Molecular Composition of Lecithins in the Primary Hepatoma Induced by 3'-Methyl-4-Dimethylaminoazobenzene

GOROH OKANO, TOYOAKI AKINO and YOHICHI MOCHIZUKI*

*The First Division, Department of Biochemistry and *Department of Pathology, Cancer Research Institute, Sapporo Medical College, Sapporo*

Okano, G., Akino, T. and Mochizuki, Y. *Molecular Composition of Lecithins in the Primary Hepatoma Induced by 3'-Methyl-4-Dimethylaminoazobenzene*. Tohoku J. exp. Med., 1977, 122 (1), 21-33 — The lecithins of the primary hepatoma induced by 3'-methyl-4-dimethylaminoazobenzene (DAB) and host liver of rat were isolated, and the individual molecular species were estimated quantitatively by combined thin-layer and gaschromatographic analysis and specific enzymic hydrolysis. The DAB-hepatoma lecithins appeared to have more specific positioning of fatty acids than the transplanted hepatoma lecithins which have been reported by Dyatlovitskaya et al. (1974). However, there were some striking differences to be noted between the DAB-hepatoma and host liver lecithins. The usual type of molecular species of lecithin, saturated-unsaturated species, was the main component in the hepatoma and host liver. The amounts of the unusual species having impaired positional specificity in fatty acid such as the saturated-saturated and the unsaturated-saturated species were high in the DAB-hepatoma when compared with host liver, although the content of the unsaturated-unsaturated species of lecithin were almost similar in both the tissues. The formation of the unusual species of lecithin in tumor tissues was discussed in relation to the impairing in the enzyme systems involved in lecithin synthesis. ——— tumor lecithin; lecithin molecular species

A number of studies have been made to gain insight into the possible connection between lipid metabolism and malignant growth and it has been suggested that the specific nature of tumor cells, the invasiveness, may be related to the surface and biochemical properties of tumor cells which mainly depend on the lipid components (Haven and Bloor 1956; Abercrombie and Ambrose 1962).

Since phospholipids exist in the form of molecular species having specific pairing of fatty acids in the cell membranes and each molecular species functions as a molecular unit, changes in the molecular composition of the phospholipid would produce important changes to the function of cell membranes.

In the liver, lecithin species containing saturated acids in the position 1 and unsaturated acids in the position 2 are common (Hanahan 1960; Veerkamp et al. 1962; Montfoort et al. 1971). However, Bergelson's group (Dyatlovitskaya

et al. 1974) has recently reported that the lecithin from the transplanted rat hepatoma is characterized by the presence of molecular species having impaired positional specificity of fatty acids such as unsaturated-saturated and unsaturated-unsaturated species, in relatively high percentages as compared with host liver. They suggested the disturbance of enzymes involved at the stage of lecithin synthesis in the hepatoma and the possible roles of the unusual lecithin species in the membrane function of the tumor cells.

Only a few investigations on the analysis of molecular species of lecithin in the malignant tumors have been reported (Dyatlovitskaya et al. 1974; Wood and Snyder 1969). The present work aims to elucidate the molecular structure of lecithin in the primary hepatoma induced by 3'-methyl-4-dimethylamino-azobenzene.

EXPERIMENTAL PROCEDURE

Animals and tissue materials

Wistar male rats, initially weighing 200 g, were fed a commercial diet containing 0.06% 3'-methyl-4-dimethylamino-azobenzene (DAB). The rats were sacrificed 24 weeks after initiation of the carcinogenic treatment. The hepatomas were removed from the liver and separated into three groups according to the macroscopic appearance, and they were diagnosed as moderately differentiated, poorly differentiated, or undifferentiated hepatomas at the microscopic levels. The hepatomas were carefully freed from surroundings as well as necrotic and hemorrhagic areas. The hyperplastic nodules were isolated as the preneoplastic samples, and the host liver sample was taken from non-tumorous portions.

Fractionation of phospholipid classes with quantitative isolation of lecithins

Lipids from host liver, hyperplastic nodules and hepatoma were extracted with twenty volumes of chloroform-methanol (2:1). The lipid fraction was washed by the method of Folch et al. (1957) using 0.9% NaCl. The lipid extract was chromatographed on DEAE-cellulose as described by Akino et al. (1970). The lipids from the column were eluted stepwise using chloroform, methanol and then chloroform-methanol (2:1) saturated with ammonia.

Neutral phospholipids eluted with methanol were fractionated on thin-layer chromatography with chloroform-methanol-water (70:30:5). Four spots were detected under U.V. light after spraying with 0.2% dichlorofluorescein in ethanol and were identified with authentic standard samples as phosphatidylethanolamine, lecithin, sphingomyelin and lysolecithin.

Acidic phospholipids eluted with chloroform-methanol (2:1) saturated with ammonia were separated on thin-layer chromatography using silica gel-H in a solvent system of chloroform-methanol-7.5N ammonia (65:35:5). Phosphatidylserine, phosphatidylinositol, cardiolipin and phosphatidylglycerol were identified with authentic standard samples.

Each spot on the plate of thin-layer chromatography was scraped and eluted with chloroform-methanol-acetic acid-water (50:39:1:10) as described by Arvidson (1968) and the extract was washed once with 50% methanol. The recovery of lecithin by this procedure was $94.2 \pm 2.3\%$ when examined by the separate experiments using radioactive lecithin samples.

Subfractionation of lecithins

The purified lecithins were subfractionated to the species of different degrees of unsaturation by thin-layer chromatography on silica gel-H impregnated with silver nitrate

(Arvidson 1968). Five bands, such as saturated+monoenoic, dienoic, trienoic, tetraenoic and pentaenoic+hexaenoic subfractions, were visualized under U.V. light after spraying with 0.2% dichlorofluorescein in ethanol. Each lecithin subfraction was recovered by the same procedure as mentioned above. The total recovery of lecithin subfractions by this procedure was $92.5 \pm 4.4\%$ when tested by the separate experiment using radioactive lecithins. The ratio of the subfractions was determined by phosphorus analysis.

Preparation of diacylglycerol acetates from lecithin subfractions

An aliquot of each lecithin subfraction obtained by silver nitrate thin-layer chromatography was hydrolyzed by phospholipase C from *Clostridium welchii* (Sigma Chem. Co.) by the procedure of Renkonen (1965). The 1,2-diacylglycerols were purified by thin-layer chromatography with hexane-ether-acetic acid (50:50:1) as solvent. After eluting from silica gel with chloroform-methanol (9:1), the diacylglycerols were acetylated in a solution of dry pyridine and acetic anhydride at room temperature for overnight according to the method of Renkonen (1966). The diacylglycerol acetates were purified by thin-layer chromatography with hexane-ether (65:35) as solvent.

Positional analysis of fatty acids in lecithin

Positional distribution of fatty acid in the glycerol moiety of lecithin was determined as follows (Toshima and Akino 1972): lecithin was dried under nitrogen stream and taken up in 5 ml of ether. The lecithin ether solution was then added with 0.2 ml of phospholipase A₂ solution containing 10 mg *Crotalus adamanteus*, 0.01 M CaCl₂ and 0.1 M Tris-buffer (pH 7.4) in a total volume of 1 ml. The mixture was shaken vigorously at room temperature for 2 hr. Fatty acid and lysolecithin thus obtained were separated by thin-layer chromatography and transesterified by BF₃-methanol.

Determination of diacyl, alkenyl-acyl and alkyl-acyl lecithins

The contents of diacyl, alkenyl-acyl and alkyl-acyl lecithins were determined by the combination of mild alkaline and acid hydrolysis methods. Lecithin samples were hydrolyzed by mild acid treatment in the following manner (Dittmer and Wells 1969): Samples were treated in 90% acetic acid at 55°C for 45 min, then the lipid layer was obtained by Folch's method (1957) and separated to lysolecithin and lecithin by thin-layer chromatography. The phosphorus ratio between lysolecithin (corresponding to alkenyl-acyl type) and lecithin (diacyl and alkyl-acyl types) was estimated. The lecithin was further treated by mild alkaline hydrolysis according to the method of Dawson (1960) and the phosphorus ratio was estimated in the water layer (corresponding to diacyl type) and in the organic layer (alkyl-acyl type).

Analytical methods

Fatty acid methylesters and diacylglycerol acetates were analyzed with a Shimadzu Gaschromatograph GC-5A by hydrogen ion detector.

An aliquot of each phospholipid or fatty acid fraction was transmethyated using BF₃-methanol as described by Morrison and Smith (1964). Fatty acid methylesters thus obtained were analyzed on a 2 m × 4 mm-o.d. Pyrex column packed with 10% diethylene glycol succinate on Shimalite W. The runs were made isothermally at 180°C with a carrier gas (nitrogen) flow rate of 60 ml/min. Peak identity of fatty acid methylesters was made by comparison with standards obtained from Sigma Chem. Co.

The diacylglycerol acetates were analyzed on a 1 m × 4 mm-o.d. Pyrex column packed with 1% Silicone OV-1 on Gaschrom Q; the temperature was manually programmed from 250–335°C at approximately 5°/min. Flow rates of nitrogen carrier gas, hydrogen and air were 120, 50 and 50 ml/min, respectively.

Peak identity of the diacylglycerol acetates was made by comparison with appropriate reference standards. The carbon number represents that of acyl carbon atoms in the molecule including that of acetic acid. Thus the peaks corresponding to dipalmitomono-

acetate and distearomonoacetate were referred to as 34 and 38 of carbon number, respectively. A peak with carbon number of 40 would, therefore, be identified as the diacylglycerol acetates containing long chain fatty acids of carbon number of 18 and 20.

Phosphorus was determined by the method of Bartlett (1959).

RESULTS

Although the hepatomas were divided into three groups macroscopically and microscopically, the chemical data of the three groups were the same, so the data of the hepatomas were combined.

The amounts of total phospholipids obtained by Folch's extraction were significantly lower in the hepatoma (22.7 ± 4.0 μ moles/wet g tissue) than in host liver (43.4 μ moles/wet g tissue) and hyperplastic nodule (41.5 μ moles/wet g tissue), which are in agreement with the data reported by various authors (Veerkamp et al. 1961; Figard and Greenberg 1962; Snyder et al. 1969). The quantitative phospholipid compositions of host liver, hyperplastic nodule and hepatoma were almost similar to the data reported earlier (Veerkamp et al. 1961; Snyder et al. 1969; Bergelson et al. 1970), the main differences being somewhat lower lecithin and substantially higher phosphatidylserine and sphingomyelin in tumor tissues, as seen in Table 1. It has been suggested that increase of sphingomyelin and phosphatidylserine in the tumor cells may be important in relation to membrane functions since they are especially prominent in plasma membrane of the cell (Haven and Levy 1941; Bergelson et al. 1970).

The contents of diacyl, alkenyl-acyl and alkyl-acyl in lecithin were determined by the combination of mild acid and alkaline hydrolysis methods. The results are summarized in Table 2. The lecithin in the hepatoma consisted of small amounts of alkenyl-acyl (approximately 2.1%) and alkyl-acyl (approximately 5.5%), and the great majority of the hepatoma lecithin was a diacyl type. The content of the plasmalogenic molecules could also be confirmed to be low, since

TABLE 1. *Phospholipid composition of host liver, hyperplastic nodule and DAB-hepatoma in rat*

Phospholipids	DAB-hepatoma	Host liver	Hyperplastic nodule
Total lipid-P (μ moles/wet g tissue)	$22.7 \pm 4.0^\dagger$	43.3*	41.5*
Lecithin	$51.8 \pm 2.2\%$	58.6%	56.1%
Phosphatidylethanolamine	29.2 ± 2.2	25.3	25.1
Sphingomyelin	8.1 ± 1.2	3.7	5.3
Lysolecithin	0.9 ± 0.4	—	2.2
Phosphatidylserine	3.2	0.7	0.7
Phosphatidylinositol	2.2	7.6	4.3
Cardiolipin	2.5	3.0	4.3
Phosphatidylglycerol	2.2	0.6	1.2
Unidentified	0.4	0.5	0.7

* The values represent the average of two cases.

† The values represent mean $\pm$ S.D. from four cases.

TABLE 2. *Class composition of lecithins of host liver and DAB-hepatoma in rat*

Classes	DAB-hepatoma	Host liver
Diacyl lecithin	92.4±0.4*%	98.1±0.1*%
Alkenyl-acyl lecithin	2.1±0.3	0.9±0.1
Alkyl-acyl lecithin	5.5±0.1	1.0±0.1

* The values represent mean±s.d. from four cases.

the peaks of dimethylacetals were found in trace on the gaschromatograms of fatty acid methylesters derived from the hepatoma lecithin. The plasmalogen content in DAB-rat hepatoma lipids has been estimated by Veerkamp et al. (1961) to be 0.21 mg/100 g wet tissue which corresponds to approximately 14% of total phospholipids, whereas that in normal liver was 4%. However, they did not estimate the plasmalogen content in individual phospholipids. Wood and Snyder (1969) have found a rather high percentage (36%) of alkyl-acyl type of lecithin in Ehrlich ascites tumor.

As mentioned above, the amounts of alkenyl-acyl and alkyl-acyl types were significantly low in the lecithins of the DAB-hepatoma as well as host liver, and so the intact lecithin molecules were used without separation of diacyl, alkenyl-acyl and alkyl-acyl types for the following analysis to estimate molecular species.

Table 3 shows the results of analysis of fatty acid of lecithin in host liver, hyperplastic nodule and the hepatoma. It was noted that the fatty acid composition of lecithin in host liver was almost similar to that in hyperplastic nodule at preneoplastic stage. Their fatty acid distribution was, however, highly specific; 87% of the saturated acids were found in position 1, whereas the

TABLE 3. *Composition and positional distribution of lecithin from host liver, hyperplastic nodule and DAB-hepatoma*

Fatty acids	DAB-hepatoma†			Host liver*			Hyperplastic nodule*		
	Total	1	2	Total	1	2	Total	1	2
14:0	0.8±0.1	1.2±0.3	tr	1.1	1.2	1.0	1.3	1.7	0.8
15:0	tr	tr	tr	0.6	0.8	tr	0.6	0.8	0.4
16:0	33.6±2.5	54.4±3.1	10.1±2.9	26.8	48.3	5.2	30.0	55.4	4.6
16:1	2.0±0.6	2.1±1.2	1.8±0.5	1.9	2.1	1.7	2.2	2.5	1.9
18:0	13.2±1.6	24.5±2.8	1.9±1.0	19.0	36.0	2.0	15.0	28.7	4.2
18:1	16.0±1.7	12.4±2.5	22.1±1.7	9.6	7.1	12.1	9.9	6.7	13.0
18:2	19.7±2.6	3.0±1.7	36.5±5.2	16.9	2.6	31.1	19.3	1.7	36.9
20:3	0.5±0.3	—	1.6±0.6	2.6	—	5.2	1.0	—	1.9
20:4+22:0	10.6±1.4	—	21.1±2.8	13.6	—	26.7	14.1	—	27.4
22:1	tr	—	tr	1.1	0.6	1.5	0.6	1.1	—
20:5	tr	—	tr	1.0	—	1.9	0.9	—	1.8
22:5	tr	—	0.5±0.4	0.7	—	1.3	0.8	—	1.6
22:6	1.7±0.6	—	3.3±1.4	4.9	—	9.7	4.2	—	8.4
Saturated	48.0	83.5	12.1	48.1	87.3	8.2	46.9	87.8	6.6
Unsaturated	51.6	16.1	87.2	52.3	12.4	91.2	53.0	12.0	92.9

* The values represent the average of two cases.

† The values represent mean±s.d. from four cases.

unsaturated acids almost selectively occupied position 2. The only exception was the acid of 18:1 which was distributed more or less uniformly between both positions. Morin (1965) found the increase in the 18:1 acid and decrease in the 18:0 acid in the lecithin fraction of rat liver at the preneoplastic stage of DAB-carcinogenesis.

As to the hepatoma lecithin, the fatty acid patterns were generally in agreement with the analysis reported for those of various tumors (Figard and Greenberg 1962; Snyder et al. 1969; Wood and Harlow 1970; Dyatlovitskaya et al. 1971). Namely, there were some striking differences in the fatty acid composition between the hepatoma and host liver lecithins. However, in comparison with the latter, the hepatoma lecithin contained less 18:0 and more 16:0 and 18:1.

The content of the 22:6 acid in host liver lecithin was approximately one-third of that in hepatoma lecithin. Although the drastic change in the percentage of 20:4 has been reported using the transplanted hepatoma, the decrease of 20:4 in hepatoma lecithin was not so significant in the present study. The less selective distribution of fatty acids in glycerol moiety of lecithin in the tumor cells has also been shown by several research groups (Gray 1963; Dyatlovitskaya et al. 1971). In DAB-

TABLE 4. *Fatty acid composition of lecithin from host liver and the positional distribution of fatty acids in each subfraction*

Subfraction	Saturated + monoenoic	Dienoic	Trienoic	Tetraenoic	Pentaenoic + hexaenoic
Phosphorus %	20.2	33.5	7.8	28.1	10.4
Fatty acid composition					
14:0	2.0	1.5	0.7	1.0	—
16:0	26.6	23.7	20.7	19.8	23.0
16:1	2.4	+	+	+	+
18:0	11.5	16.9	21.7	21.9	16.3
18:1	52.8	11.1	7.5	6.7	9.9
18:2	1.1	44.0	6.7	0.9	7.6
20:3	—	—	40.7	—	—
20:4	—	—	—	47.4	14.1
20:5	—	—	—	—	6.6
22:5	—	—	—	—	7.1
22:6	—	—	—	—	12.1
Others	3.6	2.4	1.7	2.0	3.2
Fatty acid composition of the 2nd position					
14:0	+	1.0	+	+	+
16:0	11.6	5.1	4.3	2.2	4.9
16:1	4.8	+	—	—	—
18:0	+	1.8	2.2	1.0	3.9
18:1	78.3	4.4	2.5	+	6.8
18:2	2.0	87.2	9.6	1.0	2.7
20:3	—	—	79.8	—	—
20:4	—	—	—	94.8	28.1
20:5	—	—	—	—	13.2
22:5	—	—	—	—	14.1
22:6	—	—	—	—	24.1
Others	2.6	0.5	1.6	1.0	2.2

TABLE 5. *Fatty acid composition of lecithin from DAB-hepatoma and the positional distribution of fatty acids in each subfraction*

Subfraction	Saturated + monoenoic	Dienoic	Trienoic	Tetraenoic	Pentaenoic + hexaenoic
Phosphorus %	29.9±3.2	37.0±4.1	6.1±1.1	18.7±2.4	8.3±1.3
Fatty acid composition					
14:0	2.2	1.7	1.2	1.9	+
16:0	43.0	29.5	25.9	24.8	25.7
16:1	3.0	1.7	1.2	+	+
18:0	11.2	13.1	13.3	15.8	13.2
18:1	35.8	12.8	12.5	14.1	14.3
18:2	1.3	39.5	27.0	+	5.2
20:3	—	—	15.2	—	—
20:4	—	—	—	39.6	13.1
20:5	—	—	—	—	1.1
22:5	—	—	—	—	+
22:6	—	—	—	—	21.8
Others	3.5	1.7	3.7	3.8	5.6
Fatty acid composition of the 2nd position					
14:0	1.6	+	+	+	+
16:0	34.9	11.9	11.8	9.7	10.2
16:1	4.8	2.1	—	+	—
18:0	4.8	2.7	6.3	4.3	4.9
18:1	49.6	6.6	6.5	3.9	5.6
18:2	2.2	75.3	42.9	+	3.7
20:3	—	—	30.4	—	—
20:4	—	—	—	79.1	26.1
20:5	—	—	—	—	2.0
22:5	—	—	—	—	2.1
22:6	—	—	—	—	43.5
Others	2.1	1.4	2.1	3.0	1.9

induced hepatoma, the disturbance of positional specificity of fatty acid in lecithin seemed to be not significant, although somewhat higher unsaturated acids in position 1 and about twofold of saturated acids in position 2 were found as compared with the fatty acid pattern of host liver lecithin. The fatty acid composition of lecithin was quantitatively quite similar at the different stages of DAB-hepatoma.

Lecithins were separated to subfractions according to the degrees of unsaturation by silver nitrate thin-layer chromatography. Each lecithin subfraction, that is, saturated + monoenoic, dienoic, trienoic, tetraenoic, and pentaenoic + hexaenoic ones was subjected to the analysis of (1) positional distribution of fatty acids using phospholipase A₂, and (2) carbon number distribution of diacylglycerol acetates derived from lecithin subfractions by phospholipase C.

The fatty acid composition in the whole molecule and each position of lecithins from host liver and the hepatoma are illustrated in Tables 4 and 5, respectively.

The data on carbon number obtained by analysis of diacylglycerol acetates derived from lecithin subfractions, which is shown in Table 6, provide much information needed to determine which molecular species constitute the lecithins.

TABLE 6. Carbon number composition of the diglyceride acetates derived from lecithin subfractions of host liver and DAB-hepatoma

Subfraction	Carbon number*	DAB-hepatoma	Host liver
Saturated + monoenoic	32	1.6±0.2†%	—%
	34	24.0±1.7	11
	36	56.7±3.2	63
	38	14.7±0.4	20
Dienoic	34	4.3±1.3	1
	36	58.7±2.3	50
	38	34.3±2.4	43
	40	1.7±0.7	1
Trienoic	36	9.3	4
	38	62.3	52
	40	27.3	44
Tetraenoic	38	57.3±1.2	47
	30	40.0±1.2	50
	42	1.0	—
Pentaenoic + hexaenoic	38	16.3±3.0	17
	40	58.6±0.7	67
	42	22.7±2.1	16
	44	2.3±0.3	—

* The total number of acyl carbon atoms per molecule including that of the acetic acid residue.

† Mean±S.E. $N=3$.

However, a carbon number fraction separated by gaschromatography is generally composed of a mixture of molecular species having different combinations of fatty acids. It is necessary to resolve the pairing of fatty acids in each carbon number species.

Therefore, in addition to the carbon number distribution, the data on the fatty acid composition of the lecithin subfractions were used to reconstruct the fatty acid pairing of molecular species of lecithins. Namely, the reconstitution of the composition of each lecithin subfraction recovered from silver nitrate thin-layer chromatography was carried out by the combination of carbon number distribution and fatty acid composition in each position, essentially according to the calculation method of Kuksis and Marai (1967). All the possible pairings were first listed from the fatty acid present, and then, in order to obtain the major molecular species of lecithin, according to the method of van Golde et al. (1965), the main fatty acid pairings were chosen from the data. Table 7 lists the composition of the major individual lecithin of host liver and DAB-hepatoma.

Our findings on the molecular composition of host liver lecithin are generally consistent with the data obtained from rat liver lecithin by Kuksis et al. (1968), van Golde et al. (1965) and Dyatlovitskaya et al. (1974). The majority of host liver lecithin species were a saturated-unsaturated type.

The result of the molecular composition of the hepatoma lecithins showed

TABLE 7. *Main molecular species of lecithins of host liver and DAB-hepatoma*

Position 1	Position 2	DAB-hepatoma	Host liver
16:0	16:0	4.5	1.4
16:0	18:0	0.9	+
16:0	16:1	+	0.8
16:0	18:1	11.2	10.2
18:0	18:1	3.9	2.2
18:1	16:0	6.0	2.4
18:1	18:0	+	+
14:0	18:2	1.0	+
16:0	18:2	16.8	15.0
18:0	18:2	9.4	12.6
18:2	16:0	+	+
18:1	18:1	2.7	2.5
16:0	20:4	10.6	13.0
18:0	20:4	5.6	14.6
18:2	18:2	+	+
16:0	22:5	+	+
18:0	22:5	+	+
16:0	22:6	1.3	2.3
18:0	22:6	0.9	1.0
18:1	20:4	2.5	1.0

similar profiles to that of host liver lecithin. However, there were observed some striking differences between them. In the hepatoma lecithin the saturated-unsaturated type decreased remarkably and substantial increases were found in the saturated-saturated and the unsaturated-saturated types. However, the content of the unsaturated-unsaturated species, one of the unusual lecithins, was almost similar between the hepatoma and host liver lecithins.

The results from DAB-hepatoma lecithins were quite different from those of the transplanted hepatoma lecithins reported by Dyatlovitskaya et al. (1973), who found approximately 10 times the amount of unsaturated-unsaturated species and approximately twice the amount of unsaturated-saturated species in the hepatoma lecithins as compared with normal rat liver lecithins. They also noted that the content of saturated-saturated species was almost the same in both the tissues. However, considerable amounts of saturated-saturated species, especially of the 16:0/16:0 type, were estimated in the DAB-hepatoma lecithin in the present study. In the monounsaturated subfraction of the hepatoma lecithins, the contents of the normal saturated-unsaturated species such as 16:0/18:1 and 18:0/18:1 were almost the same when compared with host liver lecithin, whereas the amounts of the reversed unsaturated-saturated species, 18:1/16:0, were comparatively high. In contrast, the percentage distributions of the reversed species, 18:2/16:0, and the diunsaturated species, 18:1/18:1, in dienoic subfraction were similar between both the tissues. The content of tetraenoic fraction decreased in the hepatoma lecithin, mainly owing to the significant decline of 18:0/20:4, whereas the decrease of 16:0/20:4 was not so remarkable. The pairing of 20:4 and other unsaturated acids to form polyenoic species has been pointed out as

characteristic combinations in the transplanted hepatoma lecithins (Dyatlovitskaya et al. 1974). However, there were no remarkable differences in such kinds of molecular species of lecithins between host liver and hepatoma, although somewhat increase of 18:1/20:4 species was observed in the DAB-hepatoma lecithin.

DISCUSSION

Dyatlovitskaya et al. (1974) have reported characteristics of molecular species of lecithins in the transplanted hepatoma. They summarized their data as follows: (1) hepatoma lecithin has lower specificity of fatty acid pairing than liver lecithin, (2) in the hepatoma lecithin the content of monounsaturated species is higher and that of 20:4 containing species is much lower than in the liver lecithin, and (3) hepatoma lecithin contains considerable amounts of unsaturated-saturated species and unsaturated-unsaturated species, which are rare or absent in the liver.

Our findings on the molecular composition of lecithins in the primary hepatoma induced by DAB were quite different from those of the transplanted hepatoma lecithins. Main differences are as follows: (1) The DAB-hepatoma lecithin appeared to have more specific positioning of fatty acids than the transplanted hepatoma lecithin which have been reported by Dyatlovitskaya et al. (1974), although oleic acid was distributed equally in both positions of lecithin, and so (2) the normal saturated-unsaturated species was the main component, but the molecular species having impaired positional specificity such as the reversed unsaturated-saturated and saturated-saturated species were noted in comparatively high contents when compared with host liver lecithin. However, the content of the unsaturated-unsaturated species, one of the unusual lecithins, was almost similar in the hepatoma and host liver lecithins.

It has been established that in the liver major lecithin species can be synthesized mainly by specific routes (Arvidson 1968; Hill et al. 1968; Kanoh 1969), that is, oligoenoic species having 18:1 or 18:2 at position 2 may be preferentially synthesized via de novo pathway and tetraenoic species mainly via direct acylation of 1-acyl lysolecithin and hexaenoic species highly via methylation of phosphatidylethanolamine, respectively. Dyatlovitskaya et al. (1974) suggested, from the facts that the reversed species of hepatoma lecithin contained 18:1 and 18:2 whereas the location of 20:4 remained strictly specific, that the impaired enzyme system involved in lecithin synthesis in the hepatoma is that of a choline phosphotransferase and 20:4 acyl-transferase system appears to be still working selectively.

In the DAB-hepatoma, the acids of 20:4 and 22:6 were located exclusively at position 2 of lecithin, and so the selectivity of incorporation of 20:4 and 22:6 into lecithins via acylation and methylation pathways seems not to be impaired in the DAB-hepatoma, although Figard and Greenberg (1962) have observed low activity of the methylating enzyme in various hepatomas. On the other hand, the proportion of the unusual lecithin species was not significantly high in DAB-

hepatoma as compared with the transplanted hepatoma lecithins, but when compared with host liver lecithins the increase in unsaturated-saturated and disaturated species was noted. The reversed lecithins might be introduced by the disturbance of fatty acyl donor selectivity of enzyme systems involved in lecithin synthesis, especially glycerophosphate acyltransferase and 1-acyl glycerophosphate acyltransferase system, because in choline phosphotransferases the selectivity of diacylglycerols as substrates is not specific for the species containing 18:1 or 18:2 at position 2 as reported by Kanoh and Ohno (1970). The glycerophosphate acyltransferase system in rat liver microsomes is specific for saturated acids at position 1 and for unsaturated acids at position 2 of phosphatidic acid, which has been reported by Yamashita et al. (1973). Very interesting was the observation in the hepatoma lecithin that the content of the reversed species such as 18:1/16:0 was increased, while that of the diunsaturated species, 18:1/18:1, remained unchanged. The data strongly suggest that when acylated with unsaturated acids at position 1, saturated acids may preferentially be incorporated into position 2 in the hepatoma. Considerable amounts of disaturated lecithin, especially 16:0/16:0, were present in DAB-hepatoma lecithin. The disaturated lecithin is only one molecular species which has been specifically implicated in physiological function (Klaus et al. 1961). This compound, particularly dipalmitoyllecithin, has been claimed to be the active surfactant in the lung. However, it has been noted that the disaturated species is contained not only in the lung but also in other tissues such as erythrocytes, spleen, kidney (Shimojo et al. 1974) and Yoshida hepatoma (Fallani et al. 1976), although its role on membrane functions in the mammalian cells is still unknown.

Acknowledgment

We wish to thank Professor Kimiyoshi Ohno for his advice in preparing this manuscript.

References

- 1) Abercrombie, M. & Ambrose, E.J. (1962) The surface properties of cancer cells: A review. *Cancer Res.*, **22**, 525-548.
- 2) Akino, T., Kanoh, H. & Shimojo, T. (1970) On the molecular species of rat liver phosphatidylinoitol. *Tohoku J. exp. Med.*, **101**, 289-297.
- 3) Arvidson, G.A.E. (1968) Structural and metabolic heterogeneity of rat liver glycerophosphatides. *Europ. J. Biochem.*, **4**, 478-486.
- 4) Bartlett, G.R. (1959) Phosphorus assay in column chromatography. *J. biol. Chem.*, **234**, 466-468.
- 5) Bergelson, L.D., Dyatlovitskaya, E.V., Torkhovskaya, T.I., Sorokina, I.B. & Gorkova, N.P. (1970) Phospholipid composition of membranes in the tumor cell. *Biochim. biophys. Acta*, **210**, 287-298.
- 6) Dawson, R.M.C. (1960) A hydrolytic procedure for the identification and estimation of individual phospholipids in biological samples. *Biochem. J.*, **75**, 45-53.
- 7) Dittmer, J.C. & Wells, M.A. (1969) Quantitative and qualitative analysis of lipids and lipid components. In: *Methods in Enzymology*, 14, edited by J.M. Lowenstein, Academic Press, New York and London, pp. 482-530.
- 8) Dyatlovitskaya, E.V., Yanchevskaya, G.V., Kolesova, N.P. & Bergelson, L.D.

- (1973). Tumor lipids. Positional distribution of fatty acids in rat liver and hepatoma-27 glycerophospholipids. *Biokhimiya*, **38**, 749-755.
- 9) Dyatlovitskaya, E.V., Yanchevskaya, G.V. & Bergelson, L.D. (1974) Molecular species and membrane forming properties of lecithins in normal liver and hepatoma. *Chem. Phys. Lipids*, **12**, 132-149.
 - 10) Figard, P.H. & Greenberg, D.M. (1962) The phosphatides of some mouse ascites tumors. *Cancer Res.*, **22**, 361-367.
 - 11) Folch, J., Lees, M. & Sloane-Stanly, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.*, **227**, 497-509.
 - 12) Gray, G.M. (1963) The lipid composition of tumor cells. *Biochem. J.*, **86**, 350-357.
 - 13) Hanahan, D.J. (1960) Phosphoglycerides. In: *Lipid Chemistry*, edited by D.J., Hanahan, John Wiley, New York, pp. 42-105.
 - 14) Haven, F.L. & Levy, S.R. (1941) The occurrence and rate of turnover of tumor sphingomyelin. *J. biol. Chem.*, **141**, 417-425.
 - 15) Haven, F.L. & Bloor, W.R. (1956) Lipids in cancer. *Advanc. Cancer Res.*, **4**, 237-314.
 - 16) Hill, E.E., Husbands, D.R. & Lands, W.E.M. (1968) The selective incorporation of ¹⁴C-glycerol into different species of phosphatidic acid, phosphatidylethanolamine, and phosphatidylcholine. *J. biol. Chem.*, **243**, 4440-4451.
 - 17) Kanoh, H. (1969) Biosynthesis of molecular species of phosphatidyl choline and phosphatidyl ethanolamine from radioactive precursors in rat liver slices. *Biochim. biophys. Acta*, **176**, 756-763.
 - 18) Kanoh, H. & Ohno (1970) Biosynthesis of lecithins and phosphatidyl ethanolamines from various radioactive 1,2-diglycerides in rat liver microsomes. *Biochim. biophys. Acta*, **218**, 249-258.
 - 19) Klaus, M.H., Clements, J.A. & Havel, R.J. (1961) Composition of surface-active material isolated from beef lung. *Proc. nat. Acad. Sci. (Wash.)*, **47**, 1858-1859.
 - 20) Kuksis, A. & Marai, L. (1967) Determination of the complete structure of natural lecithins. *Lipids*, **2**, 217-224.
 - 21) Kuksis, A., Marai, L., Breckenridge, W.C., Gornall, D.A. & Stachnyk, O. (1968) Molecular species of lecithins of some functionally distinct rat tissues. *Canad. J. Physiol. Pharmacol.*, **46**, 511-524.
 - 22) Montfoort, A., van Golde, L.M.G. & van Deenen, L.L.M. (1971) Molecular species of lecithins from various animal tissues. *Biochim. biophys. Acta*, **231**, 335-342.
 - 23) Morin, R.J. (1965) Effects of 4-dimethylaminoazobenzene on hepatic microsomal phospholipids. *Cancer Res.*, **25**, 118-123.
 - 24) Morrison, W.R. & Smith, L.M. (1964) Preparation of fatty acid methylesters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.*, **5**, 600-608.
 - 25) Renkonen, O. (1965) Individual molecular species of different phospholipid classes. II. A method of analysis. *J. Amer. Oil. Chemist's Soc.*, **42**, 298-304.
 - 26) Rendonon, O. (1966) Individual molecular species of phospholipids. III. Molecular species of ox-brain lecithins. *Biochim. biophys. Acta*, **125**, 288-309.
 - 27) Shimojo, T., Abe, M. & Ohta, M. (1974) A method for determination of saturated phosphatidylcholine. *J. Lipid Res.*, **15**, 525-527.
 - 28) Snyder, F., Blank, M.L. & Morris, H.P. (1969) Occurrence and nature of o-alkyl and o-alk-1-enyl moieties of glycerol in lipids of morris transplanted hepatomas and normal rat liver. *Biochim. biophys. Acta*, **176**, 502-510.
 - 29) Toshima, N. & Akino, T. (1972) Alveolar and tissue phospholipids of rat lung. *Tohoku J. exp. Med.*, **108**, 253-263.
 - 30) Van Golde, L.M.G., Zwaal, F.A. & van Deenen, L.L.M. (1965) Studies on the molecular species of lecithin from rat liver. *Kon. Ned. Akadem. Wetenschappen*, **68**, 255-265.
 - 31) Veerkamp, J.H., Mulder, I. & van Deenen, L.L.M. (1961) Comparative studies on the phosphatides of normal rat liver and primary hepatoma. *Z. Krebsforsch.*, **64**, 137-148.
 - 32) Veerkamp, J.H., Mulder, I. & van Deenen, L.L.M. (1962) Comparison of the fatty acid composition of lipids from different animal tissues including some tumors.

- Biochim. biophys. Acta*, **57**, 299-309.
- 33) Wood, R. & Snyder, F. (1969) Tumor lipids: Metabolic relationships derived from structural analyses of acyl, alkyl and alk-1-enyl moieties of natural glycerides and phosphoglycerides. *Arch. Biochem. Biophys.*, **131**, 478-494.
- 34) Wood, R. & Harlow, R.D. (1970) Tumor lipids: Structural analyses of the phosphatides. *Arch. Biochem. Biophys.*, **141**, 183-189.
- 35) Yamashita, S., Hosaka, K. & Numa, S. (1973) Acyl-donor sepcificities of partially purified 1-acetyllycerophosphate acyltransferase, 2-acetyllycerophosphate acyltransferase and 1-acyllycerophosphorylcholine acyltransferase from rat-liver microsomes. *Europ. J. Biochem.*, **38**, 25-31.
-