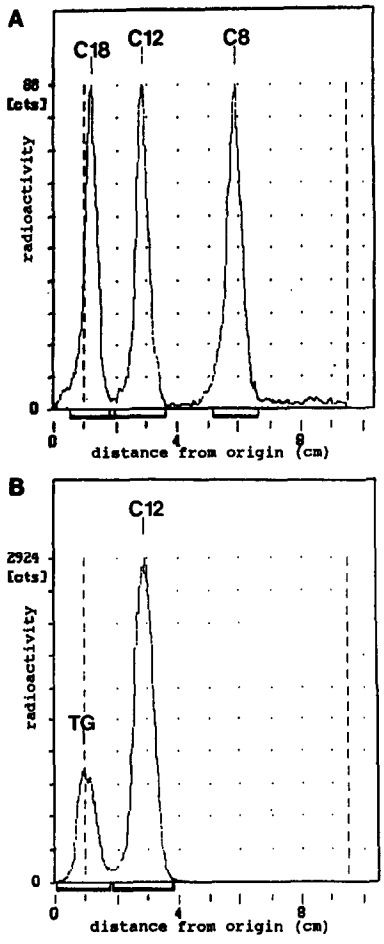

Fig. 6. Analysis of monocarboxylic acids incorporated into triacylglycerols after C12 treatment. HeLa cells were incubated with 1.76 μM [1-^{14}C]C12 in the presence of 200 μM C12. The total lipids were extracted and separated by TLC in solvent 1. After scraping the triacylglycerol spots from the plates, the triacylglycerols were extracted and hydrolysed with KOH. The monocarboxylic acids were separated by TLC in solvent 3 (B). C8, C12 and C18 were chromatographed as references (A).

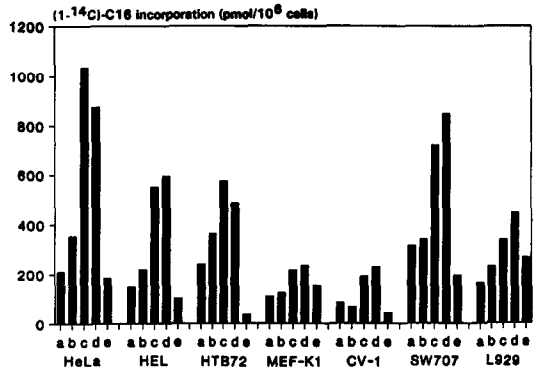

Fig. 7. Influence of the chain length of monocarboxylic acids on the synthesis of triacylglycerols in various cell lines. Different cell lines (HeLa, HEL, HTB72, MEF-K1, CV-1, SW707, L929) were incubated with 1.81 μM [1-^{14}C]-C16 and 200 μM C12 or with equimolar concentrations of C8, C11 or C15. Five hours later the total lipids were extracted and separated by TLC in solvent 2. ^{14}C Label in the triacylglycerol fraction was quantitated by radio-densitometry and molar concentrations were calculated. (a) untreated, (b) treated with C8, (c) treated with C11, (d) treated with C12, (e) treated with C15. Values are means from three cultures; SD was smaller than 10%.

two points are of importance: (1) the synthesis of triacylglycerols was preferentially stimulated by medium chain length fatty acids, such as C11 and C12, while longer and shorter chain fatty acids were either less effective or completely inert; (2) C11 and C12 stimulated the incorporation of all monocarboxylic acids, regardless of their chain length (C8, C12, C16, C18), into the triacylglycerols.

The incorporation of monocarboxylic acids into the diacylglycerols was not stimulated. This leads us to speculate that C11 and C12 may play a regulatory role in the synthesis of triacylglycerols, namely, in the last step: the synthesis of triacylglycerols from diacylglycerols catalysed by diacylglycerol acyltransferase. This agrees with the observation by Haagsman and Van Golde [17] that the stimulation of triacylglycerol synthesis following incubation of hepatocytes with palmitic acid is accompanied by an enhanced activity of diacylglycerol acyltransferase [17]. Coleman and Bell [18] observed that the highest activities of microsomal diacylglycerol acyltransferase were found when decanoyl-CoA or lauroyl-CoA was used as substrate while lower activities were observed with longer and shorter saturated fatty acids. Thus, our findings could be explained by either of two mechanisms: Firstly, medium chain length fatty acids could enhance the level of triacylglycerol per cell because diacylglycerol acyltransferase acts preferentially on medium chain acyl CoA. The other explanation could be that medium chain fatty acids enhance diacylglycerol acyltransferase activity that is optimal on the C12 substrate (induction of enzyme activity by its substrate). However, the addition of C12 led to the enhanced incorporation of all radiolabeled fatty acids (C8, C12, C16 and C18) into triacylglycerols without alteration of their chain lengths, as C8, C12 and C18 were recovered from triacylglycerols after hydrolysis. Thus, it appears that the activity of diacylglycerol acyltransferase is stimulated by C12 for all substrates. As this effect occurred in the presence of the protein synthesis inhibitor cycloheximide (data not shown), C12 seems to enhance the general activity of diacylglycerol acyltransferase rather than inducing the *de novo* synthesis of the enzyme.

The incubation of tumor cells with C11 and C12 did not affect their growth rate. In the presence of D609, however, C12 caused selective killing of tumor cells and enhanced antiviral activity [3, 10]. D609 prevents the hydrolysis of triacylglycerols (data not shown). This fact in conjunction with the stimulation of triacylglycerol synthesis by C11 and C12 generates an energetic "one-way-street" where the use of

triacylglycerols as an energy source is being precluded. This, in turn, results in a lack of energy, as the *de novo* synthesis of triacylglycerols is a rather energy-demanding process. Furthermore, the synthesis of such unusual triacylglycerols that contain C12 may be involved in alteration of the permeability and fluidity of cell membranes.

Work is in progress indicating that C12-containing triacylglycerol can indeed be detected in plasma membranes (data not shown). This finding may explain the inhibitory effect of the combined D609/C12 treatment on the replication of HIV-1 [9]. Despite viral RNA and protein synthesis in the treated cells the formation of infectious viral progeny was suppressed. It is conceivable that the altered composition of the plasma membrane may exert deleterious effects both on virus budding and on the infectivity which depends, at least in part, on the composition of the viral envelope.

Structural alterations of cell membranes (e.g. depletion of phospholipids), ATP reduction and alteration of ion flux have been postulated to be essential for necrotic cell death [19]. Indeed, the combination of C12 and D609 amplifies the activity of rhTNF and leads to necrotic cell death [2]. In this case, too, the high energy requirement for triacylglycerol production is probably implicated in cell death resulting from lack of energy. Furthermore, on the one hand the absence of glucose enhances the activity of rhTNF while, on the other, glucose is urgently required for triacylglycerol synthesis. Glycerol-3-P, which is used for triacylglycerol synthesis, originates from glycolytic dihydroxyacetonephosphate and acetyl-CoA (CoA is used for the activation of fatty acids) originates from glycolytic pyruvate. We think it likely that the enhanced synthesis of triacylglycerols by C11 and C12 without degradation contributes to a glucose and energy deficit in cells and so contributes—in the simultaneous presence of both D609 and TNF—to cell death.

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