Brit. J. Nutr. (1960), **14**, 247

Polyenoic fatty acids and cholesterol in blood, heart and liver of chicks fed on hydrogenated and unhydrogenated arachis oil

By GUNHILD HØLMER,* GUNHILD KRISTENSEN, E. SØNDERGAARD AND H. DAM

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

(*Received* 4 *August* 1959—*Revised* 2 *November* 1959)

Several investigations have been made into the polyunsaturated fatty acids of various chick tissues (Dam, Kristensen, Nielsen, Prange & Søndergaard, 1956; Dam & Nielsen, 1956; Dam, Jart, Kristensen, Nielsen & Søndergaard, 1958) and blood plasma (Bieri, Pollard & Briggs, 1957), but, as far as we are aware, only a few studies of the corresponding values for blood cells have been reported in the literature. Chevallier, Manuel, Burg & Rouillard (1950) and Chevallier, Manuel & Rouillard (1951) examined human serum and erythrocytes and found that serum contained more dienoic than tetraenoic acids and only small amounts of trienoic acids, whereas erythrocytes contained more tetraenoic than dienoic acids.

Similar results were obtained by Evans, Waldron, Oleksyshyn & Riemenschneider (1956), who studied blood plasma and erythrocytes from young male students and determined polyenes by the isomerization technique.

Further, James, Lovelock & Webb (1957) reported an in vitro synthesis of linoleic and arachidonic acids from ^{14}C-labelled acetate in human plasma and erythrocytes. In a later publication (James, Lovelock & Webb, 1959) these authors considered exchange of carbon units at the carboxyl end as an explanation of their findings.

Our studies were planned partly to examine whether the distribution of polyenoic acids in blood from chicks fed on diets without fat, with 10% hydrogenated arachis oil or with 10% unhydrogenated arachis oil, with or without 1% cholesterol was as described above for human blood, and partly to obtain an indication of whether a possible in vivo synthesis might be reflected in the contents of di- and tetra-enoic acids.

In connexion with these experiments, polyenoic acids were also determined in heart and liver and cholesterol in plasma, heart and liver.

The effects of a fasting period of about 20 h before death were also studied.

EXPERIMENTAL

The experiment without cholesterol was done about 11 weeks before that with it. In each experiment, sixty-six day-old chicks were given a commercial chick mash (Dam, Hartmann, Jacobsen & Søndergaard, 1957) for 14 days and were then distributed into groups with eleven chicks in each and fed on the experimental diets for 4 weeks.

* Former name: Gunhild Kofoed Nielsen.

The experimental diets were:

(1) A fat-free basal diet (Table 1 of Dam, Kristensen, Nielsen & Søndergaard, 1959).

(2) A diet with 10% of an hydrogenated arachis oil (m.p. 40–42°) which contained 0·1% dienoic, 0·1% preformed conjugated dienoic and no higher unsaturated acids (as determined by the method of Hammond & Lundberg, 1953).

(3) A diet with 10% of an unhydrogenated arachis oil which contained 26·1% dienoic, 1·2% trienoic, 0·3% preformed conjugated dienoic acids and only negligible amounts of higher polyunsaturated acids (as determined by the method of Hammond & Lundberg, 1953).

The fats were incorporated in the fat-free basal diet instead of the same weight of sucrose. In the diets with 1% cholesterol, the cholesterol also replaced the same weight of sucrose.

Each chick received the daily equivalent of 250 i.u. vitamin A and 20 i.u. vitamin D as an alcohol–water solution with Tween 80 (polyoxyethylene sorbitan monooleate) given as 0·1 ml twice weekly.

At the end of the 4-week experimental feeding period, half of both lots of chicks were fasted (but had access to water) for the last 20–22 h before decapitation and the other half were given food *ad lib.*

Just before each chick was killed, 5 ml blood were taken from its jugular vein and transferred to a centrifuge tube, clotting being prevented by about 0·08 ml heparin solution (5000 i.u. heparin/ml). Plasma and blood cells were separated by centrifugation for 6 min at 1260 *g*.

Samples of plasma from each chick were analysed for cholesterol by the method of Abell, Levy, Brodie & Kendall (1952). Determinations of polyenoic fatty acids were made on pooled samples (2 ml plasma from each chick in the group).

The plasma was saponified with 5 ml 30% (w/v) aqueous KOH and 10 ml 96% ethanol on a steam-bath for 3 h. The unsaponifiable matter was extracted with diethyl ether; after acidification of the extracted aqueous phase with conc. HCl (3 ml) the fatty acids were extracted with light petroleum. After drying with anhydrous sodium sulphate, the petroleum extracts were evaporated under diminished pressure at room temperature and subjected to isomerization analyses (Hammond & Lundberg, 1953).

The blood cells from the individual 5 ml blood samples were pooled for each group and washed by resuspension and centrifugation three times in Ringer solution. Fat was extracted and saponified as described by Evans *et al.* (1956).

The unsaponifiable matter and fatty acids were isolated as described above, and the polyenoic fatty acids were then determined.

Polyenoic fatty acids in heart and liver were determined as previously reported (Dam *et al.* 1956). After saponification of the tissue the cholesterol was extracted from the alkaline solution with light petroleum. After washing with water and drying with anhydrous sodium sulphate, the extract was evaporated under diminished pressure, and the cholesterol dissolved in chloroform. The cholesterol content was determined by the Liebermann–Burchard colour reaction. Two or three hearts were pooled for each determination, and three or four determinations were made for each group. The livers were analysed individually.

RESULTS AND DISCUSSION

Polyunsaturated fatty acids (*Table* 1)

Plasma. On the fat-free diet, trienoic acids accumulated in the plasma. Such an accumulation of trienes was found earlier in liver, heart and lungs (Dam *et al.* 1956). The other polyenoic acids in plasma were also distributed in about the same proportion as in organs.

When hydrogenated arachis oil was given there was no increase in trienoic acids. The content of dienoic acids was considerably lower in the non-fasted than in the fasted chicks. The other polyenes were hardly affected.

A high content of dienoic and tetraenoic acids in the plasma was caused by 10% of dietary arachis oil, whereas trienoic acids were absent and more unsaturated acids present in small amounts only.

It appears as if fasting influenced the proportions between dienoic and tetraenoic acids, favouring a higher content of tetraenoic acids.

Supplementation of the diet with 1% cholesterol did not give rise to any changes in the group on the fat-free diet or in that on the diet with 10% hydrogenated arachis oil. However, in the groups given 10% unhydrogenated arachis oil, 1% of cholesterol caused lowering of the content of tetraenoic acids, especially in the fasted chicks. This finding is in agreement with observations made on livers of chicks (Dam & Nielsen, 1956).

Erythrocytes. The marked deposition of trienoic acids ordinarily observed in organs after fat-free feeding was not found in the blood cells, whether or not the diet contained 1% cholesterol.

The hydrogenated arachis oil gave a low content of all polyenes, independent of the presence of dietary cholesterol.

Unhydrogenated arachis oil caused deposition especially of dienoic and of some tetraenoic acids, but there was no indication of a preponderance of tetraenoic acids in blood cells or of dienoic acids in blood plasma as found by Chevallier *et al.* (1951) and by Evans *et al.* (1956) for man.

The values for dienoic and tetraenoic acids in plasma and erythrocytes were relatively low, except when arachis oil had been given. Synthesis of dienes and tetraenes, as found by James *et al.* (1957) in in vitro experiments, did not seem to occur in chicks in vivo to the extent that it influenced the values found.

The depressing effect of cholesterol on tetraenoic acids found earlier for liver was not seen in the erythrocytes.

The results showed hardly any difference between the fasted and the corresponding non-fasted groups.

Heart. For the groups on the fat-free diet, neither fasting nor dietary cholesterol influenced significantly the amount of the individual polyethenoid acids. The deposition of trienoic acids normally occurring on fat-free diets was observed.

In the groups with 10% hydrogenated arachis oil in the diet the content of dienoic and perhaps that of tetraenoic acids was higher for the fasted than for the non-fasted groups with or without cholesterol supplementation.

Unhydrogenated arachis oil in the diet (10%) caused deposition of dienoic and tetraenoic acids, whereas trienoic acids were almost absent (cf. Dam *et al.* 1956).

The amount of tetraenoic acids was somewhat higher for the fasted than for the non-fasted chicks in the cholesterol-free groups, which may have been due to metabolism of less unsaturated acids during fasting.

Table 1. *Polyenoic fatty acids in plasma, erythrocytes, heart and liver of chicks fed on diets with no fat or with* 10% *hydrogenated or unhydrogenated arachis oil, with or without* 1% *cholesterol*

		Dietary characteristics and group no.											
		No fat		Hydrogenated arachis oil		Unhydrogenated arachis oil		No fat, cholesterol		Hydrogenated arachis oil, cholesterol		Unhydrogenated arachis oil, cholesterol	
Acid		1955	1956	1957	1958	1959	1960	2004	2005	2006	2007	2008	2009
Site	Type	(n.f.)	(f.)	(n.f.)	(f.)	(n.f.)	(f.)	(n.f.)	(f.)	(n.f.)	(f.)	(n.f.)	(f.)
Plasma: as percentage of total fatty acids	Dienoic	—	5·0	2·3	6·0	16·4	12·1	1·4	3·1	1·7	4·1	19·1	15·3
	Trienoic	—	9·9	1·2	1·9	−0·3	−2·6	5·3	10·1	1·0	1·7	0·9	−0·3
	Tetraenoic	—	3·6	0·7	1·8	11·1	23·6	1·1	2·6	0·6	1·4	9·7	13·0
	Pentaenoic	—	1·3	0·3	0·7	2·1	4·8	0·4	0·8	0·2	0·8	1·0	1·5
	Hexaenoic	—	1·3	0·5	0·9	1·3	3·7	0·7	1·0	0·2	0·1	1·3	1·6
	Total polyenoic	—	21·1	5·0	11·3	30·6	41·6	8·9	17·6	3·7	8·1	32·0	31·1
mg/100 ml	Total fatty	—	244	167	148	218	157	166	195	275	159	214	162
	Total polyenoic	—	51·5	8·4	16·7	66·7	65·3	14·8	34·3	10·2	12·9	68·5	50·4
Erythrocytes*: as percentage of total fatty acids	Dienoic	4·1	3·7	4·1	2·9	17·6	19·2	5·7	2·1	1·3	2·4	—	25·5
	Trienoic	2·5	2·7	1·2	0·2	−0·3	−0·3	1·7	1·8	1·0	1·6	—	0·7
	Tetraenoic	2·6	2·4	1·8	1·9	7·7	7·6	1·8	1·6	1·2	1·6	—	6·3
	Pentaenoic	1·0	0·8	0·7	0·5	1·7	1·7	0·5	0·6	0·4	0·3	—	0·8
	Hexaenoic	2·9	0·9	1·0	3·0	1·5	1·3	1·3	1·7	0·4	0·3	—	0·9
	Total polyenoic	13·1	10·5	8·8	8·5	28·2	29·5	11·0	7·8	4·3	6·2	—	34·2
Heart†: as percentage of total fatty acids	Dienoic	5·1	5·5	4·4	6·6	16·7	17·8	4·1	4·6	4·9	6·7	17·3	18·4
	Trienoic	4·1	4·9	1·9	2·3	0·8	0·4	4·2	4·3	1·7	1·5	0·4	0·4
	Tetraenoic	3·9	4·6	3·1	4·5	9·7	14·4	3·1	3·3	2·4	3·5	10·0	7·9
	Pentaenoic	0·4	0·4	0·4	0·3	0·8	1·2	0·2	0·3	0·3	0·3	0·8	0·6
	Hexaenoic	0·3	0·3	0·4	0·3	0·5	0·4	0·3	0·3	0·3	0·2	0·4	0·3
	Total polyenoic	13·8	15·7	10·2	14·0	28·5	34·2	11·9	12·8	9·6	12·2	28·9	27·6
mg/100 g	Total fatty	3110	2980	3530	2500	2730	2530	4120	3470	3460	3280	3590	5040
	Total polyenoic	427	450	353	350	1042	850	475	442	354	392	1094	1436
Liver‡: as percentage of total fatty acids	Dienoic	1·4	6·2	4·9	6·5	15·8	14·1	1·3	3·4	3·9	4·9	13·7	16·9
	Trienoic	3·6	8·8	2·2	2·5	1·1	0·0	3·1	8·5	1·6	2·3	1·2	0·1
	Tetraenoic	2·4	7·3	2·6	3·0	16·0	21·1	1·7	4·7	1·2	2·6	9·4	14·6
	Pentaenoic	0·4	1·5	0·4	0·5	2·6	3·7	0·3	1·0	0·3	0·5	1·1	1·8
	Hexaenoic	0·3	1·5	0·5	0·6	2·0	3·2	0·4	1·1	0·3	0·5	1·0	1·7
	Total polyenoic	8·1	25·3	10·6	13·1	37·5	42·1	6·8	18·7	7·3	10·8	26·4	35·1
mg/100 g	Total fatty	4990	2780	3380	2910	2460	2460	5990	3040	3780	3310	3340	3280
	Total polyenoic	356	698	357	374	931	1051	368	543	250	373	850	1132

* One determination/group. † Three determinations/group. ‡ Five determinations/group.
The small negative values for trienoic acids are due to the arbitrary nature of the method.
n.f., non-fasted; f., fasted.

For chicks fed on diets with 1% cholesterol and 10% arachis oil until the 20 h fasting period before they were killed, the content of tetraenoic acids appeared to be less than for the corresponding non-fasted chicks. Apparently the explanation is that the depressing effect of 1% cholesterol given for 4 weeks was greater than the opposite effect of about 20 h fasting.

The depression of tetraenoic acids by means of dietary cholesterol has previously been noted in livers of non-fasted chicks (Dam & Nielsen, 1956), but not in hearts.

Cholesterol supplementation did not seem to affect the content of tetraenoic acids in hearts of non-fasted chicks.

Liver. Fasting for 20 h before decapitation caused higher values for polyenes in all groups. Otherwise the results were largely the same as those found earlier (Dam *et al.* 1956; Dam *et al.* 1958).

Cholesterol (*Table* 2)

Plasma. The mean plasma-cholesterol contents of the fasted chicks fed on a fat-free diet without cholesterol or on a diet with 10% arachis oil without cholesterol (groups 1956 and 1960, respectively) were significantly higher ($P < 0.01$ and $P < 0.02$) than the mean plasma-cholesterol contents of the non-fasted chicks fed on these two diets (groups 1955 and 1959). For the chicks on a fat-free diet this finding is

Table 2. *Mean values with their standard errors for cholesterol content of plasma, heart and liver (wet weight) of chicks fed on diets with no fat or with* 10% *hydrogenated or unhydrogenated arachis oil, with or without* 1% *cholesterol*

	Dietary characteristics and group no.					
	No fat		Hydrogenated arachis oil		Unhydrogenated arachis oil	
	1955 (n.f.)	1956 (f.)	1957 (n.f.)	1958 (f.)	1959 (n.f.)	1960 (f.)
Plasma (mg/100 ml)	159±7 (10)	199±9 (11)	133±10 (11)	144±9 (11)	129±6 (11)	150±5 (10)
Heart (mg/100 g)	177±1 (3)	182±7 (4)	207±9 (4)	198±10 (3)	164±1 (3)	173±14 (2)
Liver (mg/100 g)	399±34 (5)	400±20 (5)	414±21 (5)	434±34 (5)	276±4 (4)	289±16 (4)

	Dietary characteristics and group no.					
	No fat, cholesterol		Hydrogenated arachis oil, cholesterol		Unhydrogenated arachis oil, cholesterol	
	2004 (n.f.)	2005 (f.)	2006 (n.f.)	2007 (f.)	2008 (n.f.)	2009 (f.)
Plasma (mg/100 ml)	200±13 (11)	217±10 (11)	556±65 (11)	489±51 (11)	695±54 (11)	687±55 (11)
Heart (mg/100g)	206±10 (4)	239±7 (4)	372±11 (4)	432±37 (4)	415±25 (4)	458±63 (4)
Liver (mg/100g)	684±48 (5)	549±36 (4)	1801±260 (5)	2118±330 (5)	2285±363 (5)	2537±346 (5)

n.f., non-fasted; f., fasted.
The figures in parentheses indicate the number of determinations made for each group.
The plasma- and liver-cholesterol contents were determined for each chick individually; two or three hearts were pooled for each analysis.

in agreement with earlier ones (Dam *et al.* 1959). The fasting period did not affect the plasma-cholesterol values when 10% hydrogenated arachis oil was present in the diet or when the diet was supplemented with 1% cholesterol.

When the two fats were given without cholesterol there was scarcely any difference between plasma-cholesterol values for fasted and non-fasted chicks. Both fats caused values lower than the corresponding values for the fat-free groups.

When 1% cholesterol was added to the diet, the chicks on the diet with 10% arachis

oil had somewhat higher plasma-cholesterol contents than those on the diet with 10% hydrogenated arachis oil.

Heart and liver. The fasting period did not influence significantly the cholesterol content of heart or liver, but when 1% dietary cholesterol was given the heart-cholesterol values of the fasted groups were somewhat higher than those of the non-fasted groups.

Without dietary cholesterol, the diet with hydrogenated arachis oil gave the higher heart- and liver-cholesterol values; and the diet with unhydrogenated arachis oil gave lower values than the fat-free diet. The difference between the effect of the fat-free diet and that with unhydrogenated arachis oil was most marked in the liver. With dietary cholesterol, the two diets caused nearly the same cholesterol values in heart and liver.

These differences in the heart- and liver-cholesterol contents obtained with diets with 10% hydrogenated arachis oil or 10% arachis oil correspond to those found in earlier experiments (Dam *et al.* 1956, 1958).

SUMMARY

1. Sixty-six 14-day-old chicks were divided into groups of eleven and were fed for 4 weeks on artificial diets without fat or with 10% hydrogenated or unhydrogenated arachis oil, with or without 1% cholesterol. Half of the chicks in each group were fasted for about 20 h before being killed. Polyenoic fatty acids were determined in plasma, erythrocytes, heart and liver, and cholesterol in plasma, heart and liver.

2. In the plasma, fasting favoured a higher content of all polyenoic acids except in animals on the diet with 10% unhydrogenated arachis oil, in which an increase of tetraenoic was associated with a decrease of dienoic acids. Dietary cholesterol lowered the amount of tetraenoic acids.

3. In erythrocytes, the fat-free diet did not give rise to any marked increase of trienoic acids, contrary to what occurred in plasma and tissues. The amount of dienoic acids was always greater than that of tetraenoic acids, especially when unhydrogenated arachis oil was given. Fasting or dietary cholesterol did not influence the content of tetraenoic acids.

4. In the heart, the diet with 10% unhydrogenated arachis oil caused the usual deposition of tetraenoic and dienoic acids. Fasting increased the amount of tetraenoic acids, but this effect was counteracted when the diet before fasting contained 1% cholesterol.

5. In the liver, the contents of all polyenoic acids were higher in fasted chicks.

6. Fasting had a marked influence on plasma-cholesterol content in chicks fed on a fat-free diet without cholesterol or on a diet containing unhydrogenated arachis oil without cholesterol, causing significantly higher cholesterol values than in the non-fasted chicks.

7. Without dietary cholesterol the two fats gave nearly the same content of plasma cholesterol; in heart and liver the hydrogenated arachis oil caused a greater deposi-

tion of cholesterol. With dietary cholesterol the unhydrogenated arachis oil gave a greater increase in plasma-cholesterol content, but about the same values for heart and liver cholesterol as did hydrogenated arachis oil.

REFERENCES

Abell, L. L., Levy, B. B., Brodie, B. B. & Kendall, F. E. (1952). *J. biol. Chem.* **195**, 357.
Bieri, J. G., Pollard, C. J. & Briggs, G. M. (1957). *Arch. Biochem. Biophys.* **68**, 300.
Chevallier, A., Manuel, S., Burg, C. & Rouillard, J. (1950). *C.R. Soc. Biol., Paris*, **144**, 577.
Chevallier, A., Manuel, S. & Rouillard, J. (1951). *C.R. Soc. Biol., Paris*, **145**, 924.
Dam, H., Hartmann, S., Jacobsen, J. E. & Søndergaard, E. (1957). *Acta physiol. scand.* **41**, 149.
Dam, H., Jart, A., Kristensen, G., Nielsen, G. K. & Søndergaard, E. (1958). *Acta physiol. scand.* **43**, 97.
Dam, H., Kristensen, G., Nielsen, G. K., Prange, I. & Søndergaard, E. (1956). *Acta physiol. scand.* **36**, 319.
Dam, H., Kristensen, G., Nielsen, G. K. & Søndergaard, E. (1959). *Acta physiol. scand.* **45**, 31.
Dam, H. & Nielsen, G. K. (1956). *Acta physiol. scand.* **37**, 359.
Evans, J. D., Waldron, J. M., Oleksyshyn, N. L. & Riemenschneider, R. W. (1956). *J. biol. Chem.* **218**, 255.
Hammond, E. G. & Lundberg, W. O. (1953). *J. Amer. Oil Chem. Soc.* **30**, 433.
James, A. T., Lovelock, J. E. & Webb, J. (1957). *Biochem. J.* **66**, 60P.
James, A. T., Lovelock, J. E. & Webb, J. P. W. (1959). *Biochem. J.* **73**, 106.

Printed in Great Britain