〔J. Chiba Med. Soc. 49, 399-401, 1973〕

〔原著〕

STUDIES ON FATTY ACIDS OF HUMAN LIVER.

1. PARTIAL CHARACTERIZATION OF FATTY ACIDS OF HUMAN LIVER

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(Received for publication, August 10, 1973)

SUMMARY

The fatty acids of human liver were analyzed as their methyl esters by gas-liquid chromatography.

Thirty five fatty acids, including 10 saturated, 8 monoenoic, 5 dienoic acids, and other polyunsaturated fatty acids, were characterized by the following methods: (1) comparison of their chromatographic characteristics with those of standards, (2) hydrogenation for carbon chain length analysis, (3) thin-layer chromatography on silica gel impregnated with silver nitrate for the analysis of unsaturation. (4) The molecular weight of polyunsaturated fatty acids were determined by mass-spectrometry coupled with gas-liquid chromatography.

Keywords: Fatty acids, Human liver, Characterization, Gas-liquid chromatography, Mass-spectrometry.

INTRODUCTION

The lipids of human liver have been the subjects of several investigations during recent years[1-10]. However, a detailed analysis of liver fatty acids, using new methodology, has not been reported. The purpose of the present study is to characterize the fatty acids of human liver using gas-liquid chromatography and argentation thin-layer chromatography on silica gel.

MATERIALS AND METHODS

The quality of solvents used in this study was of reagent grade.

The hepatic tissues without morphological change were acquired from five adult individuals, autopsied at National Cancer Center Hospital. Concurrently, liver tissues were obtained at surgery for histological examination and a part of this tissues was pooled for chemical analyses. No attempt was made to select one particular location of the liver lobe. The tissues were wiped gently with filter paper dipped with cold physiological saline to remove surface blood and were stored frozen at −20° in polyethylenl bag until ready for analyses. The tissue was finely homogenized in 5 m*l* of physiological saline. The total lipids were extracted with chloroform-methanol, 2/1, v/v and purified according to the method of

This work was presented in part at the 45th Meeting of Chiba Medical Society, Chiba on November 16, 1968.

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荒木英爾[1], 岡崎伸生[2]: ヒト肝臓の脂肪酸の研究 1. ヒト肝臓脂肪酸の部分的同定
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昭和48年8月10日受付

Folch[11]. Fatty acid methyl esters were produced by transmethylation with boron trifluoride-methanol at 95° under reflux and were purified on layers of Silica Gel H, 350 μ thick, by developing with 10% diethyl ether in petroleum ether. The purified esters were dissolved in chloroform and fractionated with silica gel thin-layer plates impregnated with silver nitrate using diethyl ether and petroleum ether, 95/5 and 1/1, v/v as the developing solvent. The fractions were scrapped off the plates and eluted from the adsorbent with diethyl ether and diethyl ether-methanol, 1/1, v/v. Gas-chromatographic analyses were carried out on a HITACHI-PERKIN ELMER F 6-D with flame-ionization detector. The column, 1 m×3 mm, was filled with 20% diethylene glycol succinate on Diasolid L, 100-120 mesh. The stationary phase was synthesized from ethylene glycol and succinic acid. Nitrogen, at an inlet pressure of 0.5 kg/cm^2 and with a flow rate of 40 ml/min, served as carrier gas. All samples were analyzed isothermally at 195°. The components were identified either by comparison of their retention volumes with those of palmitate and pure reference compounds[12], or from the position on graphs, where $\log_{10}$ relative retention volume is plotted against chain length for methyl esters. In addition, aliquots of the samples dissolved in methanol, were hydrogenated at room temperature with palladium-black as catalyst, and analyzed for the length of carbon chain. Polyunsaturated fatty acids were also analyzed by gas-liquid chromatography coupled with mass-spectrometry (HITACHI RMU-6 D). The spectrometer was run under the following operating conditions: ion-accelerating voltage; 1,800 V, the mass range; 2-600 M/e, electron-accelerating voltage; 70 eV, total electron-current; 80 μA, ion trap current; 4.3 mV/div.

RESULTS AND DISCUSSION

Thirty five fatty acids, including 10 saturated, 8 monounsaturated, 5 dienoic, and other polyunsaturated fatty acids, were identified in the lipids of normal human liver as shown in Table 1.

Linear relationships were observed in plots of relative retention volume versus carbon number for saturated and monounsaturated fatty acid methyl esters. As it has already been shown, positional isomers of the dienoic and polyunsaturated acids possessed different retention vol-

TABLE 1. Fatty Acids of Human Liver.

Peak No.	Relative Retention Volume*	Fatty Acid**
1.	0.36	12 : 0
2.	0.40	12 : 1
3.	0.47	12 : 2
4.	0.58	14 : 0
5.	0.67	14 : 1
6.	0.76	15 : 0
7.	0.83	14 : 2
8.	1.00	16 : 0
9.	1.17	16 : 1
10.	1.27	17 : 0
11.	1.30	17 : 1
12.	1.49	16 : 2
13.	1.70	18 : 0
14.	1.98	18 : 1
15.	2.17	19 : 0
16.	2.20	19 : 1
17.	2.53	18 : 2
18.	2.80	20 : 0
19.	2.98	18 : 3, n-6
20.	3.38	18 : 3, n-3 20 : 1
21.	3.91	21 : 0
22.	4.22	20 : 2
23.	4.40	20 : 3, n-9
24.	4.95	22 : 0
25.	5.09	20 : 3, n-6
26.	5.77	20 : 4
27.	6.45	20 : 5, n-6
28.	7.68	20 : 5, n-3
29.	8.88	22 : 3
30.	9.91	24 : 1
31.	10.20	22 : 4
32.	12.45	22 : 5, n-6
33.	12.66	22 : 5, n-3
34.	15.59	22 : 6

* Retention volumes of methyl esters of liver fatty acids relative to methyl palmitate in diethylene glycol succinate at 195°. Each figure is the mean of 15 analyses.

** Shorthand designation: chain length: number of double bonds.

umes[12]. In the minor fractions, the fatty acids with odd number of carbon atoms, such as $C_{15:0}$, $C_{17:0}$, $C_{17:1}$, $C_{19:0}$, $C_{19:1}$, and $C_{21:0}$, were detected. A compound that had the same Rf-value as hydroxy fatty acid on thin-layer chromatogram was found in the analysis of fatty acid methyl esters.

Although some of the fatty acids were overlapped with others and gave one peak, they could be separated by argentation chromatography. This chromatography, while not ideal for quantitative analyses, gave the reproducible results in our study. With further combination of techniques of structural analyses, the fatty acid identification may come to be complete.

ACKNOWLEDGMENTS

We are grateful to the staff of Application Laboratory, the Naka works, Hitachi Ltd, Ibaragi for letting us use the mass-spectrometer equipped with gas chromatograph.

This investigation has been supported in part by Grant from the Ministry of Health and Welfare to the research group for the Biochemistry of Human Cancer (Director: Dr. Yuich Yamamura).

要　　旨

ヒト肝臓の脂肪酸のガスクロマトグラフィーを行ない，1) 標準物質のクロマトグラムとの比較，2) 水素化物のガスクロマトグラフィー，3) 硝酸銀薄層クロマトグラフィーによる分画，4) 質量分析法などの成績とあわせて，飽和-10，モノ不飽和-8，ジ不飽和-5を含む35の脂肪酸を同定した。

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