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EFFECTS OF ETHYL LINOLEATE ON CHRONIC LIVER DISEASES WITH SPECIAL REFERENCE TO LIPID METABOLISM

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SUMMARY

The administration of purified ethyl linoleate supplemented with or without alpha-tocopherol or pyridoxal phosphate to chronically CCl_4 poisoned mice proved to be effective upon recovery from the liver damage. CCl_4 poisoning resulted in a dramatic decrease in total lipid content of mouse liver in each diet group and administration of ethyl linoleate promoted this tendency. Gas-liquid chromatographic analysis of mouse liver lipids indicated that the administration of ethyl linoleate to CCl_4 poisoned mice did not show increase in percentage of linoleic acid and that the administration to normal controls showed a marked increase. On the contrast to the changes of linoleic acid, significant increases in percentages of arachidonic and docosahexaenoic acids were observed in CCl_4 poisoned mouse liver and the administration of ethyl linoleate stimulated this tendency.

In clinical studies, after the administration of ethyl linoleate supplemented with or without alpha-tocopherol and pyridoxal phosphate to the patients with liver diseases, progressive clinical improvements accompanied by the satisfactory improvements in liver function tests were observed.

The percentage of serum linoleic acid in these patients having lower percentage than normals increased to the normal level with a slight elevation of serum total lipids after the administration. No apprecialble effect of supplementation of alpha-tocopherol and pyridoxal phosphate upon fatty acid metabolism were observed in these patients.

From these results, it is concluded that one can expect a satisfactory response to the administration of ethyl linoleate to the patients with chronic liver diseases.

In 1941, Patek and Post[1] indicated that the patients with liver cirrhosis responded favorably to diets which were relatively rich in protein and in fat, and also showed that no increased fatty infiltration in the liver of the patients received these diets was observed. Since the report of Patek and Post, many investigators[2,3,4,5] have confirmed by their experiences that high fat supplement to the patients with chronic liver diseases was harmless.

Thereafter, high-caloric foods rich in protein and fat, such as meat, butter,

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milk and egg which were rich in saturated fatty acids and poor in poly-unsaturated fatty acids, have generally been considered to be suitable for the dietary treatment of chronic liver diseases.

Concerning fatty acid metabolism, especialy essential fatty acid, the conversion of linoleic acid to arachidonic acid has been studied by many investigators after feeding rats with linoleate[6,7,8,9] and Steinberg[10] and Mead[11] confirmed this using C^{14}-labeled linoleic acid. In this conversion, pyridoxine has been considered to play a major role. Witten and Holman[12] and Dam[13] reported that the impairment of the conversion of linoleic acid to arachidonic acid might occur in the lack of pyridoxine, while, Swell[14] suggested that pyridoxine might act indirectly in the biosythetic scheme from linoleate to arachidonate. Kirshman and Coniglio[15] considered that pyridoxine generally affects the metabolism of fatty acids.

On the other hand, in the patients with liver diseases an increase in plasma fatty peroxide has been observed by Schauenstein[16], and a decrease in serum alpha-tocopherol which prevent the auto-oxidation of fats *in vivo*, has been observed by Takahashi.[17]

Recently, a decrease in percentage of plasma linoleic acid and an increase in that of oleic acid in patients having liver cirrhosis have been observed by Schrade[18], Okinaka[19] and Anazawa[20]. This reduction of plasma linoleic acid suggested reasonably that the supplement of linoleic acid might improve liver cirrhosis. From this point of view, Okinaka[19], Anazawa[20] and Kimura[21] administered 30 to 60 grams of safflower oil as a source of linoleic acid to patients with chronic liver diseases who had had lower level of linoleic acid than normal. The relief of the signs was obtained and the percentage of linoleic acid in plasma rose to the normal level. The flocculation tests, however, were not improved satisfactorily.

From these considerations mentioned above pyridoxine and alpha-tocopherol as well as ethyl linoleate were expected to improve chronic liver deseases. Therefore, the present study was attempted to investigate the effects of ethyl linoleate with or without supplementation of pyridoxal phosphate and alpha-tocopherol upon the animals induced chronic liver impairments and patients with chronic liver diseases with special reference to fatty acid metabolism.

ANALYTICAL PROCEDURES

Analytical procedures employed in this study were as follows: Plasma and liver cholesterols (ester form and free form) were determined by the method of Schoenheimer and Sperry modified by Sperry and Webb[22,23]. Phospholipids were determined by the method of Fiske Subbarow[24], esterified fatty acids by the method of Stern and Shapiro[25] and total lipids by the modified method of Bradgon[26].

The fatty acid composition of the plasma lipids were determined by gas-liquid chromatography[27]. Plasma and liver lipids were extracted in ether-methanol mixture reflaxing for 30 minuits at 60°C, and saponification of extracted lipids was carried out by reflaxing with 10 ml of 0.2 N KOH-methanol solution for 30 minuites at 60°C. After the removal of the unsaponifiable substances, the fatty acids which were obtained by acidfication were methylated with diazomethane. The methylated fatty acids were then taken up in ethyl ether, concentrated to the desired volume and injected into the vaporization chamber of a gas-liquid chromatograph of Shimazu Seisakusho Co. Ltd., Model GC-1 B attached with hydrogen flame ionization detector using 15% diethyl-eneglycol succinate polyester on Celite 545 (80 to 100 mesh) column.

The percentage of fatty acids was obtained by measuring the area under each peak with a planimeter.

A) Effects of dietary fats on chronic CCl_4 poisoned rats

Materials and methods

Female rats of Wister strain were used for this study. Chronic liver impairment of the rats were produced by intramuscular injection of 5% CCl_4 dissolved in olive oil in a dose of 0.25 ml per 100 g of body weight 3 times a week for 3 weeks. After the final injection, rats were divided into 4 groups according to subsequent feedings as shown in Table 1 and maintained on each experimental diet for a week. Safflower oil used in this experiments contained about 76% of linoleic acid, 13% of oleic acid, and lard contained about 27% of palmitic acid and 40% of oleic acid. Diets fed ad libitum. Food cups were removed from all cages about 12 hours before sacrifice. The rats were anesthetized with ethyl ether and blood was removed by cardiac puncture with a heparinized syringe. The livers were immediately removed, weighed, homogenized with a Potter-Elvehjem type homogenizer and extracted according to each analytical method. Plasma and liver lipid fractions were determined and fatty acid composition of plasma lipids were also analyzed by gas-liquid chromatography. Another part of livers were used for microscopic study by staining with hematoxilyn-eosin.

TABLE 1. Experimental Diets

	Standard (g)	Low Fat (g)	High Fat	
			Safflower oil (g)	Lard (g)
Starch	680	894	500	500
Casein	180	180	180	180
Butter	95	—	—	—
Safflower oil	—	—	180	—
Lard	—	—	—	190
MaCollum's Salts	40	40	40	40
Agur	10	10	10	10
Vitamins Mixture	2.5	2.5	2.5	2.5

1) Histological findings of the liver of rats fed various experimental diets after CCl_4 poisoning

As given in Table 2 marked fatty infiltration, moderate centrilobular necrosis and regeneration of the liver cells were observed in the standard diet group.

In the low-fat diet group, moderate centrilobular necrosis, fatty infiltration and atrophy of the liver cells were noted. Impairment of the liver was almost the same degree as in the standard diet group.

In the high-lard diet group, centrilobular necrosis and fatty infiltration were slight, and mild atrophy and degeneration of liver liver cells were observed.

In the group fed high safflower oil diet, some fatty infiltration and atrophy of the liver cells were observed. The liver damages were the slightest in this group.

TABLE 2. Effects of Dietary Fats on Histological Findings of CCl_4 Poisoned Rat Livers

	Standard	Fat Free	High Lard	High Saffl.
Centrilobular Necrosis	+	+	±	±
Fatty Infiltration	++	+	±	+
Cellurar Degeneration	+	++	+	−
Atrophy of Liver Cells	−	+	+	+
Regeneration of Liver Cells	+	±	+	+
Severity of Impairment	1	1	3	4

2) Influences of various experimental diets on the lipid metabolism of CCl_4 poisoned rats

a) Plasma lipid fractions

As indicated in Fig. 1 chronic CCl_4 poisoning in general caused increases in all plasma lipid fractions with the exception of free cholesterol. Total cholesterol levels in CCl_4 poisoned rats fed high-lard diet showed the highest and in rats fed high safflower oil diet were almost the same level as obtained in the standard diet group. In contrast, free cholesterol level in these rats showed a slight decrease in all groups. Increases in phospholipids were observed in CCl_4 poisoned rats of all experimental groups. The increase was remarkable in the rats fed high-fat diets and rats fed low-fat diet showed the slightest increase of all the groups.

Esterified fatty acids in CCl_4 poisoned rats showed a significant increase in each diet group.

Total lipids increased in CCl_4 poisoned rats in all groups; the highest was the group fed high-lard diet, next was the standard diet group, then, the high-safflower oil diet group and the lowest was the low-fat diet group.

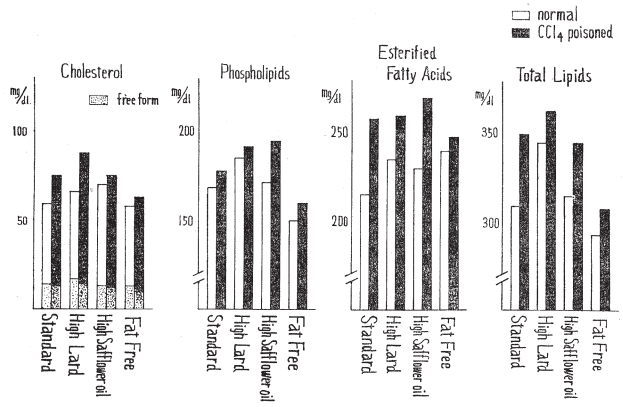

FIG. 1. Changes of plasma lipid fractions in CCl_4 poisoned rats fed various experimental diets.

b) Liver lipid fractions

As indicated in Fig. 2, total and free cholesterol contents in the liver were not influenced by CCl_4 poisoning but were slightly higher in high-fat diet groups than the standard and low-fat diet groups. CCl_4 poisoning caused a slight decrease in phospholipid contents in the liver of the rats fed high-lard, standard and low-fat diet. No increase was observed in the group fed high-safflower oil diet. Esterified fatty acid contents in the liver of non-treated rats in the high-fat diet groups were slightly higher than those of others. A significant increase was observed in the liver of CCl_4 poisoned rats fed high safflower-oil diet. Total lipid content in the liver of each diet group was proportional to esterified fatty acid contents.

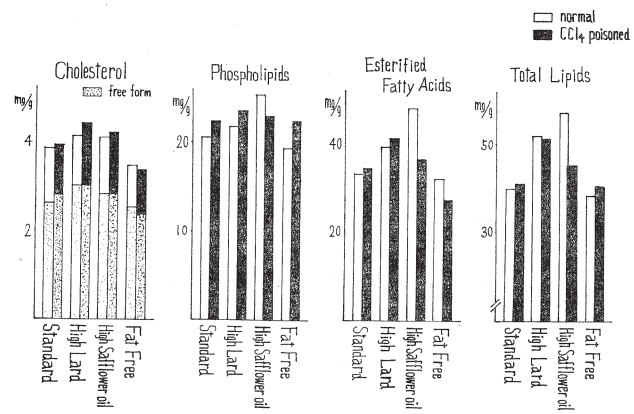

FIG. 2. Changes of liver lipid fractions in CCl_4 poisoned rats fed various experimental diets.

c) Plasma fatty acid composition

As shown in Fig. 3, in the standard diet group CCl_4 poisoning caused a significant decrease in percentage of plasma oleic and linoleic acids, while, percentages of arachidonic and docosahexaenoic acids were increased. Slight or no changes of other fatty acids were observed in CCl_4 poisoned rats.

Rats fed high-lard diet showed increases in percentages of palmitic, stearic and oleic acids and decreases in percentages of linoleic, arachidonic and docosahexaenoic acids as compared with non-CCl_4 poisoned rats fed high-lard diet.

Feeding with high-safflower oil diet for a week, significant increases in percentages of linoleic and arachidonic acids and a marked decrease in oleic acid were noted. CCl_4 poisoning resulted in significant decreases in percentages of arachidonic and docosahexaenoic acids and increases in percentages of

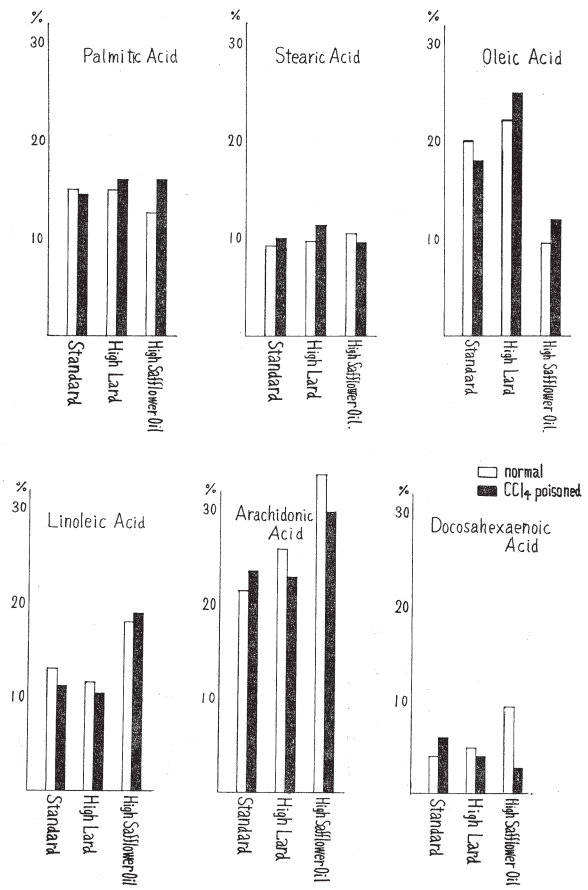

FIG. 3. Changes of fatty acid composition of plasma lipids in CCl_4 poisoned rats fed various experimental diets.

palmitic and oleic acids as compared with normals fed high safflower oil diet.

B) Effects of ethyl linoleate on chronic CCl_4 poisoned mice

Materials and methods

About 25 grams of female D-D strain mice were used for this experiment. Chronic liver impairment of the mice were induced by intramuscular injection of 5% CCl_4 in olive oil in a dose of 0.1 ml twice a week for a month. Immediately after the final injection, the mice were divided into two groups; standard diet group and ethyl linoleate supplemented group (as designated E.L.). Standard diet group was fed on commercial diet (Oriental pellet) without supplementation and E.L. group was fed on commercial diet supplemented with ethyl linoleate at 10% level (w/w). Then E.L. group was divided into 2 subgroups, alpha-tocopherol group and pyridoxal phosphate group (as designated E.L.-E and E.L.-B_6, respectively). E.L.-E group was received intramuscular injection of 250 gammas of alpha-tocopherol daily and E.L.-B_6 group was received intramuscular injection of 50 gammas of pyridoxal phosphate daily.

Ethyl linoleate used in this experiment was supplied from Ono Pharm. Co. Ltd. and its purity was 97%.

The mice were maintained on each experimental diet for 1 to 2 weeks. Diets were fed ad libitum. The mice were decapitated after over night fasting and the livers were immediately removed. Liver lipid fractions and fatty acid compositions were determined and microscopic study was also carried out.

1) Effects of ethyl linoleate on histological findings of the livers of normal mice

As indicated in Table 3, cloudy swelling of liver cells was noted only in E.L. and E.L.-B_6 in the 1st week after administration. No other remarkable pathological findings of the liver could be noted.

TABLE 3. Histological Findings of Livers of Normal Mice After the Administration of Ethyl Linoleate

Diet groups	1 Weeks				2 Weeks			
	St.	E.L.	E.L.+E.	E.L.+B_6	St.	E.L.	E.L.+E.	E.L.+B_6
Centrilobular Necrosis	−	−	−	−	−	−	−	−
Cellurar Degeneration	−	−	−	−	−	−	−	−
Cloudy Swelling	±	+	±	+	±	±	−	−
Cellurar Regeneration	−	−	−	−	−	−	−	−
Disarrangement of Liver cell Columns	−	−	−	−	−	−	−	−
Monocytic Infiltration	−	−	−	−	−	−	−	−

2) Effects of ethyl linoleate on histological findings of the livers of CCl_4 poisoned mice

As indicated in Table 4 the livers of standard diet group showed degeneration, cloudy swelling and regeneration of liver cells, disarrangement of liver cell cords and monocytic infiltration after 1 week feeding. Two weeks later, degeneration and cloudy swelling of liver cells became worse. The regeneration of liver cells and disarrangement of liver cell cords remained as the same degree as shown in the 1st week, and only monocytic infiltration decreased.

E.L. group also showed degeneration, cloudy swelling and regeneration of liver cells, disarrangement of liver cell cords and monocytic infiltration in the 1st week. Two weeks later, damage of liver cells remained unchanged, while, disarrangement of liver cell cords, monocytic infiltration and cloudy swelling of liver cells disappeared.

E.L.-E group showed severe degeneration of liver cells, mild cloudy swelling and regeneration of liver cells within the 1st week. Two weeks later, cloudy swelling remained unchanged, while, regeneration of liver cells was remarkable.

TABLE 4. Effects of the Administration of Ethyl Linoleate on Histologocal Findings of Livers of CCl_4 Poisoned Mice

Diet groups	1 Week				2 Weeks			
	St.	E.L.	E.L.+E.	E.L.+B_6	St.	E.L	E.L.+E.	E.L.+B_6
Centrilobular Necrosis	±	±	−	−	−	−	−	−
Cellurar Degeneration	+	+	++	±	++	+	±	+
Cloudy Swelling	+	++	+	±	++	+	++	++
Cellurar Regeneration	+	+	+	±	+	+	++	++
Disarrangement of Liver Cell Cords	+	+	±	−	+	−	−	+
Monocytic Infiltration	+	+	±	±	±	−	−	−

3) Changes of the liver lipid fractions after the administration of ethyl linoleate in CCl_4 poisoned mice

As indicated in Table 5 and Fig. 4 and 5, in standard diet group, slight increase in total cholesterol and significant decrease both in esterified fatty acids and in total lipids were observed in CCl_4 poisoned mouse livers in both 1st and 2nd week.

In E.L. group, there was no difference in total cholesterol and phospholipids between normal and CCl_4 poisoned mice. Esterified fatty acids decreased in the 2nd week and total lipids decreased remarkably in both the 1st and 2nd week.

TABLE 5. Changes of Liver Lipid Fractions in CCl_4 Poisoned Mice After the Administration of Ethyl Linoleate (mg/g of wet liver)

		Total choles.	Free choles.	Phospholipids	Esterified fatty acids	Total lipids
Normal-standard		3.1	2.7	38.1	53.4	84.2
CCl_4-standard	1 week	4.3	3.8	38.8	41.7	75.6
〃	2 week	3.3	2.8	34.3	42.3	53.1
Normal-E.L.	1 week	2.7	2.5	40.1	43.7	80.3
〃	2 week	3.0	2.7	40.7	51.4	65.8
CCl_4-E.L.	1 week	3.4	3.1	38.7	41.6	48.7
〃	2 week	2.9	2.6	40.2	43.5	51.7
Normal-E.L.+E.	1 week	3.5	2.9	39.8	49.6	75.1
〃	2 week	3.1	2.3	36.8	50.6	82.4
CCl_4-E.L.+E.	1 week	3.8	3.5	37.0	37.0	42.7
〃	2 week	3.4	3.0	34.0	36.7	38.9
Normal-E.L.+B_6	1 week	2.8	2.4	42.7	52.6	77.4
〃	2 week	3.8	3.0	33.1	50.9	69.6
CCl_4-E.L.+B_6	1 week	3.3	3.0	41.2	46.6	53.0
〃	2 week	3.2	2.7	35.5	38.9	42.2

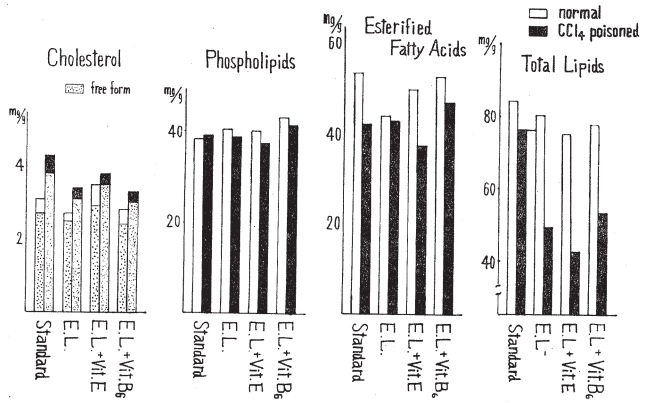

FIG. 4. Changes of liver lipid fractions in CCl_4 poisoned mice after a week administration of ethyl linoleate.

In E.L.-E group and E.L.-B_6 group, almost the same changes as in E.L. group were observed in all lipid fractions.

4) Changes of fatty acid composition of liver lipids after the administration of ethyl linoleate in CCl_4 poisoned mice

Changes of fatty acid composition of mouse liver lipids after the administra-

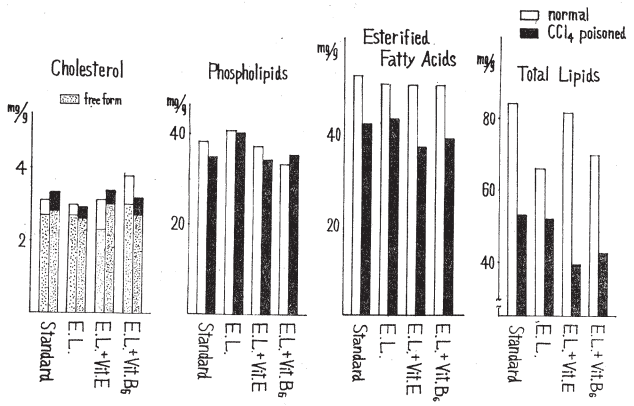

FIG. 5. Changes of liver lipid fractions in CCl_4 poisoned mice after two weeks administration of ethyl linoleate.

TABLE 6. Changes of Fatty Acid Composition of Liver Lipids After the Administration of Ethyl Linoleate in CCl_4 Poisoned Mice (%)

		14	16	16:1	18	18:1	18:2	18:3	20:4	22:6
Normal-standard		1.6	21.0	4.8	10.9	20.2	25.4	1.1	6.4	6.6
CCl_4-standard	1 week	0.8	22.5	2.3	14.9	13.8	20.7	—	11.7	12.0
〃	2 week	0.6	19.1	2.9	12.6	15.7	24.6	0.8	10.9	12.2
Normal-E.L.	1 week	0.8	16.7	2.8	10.4	11.5	37.8	1.2	7.6	9.8
〃	2 week	1.1	16.0	2.8	11.0	12.8	34.9	0.7	10.7	8.2
CCl_4-E.L.	1 week	0.6	18.5	2.8	18.0	8.5	20.8	—	18.4	11.4
〃	2 week	1.1	18.1	3.3	15.0	9.7	31.1	—	12.1	9.2
Normal-E.L.+E.	1 week	0.7	18.6	2.8	14.6	10.9	29.0	0.9	11.8	9.4
〃	2 week	1.0	13.7	3.0	7.9	13.2	47.1	1.2	5.7	5.5
CCl_4-E.L.+E.	1 week	0.7	19.2	1.6	19.2	6.7	20.1	—	18.0	14.2
〃	2 week	0.9	19.9	4.2	13.9	15.4	23.5	—	12.7	9.3
Normal-E.L.+B_6	1 week	0.8	17.3	2.7	12.5	16.4	30.4	0.6	8.4	7.9
〃	2 week	0.7	15.7	2.8	11.8	14.1	33.1	0.4	10.4	9.6
CCl_4-E.L.+B_6	1 week	0.2	20.4	1.1	16.1	8.9	22.6	—	15.7	14.3
〃	2 week	0.5	16.5	2.3	18.7	7.8	23.2	—	17.9	11.6

tion of ethyl linoleate are given in Table 6 and in Figs. 6 and 7.

CCl_4 poisoning caused significant decrease in percentages of palmitoleic and oleic acids, slight increase in stearic acid and significant increase in arachidonic and docosahexaenoic acids. Percentages of linoleic acid showed slightly lower level than normal in the 1st week but returned to almost normal level in the 2nd week.

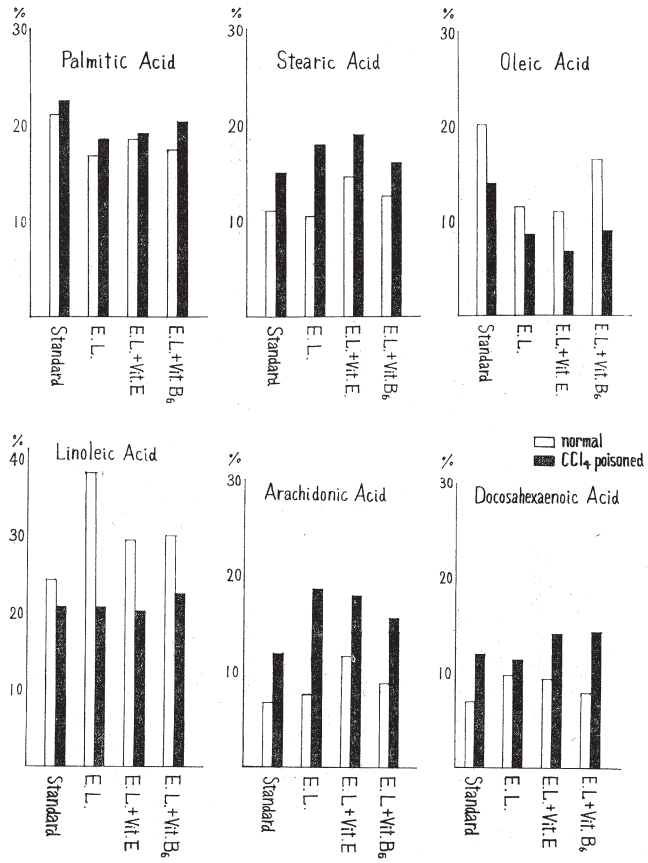

FIG. 6. Changes of fatty acid composition in livers of CCl_4 poisoned mice after a week administration of ethyl linoleate.

In E.L. group, significant increase in percentages of stearic, arachidonic and docosahexaenoic acids were observed both in the 1st and the 2nd week. Palmitoleic and oleic acids decreased moderately. Linoleic acid decreased markedly in the 1st week in spite of the administration of ethyl linoleate, but the difference between CCl_4 poisoned and normal mice became slight in the 2nd week.

In E.L.-E group, percentage of stearic acid slightly increased in the 1st week and then decreased in the 2nd week. Oleic acid decreased significantly both in the 1st and the 2nd week. Linoleic acid decreased slightly in the 1st week and markedly in the 2nd week, while, arachidonic and docosahexaenoic acids increased markedly in the 1st week and in the 2nd week they showed a tendency to decrease.

In E.L.-B_6 group, percentage of stearic acid moderately increased and oleic

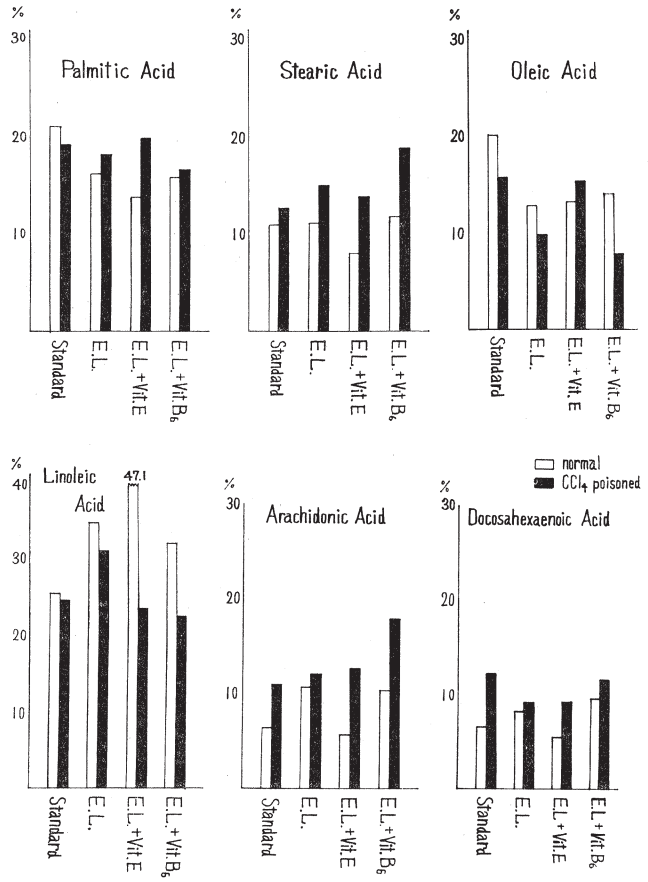

FIG. 7. Changes of fatty acid composition in livers of CCl_4 poisoned mice after two weeks administration of ethyl linoleate.

acid markedly decreased both in the 1st and the 2nd week. Percentage of linoleic acid was markedly lower than that in normals, while those of arachidonic and docosahexaenoic acids increased both in the 1st and the 2nd week.

CLINICAL STUDIES

C) Effects of ethyl linoleate on patients with chronic liver diseases

Materials and methods

Materials comprised 22 cases with liver cirrhosis and 14 cases with chronic hepatitis who were admitted to the 2nd Department of Internal Medicine, Nagoya University Hospital. They were diagnosed by the routine examinations including laparoendoscopy and needle boipsy of the liver and were placed on a diet containing about 2,400 calories, with 100 g of protein, 400 g of carbohydrate and 45 g of fat.

Twenty milliliters of ethyl linoleate was administered orally to these patients daily for a month. Successively, 20 ml of ethyl linoleate supplemented with 300 mg of alpha-tocopherol and 30 mg of pyridoxal phosphate were administered orally for a month to 17 cases with liver cirrhosis and to 13 cases with chronic hepatitist.

Effects of ethyl linoleate on clinical signs and symptoms of the patients with chronic liver diseases and on liver function tests were investigated. Simultaneously, serum lipid fractions and fatty acid compositions of the serum lipids were analyzed before and after the administration.

1) Results of ethyl linoleate on signs and symptoms of patients with liver cirrhosis

As shown in Table 7, fatigue, abdominal pain, epigastric distress and constipation were observed in 7, 5, 3, and 2 of 22 cases, respectively. After the administration of ethyl linoleate, about a half of them subsided these complaints. Anorexia was also greatly improved after the administration of ethyl linoleate. Diarrhea was observed in one case after the administration. Twelve of 13 cases whose livers had been palpated remained unchanged in their sizes and jaundice subsided in 5 to 10 cases after the administration. Tenderness of the liver, edema and ascites were also improved. Weight gain and loss were observed in 11 and in 7 cases, respectively.

TABLE 7. Effects of Ethyl Linoleate on Symptoms of Patients with Liver Cirrhosis

	Before Administration	After Administration		
		Improved	Unchanged	Aggravated
Fatigue	7/22	4	2	1
Anorexia	6/22	5	1	0
Nausea, Vomiting	1/22	1	0	0
Epigastric Distress	3/22	2	0	1
Abdominal Pain	5/22	3	2	0
Diarrhea	0/22	0	0	1
Constipation	2/22	1	1	0

Effects of Ethyl Linoleate on Signs of Patients with Liver Cirrhosis

	Before Administration	After Administration		
		Improved	Unchanged	Aggravated
Jaundice	10/22	5	5	0
Hepatomegaly	13/22	1	12	0
Tenderness of Liver	5/22	4	1	0
Splenomegaly	1/22	0	1	0
Edema	2/22	2	0	0
Ascites	4/22	3	1	0
Body Weight		11	4	7

2) Results of ethyl linoleate on signs and symptoms of patients with chronic hepatitis

As shown in Table 8, fatigue and anorexia were observed in many cases with chronic hepatitis before the administration, but after the administration of ethyl linoleate for a month, pronounced improvement of these symptoms was observed in almost all cases. Nausea, abdominal pain, epigastric distress which were also complained of some of the patients, were improved after the administration of ethyl linoleate for a month.

The liver was palpated in 13 of 14 cases of chronic hepatitis before the administration. In 5 of these 13 cases, decreases in liver size were observed after the administration and the remaining 8 the liver sizes unchanged. Tenderness of the liver was observed in 6 of 14 cases before the administration, and in 4 cases of them the complaints were improved after the administration. Jaundice observed in 3 cases were also improved after the administration of ethyl linoleate. Gain in weight was observed in 9 cases and loss in weight in 2 cases.

TABLE 8. Effects of Ethyl Linoleate on Symptoms of Patients with Chronic Hepatitis

	Before Administration	After Administration		
		Improved	Unchanged	Aggravated
Fatigue	11/14	9	2	0
Anorexia	12/14	10	2	0
Nausea, Vomiting	3/14	3	0	0
Abdominal Pain	5/14	3	2	0
Epigastric Distress	2/14	2	0	0
Gingival Bleeding	1/14	0	1	0

Effects of Ethyl Linoleate on Signs of Patients with Chronic Hepatitis

	Before Administration	After Administration		
		Improved	Unchanged	Aggravated
Jaudice	2/14	2	0	0
Hepatomegaly	13/14	5	8	0
Tenderness of Liver	6/14	4	2	0
Edema	0/14	0	0	1
Body Weight		9	3	2

3) Effects of ethyl linoleate on liver function tests of patients with liver cirrhosis

As given in Table 9, following the administration of ethyl linoleate, A/G ratio returned to the normal value in 5 of 13 patients whose A/G ratio had been abnormal. Serum total bilirubin decreased to within the normal range in 5 of 10 cases which had elevated value initially. Of 16 cases which showed abnormal B.S.P. retention at 45 minuites, 10 showed improvement after the administration. Of 8 cases which had had an increased thymol turbidity, 5

TABLE 9. Effects of Ethyl Linoleate on Liver Function Tests of Patients with Liver Cirrhosis (22 cases)

	Before Administration		After Administration		
	Abnormal	Normal	Improved	Unchanged	Aggravated
A/G	13	9	5	17	0
Total Bilirubin	10	9	5	12	2
B.S.P.	16	6	10	11	1
T.T.T.	8	10	4	11	3
C.C.F.	8	8	3	12	1
SGOT	14	8	8	10	4
SGPT	11	11	9	11	2
Al. Phos.	10	10	4	14	2

showed decrease of it. Cephalin flocculation at 24 hours was positive in 8 cases, in 4 of them it became negative. Of 14 cases having had elevation of S-GOT, 8 returned toward the normal level and 4 cases having shown the normal level at pre-administration became aggravated. Of 11 cases having had elevation of S-GPT initially, 9 returned toward the normal level. Serum alkaline phosphatase had been elevated in 10 cases and about a half of them was also improved after the administration.

4) *Effects of ethyl linoleate on liver function tests of patients with chronic hepatitis*

As given in Table 10, almost all cases showed A/G ratio of within normal range before and after the administration of ethyl linoleate. Of 5 cases having shown elevation of serum bilirubin, 4 decreased to within normal level. Of 4 cases having shown abnormal B.S.P. retention, all of them showed the improvement after the administration, while of 11 cases having shown normal B.S.P. retention, 2 became aggravated. Thymol turbidity and cephalin flocculation at 24 hours were improved satisfactorily. Serum transaminases were also satisfactorily improved and no aggravated cases were noted. All cases showed

TABLE 10. Effects of Ethyl Linoleate on Liver Function Tests of Patients with Chronic Hepatitis (14 cases)

	Before Administration		After Administration		
	Abnormal	Normal	Improved	Unchanged	Aggravated
A/G	2	9	1	9	1
Total Bilirubin	5	6	4	7	0
B.S.P.	4	9	4	7	2
T.T.T.	6	5	5	5	1
C.C.F.	4	1	4	1	0
SGOT	6	8	6	8	0
SGPT	6	8	5	9	0
Al. Phos.	1	12	1	12	0

normal level of serum alkaline phosphatase and no aggravated cases were observed after the administration.

5) Effects of ethyl linoleate on serum lipid fractions inpatients with liver cirrhosis

As shown in Table 11, the mean values of serum lipid fractions in 22 patients with liver cirrhosis were compared with those in 252 normal adults consisting of 190 males and 62 females aged 18 to 65 years old (Table 13). Total and esterified cholesterol levels in the patients with liver cirrhosis showed lower levels than those in normals. Slight decrease in phospholipids, esterified fatty acids and total lipids were also observed in the patients with liver cirrhosis.

The administration of ethyl linoleate to the patients with liver cirrhosis resulted in slight decrease in total and esterified cholesterol levels and in esterified fatty acids. Phospholipids and total lipids remained unchanged.

The administration of ethyl linoleate supplemented with alpha-tocopherol and pyridoxal phosphate also caused decrease in total and esterified cholesterols, a slight increase in phospholipids and showed minimum change in esterified fatty acids and total lipids.

TABLE 11. Changes of the Lipid Fractions After the Administration of Ethyl Linoleate in Patients with Liver Cirrhosis (mg/dl)

	Before Administration	After Administration	
		E.L.	E.L.+Vit. E and B_6
No. of cases	22	17	17
Total choles.	178	164	160
Choles. esters	129	118	108
Phospholipids	206	211	219
Esterified fatty acids	326	293	331
Total lipids	651	653	644

6) Effects of ethyl linoleate on serum lipid fractions in patients with chronic hepatitis

As shown in Table 12, the serum lipid fractions in patients with chronic hepatitis were slightly lower than those in normal adults. Total and esterified cholesterols, phospholipids and esterified fatty acids were definitely lower than the normals and they increased to the normal levels after the administration of ethyl linoleate. Total lipids also increased but still remained lower than those in the normals.

Administration of ehtyl linoleate supplemented with alphatocopherol and pyridoxal phosphate also resulted in the increase of all lipid fractions to the normal levels.

TABLE 12. Changes of the Lipid Fractions After the Administration of Ethyl Linoleate in Patients with Chronic Hepatitis (mg/dl)

	Before Administration	After Administration	
		E.L.	E.L.-Vit. E. and B_6
No. of cases	15	15	12
Total choles.	174	221	212
Choles. esters	121	160	160
Phospholipids	199	224	236
Esterified fatty acids	328	358	370
Total lipids	568	595	696

TABLE 13. Lipid Fractions in Normal Adults (mg/dl)

No. of cases	252
Total cholesterol	190±32
Cholesterol esters	150±15
Phosholipids	226±37
Esterified fatty acids	348±97
Total lipids	699±184

Fatty Acid Composition of Serum Lipids in Normal Adults (%)

No. of cases	14	16	16:1	18	18:1	18:2	18:3	20:3	20:4	20:5	22:6
219	1.9 ±0.7	22.1 ±2.1	7.6 ±1.1	8.1 ±1.8	24.1 ±4.2	27.6 ±3.6	1.2 ±0.5	0.5 ±0.3	4.8 ±1.1	0.9 ±0.6	3.2 ±1.0

7) Effects of ethyl linoleate on serum fatty acid composition in patients with liver cirrhosis and in patients with chronic hepatitis

As shown in Table 14 and Fig. 8, the fatty acid composition of serum lipids in patients with liver cirrhosis were compared with those in normals, slight increase in percentage of palmitoleic acid and decreases in stearic and linoleic acids were observed. No appreciable differences were observed between normals and patients with liver cirrhosis in percentages of other fatty acids. Ethyl linoleate administration caused increase in percentage of linoleic acid to the level seen in normal adults and slight decrease in oleic acid.

Administration of ethyl linoleate supplemented with alpha-tocopherol and pyridoxal phosphate resulted in the same changes in fatty acid composition

TABLE 14. Changes of the Fatty Acid Composition of Serum Lipids After the Administration of Ethyl Linoleate in Patients with Liver Cirrhosis (%)

	No. of cases	14	16	16:1	18	18:1	18:2	18:3	20:3	20:4	20:5	22:6
Before administration	22	2.4	23.6	10.4	6.8	24.8	23.0	0.6	0.5	4.4	0.7	2.8
After administration E.L.	17	1.9	21.4	9.5	6.5	22.9	27.8	0.8	0.6	4.9	0.5	3.0
E.L.+Vit.E. and B_6	17	1.5	20.0	8.0	6.5	21.5	26.9	1.0	0.9	4.5	0.6	2.7

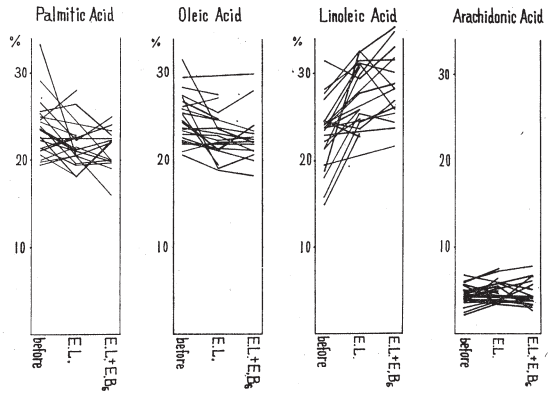

FIG. 8. Changes of fatty acid composition of serum lipids after the administration of ethyl linoleate in patients with liver cirrhosis.

TABLE 15. Changes of the Fatty Acid Composition of Serum Lipids After the Administration of Ethyl Linoleate in Patients with Chronic Hepatitis

	No. of cases	14	16	16:1	18	18:1	18:2	18:3	20:3	20:4	20:5	22:6
Before administration	15	1.9	22.4	8.8	6.6	24.8	24.0	0.7	0.7	5.2	0.9	3.4
After administration												
E.L.	13	2.1	21.4	8.6	6.6	22.1	27.2	1.2	0.6	4.6	0.9	3.4
E.L.+Vit.E. and B_6	10	2.0	22.1	8.9	7.0	21.8	27.4	1.6	0.9	5.0	0.8	2.8

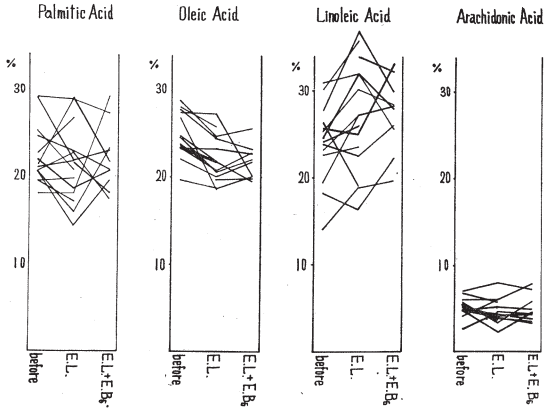

FIG. 9. Changes of fatty acid composition of serum lipids after the administration of ethyl linoleate in patients with chronic hepatitis.

as seen in ethyl linoleate administration alone. No change was observed in percentages of arachidonic acid after the administration of ethyl linoleate with or without supplementation of alpha-tocopherol and pyridoxal phosphate.

In the patients with chronic hepatitis, as shown in Table 15 and Fig. 9, percentage of linoleic acid was slightly lower than that of normals and percentages of other fatty acids were approximately equal to those observed in normals.

The administration of ethyl linoleate with or without alpha-tocopherol and pyridoxal phosphate caused a slight decrease in percentage of oleic acid and increase in linoleic acid to the level as presented in normals, and no significant change was observed in percentage of arachidonic acid.

DISCUSSION

In the present experiment, microscopic study of the liver revealed that chronically CCl_4 poisoned rats fed high-safflower oil diet showed the slightest liver impairments of the rats fed those experimental diets. Among the experimental groups, the liver of the rats fed standard and fat-free diets exhibited more serious impairments than the rats fed high-fat diets. Furthermore, the livers obtained from E.L.+E and E.L.+B_6 groups showed the slightest liver damage and remarkable regeneration of liver cells were observed.

It is, therefore, suggested from these observations that the administration of ethyl linoleate with or without supplementation with alpha-tocopherol or pyridoxal phosphate to CCl_4 poisoned mice should prove be effective on recovery from CCl_4 poisoned liver damage. Previously, Kimura[21] reported that supplement of high-butter diet to mice had a good effect on recovery from chronically CCl_4 treated liver. Tomono and Naito[28] observed that high fat administration prevented the fibrosis in the rat liver which was induced experimentally by the venous injection of the egg york solution.

Clinically, frequent complaints including fatigue, loss of appetite, epigastric distress, nausea and vomiting observed in almost all cases with liver cirrhosis disappeared after the administration of ethyl linoleate. Ascites and edema disappeared. Of 12 cases which had had jaundice, in 7 cases it disappeared during the administration and enlargement of the liver regressed in size about a half of them. Improvements in liver function tests during the administration of ethyl linoleate were also noticed in almost all cases with a few aggravated exceptions. In the previous studies concerning the improvements after the administration of fats, Mindrum and Schiff[29] showed that patients with severe fatty liver could absorb and utilize a large amount of fat in their diet, and showed progressive clinical improvement accompanied by improvement in liver function tests and elimination of fat infiltration in the liver specimens. Walker[30] also reported a case of xanthomatous biliary cirrhosis treated with ethyl

linoleate. The administration of 40 grams of ethyl linoleate resulted in the dramatic and sustained fall in the plasma level of total lipid, cholesterol, phospholipids, and also resulted in the relief of some of the signs and symptoms.

In chronic CCl_4 poisoned rats, increases of all plasma lipid fractions were observed. Marked increases were observed in plasma esterified fatty acids and total lipids, while there was a slight difference between CCl_4 poisonings and normals in plasma total cholesterol and phospholipid levels. These increases of plasma esterified fatty acid and total lipid levels were due mainly to the increase of plasma triglycerides.

Although Ito[31] stated that the administration of CCl_4 caused a marked elevation of total and phospholipids within 24 hours and a gradual fall with a recovery to normal level within a week, there was a few differences in all liver lipid fractions between normal controls and chronic CCl_4 poisonings.

In the 2nd experiment, total and free cholesterol contents of the livers of the mice treated with CCl_4 increased in each group in the 1st week, then, in the 2nd week showed gradual fall to the normal level in each group. There was no change in phospholipid content in the livers of all groups. CCl_4 poisoning resulted in a marked decrease in total lipid content of mouse livers in each group and administration of ethyl linoleate promoted this tendency. This results agreed with Kimura's report[21] in which the gradual decrease in total lipid content of the liver was observed in the studies of high-saffiower oil supplement to chronic CCl_4 poisoned rats. Tanaka[32], Tomono and Naito[28] also observed a decrease in total lipid content in rat livers with experimental liver cirrhosis. This reduction of total lipids in the liver may be due mainly to the decrease of triglyceride fraction.

Gas-liquid chromatographic analysis revealed that the administered fat largely influenced upon plasma fatty acid composition of rats whether with or without CCl_4 poisoning; high-lard supplement caused increase in percentage of plasma oleic acid and high-saffiower-oil diet caused a marked increase in that of plasma linoleic acid.

The administration of ethyl linoleate to CCl_4 poisoned mice did not show any increase in percentage of liver linoleic acid, while to normal controls the administration of ethyl linoleate showed a significant increase. On the contrast to the decrease in percentage of linoleic acid, CCl_4 poisoning caused a significant increase in percentage of arachidonic acid in the livers of the mice and the administration of ethyl linoleate acceralated this tendency. No influence in addition of alpha-tocopherol or pyridoxal phosphate to ethyl linoleate upon lipid metabolism in the liver of mouse could be appreciated.

When ethyl linoleate was administered to mice, there was a remarkable decrease in total lipid content paralleled with the decrease in percentage of linoleic acid in the livers of CCl_4 poisoned mice, while there was a slight decrease in total lipid content in the livers of normal mice and marked increases

in percentage of arachidonic and docosaxaenoic acids were observed in the livers of CCl_4 poisoned mice. The decrease in percentage of linoleic acid in the livers in CCl_4 poisoned mice may result in a relatiove increase in percentages of arachidonic and docosahexaenoic acids. Getz[33] indicated that arachidonic acid was contained chiefly in phospholipid fraction and less in triglycerides in rat livers. Consequently, the decrease in triglycerides in the liver of CCl_4 poisoned mice, as described above, might result in this marked decrease in linoleic acid and it is suggested that ethyl linoleate may prevent the deposition of fat in the liver of CCl_4 poisoned mice, and that the conversion of linoleic to arachidonic acid in the liver of CCl_4 poisoned mice may keep not to be disturbed contrary to possible expectation.

In this study, determination of serum lipid fractions in patients with chronic liver diseases revealed that all lipid fractions were lower than in normals. From an analysis of serum lipid in variety of hepatic diseases, Phillips[34] suggested that parenchymal injury caused a decrease in serum free cholesterol and cholesterol esters.

The lowering of plasma cholesterol by the administration of an adequate amount of linoleic acid has been demonstrated by many investigators.[35,36,37,38] In this study, total serum cholesterol in patients with liver cirrhosis were slightly lowered by the administration of ethyl linoleate, reversely in patients with chronic hepatitis, total serum cholesterol elevated slightly during the administration. This evidence suggests that in chronic hepatitis improvement of parenchymal injury of the liver may by accompanied with accerelated biosynthesis and esterification of cholesterol, which cause an elevation of serum total cholesterol.

Schrade[18] noted the reduction of plasma polyunsaturated fatty acid contents in 10 cases with advanced liver cirrhosis by using the spectrophotometrical method. Thereafter, a decrease in percentage of linoleic acid and an increase in that of oleic acid in patients with liver cirrhosis were found by gas-liquid chromatographic analysis of plasma lipids.

A moderate decrease in percentage of serum linoleic acid was noted in patients either with liver cirrhosis, or with chronic hepatitis, but the increase of oleic acid was not so significant as many investigators reported.[19,20]

There was no difference in percentage of arachidonic acid between normals and patients with liver diseases.

Administration of ethyl linoleate supplemented with or without alpha-tocopherol and pyridoxal phosphate to these patients caused increase in percentage of linoleic acid to the levels presented in normals and decrease in oleic acid. There was no change observed in arachidonic acid.

Although the mechanism of this decrease in percentage of serum linoleic acid and increase in that of oleic acid in patients with chronic liver diseases could not be clarified, it would be possibly suggested that the absorption of

linoleic acid from dietary fat and/or the incorporation of linoleic acid to serum lipids might be impaired in patients with chronic liver diseases.

From these results, it is concluded that the administration of ethyl linoleate can expect the satisfactory response to the patients both with liver cirrhosis and with chronic hepatitis.

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