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94. Yoshio Sakurai and Takao Iwaguchi: Fatty Acid Components of Lipid Fractions of Yoshida Sarcoma and Rat Ascites Hepatomas.*1

(*Cancer Institute, the Japanese Foundation for Cancer Research**2)

Lipid content of the ascites tumors and host liver differed much from each other. The ratios of saturated acids to the unsaturated in the neutral lipid fraction of the tumors and the liver were always less than 1. On the contrary, the similar ratios in the phospholipid fraction of the tumors were more than 1 without exception, while that of the liver were less than 1 as in the case of neutral lipid fraction.

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According to studies of Van Deenen[1] and Gier[2] on the membranes of red blood cells, there seems to be a relationship between phospholipid composition of red blood cells and their membrane permeability. One of the same authors[3] also reported a difference in the ratio of stearic acid to oleic acid in phospholipids between the primary rat hepatoma and normal host liver. On the other hand, lipid composition of various tumors has been compared by many workers. For instance, Yamakawa, *et al.*[4] reported that no profound difference in lipid composition was found between Ehrlich ascites carcinoma and sarcoma 180, while a relatively larger amount of free fatty acids was present in both tumors than in normal mouse liver, of which stearic acid was predominant. A similar comparison was made by Gray, *et al.*[5] between two tumors, Handschutz ascites carcinoma and BP8/C3H ascites sarcoma, and normal tissues of the host animals. Their results showed a far less selective distribution of fatty acids in the fraction of phospholipids and neutral lipids than was usually found in normal tissues, on which Connellan, *et al.*[6] reported their experimental results in 1965. This paper deals with analysis of fatty acids participating in the structure of lipids obtained from Yoshida sarcoma, a subline of Yoshida sarcoma resistant to nitrogen mustard (HN_2), and rat ascites hepatomas. It is a well known fact that these tumors exhibit individually different sensitivity to antitumor agents and, therefore, it was anticipated, that some difference in chemical composition of the membrane of the cells or of the intracellular particles might play a rôle in their drug sensitivity, because the difference in chemical structure of membranes may exert an unnegligible influence on their permeability.

Experimental

Animals and Materials——One million tumor cells of Yoshida sarcoma, HN_2-resistant Yoshida sarcoma and ascites hepatomas (AH-130, AH-60C, AH-7974, AH-70B,) were intraperitoneally transplanted to Donryu rat. Five to 10 days after inoculation, animals were killed by decapitation and the infiltrated mass of tumor

*1 Nishisugamo, Toshima-ku, Tokyo (桜井欽夫, 岩口孝雄).

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1) L. L. M. Van Deenen: Biochem. Biophys. Acta, **43**, 95 (1960).
2) D. Gier: Naturwiss., **48**, 54 (1961).
3) L. L. M. Van Deenen: Krebsforsch., **64**, 137 (1961).
4) T. Yamakawa: Japan. J. Exptl. Med., **32**, 289 (1962).
5) G. M. Gray: Biochem. J., **86**, 350 (1963).
6) J. M. Connellan: Biochem. J., **94**, 81 (1965).

on the omentum (5～10 g. wet weight) was extirpated. Normal rat liver was perfused with 0.85% NaCl before extirpation.

Chemicals——The methyl esters of fatty acids from Sigma Chemical Co. (U. S. A.) were used as authentic standards for gas chromatographic analysis.

Extraction and Saponification of Lipids——Five to 10 g. of tumor wet tissue was homogenized in a Waring blender at 4° for 5 min. with 25～50 ml. of Bloor's reagent[7] (95% EtOH–ether/3 : 1 v/v) and allowed to stand at room temperature for 12 hrs. The mixture was refluxed at 70～80° for 30 min. and filtered through defatted filter paper. The residue was extracted 3 times with boiling petroether (b.p. 50～60°). Thirty to 60 ml. of the combined extract was added to 60～120 ml. of 0.5% NaCl solution. The upper layer was washed with a small amount of H_2O and evaporated to a small volume under a reduced pressure. The concentrated solution was dissolved in 4～8 ml. of Me_2CO and allowed to stand at −25° for several hrs. White precipitate formed (phospholipid fraction) was separated by centrifugation at 4000 r.p.m., at −5° for 15 min. and collected. The supernatant Me_2CO solution (neutral lipid fraction) was evaporated under a reduced pressure and collected. Both fractions were respectively saponified by Wiese and Hansen's method.[8] All these procedures were carried out in nitrogen atmosphere to prevent autoxidation.

Preparation of Fatty Acid Methyl Esters——The saponified fatty acids of each lipid fraction were methylated with CH_2N_2 in ether dissolved in hexane, and kept at −25° in a dark place until used for analytical experiments.

Gas-Liquid Chromatography——Analysis of fatty acid methyl esters was carried out with Yanagimoto's GCG-3 Type gas chromatograph fitted with a catharometer, under flow of He gas at 90 ml./min. Column: 2 m. in length, 5 mm. in diameter, packed with Celite 545 impregnated with polyethylenediglycol succinate supplied by Nishio Co., Tokyo. Working temperature : 180°. Detecter temperature : 200°. The identification of effluent peaks was made by the relative retention volume with authentic standards. The relative quantities of fatty acid esters in each sample were calculated on the relative peak areas on the chromatograms determined by triangulation procedure.

Result and Discussion

Lipid content of Yoshida sarcoma, HN_2-resistant strain of Yoshida sarcoma, rat ascites hepatomas (AH-130, AH-60C, AH-7974, AH-70B), and normal rat liver is shown in Table I. The total lipid and phospholipid content of the ascites tumors and host

Table I. Lipid Content of Tumor Infiltration on the Omentum

	Yoshida sarcoma	HN_2-Resistant Yoshida sarcoma	AH-130	AH-60C	AH-7974	AH-70B	Liver
MED of Nitrogen Mustard N-oxide Hydrochloride	1		1	5	25	50	
Total Lipid[a]	34	30	90	15	57	27	53
Phospholipid[a]	21	22	47	4	35	2	42

MED : Minimum effective dose, mg./kg. a) mg./g. tumor wet weight

Table II. Fatty Acid Component of Neutral Lipid Fraction (%)

Fatty Acid	Yoshida sarcoma	HN_2-Resistant Yoshida sarcoma	AH-130	AH-60C	AH-7974	AH-70B	Liver
Myristic	4.0	3.0	6.2	8.7	3.4	8.0	1.6
Palmitic	27.4	27.6	29.0	27.0	27.0	23.5	25.5
Stearic	6.9	4.2	3.8	3.5	3.1	2.3	6.1
Palmitoleic	5.2	7.1	5.1	4.3	10.1	8.8	2.5
Oleic	34.5	34.8	32.6	38.0	33.3	33.5	28.3
Linoleic	22.0	23.3	23.3	18.5	23.1	23.9	36.0

7) Y. Hikasa : Saishin Igaku, **18**, 921 (1963).
8) D. J. Hanahan : J. Biol. Chem., **228**, 685 (1957).

Table Ⅲ. Fatty Acid Component of Phospholipid Fraction (%)

Fatty Acid	Yoshida sarcoma	HN_2-Resistant Yoshida sarcoma	AH-130	AH-60C	AH-7974	AH-70B	Liver
Myristic	7.0	6.9	5.2	5.3	6.1	7.8	1.9
Palmitic	35.7	38.3	40.6	37.5	38.0	52.6	7.3
Stearic	29.4	18.0	13.8	7.8	15.9	5.8	4.8
Palmitoleic	trace	trace	3.6	5.2	8.7	6.8	1.5
Oleic	15.5	22.5	22.0	28.4	20.8	16.5	13.2
Linoleic	12.4	14.3	14.8	15.9	10.5	10.5	31.9
Arachidonic							39.4

liver differed widely from each other. Fatty acid components of neutral lipid fractions of the tumors and normal liver are given in Table Ⅱ. Chromatographic patterns of fatty acids obtained from neutral lipid fractions of the tumors and the liver were not so different from each other. Generally speaking, the quantity of saturated acids was less than that of unsaturated acids. Among the saturated acids, palmitate was the most predominant in all cases as shown in Table Ⅱ. Fatty acids obtained by hydrolysis of phospholipid fractions are shown in Table Ⅲ. Contrary to the results obtained from neutral lipid fraction, the total content of saturated fatty acids in phospholipid fractions was far greater than that of unsaturated acids, among which palmitate was still the predominant. In the case of phospholipid of normal liver, however, the amout of unsaturated acids was far larger than that of the saturated, which is due to the characteristic appearance of a large quantity of arachidonate as shown in Table Ⅲ. In conclusion, individual lipid content differed much from each other so far as the results of this experiment with the ascites tumors and normal rat liver concerned. From these data, no characteristic figure which distinguishes the malignant tissue from the normal was observed. It is a matter of interest, however, that in normal liver, the ratio of the saturated fatty acid to the unsaturated in the neutral lipid fraction was found to be far larger than the ratio of the phospholipid fraction, while, in the tumor groups, the correlation between these two ratios appeared to be just the reverse. On the other hand, no marked differences were proved either in the quantity of neutral lipid and of phospholipid or in fatty acid patterns of both lipid fractions between the two strains of rat ascites hepatoma, AH-130 and AH-7974, the former of which is known to be very sensitive and the latter very resistant to antitumor alkylating agents.