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The Influence of Ethyl Linoleate and Ethyl Linolenate on Polyenoic Fatty Acids and Cholesterol in Tissues of Chicks

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With 3 tables

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In the animal body, linoleic and linolenic acids behave differently in several respects.

Both of these compounds can sustain growth in young rats, whereas it is reported that linolenic acid does not prevent skin symptoms of essential fatty acid deficiency, such as linoleic acid does (1).

Linolenic acid is more efficient than linoleic in lowering plasma cholesterol in hypercholesterolemic rats (2, 3). In vitamin E deficient chicks both of these fatty acids accelerate exudative diathesis, but as far as it is known linolenic acid does not cause encephalomalacia (4). It is therefore of interest to study the pattern of polyenoic fatty acids in organs of animals fed these fatty acids.

The investigations reported here are the first to be published of a series of studies carried out with diets similar to those used in certain of our studies on encephalomalacia in chicks.

In the present study, vitamin E has been added to the diet in order to secure survival of the animals for an equal length of time. It is not expected, however, that the presence or absence of vitamin E is of great importance for the polyenoic acid pattern in tissues. Unpublished studies from our laboratory with chicks fed lard showed essentially the same pattern of polyenoic fatty acids in the brain whether vitamin E was given or not.

Experimental

The preparations of ethyl linoleate and ethyl linolenate used in this study were obtained from The Hormel Institute, Austin, Minnesota, U.S.A., in September 1957 and stored in sealed ampoules at room temperature. According to the producers, the content in the linolenate of substances having trans-double bonds was approximately 15% at the time the preparation was made.

Analysis by alkali isomerization showed the following composition with respect to conjugable polyenoic acids (Sept. 1958). Linoleate: 90.3% dienoic (theory, 91.0%); I. V. 161 (theory, 164). Linolenate: 0.8% dienoic, 85.3% trienoic (theory, 90,9%); I. V. 219 (theory, 248).

From the absorption at 965 cm^{-1} in CS_2 the contents of trans-isomers (calculated as elaidic acid) were found to be as follows (July 1958):

Linoleate: about 2% trans.

Linolenate: about 50% trans.

The feeding experiment described here was carried out in September-October 1958.

Thirty-six chicks which had received a "normal" commercial starter ration (5)[1]) the first two weeks after hatching were distributed into three groups (groups nos. 2109,2110, and 2111) with 12 chicks in each and fed the experimental diets shown in table 1.

Table 1. Composition of diets (g/100 g)

Group no.	2109	2110	2111
FLEISCHMANN yeast 50 B[1])	40.00	40.00	40.00
Gelatine	3.00	3.00	3.00
Salt mixture[2])	5.17	5.17	5.17
Vitamin mixture[2])	0.10	0.10	0.10
Choline chloride	0.20	0.20	0.20
Sucrose	51.53	50.03	50.03
Ethyl linoleate	—	1.50	—
Ethyl linolenate	—	—	1.50

One mg dicalcium salt of 2-methyl-1,4-naphthohydroquinone diphosphoric acid ester ("Synkavit", Roche) and 10 mg d,l-α-tocopheryl acetate ("Ephynal", Roche) were added per 100 g of each diet. Vitamins A and D_3 were given in aqueous solution (5), 0.1 ml twice a week orally to each animal, corresponding to 250 I. U. vitamin A and 20 I. U. vitamin D_3 per day.

[1]) The content of total fatty acids in this yeast was 3.8%. Polyenoic fatty acids represented 0.8% of the total fatty acids, corresponding to 0.03% of the yeast.

[2]) DAM and SØNDERGAARD (15).

After 4 weeks on the experimental diets the chicks were fasted for 20 hours and then sacrificed by decapitation. Immediately before sacrifice blood was withdrawn from the jugular vein for determination of plasma cholesterol. Liver, heart, brain, aorta and depot fat were taken out for determination of fatty acids and cholesterol. The experimental methods are the same as described earlier (6, 7, 8).

Results and Discussion

Polyenoic fatty acids (table 2).

It should be borne in mind that the content of trans-isomers in the linolenate may have influenced the results to some extent. It is not likely, however, that the main differences between the patterns of polyenoic acids obtained with linoleate and linolenate are due to the presence of trans acids in the latter. The polyenoic acid patterns obtained with ethyl linolenate in the present experiment resemble in many respects those obtained in earlier experiments with a linseed oil containing 11.7% of dienoic and 50.6% of trienoic acids (9).

Liver. The low-fat diet (which contained 1.52% total fatty acids and 0.012% total polyenoic fatty acids) caused deposition of trienoic acid, although to a lesser extent than found in earlier experiments with a completely fat-free diet (7).

[1]) The commercial starter ration used in this experiment differs from the artificial starter ration used in certain of our experiments on encephalomalacia. The lenght of the period during which the starter ration was given is also different.

It is not believed that these differences detract from the significance of the results.

Supplementation with 1.5% ethyl linoleate gave rise to deposition of large amounts of dienoic and tetraenoic acids. Some pentaenoic acid was also deposited as a consequence of linoleate feeding, but hexaenoic remained largely at a low level as in the low-fat group.

Table 2. Polyenoic fatty acids in tissues and depot fat of chicks

Group no. Diet characteristics		2109 low-fat		2110 1.5% ethyl linoleate		2111 1.5% ethyl linolenate	
		% of total fatty acids	mg/100 g tissue	% of total fatty acids	mg/100 g tissue	% of total fatty acids	mg/100 g tissue
Liver	Dienoic[1])	5.8	223	19.2	455	3.6	112
	Trienoic	5.4	191	—0.2	—6	6.5	202
	Tetraenoic	4.8	176	21.0	496	2.6	81
	Pentaenoic	0.9	36	3.0	71	16.2	451
	Hexaenoic	0.9	29	1.4	34	4.9	139
	Total polyenoic	17.8	655	44.4	1050	33.8	985
Heart	Dienoic[1])	7.6	238	19.4	408	6.4	157
	Trienoic	3.9	122	—0.1	0	7.2	177
	Tetraenoic	5.9	184	15.7	332	6.5	159
	Pentaenoic	0.5	15	1.2	24	6.7	162
	Hexaenoic	0.3	9	0.3	6	1.0	25
	Total polyenoic	18.2	568	36.5	770	27.8	680
Brain	Dienoic[1])	1.5	29	1.2	26	0.9	16
	Trienoic	5.3	105	2.0	39	3.0	57
	Tetraenoic	8.4	165	13.2	260	8.6	162
	Pentaenoic	2.5	49	3.8	76	3.1	58
	Hexaenoic	6.3	122	7.3	144	10.2	191
	Total polyenoic	24.0	470	27.5	545	25.8	484
Depot fat	Dienoic[1])	3.1	1507	18.3	5742	2.4	855
	Trienoic	0.5	239	0.8	251	9.1	3376
	Tetraenoic	0.2	113	0.7	202	0.6	212
	Pentaenoic	0.0	19	0.1	30	0.3	111
	Hexaenoic	0.1	63	0.1	49	0.2	71
	Total polyenoic	3.9	1941	20.0	6274	12.6	4625

[1]) Preformed conjugated + conjugatable dienoic.

Liver and depot fat were analysed individually; the figures given are averages of 10 to 12 determinations. Three hearts were pooled, and the figures are averages of 4 determinations. Two brains were pooled, and averages of 6 determinations are listed in the table.

Contrary to this finding, Reiser (10) has reported that dienoic acid preferably is converted into pentaenoic acid and, possibly, to small amounts of tetraenoic acid in the liver of chicks. The reason for this difference from our results is unknown.

The deposition of di- and tetraenoic acids after linoleate feeding in the liver of chicks agrees with the finding of WIDMER and HOLMAN (11) and STEINBERG et al. (12) for rats fed linoleate.

The most striking change in the liver produced by the diet with 1.5% ethyl linolenate is a large increase in liver pentaenoic acid and a moderate increase in hexaenoic acid. The contents of dienoic and tetraenoic acids (given as % of total fatty acids or as mg/100 g tissue) were lower than found with the low-fat diet. A large increase in liver pentaenoic acid and a more moderate increase in liver hexaenoic were also found to result from feeding of linseed oil to chicks (9), but not from the feeding of peanut oil (6).

Conversion of linolenate to pentaenoic and hexaenoic acids has been examined in pooled organs from rats by STEINBERG et al. (13). The investigators suggested that C_{20}-pentaenoic, C_{22}-pentaenoic and C_{22}-hexaenoic acids were formed from linolenic acid. A possible pathway for the conversion was indicated by MEAD (14).

Heart. Here the feeding of linolenate also led to accumulation of pentaenoic acid, although to a lesser degree than in liver. Linoleate feeding resulted in deposition of di- and tetraenoic acid as in liver.

An increase in trienoic acid occurred in liver and heart after linolenate feeding compared with linoleate feeding, probably due to deposition of linolenic acid. These results also resemble those found after feeding of linseed oil (9).

Brain. Dienoic acid was low in all three groups. The amount of trienoic acid was somewhat higher for the low-fat group than for the other groups.

The value for tetraenoic acid, which is relatively high in brain tissue, was increased in the group fed ethyl linoleate, but not in the group receiving linolenate. The amount of pentaenoic acid was relatively low in all three groups.

The brain content of hexaenoic acid is relatively high. Dietary linolenate markedly increased it, but with dietary linoleate there was no significant change. This finding also resembles that found after the feeding of linseed oil (9).

Thus, the main difference in brain polyenoic fatty acids between the groups fed linoleate and linolenate was the appearance of tetraenoic acid after linoleate feeding and of hexaenoic acid after linolenate feeding.

Depot fat. The composition of the depot fat with respect to polyenoic acids is usually more directly influenced by the dietary fat than is the composition of organ fats. This was also the case in the present experiment. Linoleate resulted in deposition of dienoic, and linolenate of trienoic acid in the depot fat.

It can be seen from Table 2 that the total amount of polyenoic acids in the organs examined was somewhat higher in the linoleate than in the linolenate group. This might be due to a more rapid catabolism of linolenic acid than of linoleic, to a less efficient absorption of linolenate, or to the presence of trans-isomers in the ingested linolenate.

Cholesterol (table 3).

The cholesterol contents of heart, brain, aorta and depot fat showed no significant differences between the groups.

In the plasma, 1.5% dietary ethyl linolenate caused significantly lower plasma cholesterol levels than 1.5% dietary ethyl linoleate. This corresponds to results found for hypercholesterolemic rats by other workers (e. g., 2, 3), and to unpublished experiments from our laboratory with hamsters.

The groups fed the low-fat diet and the diet with 1.5% ethyl linoleate had the same plasma cholesterol levels.

Liver cholesterol showed significant differences between all three groups, ethyl linoleate causing the lowest average cholesterol value and the low-fat diet the highest. Thus, the effect of ethyl linoleate on cholesterol in liver was different from the effect in plasma. The same was found for hypercholesterolemic rats by PEIFER et al. (3). A depression of liver cholesterol caused by feeding 1.5% ethyl linoleate has also been found in an earlier, unpublished experiment from this laboratory with chicks.

Table 3. Average cholesterol contents of plasma, liver, heart, brain, aorta and depot fat with mean standard errors. Numbers in parentheses indicate the numbers of animals in each group

Group no.	Diet characteristics	Plasma (mg/100 ml)	liver (mg/100 g)	Heart[1] (mg/100 g)	Brain[1] (mg/100 g)	Aorta[2] (mg/100 g)	Depot fat (mg/100 g)
2109	Low-fat	218 (11) ± 11	385 (11) ± 9	175 ± 7	1123 ± 86	550 (11) ± 35	105 (9) ± 12
2110	1.5% ethyl linoleate	212 (10) ± 4	301 (12) ± 8	184 ± 25	1201 ± 17	455 (12) ± 48	123 (10) ± 12
2111	1.5% ethyl linolenate	169 (12) ± 8	349 (12) ± 7	177 ± 4	1158 ± 38	557 (12) ± 27	94 (9) ± 16

[1]) Two to three organs were pooled for an analysis. [2]) Dry weight.

Summary

Polyenoic fatty acids in liver, heart, brain and depot fat, and cholesterol in plasma, liver, heart, brain, aorta and depot fat were determined in chicks fed a low-fat diet (the fat content of which was only that present in FLEISCHMANN yeast 50 B), and the low-fat diet supplemented with 1.5% ethyl linoleate and 1.5% ethyl linolenate, respectively, through 4 weeks.

Regarding *polyenoic fatty acids*, the most pronounced findings were the following:

In the group fed linoleate, dienoic acid was deposited in liver, heart and depot fat, tetraenoic acid was deposited in liver, heart and in brain.

In the linolenate group, trienoic acid was deposited in depot fat, pentaenoic acid in liver and heart and hexaenoic in liver and brain.

The total amount of polyenoic acids in the organs examined was higher in the linoleate group than in the linolenate group.

The *cholesterol* contents of heart, brain, aorta and depot fat showed no significant differences between the groups.

In plasma and in liver, ethyl linolenate had a depressing effect on the cholesterol content compared with the low-fat diet. Ethyl linoleate had no effect on plasma cholesterol, whereas it depressed liver cholesterol to a greater extent than linolenate.

Zusammenfassung

Drei Gruppen von je 10 Küken wurden 4 Wochen lang mit künstlichen, Vitamin E supplementierten Nahrungen gefüttert. In der ersten Gruppe erhielt die Nahrung keine Zulage, in der zweiten Gruppe wurde eine Zulage von 1,5% Äthyllinoleat und in der dritten eine Zulage von 1,5% Äthyllinolenat gegeben.

Polyenfettsäuren wurden in Leber, Herz, Hirn und Depotfett mit der Alkali-Isomerisierungs-Methode (8), Cholesterin in Plasma, Leber, Herz, Hirn und Depotfett mittels der LIEBERMANN-BURCHARD- und Aorta mittels der TSCHUGAEFF-Reaktion (7) bestimmt.

Die wichtigsten Befunde bezüglich der *Polyensäuren* waren:

In der Linoleat-Gruppe wurde Diensäure in Leber, Herz und Depotfett, Tetraensäure in Leber, Herz und Hirn abgelagert.

In der Linolenat-Gruppe wurde Triensäure in Depotfett, Pentaensäure in Leber und Herz, und Hexaensäure in Leber und Hirn abgelagert.

Die Gesamtmenge der Polyensäuren in den untersuchten Organen war höher in der Linoleat-Gruppe als in der Linolenat-Gruppe.

Die Befunde bezüglich *Cholesterin* waren:

Im Vergleich mit der Nahrung ohne Fettsäurezulage führte die Linolenat-Nahrung zu niedrigeren Cholesterin-Werten in Plasma und Leber. Linoleat zeigte keine Wirkung gegenüber dem Plasma-Cholesterin, drückte aber den Cholesteringehalt der Leber mehr herab als Linolenat.

References

1. BURR, G. O., Fed. Proc. **1**, 224 (1942). — 2. HAUGE, J. G., and R. NICOLAYSEN, Acta physiol. Scand. **45**, 26 (1959). — 3. PFEIFER, J. J., F. JANSSEN, P. AHN, W. COX and W. O. LUNDBERG, Arch. Biochem. Biophys. **86**, 302 (1960). — 4. DAM, H., G. KOFOED NIELSEN, I. PRANGE and E. SØNDERGAARD, Nature **182**, 802 (1958). — 5. DAM, H., S. HARTMANN, J. E. JACOBSEN and E. SØNDERGAARD, Acta physiol. Scand. **41**, 149 (1957). — 6. DAM, H., G. KRISTENSEN, G. KOFOED NIELSEN, I. PRANGE and E. SØNDERGAARD, Acta physiol. Scand. **36**, 319 (1956). — 7. DAM, H., G. KRISTENSEN, G. KOFOED NIELSEN and E. SØNDERGAARD, Acta physiol. Scand. **44**, 67 (1958). — 8. HAMMOND, E. G. and W. O. LUNDBERG, J. Amer. Oil Chem. Soc. **30**, 433 (1953). — 9. DAM, H., G. KRISTENSEN, G. KOFOED NIELSEN and E. SØNDERGAARD, Acta physiol. Scand. **45**, 31 (1959). — 10. REISER, R., J. Nutrition **42**, 325 (1950). — 11. WIDMER, C. and R. T. HOLMAN, Arch. Biochem. Biophys. **25**, 1 (1950). — 12. STEINBERG, G., W. H. SLATON, D. R. HOWTON and J. F. MEAD, J. Biol. Chem. **220**, 257 (1956). — 13. STEINBERG, G., W. H. SLATON, D. R. HOWTON and J. F. MEAD, J. Biol. Chem. **224**, 841 (1957). — 14. MEAD, J. F., Amer. J. Clin. Nutr. **8**, 55 (1960). — 15. DAM, H. and E. SØNDERGAARD, Z. Ernährungswiss. **2**, 217 (1962).

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