The relationship between the linoleic acid content of the diet, the fatty acid composition of the plasma phospholipids and the degree of aortic atherosis in experimental rabbits

By J. H. MOORE and D. L. WILLIAMS

National Institute for Research in Dairying, Shinfield, Reading

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In a study of the relationship between diet, plasma lipid composition and aortic atherosis in experimental rabbits, Moore & Williams (1964*b*) found that irrespective of dietary treatment the degree of atheromatous degeneration was associated with elevated plasma cholesterol levels only when the levels of phospholipid in the plasma were insufficient for the molar ratio of phospholipid:free cholesterol to attain a value above unity. These observations suggested that the effect of diet on plasma phospholipid levels might play an essential part in determining the association between diet and cardiovascular degeneration in experimental animals. Since linoleic acid is an important component of the major phospholipids that are present in animal tissues (Hanahan, 1960), it is conceivable that, under certain circumstances, the level of linoleic acid in the diet may regulate to some extent the rate of phospholipid synthesis in the liver and hence the level of phospholipid circulating in the plasma. Thus, it is feasible that the relatively low levels of plasma phospholipid observed by Moore & Williams (1964*b*) in groups of rabbits given a diet containing only 0·4% maize oil may have been due to the limited amount of linoleic acid that was contained in this diet. It seemed possible that any regulatory effect of the level of linoleic acid in the diet on the level of phospholipid in the plasma might be reflected in the fatty acid composition of the plasma phospholipids. To investigate this possibility a detailed study was made of the fatty acid compositions of the phospholipid fractions isolated from the plasma samples obtained from the rabbits of Expt 2 described by Moore & Williams (1964*a*, *b*). The results of this investigation are now reported, together with an examination of the relationships between the fatty acid composition of the plasma phospholipids, the level of phospholipid and the phospholipid:cholesterol ratio in the plasma and the degree of atheromatous degeneration in the aortas of the experimental rabbits.

EXPERIMENTAL

Rabbits, diets and experimental procedure. The experimental procedures have been described in detail previously (Moore & Williams, 1964*a*, *b*) and therefore will be summarized only briefly now. Eighty-four male New Zealand White × Lop Ear rabbits, 4 months of age at the beginning of the experiment, were divided into six groups of fourteen each. The rabbits were housed in individual cages and were given

food and water *ad lib.* To 80 parts of a semi-purified, low-fat basal diet (Moore & Williams, 1964*a*) were added: for group 1, 20 parts maize oil; for group 2, 20 parts butterfat; for group 3, 10 parts maize oil and 10 parts butterfat; for group 4, 0·47 parts maize oil and 43·1 parts wheat starch; and for group 5, 10·2 parts maize oil and 21·6 parts wheat starch. The diets given to the rabbits of groups 2 and 4 contained equal amounts of linoleic acid per kcal gross energy; so did the diets given to the rabbits of groups 3 and 5 (Moore & Williams, 1964*a*). The animals in group 6 were given an ordinary commercial rabbit diet. The various diets were given to the six groups of rabbits for a period of 38 weeks, after which a sample of blood was taken from the marginal ear vein of each rabbit. The animals were then killed and the aortas were removed and fixed in 4% (w/v) formaldehyde in 0·9% (w/v) sodium chloride saturated with calcium carbonate. The aortas were stained with Sudan IV (0·05%, w/v, in ethanol–acetone–water, 35:35:30, v/v/v), and the degree of atheromatous degeneration of the intimal surface was assessed by the technique described by Moore & Williams (1964*a*).

Analytical procedure. The total fatty acid content of the various diets was determined by the method reported by Moore & Williams (1963*a*). A full account of the methods used for the extraction of the lipids from the plasma and for the chromatographic fractionation and analysis of the plasma lipids has been given by Moore & Doran (1962) and Moore & Williams (1964*b*). The composition of the fatty acids present in the various diets and in the plasma phospholipids was determined by gas–liquid chromatography (Moore & Williams, 1963*a*, 1964*c*).

Statistical analysis. From a preliminary examination of the results for the fatty acids of the plasma phospholipids it was evident that the standard deviation between animals within a diet group tended to be proportional to the diet group mean. Consequently all observational values were transformed to logarithms in order to reduce heterogeneity among the within-group variances. The tests of significance summarized in Tables 2 and 5 refer to differences between mean logarithmic values of each diet group. These mean logarithmic values are presented in Tables 2 and 5 by their antilogarithms which are the geometric means for each diet. Arithmetic means of the untransformed values are also given; they agree roughly with the geometric means which are invariably smaller. The tests of significance are based on the multiple-range test (Duncan, 1955) as modified for unequal numbers of replications (Kramer, 1956).

RESULTS AND DISCUSSION

Effect of dietary treatment on the fatty acid composition of the plasma phospholipids. The fatty acid compositions of the diets given to the six groups of rabbits are shown in Table 1 in which (and elsewhere in this paper) the shorthand designation of Farquhar, Insull, Rosen, Stoffel & Ahrens (1959) is used to denote the various fatty acids. As regards the compositions of the diets containing butterfat, it must be remembered that the short-chain fatty acids (from butyric to capric) are not determined by the particular technique of gas chromatography that was used in this study. The compositions of the major fatty acids present in the plasma phospholipids are given in Table 2. The fatty acids listed there account for about 96% of the total fatty acids measured.

Table 1. *Fatty acid contents (g/100 g) of the various diets given to the experimental rabbits*

Acid	Group 1 (20% maize-oil diet)	Group 2 (20% butter-fat diet)	Group 3 (10% maize-oil, 10% butterfat diet)	Group 4 (low-fat, high-starch diet)	Group 5 (10% maize-oil, starch diet)	Group 6 (commercial diet)
12:0	0·027	0·483	0·255	0·022	0·024	Trace
14:0	0·086	2·91	1·50	0·070	0·077	0·017
16:0	2·00	5·61	3·80	0·137	0·970	0·510
16:1	0·058	0·390	0·224	0·009	0·030	0·033
18:0	0·267	1·39	0·829	0·022	0·132	0·063
18:1	4·64	3·48	4·06	0·121	2·15	0·781
18:2	10·6	0·266	5·44	0·213	4·87	1·85

The remaining 4% consisted of small amounts of 12:0, 14:0, 15:0, 15:0 br, 17:0, 17:0 br, 18:3, 20:1, 20:2, 20:3, 22:5 and 22:6. Although the diets given to the rabbits of groups 2 and 3 contained appreciable quantities of lauric and myristic acids, neither of these acids appeared in more than trace amounts in the plasma phospholipids. This observation supports the results of Moore & Williams (1963*a*) who found that, even when hydrogenated coconut oil (containing 41% of 12:0 and 23% of 14:0) was included in the diet of rabbits for a period of 32 weeks, lauric and myristic acids remained as minor components of the liver phospholipids. Similar findings on the effects of dietary coconut oil on the lauric and myristic acid contents of the erythrocyte phospholipids of experimental rabbits have been reported by Mulder, De Gier & Van Deenen (1963). The effects of dietary treatment on the two main saturated acids (16:0 and 18:0) of the plasma phospholipids were not particularly striking, but it should be noted that the concentration of palmitic acid in the plasma phospholipids of the rabbits given the diets containing high levels of linoleic acid (groups 1, 3 and 5) were significantly lower than in the plasma phospholipids of the rabbits given the diets containing low levels (groups 2, 4 and 6). The plasma phospholipids of the rabbits in groups 2 and 4 (given the diets with the lowest levels of linoleic acid) tended to contain higher concentrations of the two major monoenoic acids (16:1 and 18:1) than did the plasma phospholipids of the other four groups of animals. Within certain limits, the linoleic acid content of the diet had a pronounced effect on the concentration of linoleic acid in the plasma phospholipids. Thus, the levels of linoleic acid in the plasma phospholipids of the rabbits in groups 2 and 4 (given diets containing 0·27 and 0·21% linoleic acid respectively) were considerably less than in the plasma phospholipids of the rabbits of groups 1, 3 and 5. However, there were no significant differences between the concentrations of linoleic acid in the plasma phospholipids of the rabbits in groups 1, 3 and 5 (given diets containing 10·6, 5·4 and 4·9% linoleic acid respectively). Similarly, the level of linoleic acid in the plasma phospholipids of the rabbits in group 6 (given a diet containing 1·9% linoleic acid) was not significantly less than the corresponding values observed for the rabbits of groups 3 and 5. In view of the fact that linoleic acid is an essential precursor in the biosynthesis of arachidonic acid in animal tissues, the observed effects of diet on the concentrations of arachidonic

Table 2. *Fatty acid composition (molar percentage of the total) of the phospholipids in the plasma of the rabbits on the various dietary treatments. Ranking of means and significance of differences by the multiple-range test (Duncan, 1955; Kramer, 1956). The mean values differ significantly (P = 0·01) except when they share a common underlining*

							Average standard error of the mean (66 df), log units
16:0							
Group no.	4	2	6	5	3	1	
Arithmetic mean	26·7	25·8	25·6	22·1	21·0	20·9	
Geometric mean	26·4	25·7	25·3	21·8	20·9	20·8	
Mean log	1·42	1·41	1·40	1·34	1·32	1·32	±0·016
16:1							
Group no.	4	2	6	3	5	1	
Arithmetic mean	1·5	0·8	0·6	0·6	0·5	0·4	
Geometric mean	1·4	0·8	0·6	0·6	0·4	0·3	
Mean log	0·14	−0·10	−0·22	−0·25	−0·35	−0·49	±0·058
18:0							
Group no.	6	3	1	5	2	4	
Arithmetic mean	21·0	20·4	20·4	20·1	18·3	17·2	
Geometric mean	20·8	20·4	20·3	20·0	18·2	17·0	
Mean log	1·32	1·31	1·31	1·30	1·26	1·23	±0·016
18:1							
Group no.	2	4	3	5	1	6	
Arithmetic mean	25·9	23·9	18·9	16·8	15·1	12·8	
Geometric mean	25·2	23·6	18·5	16·6	14·8	12·4	
Mean log	1·40	1·37	1·27	1·22	1·17	1·09	±0·027
18:2							
Group no.	1	5	3	6	2	4	
Arithmetic mean	37·9	34·9	33·6	29·3	22·2	21·5	
Geometric mean	37·6	34·0	32·8	29·0	21·4	21·2	
Mean log	1·58	1·53	1·52	1·46	1·33	1·33	±0·026
20:4							
Group no.	6	4	2	5	3	1	
Arithmetic mean	4·2	3·5	3·3	2·8	2·5	2·1	
Geometric mean	4·1	3·4	3·2	2·8	2·5	2·0	
Mean log	0·61	0·53	0·51	0·44	0·39	0·30	±0·032

Group 1, 20% maize-oil diet; group 2, 20% butterfat diet; group 3, 10% maize-oil, 10% butterfat diet; group 4, low-fat, high-starch diet; group 5, 10% maize-oil, starch diet; group 6, commerical diet.

Certain of the animals died during the experiment. The numbers of animals at the end of the experiment were: group 1, 12; group 2, 13; group 3, 13; group 4, 12; group 5, 10; group 6, 12.

acid in the plasma were somewhat unexpected. It seemed rather anomalous to find that the concentration of arachidonic acid in the plasma phospholipids of the rabbits given the high-starch diet (group 4) was significantly greater than in the plasma phospholipids of the rabbits given the diet containing 20% maize oil (group 1). It should be noted that the concentrations of the C_{20} and C_{22} polyunsaturated acids in

the plasma phospholipids of the rabbits were consistently lower than the concentrations of these acids that have been reported for the plasma phospholipids of man (e.g. Nelson, 1962) and various experimental animals (e.g. Guidry, Geer & Robertson, 1964; Privett & Blank, 1964).

Interrelationships between the various fatty acids present in the plasma phospholipids. Lecithin accounted for more than 70% of the total phospholipids in the plasma of the rabbits on all six dietary treatments (Moore & Williams, unpublished observations). It has been established that the lecithin obtained from most natural sources consists predominantly of molecules with saturated fatty acids occupying the α-position and unsaturated acids occupying the β-position (Hanahan, 1960; Hanahan, Brockerhoff & Barron, 1960; Moore & Williams, 1963*b*, 1964*c*). It might therefore be expected that the plasma phospholipids of the experimental rabbits would contain approximately equimolar concentrations of saturated and unsaturated fatty acids. In fact, only in the rabbits of group 6 (given the commercial diet) was a value of 1·0 obtained for the saturated:unsaturated fatty acid ratio in the plasma phospholipids. As may be seen from Table 2, there was a slight excess of unsaturated acids over saturated acids in the plasma phospholipids of the rabbits in groups 1–5. In view of the positional distribution of saturated and unsaturated fatty acids in the lecithin molecule, it follows that any changes in the concentrations of the individual unsaturated fatty acids of lecithin will be confined generally to those acids situated in the β-position and that changes in the concentrations of the individual saturated acids will be confined generally to those acids situated in the α-position. Thus, if only the major fatty acids of the plasma phospholipids are considered, an increase in the concentration of linoleic acid should coincide with a decrease in the concentration of oleic acid, and an increase in the concentration of palmitic acid should coincide with a decrease in the concentration of stearic acid. Examination of the concentrations of the four major fatty acids present in the plasma phospholipids of the individual rabbits on all six dietary treatments revealed highly significant inverse rectilinear relationships between the concentrations of stearic and palmitic acids and between the concentrations of oleic and linoleic acids (Table 3). The nature of these two relationships is consistent with the results obtained by Hanahan & Blomstrand (1956) from experiments in which various ^{14}C-labelled fatty acids were administered to rats. Saturated ^{14}C-labelled fatty acids were found to exchange with the fatty acids present in the α-position of the liver lecithin, whereas unsaturated ^{14}C-labelled fatty acids were found to exchange with the fatty acids present in the β-position. The relationship found between the concentrations of oleic and linoleic acids (Table 3) lends support to the observations of Mulder *et al.* (1963) who noted that the replacement of coconut oil by maize oil in the diet of rabbits resulted in a marked increase in the concentration of linoleic acid and a corresponding decrease in the concentration of oleic acid in the β-position of the phosphatidyl choline and phosphatidyl ethanolamine isolated from the erythrocytes. However, there appeared to be a limit to the extent to which linoleic acid could replace oleic acid in the plasma phospholipids of the rabbit. The results given in Table 2 clearly show that an increase in the level of linoleic acid in the diet from 4·9 to 10·6% did not result either in a significant increase in the concentration of

Table 3. *Relationships between the major fatty acids present in the plasma phospholipids of the experimental rabbits*

Comparison		Regression equation	Standard error of slope	Correlation coefficient
y	*x*			
18:0	16:0	$y = 27{\cdot}19 - 0{\cdot}359x^{***}$	$\pm 0{\cdot}0855$	$-0{\cdot}448^{***}$
18:1	18:2	$y = 34{\cdot}83 - 0{\cdot}531x^{***}$	$\pm 0{\cdot}0610$	$-0{\cdot}723^{***}$
18:0	18:2	$y = 15{\cdot}48 + 0{\cdot}132x^{**}$	$\pm 0{\cdot}0411$	$0{\cdot}359^{**}$
16:0	18:1	$y = 19{\cdot}39 + 0{\cdot}228x^{**}$	$\pm 0{\cdot}0670$	$0{\cdot}364^{**}$
16:0	18:2	$y = 33{\cdot}86 - 0{\cdot}340x^{***}$	$\pm 0{\cdot}0371$	$-0{\cdot}739^{***}$
18:0	18:1	$y = 25{\cdot}20 - 0{\cdot}304x^{***}$	$\pm 0{\cdot}0477$	$-0{\cdot}607^{***}$

** $0{\cdot}01 > P > 0{\cdot}001$. *** $P < 0{\cdot}001$.

linoleic acid or in a significant decrease in the concentration of oleic acid in the plasma phospholipids.

Further comparison of the concentrations of the four major fatty acids present in the plasma phospholipids of the individual rabbits disclosed significant positive correlations between the concentrations of palmitic and oleic acids and between the concentrations of stearic and linoleic acids (Table 3). These relationships, although obtained for the total plasma phospholipids, suggest that lecithin molecules with palmitic acid in the α-position tended to contain oleic acid in the β-position and that those molecules with stearic acid in the α-position tended to contain linoleic acid in the β-position. Little is known about the possible association that may exist between the acids present in the α- and β-positions of lecithin for the simple reason that as yet there are no methods available for the separation of different molecular species of lecithin. The important stage in the biosynthesis of lecithin that determines its characteristic fatty acid composition is the stepwise acylation of α-glycerophosphate to form phosphatidic acid (Kennedy, 1957, 1961; Rossiter & Strickland, 1960). Although there is at present no information on the order in which the two hydroxyl groups of α-glycerophosphate are acylated, it is tempting to suggest that the identity of the first fatty acids to be incorporated into the molecule may influence to some extent the identity of the second fatty acid molecule that eventually becomes esterified with the remaining hydroxyl group. The findings of this investigation may be explained if, for example, the presence of linoleic acid in the β-position encouraged the introduction of stearic acid or, alternatively, inhibited the introduction of palmitic acid into the vacant α-position. On the other hand, the saturated fatty acid in the α-position might be the determining one. However, such an explanation must remain entirely speculative until the detailed mechanism of the acylation of α-glycerophosphate has been elucidated.

Relationships between the fatty acid composition of the plasma phospholipids, the level of phospholipid in the plasma and the degree of aortic atherosis. The degrees of aortic atherosis and the compositions of the plasma lipids in the six groups of experimental rabbits have been described in detail by Moore & Williams (1964*a*, *b*). However, for convenience in the present discussion the essential findings of these two previous reports are summarized in Table 4. It may be seen that extensive degeneration of the aorta occurred only in the rabbits of groups 2 and 4 in which the concentration of

Table 4. *Degrees of atherosis in the aorta* and concentrations of total cholesterol and phospholipid in the plasma of the rabbits on the various dietary treatments*

(Mean values with their standard errors)

	Group 1 (20% maize-oil diet)	Group 2 (20% butterfat diet)	Group 3 (10% maize-oil, 10% butterfat diet)	Group 4 (low-fat, high-starch diet)	Group 5 (10% maize-oil, starch diet)	Group 6 (commercial diet)
Degree of atherosis	1·61 ± 0·8	18·9 ± 5·3	2·0 ± 0·6	22·9 ± 7·5	1·32 ± 0·6	1·65 ± 0·46
Total cholesterol (mg/100 ml plasma)	44·9 ± 6·7	151 ± 8·5	83·8 ± 4·2	85·4 ± 6·2	55·7 ± 3·9	50·5 ± 5·0
Phospholipid (mg/100 ml plasma)	83·2 ± 7·9	163 ± 9·0	132 ± 5·5	72·1 ± 5·2	71·9 ± 4·5	67·3 ± 4·2

* Arbitrary scale (see Moore & Williams, 1964*a*).

phospholipid in the plasma was approximately equal to or slightly less than the concentration of cholesterol. Only slight aortic atherosis occurred in the rabbits of the other four groups in which the concentration of phospholipid in the plasma was greater than the concentration of cholesterol. In fact, the degree of aortic atherosis appeared to be related to the phospholipid:free cholesterol ratio in the plasma (Moore & Williams, 1964*b*). A particularly interesting comparison is that between group 3 (slight atherosis) and group 4 (extensive atherosis). The plasma cholesterol levels in these two groups of rabbits were very similar but the level of phospholipid in the plasma of the rabbits in group 3 was almost twice the corresponding level in the plasma of the rabbits of group 4. These findings seem to imply that when rabbits are given a hypercholesterolaemic diet, there is a tendency for the level of phospholipid in the plasma to increase, possibly in an effort on the part of the animal to maintain a stable lipoprotein system in the blood. Any increase in the level of phospholipid in the plasma will presumably be due to an increase in the synthesis of phospholipid in the liver. Nevertheless, this can occur only if the diet contains sufficient quantities of certain essential precursors, e.g. linoleic acid, that may be necessary to answer the requirements for the higher rate of phospholipid synthesis. In this respect it should be noted that Mead & Fillerup (1957) found that, when ^{14}C-labelled stearate, oleate and linoleate were administered orally to rats, the labelled stearate and oleate were rapidly incorporated into the plasma triglycerides but the labelled linoleate was rapidly incorporated into the plasma phospholipids. Thus, the levels of linoleic acid in the hypercholesterolaemic diets given to the rabbits of groups 2 and 4 may not have been sufficient to support rates of phospholipid synthesis in the liver that would have been necessary to ensure that the molar ratio, phospholipid:free cholesterol in the plasma did not fall below 1·0 or thereabouts (Moore & Williams, 1964*b*). It was of interest therefore to find that the fatty acid compositions of the plasma phospholipids in the two groups of rabbits with extensive atheromatous degeneration of the aorta were characteristically different from those of the plasma phospholipids in the four groups of rabbits that were relatively free from aortic atherosis (Table 2). This difference is most evident when the linoleic:oleic acid ratios of the plasma phospholipids of the various groups of rabbits are compared. These values are given in Table 5 from which it is clear that the

linoleic:oleic acid ratios in the plasma phospholipids of the rabbits in groups 1, 3, 5 and 6 (slight atherosis and high phospholipid:cholesterol ratios in the plasma) were about two to three times greater than the corresponding ratios in the plasma phospholipids of the rabbits in groups 2 and 4. The palmitic:stearic acid ratios (also given in Table 5) in the plasma phospholipids of the rabbits in groups 2 and 4 were greater, but only at the 5% level of significance, than the corresponding ratios in the plasma phospholipids of the rabbits in the remaining four groups. However, since palmitic and stearic acids may arise by endogenous synthesis, it is unlikely that the nature of the diet can have any appreciable direct effect on the proportions of stearic and palmitic acids in the plasma phospholipids. As pointed out previously (p. 608), it is likely that the ratio of these two saturated acids is determined indirectly by the effect of the linoleic acid content of the diet on the ratio of the two major unsaturated acids in the plasma phospholipids.

Table 5. *Linoleic acid:oleic acid and palmitic acid:stearic acid ratios in the phospholipids of the plasma of the rabbits on the various dietary treatments. Ranking of means and significance of differences by the multiple-range test (Duncan, 1955; Kramer, 1956). The mean values differ significantly except when they share a common underlining)*

	Linoleic acid:oleic acid ratio						Average standard error of the mean (66 df), log units
Group no.	1	6	5	3	4	2	
Arithmetic mean	2·64	2·46	2·15	1·92	0·98	0·94	
Geometric mean	2·54	2·35	2·05	1·78	0·89	0·85	
Mean log	0·41	0·37	0·31	0·25	−0·045	−0·071	±0·049
							0·01 > *P* > 0·001
	Palmitic acid:stearic acid ratio						
Group no.	4	2	6	5	3	1	
Arithmetic mean	1·60	1·61	1·26	1·12	1·04	1·03	
Geometric mean	1·55	1·51	1·22	1·09	1·03	1·02	
Mean log	0·19	0·18	0·086	0·038	0·011	0·010	±0·031
							0·01 > *P* > 0·001
							0·05 > *P* > 0·01

In conclusion, it seems possible that the protective action of high levels of dietary linoleic acid against aortic atherosis in the rabbit may be due to two quite separate effects on the concentrations of blood lipids. Firstly, the results obtained with the rabbits of groups 3 and 4 suggest that high levels of linoleic acid in the diet facilitate the maintenance of elevated levels of plasma phospholipid under circumstances in which the plasma also contains elevated levels of cholesterol. Secondly, as is well illustrated by the results obtained with the rabbits of groups 1 and 5, diets rich in linoleic acid possess pronounced hypocholesterolaemic properties. The overall result of these two effects is that the phospholipid:free cholesterol ratio in the plasma remains high and, possibly owing to the presence of a relatively stable lipoprotein complex in the blood, little or no atheromatous degeneration occurs in the aorta and coronary arteries. The possible mechanism of the hypocholesterolaemic effect of diets rich in linoleic acid will be discussed in a communication to be submitted later.

SUMMARY

1. Groups of male rabbits (ten to thirteen per group) were given *ad lib.* a diet consisting of 80 parts of a low-fat basal ration to which were added for group 1, 20 parts maize oil; for group 2, 20 parts butterfat; for group 3, 10 parts maize oil and 10 parts butterfat; for group 4, 0·47 parts maize oil and 43 parts wheat starch; and for group 5, 10·2 parts maize oil and 21·6 parts wheat starch. The animals in group 6 were given an ordinary commercial diet.

2. After the animals had been given the various diets for a period of 38 weeks a large sample of blood was taken from each animal. The animals were then killed and the degree of atheromatous degeneration was determined after the aortas had been stained with Sudan IV. The fatty acid composition of the plasma phospholipids was determined by gas–liquid chromatography.

3. In all the groups, the major fatty acids present in the plasma phospholipids were stearic, palmitic, oleic and linoleic. Although linoleic acid tended to replace oleic acid in the plasma phospholipids when the rabbits were given high levels of linoleic acid in the diet, there appeared to be a limit to the extent to which the concentration of linoleic acid in the plasma phospholipids increased. There were highly significant inverse relationships between the concentrations of oleic and linoleic acids and between the concentrations of palmitic and stearic acids in the plasma phospholipids.

4. There were significant positive correlations between the concentrations of palmitic and oleic acids and between the concentrations of stearic and linoleic acids. Since lecithin was the major component of the plasma phospholipids these correlations suggest that when the β-position of lecithin is occupied by oleic acid the α-position tends to be occupied by palmitic acid and that when the β-position is occupied by linoleic acid the α-position tends to be occupied by stearic acid.

5. In the plasma phospholipids of the two groups of rabbits with extensive atheromatous degeneration of the aorta, the linoleic:oleic acid ratio in the plasma phospholipids was considerably greater than the corresponding ratio observed for the plasma phospholipids of the remaining four groups of rabbits in which little or no aortic atherosis occurred.

6. The relationships between the level of linoleic acid in the diet, the level of phospholipid and the phospholipid:cholesterol ratio in the plasma and the extent of atheromatous degeneration of the aorta are discussed. It is suggested that, under certain circumstances, the level of linoleic acid in the diet may limit the rate of phospholipid synthesis in the liver and hence the level of phospholipid circulating in the plasma.

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