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Effects of palmitic, oleic, and linoleic acids on hepatic and vascular lipid deposits*

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SUMMARY

Hepatic and vascular lipid deposits were produced in rats by a diet containing hydrogenated coconut oil plus cholesterol. The effects of different amounts of palmitic, oleic, and linoleic acids on these deposits were determined by histological examination and chemical analysis using silicic acid and gas-liquid chromatography. Marked regression of hepatic sterol esters and triglycerides was noted in the animals fed 5% or more of linoleate, but no effect on coronary lipid deposits was observed. When compared to a fat-free diet, oleate seemed to have no effect, whereas palmitate prevented the regression of hepatic sterol esters. The alterations in the fatty acid pattern of all the hepatic lipid fractions produced in animals by the cholesterol and coconut oil diet were reversed by 5% or more of dietary linoleate. There is suggestive evidence that linoleate induces increased synthesis of arachidonate. The latter fatty acid may then be incorporated into the structure of some phospholipids, which probably have a vital function in the transport of cholesterol and glycerides.

Lipid infiltration of the liver of rats fed moderate amounts of cholesterol has been demonstrated in several investigations (1–3). This excess lipid has been found to consist primarily of cholesterol esters and glycerides (4). The cholesterol ester content of rat liver can be greatly increased, and vascular lipid deposition produced, by increasing the absorption of excess dietary cholesterol by the addition of bile salts (5, 6). Choline reduces triglyceride accumulation in rats on cholesterol diets (4), but the cholesterol esters appear to be resistant to choline, as well as to other lipotropic agents (3, 4, 7). Indeed, Ridout et al. (4) found that liver cholesterol esters were slightly increased when dietary choline was highest.

Essential fatty acid deficiency has been demonstrated to produce fatty livers in rats (8, 9). Engel (8) found that this occurred even though the diet contained adequate choline. He concluded that essential fatty acids were necessary for choline to properly function as a lipotropic agent. Synthesis of cholesterol in the livers of rats deficient in essential fatty acids (EFA)[1] is decreased (10), suggesting that the accumulation of cholesterol in these livers is due to a defect in the transport mechanism.

Since excess dietary cholesterol is known to accentuate the pathological features of EFA deficiency (11, 12), it is probable that part of the fatty infiltration of the liver induced by cholesterol alone has a biochemical etiology similar to that of EFA deficiency. The investigations of the effects of choline and other lipotropic agents described above suggest that the mechanisms of cholesterol ester deposition and removal in cholesterol-fed rats differ from those for triglyceride regulation. The aims of the present study were to determine the effects on both these lipid classes of the administration of various saturated and unsaturated long-chain fatty acids, and thereby to elucidate further the mechanisms regulating the hepatic levels of cholesterol and glycerides. The fatty acids were administered both as the natural vegetable oils (mixed triglycerides) and as purified methyl esters, so as to distinguish the specific effects attributable to each

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[1] Abbreviations: EFA = essential fatty acid(s), CNO = coconut oil, CSO = cottonseed oil.

component of the oil. Histological studies were performed to determine the beneficial or detrimental effects of these fatty acids on the vascular lipid deposits induced by dietary cholesterol. Tocopherol, because of its known antioxidant properties, was administered in some experiments to investigate the possibility of its enhancement of the effect of dietary linoleate.

METHODS

Four-month-old male albino rats of our colony (USC strain) were divided into dietary groups of five each. The basal diet consisted of sucrose, casein, cellulose, a salt mixture, choline, and a vitamin mixture[2] (6). Groups I and II received a fat-free diet, and groups III-IX a diet containing 20% hydrogenated coconut oil (CNO), 3% cholesterol, and 1% cholic acid. After 14 weeks on the diets, groups I and III were killed by intraperitoneal injection of sodium pentobarbital (Nembutal); hearts and portions of liver were placed in 10% formalin, and the remainders of the livers were immediately frozen for later chemical analysis. Groups II and IV were then continued on their original diets. Group V was changed to a fat-free diet; group VI to a diet containing 20% cottonseed oil (CSO); and groups VII–IX to a fat-free diet plus oral supplements of 100 mg/animal/day of methyl oleate (VII), methyl linoleate (VIII), or methyl linoleate plus 10 mg/day of α-tocopherol acetate (IX). After 10 weeks, all the animals were killed and tissues taken by methods identical to those described for groups I and III. In a second experimental series, groups X–XV were given the diet containing hydrogenated coconut oil, cholesterol, and cholic acid for eight weeks, at which time the rats of group X were killed and tissues taken as previously described. Group XI was then continued on the original diet, XII changed to a fat-free diet, XIII to a 5% methyl palmitate diet, XIV to a 5% methyl oleate diet, and XV to a 5% methyl linoleate diet. All animals were killed after 3 weeks and tissues taken as described above.

Frozen sections were prepared from the formalin-fixed hearts and liver slices and stained for lipids by the Oil Red O method. In addition to the usual light microscopy, the tissues were examined under the polarizing microscope for birefringent refractile crystals characteristic of cholesterol.

Individual samples of each liver were lyophilized and the total lipids were extracted and weighed as described by Morin et al. (6). Fractionation of total lipids into hydrocarbons, sterol esters, triglycerides, free sterols, mono- and diglycerides, and phospholipids was accomplished by silicic acid chromatography (6). Analysis of the fatty acid composition of the sterol esters, triglycerides, and phospholipids by gas-liquid chromatography was also done by methods previously described (6).

The probability that apparent differences in the results were due to chance was calculated by the t test, and only those differences where $p < 0.01$ have been commented on as significant (except in those cases where p values are specifically stated).

RESULTS

Morphological Studies. Liver sections from animals fed the diet containing coconut oil plus cholesterol for 14 weeks showed a dense, uniform lipid infiltration throughout the liver lobule. The lipid was in the form of droplets and large globules, located in the parenchymal and Kupffer cells and extracellularly. Under the polarizing microscope, large refractile granules and crystals of cholesterol were demonstrable in all portions of the liver lobule. The livers of animals that were changed to a fat-free diet for the following 10 weeks (group V) showed a somewhat decreased amount of lipid, visible as irregularly distributed droplets in the peripheral half of the lobule. Polarizing microscopic examination revealed the persistence of refractile granules of cholesterol. Liver sections from the group changed to a diet containing cottonseed oil (VI) showed a marked depletion of demonstrable lipid. There was also a concomitant loss of refractile granules. Livers from group VII, the animals changed to a fat-free diet supplemented with 100 mg/day of methyl oleate, showed some regression of lipid infiltration as compared to group III, but there was a persistence of stainable lipid in the peripheral third of the lobule. A similar pattern, with a somewhat greater decrease in lipid, was observed in the livers of animals of groups VIII and IX, given diets supplemented with 100 mg/day of methyl linoleate.

Livers from animals of group X (CNO + chol., 8 weeks) showed a pattern of lipid infiltration similar to that of group III. No regression of lipid droplets and globules or refractile granules of cholesterol was observed in the livers of the group changed to 5% palmitate (XIII), and only a slight depletion was noted in the fat-free group (XII). Livers of the 5% oleate group

[2] The vitamin mixture had the following composition: Vitamin Test Casein, 61.35%; p-aminobenzoic acid, 2.42%; inositol, 2.0%; α-tocopherol acetate, 1.3865%; ascorbic acid, 0.8%; thiamine, 0.288%; Ca-pantothenate, 0.24%; niacin 0.24%; vitamin B_{12} triturate, 0.24%; riboflavin, 0.11%; pyridoxine, 0.108%; crystalets (5,000 USP units vitamin A/g, 50,000 USP units vitamin D/g), 0.052%; folic acid, 0.046%; menadione, 0.022%; biotin, 0.016%.

TABLE 1. LIVER LIPID COMPOSITION

Diet⟶	Fat-Free, 14 Weeks	Fat-Free, 24 Weeks	CNO + Chol., 14 Weeks	CNO + Chol., 24 Weeks	CNO + Cholesterol, 14 Weeks: + Fat-Free, 10 Weeks	+ CSO, 10 Weeks	+100 mg/d Oleate, 10 Weeks	+100 mg/d Linoleate, 10 Weeks
Group No.⟶	I	II	III	IV	V	VI	VII	VIII
Total lipid, mg/g*	294.4 ± 29.7‡	386.3 ± 45.9	676.2 ± 28.0	1103.7 ± 44.1	559.8 ± 45.8	226.3 ± 5.9	474.2 ± 46.6	358.6 ± 36.4
Sterol ester, %†	7.7 ± 3.3	15.5 ± 4.6	34.0 ± 1.9	40.0 ± 9.0	31.4 ± 5.5	4.3 ± 1.2	26.7 ± 6.1	16.9 ± 7.8
mg/g	22.6 ± 9.8	59.7 ± 17.9	230.2 ± 12.6	441.0 ± 99.2	175.7 ± 30.5	9.8 ± 2.7	126.5 ± 29.1	60.5 ± 28.1
Triglyceride, %	22.5 ± 2.8	39.2 ± 4.1	29.2 ± 1.7	26.0 ± 1.9	35.5 ± 3.4	23.1 ± 2.4	34.9 ± 5.6	38.9 ± 6.0
mg/g	66.2 ± 8.1	151.3 ± 15.9	197.6 ± 11.6	287.0 ± 21.2	198.7 ± 18.8	52.3 ± 5.5	165.3 ± 26.7	139.4 ± 21.4
Free sterol, %	6.1 ± 0.1	6.2 ± 0.8	6.3 ± 0.4	3.7 ± 0.3	6.2 ± 0.7	5.6 ± 0.5	6.5 ± 0.7	7.5 ± 1.1
mg/g	17.8 ± 0.3	24.0 ± 3.0	42.5 ± 2.5	41.1 ± 3.3	34.8 ± 3.9	12.6 ± 1.1	30.9 ± 3.4	26.8 ± 4.1
Phospholipid, %	59.9 ± 6.1	34.8 ± 3.3	24.6 ± 2.9	15.1 ± 1.3	23.2 ± 2.5	62.4 ± 2.7	27.7 ± 3.6	33.0 ± 7.2
mg/g	176.3 ± 17.8	134.4 ± 12.7	166.1 ± 19.3	166.2 ± 14.7	130.0 ± 13.8	141.2 ± 6.1	131.4 ± 17.0	118.5 ± 25.8

* mg/g of nonlipid dry weight.
† % of total lipid.
‡ Standard deviation = $\sqrt{\frac{\Sigma d^2}{n-1}}$

(XIV) were relatively free of lipid but showed the persistence of slightly excessive amounts of lipid droplets and globules uniformly distributed in the peripheral areas of the liver lobules. A complete regression of lipid infiltration, identical to that of the cottonseed oil group, was observed in the group changed to 5% linoleate (XV).

The coronary arteries of the animals of group III (CNO + chol., 14 weeks) and X (CNO + chol., 8 weeks) showed lesions consisting of intimal thickening, narrowing of the lumen, and lipid infiltration of both the intima and media. None of the subsequent diets in either series appeared to have a regressive effect on the lesions.

No effect of excess dietary tocopherol was noted in any of the histological or chemical studies; the results of group IX were identical to VIII.

Liver Lipid Fractionation. Total lipid analyses and silicic acid chromatography data are summarized in Tables 1 and 2.

Comparison between the amounts of total lipids in the livers of animals in group III (CNO + chol., 14 weeks) and groups V–IX (changed to diets without cholesterol) reveals significant decreases in all the latter groups. Regression of total lipids to levels less than half of those found in group III occurred in the group changed to cottonseed oil (VI). Smaller, but significant decreases occurred in the groups given linoleate supplements (VIII and IX). The least amounts of regression were observed in the fat-free (V) and oleate (VII)

TABLE 2. LIVER LIPID COMPOSITION

Diet⟶	CNO + Chol., 8 Weeks	CNO + Chol., 11 Weeks	CNO + Cholesterol, 8 Weeks: + Fat-Free, 3 Weeks	+5% Palmitate, 3 Weeks	+5% Oleate, 3 Weeks	+5% Linoleate, 3 Weeks
Group No.⟶	X	XI	XII	XIII	XIV	XV
Total lipid, mg/g*	331.1 ± 26.2‡	385.5 ± 34.1	296.3 ± 38.2	343.7 ± 18.7	287.1 ± 13.7	232.5 ± 10.2
Sterol esters, %†	15.3 ± 4.6	20.7 ± 3.1	5.3 ± 0.8	12.6 ± 3.3	5.6 ± 1.4	3.2 ± 0.3
mg/g	50.7 ± 15.1	79.9 ± 2.2	15.8 ± 12.0	43.3 ± 11.3	16.0 ± 4.2	7.4 ± 0.7
Triglycerides, %	27.0 ± 6.6	28.7 ± 5.4	37.2 ± 7.9	33.8 ± 6.0	25.4 ± 6.3	16.6 ± 1.3
mg/g	89.4 ± 22.0	109.0 ± 23.5	110.1 ± 20.6	116.3 ± 20.8	72.9 ± 18.2	38.7 ± 3.1
Free Sterol, %	5.3 ± 0.6	5.6 ± 0.7	6.6 ± 1.4	6.5 ± 0.5	4.9 ± 1.2	5.0 ± 0.7
mg/g	17.5 ± 1.2	21.4 ± 4.1	19.6 ± 2.5	22.4 ± 1.6	14.0 ± 3.6	11.6 ± 1.7
Phospholipids, %	48.4 ± 7.9	40.1 ± 8.1	46.2 ± 9.7	43.2 ± 6.4	60.6 ± 8.1	71.5 ± 2.8
mg/g	160.4 ± 26.3	154.7 ± 28.7	136.9 ± 31.2	148.7 ± 21.9	174.0 ± 23.1	166.1 ± 6.6

* mg/g of nonlipid dry weight.
† % of total lipid.
‡ Standard deviation = $\sqrt{\frac{\Sigma d^2}{n-1}}$

TABLE 3. FATTY ACID COMPOSITION OF LIVER STEROL ESTERS*

Diet →	CNO + Chol., 14 Weeks	CNO + Cholesterol, 14 Weeks: + Fat-Free, 10 Weeks	+ CSO, 10 Weeks	+100 mg/d Oleate, 10 Weeks	+100 mg/d Linoleate, 10 Weeks	CNO + Chol., 8 Weeks	CNO + Cholesterol, 8 Weeks: + Fat-Free, 3 Weeks	+ Palmitate, 3 Weeks
Group No. →	III	V	VI	VII	VIII	X	XII	XIII
Fatty Acid								
14:0	1.5 ± 0.4	1.0 ± 0.2	2.9 ± 1.3	0.9 ± 0.1	1.4 ± 0.2	1.4 ± 0.5	1.2 ± 0.7	1.7 ± 0.8
16:0	26.7 ± 11.0	30.2 ± 1.5	21.0 ± 1.3	31.1 ± 2.2	35.9 ± 4.6	18.3 ± 3.7	30.8 ± 2.3	34.0 ± 2.3
16:1	17.6 ± 3.7	12.3 ± 1.9	8.9 ± 2.3	11.0 ± 2.5	12.3 ± 1.7	21.4 ± 2.5	15.4 ± 3.5	11.3 ± 1.1
18:0	2.5 ± 0.2	2.3 ± 0.2	5.4 ± 0.3	2.6 ± 1.1	2.6 ± 0.7	2.2 ± 0.4	2.7 ± 0.5	2.4 ± 0.4
18:1	49.6 ± 7.3	52.7 ± 2.3	28.8 ± 3.3	53.8 ± 0.7	46.2 ± 2.5	49.9 ± 3.3	45.2 ± 7.0	49.3 ± 1.3
18:2	2.0 ± 0.2	1.5 ± 0.4	23.5 ± 5.9	0.7 ± 0.2	1.3 ± 0.4	5.7 ± 3.7	2.8 ± 2.0	1.4 ± 0.6
20:3†	Tr.‡	1.0 ± 0.3	...	Tr.	...	Tr.	Tr.	Tr.
20:4	...	...	8.5 ± 1.0	...	1.0 ± 0.1	2.1 ± 1.0	3.8 ± 2.4	...

* Percentage of total fatty acids, including standard deviation.
† 5,8,11-eicosatrienoic acid.
‡ Tr. = Trace (present, but too small to measure).

groups; the magnitude of regression in these two groups was approximately the same.

Sterol esters of the group changed to cottonseed oil (VI) showed an 8-fold decrease, expressed as percentage of total lipid, and a 24-fold decrease in absolute concentration, as compared to group III (CNO + chol., 14 weeks). Both groups given linoleate supplements (VIII and IX) also showed decreases, although of lesser magnitude.

The results in groups I and II (fat-free, 14 and 24 weeks) indicate that EFA deficiency alone caused a progressive increase in liver sterol esters. As the diet containing hydrogenated coconut oil plus cholesterol is deficient in essential fatty acids, part of the sterol ester accumulation in these animals is probably due to this deficiency. The extent of sterol ester regression seen in the groups fed cottonseed oil or linoleate was much greater than the amount of sterol ester deposition that could have been produced by a fat-free diet containing no cholesterol. An additional complexity, however, is that both hydrogenated coconut oil and cholesterol will accentuate EFA deficiency (13, 11); in the present study, therefore, the exact proportion of sterol-ester deposition due to EFA deficiency, and that due directly to the excess dietary cholesterol, cannot be determined.

Triglycerides expressed as a fraction of total liver lipids were not different in groups III (CNO + chol., 14 weeks) and VI (cottonseed oil), but the absolute concentration in group VI was less than half that in group III. Both groups given linoleate supplements (VIII and IX), compared to group III, showed higher relative amounts but similar absolute concentrations of triglycerides.

Free sterols showed little decrease in percentage (of total lipids) but a significant decrease in absolute concentration (mg/g nonlipid dry weight) in group VI as compared to III.

The percentage of phospholipids was greatly increased in group VI and increased to a lesser extent in groups VIII and IX over group III. When calculated as milligrams per gram of nonlipid dry weight, there is no significant difference in the amounts of phospholipids among any of the groups.

In the second experimental series, the livers of group XV, in which the 8-week CNO + chol. diet was followed by three weeks on 5% linoleate, showed significantly less total lipid than group X (CNO + chol., 8 weeks).

Both percentages and amounts of sterol esters were decreased in groups XII (fat-free), XIV (5% oleate), and XV (5% linoleate) as compared to group X, with the linoleate-fed group showing the greatest decrease. Group XIII, on 5% palmitate, showed a higher percentage and amount of sterol esters than the fat-free group (XII).

Triglycerides showed no change in percentage or amount in the oleate-fed group (XIV), were decreased ($p < 0.05$) in the linoleate-fed group (XV), and showed a possible increase in the fat-free and palmitate groups ($p < 0.2$) as compared to group X (CNO + chol., 8 weeks).

The amount of free sterols was less in the linoleate-fed group (XV) than in group X.

Phospholipids of groups XII (fat-free) and XIII (palmitate) were similar to group X, whereas groups XIV (oleate) and XV (linoleate) showed increases in percentages.

TABLE 4. FATTY ACID COMPOSITION OF LIVER PHOSPHOLIPIDS*

Diet→	CNO + Chol., 14 Weeks	CNO + Cholesterol, 14 Weeks: + Fat-Free, 10 Weeks	+ CSO, 10 Weeks	+100 mg/d Oleate, 10 Weeks	+100 mg/d Linoleate, 10 Weeks
Group No.→	III	V	VI	VII	VIII
Fatty Acid					
14:0	0.7 ± 0.0	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1
16:0	19.8 ± 1.8	16.7 ± 0.2	17.1 ± 1.0	18.4 ± 0.8	18.4 ± 1.2
16:1	8.3 ± 3.7	6.2 ± 0.2	Tr.	5.6 ± 0.4	5.8 ± 0.6
18:0	22.7 ± 5.4	21.4 ± 0.3	29.1 ± 1.9	21.7 ± 1.1	23.4 ± 2.8
18:1	21.4 ± 6.6	20.9 ± 1.1	5.5 ± 0.6	20.7 ± 1.4	18.4 ± 0.8
18:2	6.3 ± 1.6	2.7 ± 0.2	14.4 ± 0.9	2.3 ± 0.4	3.3 ± 0.3
18:2(?)	Tr.§	1.2 ± 0.2	Tr.	0.8 ± 0.2	0.8 ± 0.5
20:1	0.5 ± 0.2	0.7 ± 0.3	Tr.	Tr.	0.7 ± 0.3
20:3†	7.5 ± 1.5	22.0 ± 2.2	...	21.0 ± 1.1	9.6 ± 0.8
20:3‡	...	...	1.2 ± 0.4	...	S.i.‖
20:4	13.0 ± 3.0	7.3 ± 1.0	26.6 ± 2.3	8.8 ± 1.7	17.2 ± 1.0
22:4	...	...	1.4 ± 0.1	...	...
22:5	Tr.	Tr.	4.7 ± 1.5	Tr.	2.5 ± 0.7
22:6	Tr.	Tr.	Tr.	...	Tr.

* Percentage of total fatty acids, including standard deviation.
† 5,8,11-eicosatrienoic acid.
‡ 8,11,14-eicosatrienoic acid.
§ Tr. = Trace (present, but too small to measure).
‖ S.i. = Separation inadequate (but present in >1% amounts).

The fatty acid composition of the sterol esters and phospholipids of several significant groups are shown in Tables 3 and 4.

Sterol Esters. Group VI (cottonseed oil) showed decreased percentages of palmitoleic and oleic and marked increases in linoleic and arachidonic acids when compared to group III (CNO + chol., 14 weeks). Arachidonic acid was also higher in group VIII (linoleate) than in group III.

The sterol esters of group XIII (5% palmitate) showed a significantly lower percentage of arachidonic acid than groups X (CNO + chol., 8 weeks) and XII (fat-free).

Triglycerides. Similar decreases in percentages of palmitoleic and oleic acids and increases in linoleic and arachidonic acids, as in the sterol esters, are seen in group VI as compared with group III; in addition, there is a decrease in 5,8,11-eicosatrienoic acid. Also notable is the appearance in group VI of small amounts of an octadecatrienoic acid and 8,11,14-eicosatrienoic acid. (The eicosatrienoic-acid isomers were identified by comparison of their gas chromatographic retention times with those of known standards.)

Phospholipids. As in the previous fractions, group VI shows lower percentages of the monoenoic acids and higher linoleic and arachidonic acids than group III. The 5,8,11-eicosatrienoic acid seen in group III is not present in group VI. In addition, group VI has 8,11,14-eicosatrienoic acid and higher docosatetraenoic and docosapentaenoic acids than group III. Both linoleate-supplemented groups, VIII and IX, show increased percentages of linoleic acid; similar 5,8,11-eicosatrienoic acid; and increased 8,11,14-eicosatrienoic, arachidonic, and docosapentaenoic acids as compared with group III. An octadecadienoic acid other than linoleic appeared in small amounts in most of the groups and seemed to be highest in the group maintained on coconut oil and cholesterol for the full 24 weeks (IV).

DISCUSSION

It is evident from the histological and chemical results that cottonseed oil produced a marked regression of the hepatic sterol ester and triglyceride deposits that resulted from dietary coconut oil plus cholesterol. In a previous study (14), the ability of various oils, including cottonseed oil, to remove cholesterol from the liver was found to vary considerably, although all oils had a high linoleate content. When the componenents of cottonseed oil (i.e., palmitate, oleate, and linoleate) were fed in 5% amounts to rats previously fed the diet containing coconut oil plus cholesterol, only linoleate was found to accelerate lipid regression. Oleate seemed to have no effect, and palmitate an inhibiting effect, on the regression of hepatic sterol

ester deposits as compared to a fat-free diet. The fact that there was a lower percentage of arachidonic acid in the palmitate group (XIII) than either group X (CNO + chol., 8 weeks) or group XII (fat-free) suggests that during the 3 weeks on palmitate there may have occurred additional deposition of cholesterol as esters of saturated or monoenoic fatty acids, or that there was a transesterification of the latter fatty acids with the polyunsaturated cholesterol esters in the liver.

Supplementing a fat-free diet with 100 mg/day of linoleate appeared to accelerate lipid removal from the liver somewhat, but, evidently, the amount administered was insufficient for a maximum effect over the time period studied.

Some regression of sterol esters, but not triglycerides, occurred on the fat-free diet. It seems probable, therefore, that triglyceride deposition is more closely related to EFA deficiency than is sterol ester deposition.

Regression of lipid deposits in the liver but not in the arterial wall may be explained by a more rapid removal from the liver due to its greater vascularity. It is possible that a longer period of administration of linoleate would produce a regression of lipid deposits in the coronary arteries comparable to that in the liver.

It has been shown in previous investigations that EFA deficiency produces increases in monoenoic and 5,8,11-eicosatrienoic acids and decreases in linoleic and arachidonic acids in the liver (15, 16). These alterations are accentuated by dietary cholesterol (6). In the present study, this pattern, produced by a diet both deficient in essential fatty acids and containing excess cholesterol and saturated fat, was reversed in all the lipid fractions studied by the dietary administration of 5% or more linoleate.

The phospholipids of the groups receiving only 100 mg/animal/day of linoleate show decreased percentages of linoleate but increased percentages of arachidonate as compared to the group on coconut oil and cholesterol for 14 weeks. This suggests that the primary function of linoleate is in the synthesis of arachidonate and that linoleate will only accumulate in the liver when there is sufficient arachidonate incorporated into liver and serum phospholipids. The appearance in both linoleate-supplemented groups of 8,11,14-eicosatrienoic acid, which has been postulated as an intermediate in the formation of arachidonate from linoleate (17), provides further evidence for active arachidonate synthesis in these groups.

Phospholipids have been implicated in the transport of cholesterol and glycerides in the circulation. The lipotropic effect produced by choline administration has been attributed to the increased phospholipid formation it stimulates (18). In addition to choline, highly unsaturated fatty acids are a characteristic feature of some phospholipid molecules. It seems likely that dietary linoleate acts as a substrate in the synthesis of arachidonate, which, in turn, is incorporated into the structure of some phospholipids and thereby acts in lipid transport in a manner similar to choline.

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