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Fatty acids of the triglycerides and phospholipids of HeLa cells and strain L fibroblasts*

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SUMMARY

Lipids of strain L fibroblasts (mouse), grown in chemically defined medium, contained approximately 20% 16:0, 5% 16:1, 20% 18:0, and 50% 18:1 acids; and small amounts of 12:0, 14:0, 15:0, 15:1, and 20:0 acids. No polyunsaturated acids, such as 18:2, were present. The distribution of the fatty acids in the phospholipids and triglycerides was similar. When grown in the presence of dialyzed serum, 18:2 acid was found in the cells and replaced 18:1 acid. Lipids of two lines of HeLa cells (human), grown with serum present, contained at least 20 fatty acids of which 16:0, 18:0, 16:1, 18:1, and 18:2 were dominant. The phospholipids contained considerably more 18:2 acid than did the triglycerides, the principal unsaturated acid in which was 18:1. Cells contaminated with pleuropneumonia-like organisms also showed this difference in distribution.

Of the major constituents of mammalian cells cultivated continuously *in vitro*, the lipids have probably received the least attention. Lipid accumulation by cells in tissue culture is well known (1) and the influence of saturated and unsaturated fatty acids on the accumulation of lipids by cells from aorta explants has been reported (2). That cholesterol can be synthesized by at least one strain of cells grown in continuous culture has been shown (3). Utilization of emulsified triglycerides (4) and serum lipids (5) has been demonstrated. The present study deals with the fatty acid composition, as determined by gas-liquid chromatography, of two lines of HeLa cells, derived from a human carcinoma, and of strain L mouse fibroblasts. The latter cells were grown both in the presence and absence of serum and afforded the opportunity to investigate such problems as the octadecadienoic acid content of multiplying mammalian cells grown with and without an exogenous supply of this acid.

EXPERIMENTAL METHODS

Materials and Methods. Stock cultures of HeLa cells[1] and strain L fibroblasts[2] were grown at 36° in 250-ml bottles containing 10 ml of medium. Eagle's medium (6) containing 10% dialyzed horse serum was used with the HeLa cells, and Waymouth's completely chemically defined medium (7) with the strain L cells.[3] Subculturing of the HeLa cells was accomplished with trypsin (8) and that of the L cells by scraping. Inocula of approximately 2×10^6 and 4×10^6 cells were used with the HeLa and L cells, respectively. Smaller inoccula of L cells resulted in slow multiplication rates. The medium was changed every second day. In order to obtain sufficient lipid for analysis, the cells in five to six bottles were pooled.

All cell cultures were tested once or twice a week for possible contamination with pleuropneumonia-like organisms (including the L forms of bacteria) (9). Of several methods tried for this purpose, we have found the Diene's agar block procedure as given by Madoff (10) the most reliable. On numerous occasions, results were negative after 4 to 6 days incubation but positive by 10 to 11 days. The longer test period, therefore, has been used routinely. As has been observed (11), the presence of such contamination did not necessarily reveal itself by adversely affecting the cells. In the present study, cells purposely heavily contaminated promptly rounded up and came off the glass. Except where otherwise noted, all cells used for analysis

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[1] Two lines of HeLa cells were kindly provided by Dr. R. S. Chang, Department of Microbiology, Harvard School of Public Health. These were designated JHC1 and DM, respectively.

[2] Strain L fibroblasts propagated in chemically defined medium were generously provided by Dr. Charity Waymouth, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

[3] In a personal communication, Dr. Charity Waymouth kindly supplied the formula of a modification of her medium MB 752/1, designated ME 77/3, which included $FeSO_4$ (0.05 mg/100 ml).

were uncontaminated as judged by the preceding test.

Lipid analyses were done on cells from which the original medium had been removed and that were washed with fresh corresponding serum-free medium either before or after removal from the glass. The cells were extracted with chloroform-methanol (12), using two 7-ml portions of extraction mixture for approximately 100 mg of original packed cells. After the evaporation of the the solvents under N_2, the crude lipid was extracted once with 5 ml of warm $CHCl_3$ and once with 1 ml $CHCl_3$. Additional extractions were found unnecessary. The lipids were fractionated on silicic acid columns (13) to yield the cholesterol esters, triglycerides plus unesterified fatty acids, and phospholipids. In a number of cases the total fatty acids of the unfractionated cell lipids were also analyzed for their fatty acid components. The quantities of lipid present in the cholesterol ester fraction were too small for suitable analysis and were omitted. The amount of unesterified fatty acids was negligible and has been included under the heading of triglycerides. In addition to the data reported in the tables, many other gas-liquid chromatographic analyses were done corroborating those presented here.

Methyl esters of the fatty acids of the various fractions were prepared by the method of Stoffel *et al.* (14), omitting the microsublimation step, and the gas-liquid chromatographic analyses of both hydrogenated and nonhydrogenated samples were done with a Research Specialties instrument employing a strontium-90 ionization detector and argon gas. With the limited amounts of cells available, the instrument was operated near its maximum sensitivity with settings of 1.5 kv and an attenuation of 20. A 4- or 6-foot $^1/_4$-inch i.d. U-shaped stainless-steel column packed with 20% (w/w) Craig diethyleneglycol succinate[4] on Chromosorb W[5] was employed at 180° with a flow rate of 60 to 100 ml per minute. Analyses were also done using Apiezon-L[6] as the liquid phase at a temperature of 200° or 230°. Percentage composition was calculated by determining the areas under the peaks. Detector response was standardized at frequent intervals using two known mixtures of the methyl esters of lauric, myristic, palmitic, stearic, palmitoleic, oleic, and linoleic acids.[7] The mixtures differed in that one approximated the composition of the fatty acids of HeLa cells and the other that of L fibroblast fatty acids. The standards were analyzed under the same conditions as the unknowns and the same detector voltage was used. Agreement between the calculated and observed relative composition was routinely better than 2% for each component. As the detector response to other concentrations of the esters was not determined, however, the quantitative results given in the present paper must be considered to be approximate. Furthermore, no reliable data are available on the extent of conversion of the sphingomyelins of tissue culture cells to esters by the methylation procedure used.

The carbon numbers of the fatty acids were determined from semilogarithmic curves as described by Woodford and van Gent (15). Standard methyl esters used included those of lauric, myristic, pentadecanoic, palmitic, palmitoleic, stearic, arachidic, behenic, oleic, linoleic, and arachidonic acids. Published carbon numbers (15, 16) and retention times (17) were used to identify provisionally the other esters (Table 1). In the tables, the unsaturated acids were not given their specific names because it was recognized that, in spite of correct constants using both nonpolar and polar column packing materials, absolute identification must rely on

TABLE 1. FATTY ACID COMPOSITION OF HELA CELL (JHC1) TRIGLYCERIDES AND PHOSPHOLIPIDS AS DETERMINED BY GAS LIQUID CHROMATOGRAPHY*

Fatty Acid	Carbon Number			Percentage Composition	
	Present Study	Reference 16	Reference 15	Neutral Lipid	Phospholipid
14:0	14.0	14.0	14.0	2	1
15:0	15.0	—	15.0	trace	<1
16:br†	15.7	15.75	15.6	<1	2
16:0	16.0	16.0	16.0	25	18
16:1	16.33	16.5	16.4	3	5
17:0†	16.96	—	17.0	trace	<1
17:1†	17.3	17.32	17.3	<1	<1
18:br†	17.5	—	17.6	<1	<1
18:0	18.0	18.0	18.0	12	12
18:1	18.3	18.32	18.3	38	30
18:2	18.8	19.02	18.9	9	25
18:3	19.56	19.6	19.65	1	trace
20:0	20.0	20.0	20.0	1	<1
20:1†	20.2	—	20.3	4	<1
20:2†	20.7	20.65	20.7	—	1
21:0	21.0	21.0	21.0	1	3
21:1†, 20:3†	21.4	21.42	—	—	1
20:4	21.6	—	21.6	—	<1

* Based on analyses using diethylene glycol succinate at 182°. To corroborate these results, hydrogenated samples (17) were also analyzed with an Apiezon-L packed column at 230°.

† Adequate standards were not available for these acids and their provisional identification is based on published constants (15, 16, 17).

[4] Wilkens Instrument and Research, Inc., Walnut Creek, California.

[5] See footnote 4.

[6] See footnote 4.

[7] The methyl esters were obtained from the Hormel Institute, Austin, Minnesota, and Applied Science Laboratories, Inc., 140 North Barnard St., State College, Pennsylvania.

degradation procedures. Unfortunately, these require larger lipid samples than are obtainable in this laboratory.

RESULTS

As determined by gas-liquid chromatography, HeLa cells contained a fairly wide spectrum of fatty acids (Table 1). In spite of the different conditions employed, the carbon numbers of the acids agreed well with those published by Woodford and van Gent (15), Johnston and Kummerow (16), and those calculated from the data of Farquhar *et al.* (17). A number of minor components, for which no adequate standards were available, were also present and comprised about 5% to 6% of the total. Stearic, palmitic, 18:1 (presumedly oleic), and 18:2 (presumedly linoleic) acids comprised about 85% of the acids of both the triglyceride and phospholipid fractions. The most prominent of the triglyceride fatty acids was oleic with palmitic acid next in importance in the JHC1 cells. Analysis of the DM strain showed no significant differences from that of the JHC1 strain. The amount of 18:2 acid did not reach that reported by Bailey *et al.* (5).

The contamination of tissue culture cells with pleuropneumonia-like organisms is a frequent occurrence (11). Analyses of two contaminated cultures showed no significant differences in fatty acid composition.

Strain L fibroblasts grown in chemically defined medium contained no polyunsaturated fatty acids (Table 2). Approximately 95% of the total fatty acids was comprised of 16:0, 16:1, 18:0, and 18:1, with the last constituting over 50% of the total. Stearic and palmitic acids were present in about equal amounts. The relative ratio of saturated to unsaturated acids was fairly similar to that found in the HeLa cell lipids. That the fatty acids present in dialyzed serum can affect the fatty acids found in strain L fibroblasts is shown by the results in Table 3. Cells grown in serum-free medium were devoid of 18:2 acids (Table 2). Cells grown in the presence of 10% dialyzed serum for 144 hours contained approximately 15% of this acid and exhibited a fatty acid distribution pattern similar to that of the HeLa cell lipids (Table 1). When cells that had been with serum for 72 hours were grown in its absence for an additional 72 hours, a low 18:2 acid content was found. It is of some interest that the sums of the C_{18} unsaturated acids were quite similar in all three cases.

DISCUSSION

Under the conditions employed in these experiments, strain L fibroblasts grown in chemically defined media contained predominantly 16:0, 16:1, 18:0, and 18:1 acids but were devoid of polyunsaturated acids such as linoleic and arachidonic. The lack of polyunsaturated acids indicates that these cells either cannot synthesize these acids or synthesize them in amounts too small to be detected by the sensitive method employed. The largest samples used were such that the major peaks had to be obtained at sensitivities 20 to 50 times less than those of the minor components, and even under such conditions no polyunsaturated acids were detected. No requirement for polyunsaturated acids by cells in tissue culture has been shown. Evans and co-workers (18) added linoleic acid to earlier chemically defined media for strain L cells but have since abandoned its use (19).

The distribution of the major acids of HeLa cells resembled that of the fibroblasts but included substantial amounts of polyunsaturated acids. The monoenoic acids were probably palmitoleic and oleic since they had the proper carbon numbers as determined with both the DEGS and Apiezon-L columns based on results

TABLE 2. PRINCIPAL FATTY ACIDS OF TRIGLYCERIDES AND PHOSPHOLIPIDS OF STRAIN L FIBROBLASTS GROWN IN SERUM-FREE MEDIUM

Fatty Acid	Percentage Composition			
	Neutral lipids		Phospholipids	
	Lot 1	Lot 2	Lot 1	Lot 2
14:0	trace	trace	1	trace
15:1	1	2	3	—
16:0	11	14	16	12
16:1	2	4	4	4
18:0	22	19	18	20
18:1	64	61	58	64

TABLE 3. FATTY ACID COMPOSITION OF STRAIN L MOUSE FIBROBLAST LIPIDS AND THE EFFECT OF SERUM LIPIDS

Fatty Acid	Percentage Composition		
	Grown in Serum-free Medium*	Grown 72 Hours with Serum,† then 72 Hours Without Serum*	Grown 144 Hours with Serum†
14:0	2.5	1.5	1.1
15:1	<1	<1	<1
16:0	22	19	21
16:1	11	12	7.1
18:0	10	9	14
18:1	54	56	40
18:2	0	2	15

* Waymouth's medium.
† Waymouth's medium plus 10% dialyzed horse serum.

with standards and with published values. In spite of the high temperatures employed (230° with Apiezon-L), esters having retention times longer than arachidonic were not observed.

Strain L cells utilized polyunsaturated acids contained in the serum lipids (Table 3). This is in keeping with the utilization of emulsified triglycerides (4), serum lipids (5), and unesterified radioactive fatty acids[8] by tissue culture cells, and probably explains the occurrence in the HeLa cell of many of the fatty acids found in mammalian cells (15, 16, 17).

Whether the serum lipids are incorporated into the cells as such or are degraded to yield fatty acids that are then incorporated into more complex forms, is unknown. Thus, the higher 18:2 acid content of the HeLa phospholipids as compared with the triglycerides may reflect retention of intact serum phospholipids rather than a selective function of the cells in phospholipid synthesis.

The ratio of unsaturated fatty acids to saturated fatty acids was fairly well maintained by both cell strains. This was most apparent with the strain L cells grown in the presence and absence of serum where 18:2 acid replaced 18:1 acid. The extent to which such replacement can occur remains to be determined, as does the influence of exogenous fatty acids on the production of endogenous forms.

As larger quantities of HeLa and strain L lipids become available, it will be possible to employ degradation procedures to ascertain the complete structure of the constituent fatty acids. Cells grown in tissue culture afford a unique system for the study of lipid metabolism. This is especially true of cells grown in chemically defined media where exogenous lipids can be eliminated or regulated at will.

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[8] Unpublished data.

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