Biochemical Changes of the Lipid in Biopsied Livers of Patients with Malignant Neoplastic Diseases

ICHIRO NAKAZAWA and SHOICHI YAMAGATA

Department of Internal Medicine, Tohoku University School of Medicine, Sendai

NAKAZAWA, I. and YAMAGATA, S. *Biochemical Changes of the Lipid in Biopsied Livers of Patients with Malignant Neoplastic Diseases*. Tohoku J. exp. Med., 1971, **103** (2), 129-139 — In order to clarify the biochemical differences between the malignant neoplastic and benign diseases, the livers of the patients were biopsied under laparoscopy. One half of each sample was subjected to histologic examination and the other half to lipid analysis by means of a thin layer chromatography (TLC) and a gas liquid chromatography (GLC). The fatty acid profile of the total lipid prepared from the livers of 19 malignant neoplastic cases showed a higher content of $C_{20:4}$ and a lower content of $C_{14:0}$ and $C_{18:1}$ compared with that of the 14 benign cases. Furthermore, the total lipid fraction was separated to triglyceride and phospholipid fractions by TLC. In the livers of the 19 malignant cases, the amount of fatty acid components in the phospholipid fraction was higher than normal value, but the amount of fatty acid components in the triglyceride was lower than normal value.

More significant differences were observed when the ratio of the fatty acid content of the phospholipid fraction to that of the triglyceride was calculated for each patient. These ratios were over 2.20 in 19 out of 22 malignant cases, but were under 2.19 in 20 benign cases. Moreover, a case of relatively early pancreatic cancer and a case of early gastric cancer included in malignant cases mentioned above also showed ratios over 3.00. These findings will be applicable for the clinical diagnosis of the malignant neoplastic diseases. ——— malignant neoplastic diseases; phospholipid; fatty acid

We have been studying biochemical changes in animals bearing malignant tumors and in their malignant neoplastic tissues in order to establish a biochemical diagnostic method and to find a clue to chemotherapy against cancer.

Although some authors reported on the biochemical substances particular to cancerous tissues or cancerous patients, no such substance has been confirmed by other investigators.^{1,2} The studies recently reported on isozymes suggest the possibility that a biochemical diagnosis may be applicable for the case of relatively advanced cancerous patients. There was no report on the usefulness of lipid-chemical analysis of the liver for detection of cancerous diseases.

Before we discuss the lipid-chemical differences between malignant and non-malignant cases in human liver tissues, it is necessary to study whether

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Abbreviations: The fatty acids are designated by chain length and number of double bonds. For example, $C_{20:4}$ stands for a fatty acid with 20 carbon atoms and 4 double bonds.

lipid-chemical differences between hepatic and non-hepatic diseases exist in human liver tissues or not because human liver tissues have been used as experimental materials. In this report we deal with the biochemical changes of the lipid in the biopsied livers from patients with malignant neoplastic diseases.

MATERIALS AND METHODS

Reagents

All organic solvents used were redistilled. Benzene, n-hexane, diethylether, and petroleum-ether were stored with sodium wire. As for other chemicals, extra pure reagents were used. Silica Gel G (Merk) was used for a thin-layer chromatography (TLC). Standards for a gas liquid chromatography (GLC) were obtained from Applied Science Laboratories, Inc., State College, Pennsylvania, USA.

Liver Specimens

Human liver tissues were obtained by needle biopsy under laparoscopy, for the safety of the patients and for the acquirement of good samples from the livers. The method of laparoscopy has already been reported by Yamagata *et al.*³ Each biopsied specimen was divided into two parts in order to examine them both histologically and biochemically. The following cases were used for this study. As genuine malignant cases, the following 29 cases were studied: 12 cases of gastric cancer, 1 case of gastric sarcoma, 3 cases of cancer in the large intestine, 2 cases of pancreatic cancer, 3 cases of hepatoma, 1 case of duodenal cancer, 3 cases of malignant lymphoma, 1 case of hypernephroma, 1 case of uterine cancer, 1 case of multiple myeloma and 1 case of ovarian cancer.

As malignant cases with benign hepatic diseases, the following 5 cases were studied: 1 case of gastric cancer with liver cirrhosis, 3 cases of gastric cancer with hepatitis, and 1 case of hepatoma with liver cirrhosis.

As benign hepatic diseases, the following 37 cases were used: 25 cases of hepatitis, 4 cases of liver cirrhosis, and 8 cases of fatty liver.

As benign cases without hepatic diseases, the following 18 cases were used: 2 cases of enteric ulcer, 2 cases of cholelithiasis, 6 cases of diabetes mellitus, 2 cases of psychiatric diseases, 1 case of a healthy man, 1 case of gastritis, 1 case of regional enteritis, 1 case of gastric myoma, 1 case of peritoneal abscess, and 1 case of nephritis.

The biopsied liver specimens used for lipid-chemical analysis weighed about 10 to 30 mg. When liver specimens were less than 10 mg in weight, analysis of the total lipid fraction or fractionation by TLC was taken.

Extraction and Chromatography

The biopsied liver specimen was homogenized with 1 ml of CHCl_3 -methanol by a Potter-type glass homogenizer after weighing the liver specimen.⁴ Homogenate thus obtained was filtered through the filter paper (Whatmann No. 2), from which fat had been completely washed out by extracting solvent before filtration. The filtrate was dried at 38°C in N_2 gas under reduced pressure (at about 10 to 20 mm Hg). After addition of a solvent to the residue, as shown in Table 1, the solution was divided into two precisely measured parts.

A part of the solution was used for the GLC analysis of the total lipid fraction, and the other was used for lipid fractionation by TLC. The solution to be used for the analysis of the total lipid fraction was dried under the same conditions as mentioned above and was hydrolyzed by 10% KOH-methanol solution at 37°C for 48 hours.

After addition of distilled water, non-saponifiable substances were removed with n-hexane. The water phase was then acidified to a pH below 2 by addition of 6 N HCl, then added with an equal volume of n-hexane to extract the fatty acids. The extraction was repeated three times. The n-hexane solutions were collected and concentrated to dryness using a vacuum pump. Thereafter, the residue was methylated by addition of diazomethane in ethylether.

Before applying to gas chromatography, a definite quantity of methyl erucate was added to the samples as an internal standard.

For GLC, a Shimazu gas chromatograph (GC-I-B type) with FID was used and stainless columns were used in the dual system. The length of the column was 1.5 m and its diameter was 4 mm. The column was filled with Shimalite soaked with 5% diethylene glycol succinate polyester (purchased from Shimazu Co., Ltd., Tokyo, Japan). The column temperature was 185°C, the flow rate of N₂ gas was 90 ml per minute, and the flow rate of H₂ gas was 65 ml per minute.

The composition of the fatty acids in the total lipid fraction was examined in 19 malignant cases which were not accompanied with benign hepatic diseases (8 cases of gastric cancer, 1 case of gastric sarcoma, 2 cases of pancreatic cancer, 1 case of colonic cancer, 1 case of rectal cancer, 2 cases of malignant lymphoma, 1 case of duodenal cancer, 1 case of hepatoma, 1 case of uterine cancer and 1 case of multiple myeloma), in 14 non-malignant cases which were not accompanied with any hepatic diseases (1 case of a healthy man, 2 cases of cholelithiasis, 4 cases of diabetes mellitus, 2 cases of enteric ulcer, 1 case of gastric myoma, 1 case of irritable colon, 1 case of nephritis, 1 case of peritoneal abscess and 1 case of neurosis), and in 28 cases of benign hepatic diseases including 2 cases with gastric cancer, 22 cases of hepatitis, 4 cases of liver cirrhosis, 1 case of hepatitis chronica with gastric cancer and 1 case of liver cirrhosis with gastric cancer). Furthermore, in 19 malignant neoplastic cases which were not accompanied with any benign hepatic diseases (10 cases of gastric cancer, 1 case of gastric sarcoma, 2 cases of colonic cancer, 2 cases of pancreatic cancer, 1 case of hypernephroma, 1 case of uterine cancer, 1 case of hepatoma and 1 case of ovarian cancer), in 11 non-malignant cases which were not accompanied with hepatic diseases (2 cases of enteric ulcer, 3 cases of diabetes mellitus, 1 case of gastritis, 1 case of regional enteritis, 1 case of gastric myoma, 1 case of peritoneal abscess, 1 case of neurosis and 1 case of epilepsy), in 17 cases of benign hepatic diseases (7 cases of hepatitis, 2 cases of liver cirrhosis, 8 cases of fatty liver) and in 3 malignant neoplastic cases accompanied with benign hepatic diseases (2 cases of hepatitis with gastric cancer and 1 case of liver cirrhosis with hepatoma), the total lipid fraction was separated into phospholipid fraction, triglyceride fraction, cholesterol ester fraction, diglyceride fraction and free fatty acid fraction by thin-layer chromatography (TLC).⁵

As a solvent system of TLC, a mixture of petroleum ether, diethylether and acetic acid (90:10:1 v/v) was used. On a plate (20 × 5 cm), silicic acid was pasted in the thickness of 0.25 mm, dried and kept at 140°C for 3 to 4 hours. After cooling, a part of the total lipid fraction of liver specimens weighing more than 10 mg was in some cases fractionated by TLC; the samples were spotted on the plate linearly and developed for 30 minutes at room temperature. Then the plate was dried in air and sprayed by 0.2% 2',7'-dichloro-fluorescein ethanol solution. Separated fractions were marked under ultraviolet ray and the silicic acid layer of each fraction was scraped off the plate and treated with 1 ml of 10% KOH in 95% methanol, then the fatty acid was extracted and methylated and analyzed by GLC as described in Table 1.

RESULTS

Profile of the fatty acid in the total lipid

The average values obtained from 19 malignant neoplastic cases, 26 cases of benign hepatic diseases and 2 cases of benign hepatic diseases with gastric cancer, and 14 non-malignant cases without benign hepatic diseases (the control group) were shown in Table 2. In the cases of the benign hepatic diseases, as shown in Table 2, remarkable changes were recognized, compared with the values of the control group, so that they should be distinguished from the control group. According to statistical calculation, they showed an increase in percentage of C_{14:0}.

TABLE 1. *Extraction of lipid and methylation of fatty acid* TABLE 2. *Profile of fatty acid in total lipid fraction*
(Percentage)

Fatty acid	Malignant cases (Mean of 19 cases)	Control cases (Mean of 14 cases)	Benign hepatic cases (Mean of 28 cases)
C _{14:0}	0.69±0.40*	1.04±0.30*	1.43±0.51*
C _{16:0}	27.7 ±2.7	27.6 ±3.1	27.1 ±3.2
C _{18:1}	3.8 ±0.7	4.5 ±0.9	6.5 ±2.0
C _{18:0}	14.6 ±1.4	13.8 ±1.7	11.2 ±1.6
C _{18:1}	16.1 ±2.4	18.2 ±2.7	21.9 ±3.5
C _{18:2}	15.8 ±3.1	16.4 ±3.8	14.0 ±3.2
C _{18:3}	0.56±0.23	0.65±0.23	0.78±0.25
C _{20:3}	1.09±0.38	1.09±0.17	1.08±0.31
C _{20:4}	8.5 ±1.6	7.4 ±1.6	6.1 ±1.4
C _{22:5}	9.0 ±1.9	9.4 ±2.3	7.4 ±2.1

* Standard deviation

$C_{16:1}$ and $C_{18:1}$ and decrease in percentage of $C_{18:0}$, $C_{18:2}$, $C_{20:4}$ and $C_{22:5}$. In the group of benign hepatic cases in Table 2 are also included 2 cases of the benign hepatic diseases with malignant neoplastic diseases, one of which was also presented in Table 7 as case No. 21, because they also showed the same patterns as those for the benign hepatic diseases and therefore they should be classified under the group of benign hepatic diseases. In other words, the fatty acid composition of total lipid of the liver specimens obtained from the malignant cases did not give us any information on malignancy when they were combined with the benign hepatic diseases. Thus, it is necessary to examine liver specimens histologically before we deal with the profile of the fatty acid in the total lipid fraction.

In the malignant cases, as shown in Table 2, they showed a significant increase in the percentage of $C_{20:4}$, and a significant decrease in the percentage of $C_{14:0}$ and $C_{18:1}$ when compared with the control group.

Profile of the fatty acid in the phospholipid fraction

The average values obtained from 17 malignant neoplastic cases, and 11 benign cases without hepatic diseases and 7 cases of benign hepatic diseases (hepatitis and liver cirrhosis) were shown in Table 3.

In 17 malignant cases, a significant decrease in the percentage of $C_{14:0}$ was recognized statistically, compared with that of the control group. In 2 malignant cases with benign hepatic diseases (hepatitis and liver cirrhosis, respectively), which were not shown in Table 3, a significant increase in the percentage of $C_{16:1}$ and $C_{18:1}$ was recognized compared with those obtained from 7 benign hepatic diseases. In 7 cases of benign hepatic diseases, there was no significant difference recognized in the percentage of the fatty acids, compared with the control group.

Profile of the fatty acid in the triglyceride fraction

The average values obtained from 17 malignant cases and 11 benign cases without hepatic diseases (the control group) and 7 cases of benign hepatic diseases were shown in Table 4. There were no significant differences in the percentage of the fatty acid composition between the malignant cases and the control group. In 7 cases of benign hepatic diseases (hepatitis and liver cirrhosis), there was a significant decrease recognized in the percentage of $C_{18:2}$, compared with the control group. In 2 cases of benign hepatic diseases with malignant neoplastic diseases, a significant increase in the percentage of $C_{16:1}$ was recognized, compared with 7 cases of benign hepatic diseases without malignant neoplastic diseases.

Content of the fatty acid in the phospholipid fraction of the human liver

The results obtained from 19 malignant cases without benign hepatic diseases, 11 non-malignant cases without hepatic diseases (the control group), 9 cases of benign hepatic diseases without malignant neoplastic diseases and 3 malignant cases with benign hepatic diseases were shown in Tables 5, 6 and 7.

The mean value of 19 malignant cases was 15.08 ± 2.16 mg/g liver and showed a significant increase ($P < 0.005$), compared with the mean value of 11 non-malignant cases (12.24 ± 1.96 mg/g liver). The mean value of 9 cases of benign hepatic diseases was 10.43 ± 2.23 mg/g liver and showed a significant decrease ($P < 0.05$), compared with the mean value of 11 non-malignant cases.

Content of the fatty acid in the triglyceride fraction of the human liver

The results obtained from 19 malignant cases without benign hepatic diseases, 11 non-malignant cases without benign hepatic diseases, 9 cases of benign hepatic diseases without malignant diseases and 3 cases of benign hepatic diseases with malignant diseases were shown in Tables 5, 6 and 7. The mean value of 19 malignant cases was 5.05 ± 1.79 mg/g liver and showed a significant decrease ($P < 0.001$), compared with the mean value of 11 non-malignant cases (8.07 ± 2.26 mg/g liver). The mean value of 9 cases was 6.76 ± 1.70 mg/g liver and did not show a significant difference, compared with the mean value of 11 non-malignant cases.

DISCUSSION

There are some reports on the profile of fatty acids in the animal livers, and also a few papers on the profile of fatty acids in the human livers. But they have usually been studied on liver specimens autopsied or biopsied during surgical operations.^{6,7} As is known, when surgical operations are performed, free fatty acids mobilized from depot fat increase in the blood stream as a reaction to the stress of surgical operations and they are incorporated into liver tissues. For example, the liver specimen obtained immediately after laparotomy, showed a profile of fatty acids which differed from that of the liver specimen obtained just before the end of a surgical operation, even in the same patient.⁷ Therefore, in order to study the profile of fatty acids in the human liver tissues, it is necessary to biopsy the human livers under a stable condition. The needle biopsy of the liver under laparoscopy presented us liver specimens ideal for analysis of the fatty acids in liver lipids.

By using erucate as an internal standard of gas chromatography we were able to measure the content of fatty acids in each fraction; erucate did not appear to be contained in the human liver tissues at the level of this work. As described before, a half of each biopsied liver specimen was examined histologically and diagnosed pathohistologically.

As conjectured, we recognized differences between the hepatic diseases and the non-hepatic diseases in the fatty acid composition of the total lipid. As shown in Table 2, in the benign hepatic diseases (liver cirrhosis and hepatitis) the following characteristics were recognized when compared with the control group.

1. A most remarkable increase in the percentage of $C_{16:1}$ ($P < 0.001$)
2. A remarkable increase in the percentage of $C_{18:1}$ ($P < 0.005$)
3. An increase in the percentage of $C_{14:0}$ ($P < 0.01$)

4. A most remarkable decrease in the percentage of $C_{18:0}$ ($P<0.001$)
5. A remarkable decrease in the percentage of $C_{20:4}$ ($P<0.01$)
6. A decrease in the percentage of $C_{22:5}$ ($P<0.01$) and $C_{18:2}$ ($P<0.025$)

It was very interesting to notice that two cases of gastric cancer with benign hepatic diseases (a case with hepatitis and a case with liver cirrhosis) showed the same pattern as in the cases of the benign hepatic diseases.

In 19 malignant neoplastic cases, a decrease in the percentage of $C_{14:0}$ was highly significant ($P<0.01$) and a decrease in the percentage of $C_{18:1}$ was significant ($P<0.05$), compared with the cases of the control group. Therefore, it is necessary to examine the human biopsied liver tissues histologically when we deal with the fatty acid composition of the total lipid fraction in the human biopsied livers.

In order to determine the lipid fractions which are responsible for the above-mentioned changes in the fatty acid composition of the total lipid fraction, a part of the total lipid fraction in each case was fractionated by TLC into the phospholipid fraction, the triglyceride fraction, the cholesterol ester fraction and other fractions, and the fatty acid composition of each fraction was examined by GLC. As shown in Table 3, in the phospholipid fraction of 17 malignant cases without benign hepatic diseases, a decrease in the percentage of $C_{14:0}$ was statistically significant ($P<0.05$), compared with 11 cases of the control group. As shown in Table 4, in the triglyceride fraction of 17 malignant cases without benign hepatic diseases, there was no significant difference in the fatty acid composition, compared with 11 cases of the control group.

In the phospholipid fraction of 7 cases of benign hepatic diseases (hepatitis and liver cirrhosis) there was no significant difference in the fatty acid composition, compared with the control cases. But in the triglyceride fraction of 7 cases of benign hepatic diseases (hepatitis and liver cirrhosis), a significant decrease ($P<0.025$) in the percentage of $C_{18:2}$ was observed.

As can be recognized in Table 7, the phospholipid fatty acid content of human liver tissues in 19 malignant cases was 15.08 ± 2.16 mg/g liver. It showed a highly significant increase ($P<0.005$), compared with the normal value ($12.24 \pm$

TABLE 3. *Profile of fatty acid in phospholipid fraction*
(Percentage)

Fatty acid	Malignant cases (Mean of 17 cases)	Control cases (Mean of 11 cases)	Benign hepatic cases (Mean of 7 cases)
$C_{14:0}$	$0.28 \pm 0.09^*$	$0.35 \pm 0.12^*$	$0.47 \pm 0.15^*$
$C_{16:0}$	28.1 ± 1.6	26.8 ± 3.3	26.9 ± 2.7
$C_{16:1}$	2.7 ± 0.53	2.5 ± 0.85	3.1 ± 0.39
$C_{18:0}$	17.1 ± 1.5	16.9 ± 1.5	18.0 ± 1.3
$C_{18:1}$	10.8 ± 1.7	11.0 ± 1.5	12.2 ± 1.2
$C_{18:2}$	14.9 ± 2.5	16.5 ± 3.7	14.7 ± 1.9
$C_{18:3}$	0.32 ± 0.14	0.37 ± 0.14	0.35 ± 0.28
$C_{20:3}$	1.26 ± 0.39	1.25 ± 0.27	1.46 ± 0.14
$C_{20:4}$	10.0 ± 1.4	9.3 ± 1.0	9.4 ± 1.3
$C_{22:5}$	11.2 ± 1.7	12.0 ± 1.9	12.0 ± 1.2

* Standard deviation

TABLE 4. *Profile of fatty acid in triglyceride fraction*
(Percentage)

Fatty acid	Malignant cases (Mean of 17 cases)	Control cases (Mean of 11 cases)	Benign hepatic cases (Mean of 7 cases)
C _{14:0}	2.2 ±0.8*	2.3 ±0.7*	2.9 ±0.9*
C _{16:0}	29.3 ±4.2	29.8 ±3.1	30.4 ±3.9
C _{18:1}	8.0 ±1.6	7.6 ±1.7	8.3 ±0.9
C _{18:0}	4.2 ±0.8	4.1 ±1.1	5.1 ±1.2
C _{18:1}	37.3 ±2.8	36.0 ±3.0	36.5 ±2.1
C _{18:2}	15.8 ±4.4	17.2 ±3.4	13.4 ±2.6
C _{18:3}	0.92±0.37	1.14±0.34	1.05±0.24

* Standard deviation

TABLE 5. *Contents and ratio of phospholipid and triglyceride*
in non-malignant cases

Case No.	Clinical diagnosis	Fatty acid content*		
		Phospholipid	Triglyceride	Ratio
1	Duodenal ulcer	16.08 mg	7.36 mg	2.185
2	Epilepsy	10.67	5.44	1.961
3	Gastritis	10.90	5.62	1.939
4	Enteric ulcer	9.82	5.79	1.696
5	Neurosis	15.01	9.00	1.673
6	Peritoneal abscess	11.85	7.41	1.598
7	Diabetes mellitus	11.66	7.53	1.549
8	Gastric myoma	9.79	6.45	1.517
9	Regional enteritis	13.90	11.13	1.249
10	Diabetes mellitus	12.74	11.01	1.157
11	Diabetes mellitus	12.21	12.00	1.018

* In mg per 1 g of wet liver

TABLE 6. *Contents and ratio of phospholipid and triglyceride*
in benign hepatic cases

Case No.	Clinical diagnosis	Fatty acid contents*		
		Phospholipid	Triglyceride	Ratio
1	Hepatitis	8.20 mg	3.81 mg	2.152
2	Hepatitis chronica	11.10	5.98	1.856
3	Hepatitis	8.03	4.34	1.849
4	Hepatitis	13.68	8.15	1.679
5	Hepatitis	9.18	5.73	1.602
6	Liver cirrhosis	13.02	8.71	1.495
7	Hepatitis	9.75	7.19	1.356
8	Hepatitis chronica	13.09	10.34	1.266
9	Liver cirrhosis	7.84	6.57	1.193

* In mg per 1 g of wet liver

1.96 mg/g liver). In 9 cases of benign hepatic diseases the phospholipid fatty acid content of liver tissues was 10.43 ± 2.23 mg/g liver and it showed a significant decrease ($P < 0.05$), compared with the above mentioned normal value.

As can be recognized in Table 7, the triglyceride fatty acid content of human

TABLE 7. *Contents and ratio of phospholipid and triglyceride in malignant neoplastic cases*

Case No.	Clinical diagnosis	Fatty acid content*		
		Phospholipid	Triglyceride	Ratio
1	Gastric cancer	17.35 mg	2.62 mg	6.625
2	Gastric cancer	15.51	2.83	5.486
3	Gastric cancer	11.09	2.25	5.007
4	Gastric sarcoma	20.25	4.30	4.706
5	Gastric cancer	15.30	3.45	4.429
6	Pancreatic cancer	14.76	4.31	3.425
7	Ovarian cancer	15.81	4.89	3.237
8	Gastric cancer	16.42	5.17	3.173
9	Gastric cancer	13.94	4.51	3.091
10	Colonic cancer	13.58	4.47	3.038
11	Pancreatic cancer	15.06	4.96	3.036
12	Colonic cancer	14.49	4.85	2.988
13	Gastric cancer	13.47	5.08	2.652
14	Gastric cancer	16.93	7.17	2.360
15	Hepatoma	13.69	5.84	2.344
16	Uterine cancer	10.72	4.75	2.218
17	Hypernephroma	15.33	6.93	2.212
18	Gastric cancer	16.18	8.24	1.964
19	Gastric cancer	16.63	9.42	1.761
20	Hepatoma with liver cirrhosis	6.95	1.88	3.691
21	Gastric cancer with hepatitis	10.22	3.06	3.340
22	Gastric cancer with hepatitis	16.42	10.57	1.553

* In mg per 1 g of wet liver

liver tissues in 19 malignant cases was 5.05 ± 1.79 mg/g liver and it showed a highly significant decrease ($P < 0.001$), compared with the normal value (8.07 ± 2.26 mg/g liver). In 9 cases of benign hepatic diseases it was 6.76 ± 1.70 mg/g liver and did not show any significant difference, compared with the above-mentioned normal value.

As can be recognized in Tables 3 and 4, in the phospholipid fraction the percentage values of $C_{14:0}$, $C_{16:1}$ and $C_{18:1}$ were much smaller than those of the triglyceride fraction, but the percentage value of $C_{20:4}$ was much larger than the one of the triglyceride fraction which was less than 1 percent in each case of all groups. Because the sums of the phospholipid fatty acid contents and the triglyceride fatty acid contents in our cases amounted to about 60 to 80 per-cent of the total lipid fatty acid contents in human liver tissues, it seemed that the increase of the phospholipid fatty acid content and the decrease of the triglyceride fatty acid content could contribute to the changes of the fatty acid composition of the total lipid fraction in malignant cases. Therefore, the ratio of the phospholipid fatty acid content to the triglyceride fatty acid content was calculated in each case.

As shown in Table 7, in 19 malignant cases without benign hepatic diseases, the ratio of the phospholipid fatty acid content to the triglyceride fatty acid content

scattered between 1.761 and 6.625. In 11 cases of the control group, these ratios did not exceed 2.20. It is very interesting that the ratios of one case of gastric cancer with chronic hepatitis (case No. 21) and one case of hepatoma with liver cirrhosis (case No. 20) were 3.340 and 3.691, respectively. These high ratios suggest that 2 malignant cases with benign hepatic diseases (hepatitis and liver cirrhosis) belong to the same group as malignant cases without benign hepatic diseases when we consider the ratio of the phospholipid fatty acid content to the triglyceride fatty acid content, although these two cases were classified into the group of benign hepatic diseases in Table 2 on account of the data of the fatty acid composition of the total lipid fraction. But one case of gastric cancer with hepatitis showed a value lower than 2.20.

Therefore, in 19 cases of 22 malignant cases the ratio of the phospholipid fatty acid content to the triglyceride fatty acid content was over 2.20 which was the upper limit of the 20 benign cases (11 cases of the control group and 9 cases of benign hepatic diseases without fatty liver). Because one case of early gastric cancer showed a ratio of 4.429 and one case of early pancreatic cancer showed a ratio of 3.036, such calculation of the ratio of the phospholipid fatty acid content to the triglyceride fatty acid content in the biopsied human liver tissues is believed to be useful for the diagnosis of malignant neoplastic diseases.

In one case of gastric cancer described herein, it was confirmed by histologic examination of the excised cancerous tissue that cancerous infiltration was chiefly localized at the gastric mucosa and only a small part of it protruded into the gastric submucosal tissue. One case of early pancreatic cancer was so early that it was misdiagnosed macroscopically as pancreatitis chronica at the first surgical operation, and the later histologic examination of the tentatively excised pancreatic tissue clarified a malignant change in the small localized area so that reoperation was done and the rest of the pancreatic tissue was excised and the cancerous tissue was recognized in the pancreatic tissue excised.

In 2 cases of gastric cancer and 1 case of gastric cancer with hepatitis in which the ratios were shown to be below 2.20, we found the values of the phospholipid fatty acid content over 16.10 mg/g liver which was the upper limit in 20 non-malignant cases. The fact that the phospholipid fatty acid content in the liver tissue tends to increase in malignant cases would help the clinical diagnosis of malignant neoplastic diseases. Therefore, the lipid-chemical examination of human biopsied liver should be useful for the clinical diagnosis of malignant neoplastic diseases.

But there are some restrictions in the use of this method for the clinical diagnosis of the malignant neoplastic diseases. Firstly, in 8 cases of the fatty liver the ratios of the phospholipid fatty acid content to the triglyceride fatty acid content were lower than 1.0 because the triglyceride fatty acid contents in the cases of the fatty liver were over 30 mg/g liver but the phospholipid fatty acid contents did not exceed 16 mg/g liver. Therefore, the ratio over 1.00 cannot be expected in the cases of the fatty liver even if they had been associated with

malignant neoplastic diseases. However, we have never experienced such cases, so that it is unknown at present what kind of lipid-chemical changes would actually occur in those cases.

Secondly, the age is important. Our present study was concerned with the adult patients whose ages were above 18 years, but recently we have experienced a case of a 14-year-old girl suffering from hepatitis. It was confirmed by histologic examination that this case was at almost healing stage. She had fallen ill three months ago. In this case, the phospholipid fatty acid content was within normal range (11.02 mg/g liver) while the triglyceride fatty acid content was 2.278 mg/g liver which was considerably lower than the values in adult control cases. But the ratio of the former to the latter was 4.827. The fatty acid composition of the total lipid fraction in her liver tissue was different from those of adult cases. The reason why such a difference occurred is unclear at present, but the endocrine factors should be considered because she was at puberty. At any rate, a further study is necessary for children and the above-mentioned discussion should be limited to the adult cases. Of course, a frequency of the malignant neoplastic diseases is very high at the older ages, compared with children.

Thirdly, the medicines which affect the lipid metabolism should not be used just before or when the liver biopsy is taken. For example, 3 cases of ulcerative colitis which were on the steroid therapy showed an increase in the fatty acid content of the triglyceride fraction; values being over 20 mg/g liver.

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