Polyenoic fatty acids and cholesterol in chicks given 9-*cis*-12-*cis* linoleate, 9-*trans*-12-*trans* linoleate or 9-*trans*-11-*trans* linoleate

By G. HØLMER, G. KRISTENSEN, E. SØNDERGAARD AND H. DAM

Danish Fat Research Institute and Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen

(*Received* 6 *December* 1961—*Revised* 16 *February* 1962)

We have previously studied the influence of dietary hydrogenated arachis oil on the deposition of polyenoic fatty acids and cholesterol in various tissues of chicks (Dam, Jart, Kristensen, Nielsen & Søndergaard, 1958) and found mainly the same pattern of polyenoic fatty acids as with a fat-free diet. However, the content of non-conjugated trienoic acid in liver and heart was somewhat lower than in the fat-free groups.

The cholesterol contents of the organs examined were not influenced by dietary hydrogenated arachis oil when the diet was free from cholesterol.

In the experiments reported here, which were carried out in connexion with some studies on vitamin E deficiency, we fed chicks on a 'low-fat' diet containing 1·5% 9-*cis*-12-*cis* ethyl linoleate, 1·5% 9-*trans*-11-*trans* ethyl linoleate, 1·5% 9-*trans*-12-*trans* ethyl linoleate, respectively, and the low-fat diet itself in order to study the fate of these acids in the tissues and their influence on cholesterol metabolism. The designation 'low-fat' was chosen instead of 'fat-free' because one of the ingredients, Fleischmann yeast 50B, contained 3·8% total fatty acids.

EXPERIMENTAL

Day-old New Hampshire × White Leghorn chicks were given a commercial chicken mash (Karat, Karensmølle A/S, Copenhagen) for 8 days. They were then distributed into four groups with eleven chicks in each and given the experimental diets for 40 days. The compositions of the diets are shown in Table 1.

Further, each chick received the daily equivalent of 250 i.u. vitamin A and 20 i.u. cholecalciferol as an ethanol–water solution stabilized with Tween 80 (polyoxyethylene sorbitan mono-oleate). This solution (0·1 ml) was given twice weekly.

At the end of the experiment the chicks were fasted (but had access to water *ad lib.*) for the last 20–22 h before decapitation. Just before the chick was killed, 2 ml blood were taken from the jugular vein and heparinized with about 0·8 ml heparin solution (5000 i.u. heparin/ml). The plasma was separated from the cells by centrifugation for 6 min at 1260 ***g***. The total cholesterol content of the plasma was determined by the method described by Herrmann (1957).

Liver, heart and brain were taken out and examined for polyenoic fatty acids, *trans* fatty acids and cholesterol.

The tissues were saponified with 30% (w/v) aqueous KOH (4 ml/g tissue) and 96% ethanol (1·6 ml/g tissue) on a steam-bath for 2½–3 h. The unsaponifiable matter was extracted with diethyl ether. The extracts were washed with water, dried over anhydrous sodium sulphate and filtered. After filtration the extracts were taken to dryness under reduced pressure and dissolved in chloroform. Cholesterol was determined in a measured portion by the Liebermann–Burchard reaction.

Table 1. *Composition of the diets (g/kg) (all vitamin E-free)*

Ingredient	Group no. 2335	2336	2337	2340
Fleischmann yeast 50B*	400	400	400	400
Gelatin	30	30	30	30
Salt mixture no. 4†	51·7	51·7	51·7	51·7
Vitamin mixture no. 4‡	1	1	1	1
Choline chloride	2	2	2	2
Sucrose	500·3	500·3	500·3	515·3
9-*cis*-12-*cis* ethyl linoleate§	15	—	—	—
9-*trans*-11-*trans* ethyl linoleate‖	—	15	—	—
9-*trans*-12-*trans* ethyl linoleate¶	—	—	15	—
Synkavit,** 1 × 10 mg	+	+	+	+

* From the Standard Brands Incorporated, New York, N.Y.; contained 3·8% total fatty acids and 0·8% of these were polyenoic fatty acids.

† Consisted of: secondary calcium phosphate ($2H_2O$) 2800 g, calcium carbonate 875 g, desiccated magnesium sulphate (*Ph.Dan.*) 404 g, potassium chloride (*Ph.Dan.*) 460 g, sodium chloride (*Ph.Dan.*) 500 g, ferric citrate (about 17·5% Fe) 100 g, manganese sulphate (water-free) 23 g, cupric sulphate ($5H_2O$) (*Ph.Dan.*) 2 g, zinc sulphate ($7H_2O$) (*Ph.Dan.*) 1 g, aluminium sulphate ($18H_2O$) (*Ph.Dan.*) 1 g, magnesium silicate (*Ph.Dan.*) 1 g, diiodotyrosine (*Ph.Dan.*) 1 g, cobalt carbonate 0·05 g; total 5168·05 g.

‡ Consisted of: thiamine hydrochloride 3 mg, riboflavin 4 mg, nicotinic acid 50 mg, calcium pantothenate 12 mg, pyridoxine hydrochloride 3·5 mg, biotin 0·1 mg, folic acid 2·0 mg, sucrose 925·4 mg; total 1000·0 mg.

§ From the Hormel Institute, Austin, Minnesota. Analytical values for this ester were: iodine value 163·5 (theoretical 164·0), conjugated dienoic acid content < 0·07%, conjugated trienoic acid content < 0·01%.

‖ Prepared by Mr Aage Jart (Danish Fat Research Institute) from ricinoleic acid by elaidinization, dehydration and esterification. The purity was 97·5%.

¶ From the Hormel Institute, Austin, Minnesota. The purity was 98%.

** Dicalcium salt of 2-methyl-1,4-naphthahydroquinone diphosphoric acid ester (Roche Products Ltd).

Ph.Dan., *Pharmacopoeia Danica*, 9th ed. Copenhagen 1948.

The residue from the diethyl ether extraction was acidified with conc. HCl, and the liberated fatty acids were extracted with light petroleum. The extracts were combined, washed with water and dried over anhydrous sodium sulphate. After filtration, the light petroleum (b.p. 40–60°) extracts were evaporated to dryness under reduced pressure and analysed for polyethenoid structure by alkali isomerization, mainly as described by Hammond & Lundberg (1953).

The *trans* fatty acids were determined by infrared spectrophotometry in a double-beam instrument (Perkin–Elmer, Model 21). A solution of about 15% fatty acids in carbon disulphide was used for the measurements. The content of *trans* acids was determined by the base-line technique and calculated as percentage of elaidic acid.

RESULTS AND DISCUSSION

Weight gain

The dietary supplement of 9-*trans*-11-*trans* ethyl linoleate depressed the rate of weight gain to some extent. Thus, the mean values for weights after 5 weeks of experimental feeding were: for the low-fat group 201 g, for the 9-*cis*-12-*cis* ethyl linoleate group 182 g, for the 9-*trans*-12-*trans* ethyl linoleate group 173 g, and for the 9-*trans*-11-*trans* ethyl linoleate group 163 g.

Table 2. *Polyenoic fatty acids in liver, heart and brain of chicks, as percentage of total fatty acids*

	Group no. and dietary fat			
Type of acid	2335 1·5% 9-*cis*-12-*cis* ethyl linoleate	2336 1·5% 9-*trans*-11-*trans* ethyl linoleate	2337 1·5% 9-*trans*-12-*trans* ethyl linoleate	2340 low-fat
	Liver			
Dienoic	14·6	5·1	21·8	4·7
Trienoic	0·6	4·5	2·1	9·6
Tetraenoic	21·5	3·6	4·0	6·1
Pentaenoic	2·4	0·8	0·8	1·1
Hexaenoic	1·5	0·8	0·7	1·1
Preformed conjugated dienoic	1·2	4·8	0·7	0·8
Total polyenoic	41·8	19·6	30·1	23·4
	Heart			
Dienoic	14·4	6·9	17·0	6·5
Trienoic	0·7	3·5	1·5	7·8
Tetraenoic	17·5	5·3	5·3	6·4
Pentaenoic	1·4	0·6	0·5	0·6
Hexaenoic	0·4	0·2	0·3	0·3
Preformed conjugated dienoic	1·5	3·2	0·8	0·6
Total polyenoic	35·9	19·7	25·4	22·2
	Brain			
Dienoic	0·3	0·2	2·6	0·2
Trienoic	1·2	5·1	4·0	5·4
Tetraenoic	10·2	6·6	7·4	7·5
Pentaenoic	3·9	2·1	2·0	2·4
Hexaenoic	6·7	6·7	6·9	7·1
Preformed conjugated dienoic	1·0	2·2	1·0	0·9
Total polyenoic	23·7	22·9	23·9	23·5

Each value represents the mean for eight determinations.

Polyunsaturated fatty acids (*Table* 2)

Liver. The low-fat diet caused typical accumulation of trienoic acid together with fair amounts of dienoic and tetraenoic acids, whereas pentaenoic and hexaenoic acids were present in smaller amounts. The content of preformed conjugated dienoic acid is given separately in Table 2 and was less than 1%.

9-*cis*-12-*cis* ethyl linoleate (1·5% of the diet) increased the amount of conjugatable dienoic acid considerably and gave rise to a marked deposition of the conversion

product, arachidonic acid, whereas the value for trienoic acid was depressed almost to zero.

The contents of penta- and hexa-enoic acids were slightly increased compared with the values obtained on the low-fat diet. That of preformed conjugated dienoic acid was, similarly, slightly increased.

The liver fatty acids from chicks given 1·5% 9-*trans*-11-*trans* ethyl linoleate showed a distinct accumulation of trienoic acid, although less than those from the low-fat group. This may be a sign of the inability of the conjugated *trans-trans* acid to act as essential fatty acid (EFA). The ratio trienoic:tetraenoic acids (considered by Holman (1960) as a measure of EFA deficiency) was about 1·60 for the low-fat group and 1·25 for the group receiving 9-*trans*-11-*trans* ethyl linoleate. Similarly, Aaes-Jørgensen (1958) has found deposition of trienoic fatty acids in the heart and testis of rats fed on diets containing 1% *trans-trans* ethyl linoleate.

The value for preformed conjugated dienoic acid in the liver fat was, as might be expected, much higher than for the other groups. This finding means that dietary 9-*trans*-11-*trans* ethyl linoleate can be absorbed and deposited in the body. The figures for tetra-, penta- and hexaenoic acids indicate that 9-*trans*-11-*trans* ethyl linoleate had not been converted into conjugatable acids of higher degree of unsaturation.

The livers of the chicks given 1·5% 9-*trans*-12-*trans* linoleate showed a very interesting pattern of EFA. The content of dienoic acid, probably 9-*trans*-12-*trans* linoleate, was even higher than that of dienoic acid in liver fat from the corresponding group with 1·5% dietary 9-*cis*-12-*cis* linoleate. However, the presence of some trienoic acid (about 2·1%) indicated that there was some lack of EFA, although not to the same marked degree as in the group receiving the low-fat diet. It may have been due to a sparing effect of the 9-*trans*-12-*trans* linoleate on 9-*cis*-12-*cis* linoleate.

It is evident that conversion into arachidonic acid did not occur. The amount of tetraenoic acid found was most likely present in the chick before the experimental feeding. The failure to form tetraenoic acid might be due to steric hindrance caused by the *trans* double bonds or, more likely, to the specificity of the enzyme system in the chick liver.

The content of penta- and hexa-unsaturated acids was similar to that found for the low-fat group and not as high as for the 9-*cis*-12-*cis* linoleate group. The conversion into these acids also seemed inhibited.

In contrast to the results for the different organs of chicks, Holman (1951) reported that there was an increase in tetraenoic and hexaenoic acids of total carcass fat in rats fed on linolelaidate (*trans-trans* linoleate).

That the low value for tetraenoic acid found in our experiments was not simply due to slow isomerization of *trans-trans* double bonds was ascertained by examining the influence of longer isomerization times on the specific absorption at 315 mμ.

A gas chromatographic study of the same problem was also made. The pooled liver fatty acids from the 9-*trans*-12-*trans* linoleate group were converted into methyl esters, which were then separated on columns of succinate polyester ($\frac{1}{4}$ in. × 6 ft) and silicone grease ($\frac{1}{4}$ in. × 6 ft). Only small amounts of C_{20} unsaturated acids were found,

indicating that conversion of *trans* linoleate into *trans* arachidonate did not take place. The small amount observed must have represented C_{20} acids present before the experimental diet was given.

Exclusion of vitamin E from the diet does not seem to have influenced the interconversion processes. In our experiments, the diet with 1·5% 9-*cis*-12-*cis* ethyl linoleate caused nearly the same distribution and conversions as had been found in our laboratory when the same diets were supplemented with vitamin E (unpublished results).

Heart. The distribution of polyenoic fatty acids was nearly the same as described for liver, the only exception being that 1·5% 9-*trans*-11-*trans* ethyl linoleate caused a less marked deposition of trienoic acid in the heart than in the liver, whereas the corresponding values for dienoic and tetraenoic acids were somewhat higher. The ratio trienoic:tetraenoic acids was only 0·66, against 1·22 for the low-fat group.

The difference between liver and heart in this respect may be related to the different functions of these organs, the liver being an organ in which conversion processes predominantly take place and the heart an organ in which some of the conversion products are deposited later. The content of preformed conjugated dienoic acid was high after ingestion of 9-*trans*-11-*trans* ethyl linoleate, as was also found for liver. (This finding differs from that of Aaes-Jørgensen (1958) with rats in which deposition of preformed conjugated dienoic acid was high in adipose tissue but not in heart, brain or testis.)

Brain. As a sign of EFA deficiency the values for trienoic acid in the low-fat animals were elevated. The contents of tetraenoic and hexaenoic acids were high, as always in brain. Also the values found for pentaenoic acid were somewhat higher than in the other tissues, whereas dienoic acid was almost absent.

Almost the same pattern was found when 9-*trans*-11-*trans* ethyl linoleate was given, but then the value for preformed conjugated dienoic acid was increased, as in the organs mentioned.

When 1·5% 9-*cis*-12-*cis* ethyl linoleate was given, the amount of dienoic acid was almost unchanged, of trienoic decreased and of tetraenoic and pentaenoic increased. Hexaenoic acid was unchanged in amount or slightly decreased, compared with the low-fat group. The ratio trienoic:tetraenoic acid was only 0·12 against 0·72 for the low-fat group.

Finally, the giving of 9-*trans*-12-*trans* ethyl linoleate showed that some dienoic acid was deposited, contrary to what was found in all the other groups. The determinations of *trans* acids further described below showed that about 2–3% of *trans* fatty acids calculated as elaidic acid were present in this group, suggesting that the dienoic acid found by isomerization was, at least in part, the 9-*trans*-12-*trans* acid.

The content of trienoic acid was nearly the same as for the low-fat group or slightly lower, indicating that the 9-*trans*-12-*trans* ethyl linoleate had only a small effect as EFA, if any at all. The higher polyenes (tetra-, penta- and hexa-) reached the same values as for the low-fat group.

Trans *fatty acids* (*Table* 3)

Livers. In the livers from the groups receiving either the low-fat diet or 1·5% 9-*cis*-12-*cis* ethyl linoleate, no *trans* fatty acids were detected.

Fatty acids from chicks receiving 9-*trans*-11-*trans* ethyl linoleate showed a peak at 986 cm^{-1} (10·14 μ), corresponding to conjugated *trans* double bonds, whereas any absorption maximum at 967 cm^{-1} (10·35 μ) from isolated double bonds was absent. The *trans* peak represented, when calculated as elaidic acid, 11% of the total fatty acids.

Table 3. Trans *fatty acids in liver and brain of chicks* (*pooled for each group*), *expressed as percentage of elaidic acid in total fatty acids*

Type of acid	Group no. and dietary fat: 2335 1·5% 9-*cis*-12-*cis* ethyl linoleate	2336 1·5% 9-*trans*-11-*trans* ethyl linoleate	2337 1·5% 9-*trans*-12-*trans* ethyl linoleate	2340 low-fat
		Liver		
Isolated *trans*	0	0	29	0
Conjugated *trans*	0	11	0	0
Total *trans*	0	11	29	0
		Brain		
Isolated *trans*	0	0	2–3	0
Conjugated *trans*	0	0	0	0
Total *trans*	0	0	2–3	0

The liver fatty acids from the chicks in the group receiving 9-*trans*-12-*trans* ethyl linoleate showed a high peak at 967 cm^{-1} (10·35 μ) due to isolated bonds. No peak for conjugated *trans* compounds was present at 986 cm^{-1} (10·14 μ).

The absorption maximum found was equivalent to 29% *trans* fatty acids (calculated as elaidic acid).

Brain. In the brain, *trans* fatty acids were present only in chicks from the group that had received 1·5% 9-*trans*-12-*trans* ethyl linoleate. In this group, a peak at 967 cm^{-1} (10·35 μ) corresponded to about 2–3% *trans* fatty acids.

No detectable amount of *trans* acid was found in the group receiving conjugated *trans* bonds in the form of 9-*trans*-11-*trans* ethyl linoleate. This is in contrast to the deposition of this acid in the liver.

The finding that the 9-*trans*-12-*trans* isomer of linoleic acid, but not linoleic acid itself, can be deposited in the brain seems surprising and requires further study.

Cholesterol

The (fasting) cholesterol contents of plasma, liver, heart and brain are shown in Table 4.

Plasma. There was no significant difference between the plasma cholesterol contents of the four groups in this experiment, but 1·5% 9-*cis*-12-*cis* ethyl linoleate tended to give a lower average value than did the other fats.

This finding resembles that in a similar experiment (not published) with chicks receiving diets containing vitamin E. Here also we found no significant difference in the plasma cholesterol concentrations of a low-fat group and of a group fed on a diet supplemented with 1·5% 9-*cis*-12-*cis* ethyl linoleate.

Table 4. *Mean values with their standard errors for (fasting) cholesterol contents of plasma, liver, heart and brain of chicks, expressed as mg/100 ml plasma and mg/100 g tissue*

Group no.	Dietary fat	Plasma	Liver	Heart	Brain
2335	1·5% 9-*cis*-12-*cis* ethyl linoleate	144 (11) ± 14	321 (10) ± 13	155 (9) ± 19	1206 (9) ± 21
2336	1·5% 9-*trans*-11-*trans* ethyl linoleate	182 (11) ± 13	592 (8) ± 31	156 (9) ± 15	1164 (9) ± 32
2337	1·5% 9-*trans*-12-*trans* ethyl linoleate	174 (11) ± 17	710 (10) ± 60	224 (9) ± 11	1185 (8) ± 29
2340	None	171 (11) ± 8	455 (10) ± 26	156 (10) ± 9	1088 (9) ± 47

Numbers in parentheses show the numbers of animals in each group.

Liver. The group fed on a diet containing 1·5% 9-*cis*-12-*cis* ethyl linoleate had a significantly lower liver cholesterol content ($P < 0.001$) than the three other groups receiving the low-fat diet, 1·5% 9-*trans*-11-*trans* ethyl linoleate or 1·5% 9-*trans*-12-*trans* ethyl linoleate. Both 1·5% 9-*trans*-11-*trans* ethyl linoleate and 1·5% 9-*trans*-12-*trans* ethyl linoleate caused significantly higher liver cholesterol contents than did the low-fat diet, but there was no significant difference between the liver cholesterol contents of the groups fed on these two fats.

Heart. The hearts of the groups receiving 1·5% 9-*cis*-12-*cis* ethyl linoleate, 1·5% 9-*trans*-11-*trans* ethyl linoleate and the low-fat diet had the same cholesterol level, significantly lower than that of the group receiving 1·5% 9-*trans*-12-*trans* ethyl linoleate.

The finding that 1·5% 9-*trans*-12-*trans* ethyl linoleate had an elevating effect on cholesterol content in liver and heart, but not in plasma, resembles the results of an earlier experiment with chicks receiving 10% hydrogenated arachis oil containing 46% *trans* acid, calculated as elaidic acid (Hølmer, Kristensen, Søndergaard & Dam, 1960).

Brain. As would be expected, there was no significant difference in brain cholesterol between the four groups.

SUMMARY

1. The deposition of polyenoic fatty acids, total *trans* fatty acids and cholesterol in chicks after feeding on a low-fat diet alone or supplemented with 1·5% 9-*cis*-12-*cis* ethyl linoleate, 1·5% 9-*trans*-11-*trans* ethyl linoleate or 1·5% 9-*trans*-12-*trans* ethyl linoleate was studied.

2. In the liver and heart of chicks, the giving of 1·5% 9-*trans*-11-*trans* ethyl

linoleate caused deposition of trienoic acid, as found in essential fatty acid deficiency, and the value for preformed conjugated dienoic acid was much higher than in groups fed on a low-fat diet or a diet containing 9-*cis*-12-*cis* ethyl linoleate.

Chicks receiving 9-*trans*-12-*trans* ethyl linoleate had a high content of dienoic acid in liver and heart, probably 9-*trans*-12-*trans* linoleate, but conversion into a *trans*-arachidonic acid did not occur.

3. In the brain, 9-*cis*-12-*cis* ethyl linoleate gave an increased content of arachidonic acid and a decreased amount of trienoic acid, compared with the groups fed on the low-fat diet, 1·5% 9-*trans*-11-*trans* ethyl linoleate or 1·5% 9-*trans*-12-*trans* ethyl linoleate. The group receiving 9-*trans*-12-*trans* ethyl linoleate showed some conjugatable dienoic acid present, but it was nearly absent from the other three groups.

4. *Trans* fatty acids were found in the liver of chicks receiving either 9-*trans*-11-*trans* ethyl linoleate or 9-*trans*-12-*trans* ethyl linoleate, but in the brain only the latter gave rise to detectable amounts of *trans* fatty acids.

5. No effect of the dietary fats was found on the plasma and brain cholesterol contents.

6. In the liver, both 1·5% 9-*trans*-12-*trans* ethyl linoleate and 1·5% 9-*trans*-11-*trans* ethyl linoleate caused significantly higher cholesterol levels than did 1·5% 9-*cis*-12-*cis* ethyl linoleate; 9-*trans*-12-*trans* ethyl linoleate gave the highest liver cholesterol value.

7. In the heart, cholesterol content was affected only by the diet containing 1·5% 9-*trans*-12-*trans* ethyl linoleate, which caused a significant increase compared with the other diets.

REFERENCES

Aaes-Jørgensen, E. (1958). *J. Nutr.* **66**, 465.
Dam, H., Jart, Aa., Kristensen, G., Nielsen, G. K. & Søndergaard, E. (1958). *Acta physiol. scand.* **43**, 97.
Hammond, E. G. & Lundberg, W. O. (1953). *J. Amer. Oil Chem. Soc.* **30**, 433.
Herrmann, R. G. (1957). *Proc. Soc. exp. Biol., N.Y.*, **94**, 503.
Holman, R. T. (1951). *Proc. Soc. exp. Biol., N.Y.*, **76**, 100.
Holman, R. T. (1960). *J. Nutr.* **70**, 405.
Hølmer, G., Kristensen, G., Søndergaard, E. & Dam, H. (1960). *Brit. J. Nutr.* **14**, 247.

Printed in Great Britain