The effect of a low-fat maternal diet on neonatal rats

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1. Rats were raised on a low-fat diet containing 6 g fat/kg. Females of the second generation were bred and only 11 % of their pups survived to weaning age compared with a 66 % survival for control pups. Pups were killed 8–12 h after birth and their tissues were analysed.

2. Pups in the low-fat group had smaller body, brain and liver weights than control pups; the lipid contents of body, brain and liver were also significantly less.

3. In the liver triglycerides from the control group the C_{20} and C_{22} polyenoic fatty acids constituted 33 % of the total fatty acids. The liver triglyceride concentration in the low-fat group was lower and the concentration of the long-chain polyenoic fatty acids in this fraction was 20 % of the control value. The milk fatty acids from the low-fat group contained only 33 % as much of the C_{18} to C_{22} polyenoic fatty acids compared with the control group.

4. In the brain lipids from the low-fat group, changes in the fatty acid composition were less marked than in the liver lipids. In these experiments there were only small amounts of 20:3ω9 in the tissue lipids; the ratio to 20:4ω6 was less than 1.

5. These changes are discussed in relation to the influence of dietary lipids on tissue growth especially of lipid-rich tissues such as the brain.

Considerable attention has been devoted to the effects of dietary protein and energy restriction on brain development in rats. The rat brain develops largely during the first 3 weeks after birth, and dietary restrictions have usually been imposed on the mothers just before the birth of the young and during the suckling period. The results suggest that protein and energy restriction can affect brain chemistry, myelination and function (Dobbing, 1965–6; Eichenwald & Fry, 1969; Adlard & Dobbing, 1971; Baird, Widdowson & Cowley, 1971; Simonson, Stephan, Hanson & Chow, 1971; Smart & Dobbing, 1971; Zamenhof, Van Marthens & Grauel, 1971).

We distinguish between two types of lipid found in animals: (1) storage and (2) structural. This differentiation is based on the recognition by histologists by the end of the last century of 'visible' and 'invisible' fats. The visible fats are known to be largely triglyceride and are found in body depots. The structural or invisible fats are found in cellular and subcellular membranes, and in certain enzymes, usually in conjunction with proteins but in certain specialized tissues, such as myelin, the structural lipids appear to form matrices without protein. The structural lipids include phosphoglycerides, sphingolipids and some neutral lipid including cholesterol. It is of general nutritional interest that mammalian phosphoglycerides are consistently rich in polyenoic fatty acids but this is not necessarily so for the triglycerides.

The brain, unlike other organs, contains more structural lipid than protein; in the rat more than half of the brain lipids are in the myelin fraction (Cuzner, Davison & Gregson, 1965). Rat myelin lipids contain long-chain saturated and monounsaturated

fatty acids (e.g. 20:1, 24:0 and 24:1) (Kishimoto, Agranoff, Radin & Burton, 1969) and, since these acids can be made *de novo* in the body, it might be thought that brain development would be independent of exogenous lipids. On the other hand, the structural lipids of the grey matter of rat brain are rich in long-chain polyenoic fatty acids (e.g. 20:4 and 22:6) (Kishimoto *et al.* 1969); these fatty acids are metabolites of the C_{18} fatty acids which cannot be made by the rat and must be obtained in the diet (Holman, 1968, 1970). The infant rat accumulates about 70% of its total polyenoic fatty acids in the brain by the end of the first 20 d after birth (Sinclair & Crawford, 1972).

In view of these facts it is not surprising that lipid restriction can affect the growth and development of the brain in rats. Rats fed on fat-free diets during pregnancy have been shown to have offspring with brains that were smaller than those of control rats (Steinberg, Clarke & Ramwell, 1968; Galli, White & Paoletti, 1970; White, Galli & Paoletti, 1971) and also a reduced concentration of the long-chain polyenoic fatty acids in the brain ethanolamine phosphoglyceride (EPG) fraction. In the experiments of Galli *et al.* (1970) and White *et al.* (1971) the fat-free diet was offered to the rats during the last 5 d of pregnancy and during lactation. Rat milk contains linoleic and linolenic acids together with their longer-chain metabolites (Sinclair & Crawford, 1972), and in lipid-deficiency experiments in the rat it takes up to 10 weeks to reduce tissue concentrations of the essential polyenoic fatty acids (Sinclair & Collins, 1968). Therefore, deprivation of lipid during late pregnancy is unlikely to induce severe deficiency during the period of most active accumulation of polyenoic acids in the developing brain. It is interesting that White *et al.* (1971) found that the effect on brain weight under their conditions could be reversed by feeding with lipid during later life, but that learning ability was irreversibly impaired (Paoletti & Galli, 1972).

We have examined a more extreme situation. Female rats were reared through two generations on a low-fat diet and the brain and liver lipids of the third generation pups were examined.

METHODS

Male and female rats of the Wistar strain were raised from weaning to maturity on semi-synthetic diets. The control diet (Table 1) contained 60 g fat/kg. The experimental diet was made without the fat and the amounts of glucose and starch were each increased by 30 g/kg. This diet contained 6 g of chloroform–methanol-soluble material (associated with the casein and the starch)/kg; in this low-fat diet, linoleic and linolenic acids accounted for 0·27 and 0·057% of the energy respectively. The control diet contained 5·0 and 1·0% of the energy as linoleic and linolenic acids respectively.

After 6 months on the diets, the rats were mated and the resulting pups were weaned on to the same diet as their dams. Six female rats on each diet were reared until about 4 months of age and then mated with male rats from the stock colony and the newborn pups were examined (i.e. the third generation on the diets.) There were many early deaths in the litters whose mothers were fed on the low-fat diet. A similar observation was reported in the early 1950s (Kummerow, Pan & Hickman, 1952; Deuel, Martin &

Table 1. *Composition of control diet*

Ingredient	(g/kg)
Glucose	365
Potato starch	250
Casein (Casein C; Glaxo Laboratories)	250
Fat (soya-bean oil:linseed oil, 5:1)	60
Methyl cellulose	50
Mineral salts*	25
Choline	1·0
α-Tocopherol acetate	0·44
Cysteine	0·2

The following vitamins were added to the diet: (mg/kg) thiamin, 5·6; riboflavin, 9·0; pyridoxine, 5·6; nicotinic acid, 45·0; biotin, 0·5; folic acid, 2·3; *p*-aminobenzoic acid, 112·5; inositol, 112·5; calcium pantothenate, 33·8; cyanocobalamin, 0·5; menaphthone, 0·8—and (μg/kg) vitamin A, 270·6 retinol equivalent; vitamin D, 2·5 cholecalciferol equivalent.

* Contained (g/kg diet): $CaCO_3$, 1·103; $Ca_3(PO_4)_2$, 10·290; K_2HPO_4, 6·615; anhydrous Na_2HPO_4, 3·900; NaCl, 0·588; KI, 0·029; $MgSO_4.7H_2O$, 1·470; MgO, 0·588; ferric citrate. $5H_2O$, 0·294; $CuSO_4.5H_2O$, 0·015; $MnSO_4.4H_2O$, 0·103; $ZnCO_3$, 0·015.

Alfin-Slater, 1954) and it was shown that the survival of the pups was dependent upon corn oil or linoleic acid being present in the dam's diet. In rats, essential fatty-acid deficiency leads to the development of scaly skin on the tail and feet, and to changes in the appearance of body hair (Holman, 1968); however, in the present experiments these symptoms were not observed and the external appearance of the animals was indistinguishable from controls, Also, the concentration of 20:3ω9 in the liver phospholipids from both first and second generation rats was considerably less than the concentration of 20:4ω6. Thus it seemed that these rats were only partially deficient in the essential fatty acids. Apart from the diet as a source of linoleic and linolenic acids, it was established that the essential fatty acids were present in the faecal lipids of these rats. The animals were kept in cages with raised screen bottoms; however, it has been reported that this type of caging does not totally prevent coprophagy (Holman, 1968). There was no clinical reason to suspect failure of absorption of fat-soluble vitamins from this diet. Knowing that the survival rate was poor for the low-fat group, we examined pups from both groups shortly after birth.

Living pups (8–12 h after birth) were selected at random from each dietary group; a total of thirty-six pups from the control group and thirty-five from the experimental group were killed. Lipid analyses were made on eighteen pups from each group and brain DNA determinations on a further sixteen pups from each group. After decapitation of the pups, their livers, brains, and stomach contents were removed, weighed and homogenized in chloroform–methanol (2:1, containing 10 mg/l of 2:6 di-t-butyl-*p*-cresol as an antioxidant). The lipids were extracted as described by Folch, Lees & Stanley (1957) and the amount of lipid was determined gravimetrically. Neutral lipids were separated from total lipids by thin-layer chromatography (TLC) (solvent: light petroleum (b.p. 40–60°)–diethyl ether–glacial acetic acid (85:15:5, by volume)); phospholipids were separated by TLC using as the solvent: chloroform–methanol–7 M-ammonia (65:15:5, by volume). The lipid fractions were detected with dichlorofluorescein and eluted from the chromatograms with solvent (light petroleum (b.p.

40–60°)–benzene-chloroform–methanol) (1:1:2:1, by volume)). The solvent was evaporated under a stream of N_2 and the fractions were dried and weighed.

The methyl esters of the fatty acids from the different lipid fractions were prepared by treating the lipids with 0·9 M-H_2SO_4 in methanol for 3 h at 70° in a sealed tube. Samples of total lipids from brain, liver and stomach contents were also subjected to the same transesterification treatment; however, in this instance the methyl esters were purified by TLC (solvent: light petroleum (b.p. 40–60°)–diethyl ether–glacial acetic acid (90:10:1, by volume)) before gas–liquid chromatography (GLC).

GLC was carried out as described previously (Sinclair & Crawford, 1972) in a Pye series 104 chromatograph (W. G. Pye & Co. Ltd, Cambridge). We routinely used two stationary phases, ethylene glycol succinate polyester and polyethylene glycol adipate; with the latter, the saturated and monounsaturated esters have a higher retention value than on the former. A third stationary phase, Apiezon-L, was also used for confirmation of the identity of some esters; using this phase the elution order of saturated, mono- and poly-unsaturated acids is reversed, providing information on chain length and degree of unsaturation. The stationary phases were supplied by Applied Science Laboratories, Pennsylvania.

The brain DNA content was estimated by the method described by Logan, Mannell & Rossiter (1952).

RESULTS

Effect of low-fat diet on survival of litters

Table 2 shows that the number of pups per litter was about the same in each group. The dams on the low-fat diet sometimes ate several pups per litter or failed to show interest in their offspring, and only 11 % of their pups survived to weaning age; 90 % of the deaths occurred in the 1st week. In the control group 66 % of the pups survived to weaning and only half the deaths were in the 1st week.

In both groups each dam had at least two litters and in the low-fat group there was no indication of progressive changes in the successive litters of any one dam. In the control group, pups from seventeen of the nineteen litters survived to weaning whereas in the low-fat group pups from only three litters from different dams survived to this age.

Effect of diet on tissues of newborn pups

The pups in the low-fat group were significantly smaller than the control pups at birth, and their brain and liver weights were also less (Table 3).

The concentration of liver lipids in the low-fat group was 60 % of the control group; most of this difference could be accounted for by the lower concentration of liver triglycerides (Table 4). Table 5 gives the fatty acid composition of different liver lipid fractions. In the total liver fatty acids of the low-fat group there was an increase in the saturated and monounsaturated fatty acids and a decrease in the C_{18} polyenoic fatty acids and their long-chain metabolites. There was a rise in 20:3ω9 which is characteristic of essential fatty acid deficiency in the rat (Holman, 1968). The pattern of fatty acids in the liver EPG and choline phosphoglycerides (CPG) from the low-fat group was similar to the total fatty acid pattern. In the control pups the EPG con-

Table 2. *Survival of newborn pups from female rats fed on a low-fat diet*

Criterion	Control group	Low-fat group
No. of dams	6	6
No. of litters	19	19
Pups/litter*	8·9 ± 0·8	8·1 ± 0·6
Total no. of pups:†		
At birth	133 (100 %)	119 (100 %)
At 7 d	112 (84 %)	22 (19 %)
At 21 d	88 (66 %)	13 (11 %)

* Mean values with their standard errors.
† These figures do not include pups taken for analysis (see Methods).

Table 3. *Body-weights, liver and brain weights of newborn pups from rats fed on a low-fat diet*

(Mean values with their standard errors for twenty pups/group)

	Control group		Low-fat group	
	Mean	SE	Mean	SE
Body-wt (g)	5·9	0·1	4·5	0·1***
Liver wt (g)	0·31	0·01	0·24	0·02*
Brain wt (g)	0·28	0·01	0·22	0·01***
Liver: body-wt (%)	5·3	0·2	5·3	0·2
Brain: body-wt (%)	4·8	0·1	4·9	0·2

Significance of differences between dietary treatments: * $P < 0·05$; *** $P < 0·001$.

Table 4. *Analyses of liver lipids from newborn pups of rats fed on a low-fat diet*

(Mean values with their standard errors; livers from two rats were pooled per sample. The number of samples is shown in parentheses)

		Liver lipids (g/kg fresh liver)			
	Liver wt (g)	Total lipid	Total cholesterol	Triglyceride	Phospholipid
Control group	0·33 ± 0·02 (9)	38·2 ± 3·2 (9)	7·6 ± 0·9 (5)	12·8 ± 0·8 (5)	18·7 ± 3·2 (5)
Low-fat group	0·27 ± 0·02 (9)*	22·4 ± 1·6 (9)***	8·3 ± 0·4 (5)	2·6 ± 0·4 (5)***	12·2 ± 2·3 (5)

Significance of differences between dietary treatments: * $P < 0·05$; *** $P < 0·001$.

tained more $\omega 3$ than $\omega 6$ fatty acids; in the low-fat group the concentration of the $\omega 3$ acids in the EPG was half the control values, whereas there was little change in that of the $\omega 6$ acids. In the CPG fraction from the low-fat group the concentrations of both $\omega 6$ and $\omega 3$ acids were about half those in the controls. The fatty acids of the liver triglycerides were more affected by the diet than were the phospholipids. The liver triglycerides from the newborn control pups were particularly rich in long-chain polyenoic fatty acids (33 % of C_{20} and C_{22} polyenes) and in the triglycerides from the pups in the low-fat group the concentration of the C_{18}–C_{22} polyenoic fatty acids was only about 20 % of the controls.

Table 5. *Fatty acid composition (expressed as parts/10^3 of total) of liver lipids from newborn pups of rats fed on a low-fat diet*

Fatty acid	Total fatty acids		Triglycerides		Choline phosphoglycerides		Ethanolamine phosphoglycerides	
	Control	Low-fat	Control	Low-fat	Control	Low-fat	Control	Low-fat
14:0	24	11	27	119	7	13	—	—
16:0	207	234	248	247	327	284	180	168
16:1	20	55	22	72	13	67	11	42
18:0	98	122	26	74	110	147	174	175
18:1	118	246	209	374	75	229	58	152
18:2ω6	123	43	118	24	75	37	55	27
18:3ω3+20:1	13	5	14	9	2	1	1	0·4
20:3ω9	1	46	—	15	—	52	—	50
20:3ω6	8	8	11	5	4	—	4	—
20:4ω6	162	97	98	13	227	69	190	195
20:5ω3	30	14	32	11	5	10	12	16
22:4ω6	13	8	24	6	3	5	9	6
22:5ω6	4	15	5	4	1	11	4	14
22:5ω3	35	4	40	3	20	2	26	4
22:6ω3	145	84	117	15	130	67	273	135
Total ω6	310±13	171±17	256±8	52±13	310±12	122±46	262±18	242±33
Total ω3	223±17	107±11	203±21	36±12	156±14	79±23	312±17	154±23

Table 6. *Fatty acid composition (expressed as parts/10^3 of total) of milk lipids from the stomach contents of rats soon after birth*

(Mean values for four rats/group: totals for ω6 and ω3 are means with their standard errors)

Fatty acid	Control	Low-fat
12:0	23	130
14:0	25	146
16:0	238	276
16:1	44	50
18:0	40	29
18:1	270	263
18:2ω6	203	52
18:3ω3	36	17
20:0	8	1
20:1	9	2
20:3ω9	—	3
20:3ω6	7	3
20:4ω6	37	11
20:5ω3	13	3
22:4ω6	11	4
22:5ω6	< 1	2
22:5ω3	15	3
22:6ω3	22	6
Total ω6	258±8	72±21
Total ω3	86±3	29±9

Table 7. *Some brain measurements in newborn pups of rats given a low-fat diet*

(Mean values with their standard errors; brains from two rats were pooled/sample. The number of samples is shown in parentheses)

		Brain lipids (g/kg fresh brain)			DNA-phosphorus (mg/kg fresh brain)
	Brain wt (g)	Total lipid	Neutral lipid	Phospholipid	
Control group	0·28 ± 0·01 (8)	26·9 ± 1·1 (8)	7·1 ± 0·5 (4)	20·2 ± 1·4 (4)	235 ± 5 (8)
Low-fat group	0·22 ± 0·02 (8)*	28·0 ± 0·6 (8)	8·4 ± 0·8 (4)	19·4 ± 1·2 (4)	245 ± 6 (8)

Significance of differences between dietary treatments: * $P < 0.05$.

Table 8. *Fatty acid composition (expressed as parts/10^3 of total) of brain lipids from newborn pups of rats given a low-fat diet*

(Mean values for five rats/group: totals for ω6 and ω3 are means with their standard errors)

Fatty acid	Total fatty acids		Ethanolamine phosphoglycerides	
	Control	Low-fat	Control	Low-fat
14:0	56	20	88*	92*
16:0	312	366	93	116
16:1	36	41	11	17
18:0	148	138	184	192
18:1	127	158	72	101
18:2ω6	22	6	9	4
18:3ω3 + 20:1	2	4	2	2
20:3ω9	—	14	—	22
20:3ω6	3	4	5	—
20:4ω6	119	90	194	152
20:5ω3	4	10	9	7
22:4ω6	27	18	54	38
22:5ω6	17	28	28	55
22:5ω3	4	1	8	3
22:6ω3	122	98	248	196
Total ω6	188 ± 12	150 ± 10	290 ± 16	249 ± 25
Total ω3	132 ± 7	113 ± 3	261 ± 17	206 ± 14

* 16:0 + 18:0 aldehyde.

The fatty acids of liver triglycerides usually reflect dietary fatty acids (Brockerhoff, Hoyle & Hwang, 1967). In the rat, milk lipids extracted from the stomach contents contain significant quantities (about 10%) of the C_{20} and C_{22} polyenoic fatty acids (Table 6) and the content of these fatty acids was considerably lower in the milk lipids of pups whose mothers were fed on the low-fat diet.

It was noticed that the stomachs of the newborn pups in the low-fat group were often almost empty, and it is possible that some of the changes in these pups may have been due to lack of food. To test this, two newborn control pups (2 h after birth) were left with the mother and two others were taken away and kept warm. After 7 h both pairs were killed and their tissues analysed; this starvation had no measurable effect on the body, liver and brain weights or on the concentration of liver lipids. The

Table 9. *Carcass lipids from newborn pups of rats given a low-fat diet*

(Mean values for two rats/group)

		Control group	Low-fat group
Carcass* wt (g)		4·96	4·28
Water (g/kg)		850	830
Total lipid	g/kg dry carcass	13·0	8·6
Cholesteryl ester		1·3	0·8
Triglyceride		2·8	0·7
Free fatty acids		1·6	1·1
Cholesterol		2·7	1·8
Phospholipids		4·7	4·2

* Body minus brain, liver and stomach contents.

concentration of the $\omega 6$ fatty acids in the liver triglycerides from the starved pups were about 50% less; however, there was no difference in the concentration of the $\omega 3$ polyenoic fatty acids.

The brain weight of the pups in the low-fat group was less by 20%; the amounts of lipid and DNA per brain were also smaller compared with the control group. The amount of DNA in an organ represents the number of cells in that tissue (Winick, 1968). When the lipid and DNA contents were expressed per kg fresh weight of the brain, there was no difference between the two groups (Table 7). The ratio of neutral lipid to phospholipid was the same in each group.

In both the total brain lipids and the EPG fraction the amounts of the $\omega 6$ and $\omega 3$ fatty acids were smaller in pups of the low-fat group (Table 8); the differences in the brain fatty acids were less marked than those observed for the liver fatty acids.

After the removal of the brain, liver and stomach contents from two newborn pups in each group, the lipids were extracted from the remainder of the carcasses. In the low-fat group the total lipid content of the carcass was markedly less; half of the difference could be accounted for by the smaller triglyceride concentration (Table 9).

DISCUSSION

The long-chain fatty acids from the $\omega 6$ and $\omega 3$ series are most commonly found in membrane structural lipids from mammalian cells. It has been suggested that the structural lipids are important for maintenance of membrane structure and integrity (Holman, 1968, 1970; Sinclair & Collins, 1970). Decreased amounts of $\omega 6$ and $\omega 3$ fatty acids have been found in the tissue phospholipids of rats fed on fat-free diets (Holman, 1968, 1970); under these conditions changes consistent with the alteration of the properties and function of membranes have also been observed (Snipes, 1968; Hoilund, Sundberg, Herbst & Parkin, 1970; Holman, 1970; Seiler & Hasselbach, 1971). In our experiments with pregnant rats we have examined the effect of low-fat diets on the formation of brain tissue in the offspring. Brain is perhaps the most membrane-rich tissue of the body.

In the rat, brain development commences early in foetal life but quantitatively the

most active period of development is during the first three weeks after birth. The liver lipids of the newborn rat and the milk of the mother contain long-chain polyenoic acids (C_{20}–C_{22}) and it is the long-chain polyenoic acids which have been found to accumulate in the rat brain in the first 3 weeks after birth (Sinclair & Crawford, 1972). During much of this period the rat pup is entirely dependent on milk for its nutrients; therefore the polyenoics incorporated into the developing brain will have come from the maternal system, during foetal growth, and the milk, after birth. The liver of the newborn pup contains approximately one-quarter of the total body content of arachidonate plus docosahexaenoate (A. J. Sinclair, unpublished observations); it may be said that the rat is born with a significant store of long-chain polyenoics for brain development.

In the newborn pups from the low-fat group the liver weight and the total lipid content were less than in the control pups. In particular, there was a lower concentration of liver triglycerides in the low-fat group and there was significantly less of the long-chain polyenoic acids within the triglycerides; the loss of polyenoic acids was less noticeable in the liver phospholipids. In all the liver fractions both the $\omega 3$ and $\omega 6$ acids were reduced to a similar extent in the low-fat group except in the ethanolamine phosphoglycerides where there was a relatively greater loss of the $\omega 3$ acids.

It was of special interest that the liver triglycerides from the control group contained 33 % of the total fatty acids as the C_{20}–C_{22} polyenoates. Liver triglycerides from adult rats given the control diet are rich in the C_{18} polyenoic acids (32 %) and contain only small amounts of the longer-chain derivatives (9 %) (A. J. Sinclair, unpublished observations). In general, it is known that the fatty acid composition of tissue triglycerides, but not tissue phospholipids, reflects the fatty acids of the diet and that depot triglycerides act as an energy store. Therefore the appearance of significant quantities of long-chain polyenoic acids in the liver triglycerides of the newborn rats may not only reflect the environmental conditions of the developing foetus but also provide an important store for the immediate postnatal development. As the brain structural lipids do not contain the C_{18} polyenoic fatty acids, but only the C_{20} and C_{22} polyenoic acids (Kishimoto *et al.* 1969), it is again of interest that the supply of these acids is maintained in the rat milk during the time when these acids are accumulating in the rat brain (Sinclair & Crawford, 1972).

It is likely that the liver plays a great part in both the synthesis and storage of structural lipids from dietary precursors. It is tempting to speculate that the lack of polyenoic acids in the livers from the low-fat group might be a determining factor in limiting the development of tissues, such as the brain, which are particularly dependent on lipids. Although some pups can be expected to die from accidental causes, the exceptionally high mortality in the low-fat group may be due primarily to the reduced availability of lipid for energy and essential structural purposes.

The significant differences in body-weights of pups in the two groups makes it probable that the experimental animals had a reduced energy intake or increased energy expenditure or both. Measurement of food intake in the dams in a 2-week period did not reveal any significant differences in their energy intake, and the litters of those having the low-fat diet were smaller. Thus any energy restriction seems likely to result

from an increased metabolic rate in dams or pups or both. Such changes have been reported in essential fatty acid deficiency in male rats (Holman, 1968).

An unexpected finding in our low-fat group was the relatively low concentrations of the eicosatrienoic acid (20:3ω9); this acid is derived from oleic acid and it has been reported to occur in substantial amounts in rats fed on fat-deficient diets (Holman, 1968). In rats raised from weaning to maturity on fat-free diets, the ratio of 20:3ω9 to 20:4ω6 may exceed 3 in the liver lipids (Mohrhauer & Holman, 1963). In general, the appearance of this unusual acid has been considered as an indicator of essential fatty acid deficiency. In our experiments only small amounts of 20:3ω9 were observed in the tissues of the young in the low-fat group: the ratio of 20:3ω9 to arachidonic acid was less than 1. The importance of this finding is the demonstration that considerable changes can take place in the body, liver and brain weights before the appearance of substantial amounts of 20:3ω9.

It may be that the reason for the difference between our findings with respect to 20:3ω9 and those of other workers using 'fat-free' diets is that our diet contained a small amount of essential fat. As it is virtually impossible to eat a completely fat-free diet under natural conditions (Crawford & Sinclair, 1972), the marginal deficiency may be more relevant to practical nutrition which is concerned with marginal rather than total deficiencies. If developmental changes can occur in the rat before a marked rise in 20:3ω9 in the tissues it would be important to establish other biochemical measurements which could provide an earlier index of lipid malnutrition.

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