Biochem. J. (1965) **97**, 620

The Effect of Diet on the Fatty Acid Compositions of Serum, Brain, Brain Mitochondria and Myelin in the Rat

BY L. RATHBONE*

Department of Chemical Pathology, Guy's Hospital Medical School, London, S.E. 1

(*Received* 20 *April* 1965)

1. Three groups of female rats (8–12 weeks old) were maintained respectively on a linoleic acid-rich diet, a linoleic acid-poor predominantly saturated-fatty acid diet and a normal diet. Changes in the fatty acid compositions of serum, brain, brain mitochondria-rich fraction and myelin were observed. 2. Of the serum fatty acids, linoleic acid showed the greatest change in the percentage of the total acids in response to diet; the change in the proportion of oleic acid was considerable. The percentages of arachidonic acid in serum fatty acids in the groups on the linoleic acid-rich and linoleic acid-poor diets were similar, but higher than those in the normal group. 3. Changes in the proportions of linoleic acid, arachidonic acid and docosahexaenoic acid occurred in brain fatty acids that to some extent paralleled those occurring in the serum. Changes in the proportions of most other acids in the serum fatty acids were not accompanied by corresponding changes in the brain fatty acids. 4. The percentage fatty acid compositions of a mitochondria-rich fraction and myelin are given, and changes in the relative proportions of linoleic acid, arachidonic acid and possibly some docosapolyenoic acids were demonstrated to occur as a result of diet. 5. The results are discussed in relation to the possible aetiology of multiple sclerosis.

It has been suggested that an excess of saturated fat in the diet may predispose susceptible persons to multiple sclerosis (Swank, 1950; Swank, Lerstad, Strøm & Backer, 1952). Sinclair (1956) has stated that a deficiency of essential polyunsaturated fatty acids may be a factor in the aetiology of multiple sclerosis. In view of the effect of saturated fat in increasing the requirement for essential fatty acids, at least in the rat (Peifer & Holman, 1959; Holman, 1960), these two suggestions may not be mutually exclusive. The recent demonstration that the percentage of free plus total esterified linoleic acid in serum lipids is decreased in patients with multiple sclerosis, the decrease being proportional to the severity of the disease (Baker, Thompson & Zilkha, 1964), indicates that a relative deficiency of linoleic acid may be of aetiological importance, although whether in these cases the decrease is the result or cause of the disease is unknown.

To understand more fully the interrelationship between dietary, serum and brain fatty acids, these were measured in groups of rats maintained on various diets. Similar studies of the effect of dietary fatty acids on the composition of rat-brain fatty acids have been reported (Witting, Harvey, Century & Horwitt, 1961; Mohrhauer & Holman, 1963). The present work differs from these studies in that the animals were older, being 8–12 weeks old instead of weanlings. Rats of the age used in this study remained free from the clinical signs (see Aaes-Jørgensen, 1961) and the serum from chemical indications of essential fatty acid deficiency (Holman, 1960) when maintained on the diet with the low content of linoleic acid. The process of myelination, moreover, was more advanced in these animals.

* Present address: Division of Metabolic Research, Chicago Medical School Institute for Medical Research, Chicago 12, Ill., U.S.A.

EXPERIMENTAL

Animals. Female rats of the Wistar strain, bred at Guy's Hospital Medical School, were used. Rats of 160–190g., and 8–12 weeks of age (average age 9–10 weeks), were divided into two groups of 18 animals and given a basic diet to which either coconut oil or sunflower-seed oil was added to constitute 15% (w/w) or approx. 28% of the total calories. Carbohydrate and protein constituted approx. 47 and 26% of the total calories respectively. Details of the diet were (w/w): sunflower-seed oil (Medina Refinery Ltd., London) or coconut oil (Evans Medical Ltd., Speke, Liverpool), 15%; starch (maize) (Evans Medical Ltd.), 45%; sucrose (Tate and Lyle Ltd.), 10%; Casilan (calcium caseinate, 90% of protein; Glaxo Laboratories Ltd., Greenford, Middlesex), 21%; salt mixture (Hegsted, Mills, Elvehjem & Hart, 1941) in which $CaHPO_4$,-

$2H_2O$ was replaced by an equal weight of $Ca_3(PO_4)_2$ and dietary iron was provided by ferric citrate pentahydrate, 5%; dried brewer's yeast (Evans Medical Ltd.), 4%.

Rats were fed *ad lib.*, 15g. of food being provided/animal/day. A third group of 13 rats was given a diet consisting of normal rat cake (N.E. Agricultural Co-operative Society, Aberdeen), containing 7·3% (w/w) of fat; fat, carbohydrate and protein constituted approx. 17, 55 and 29% of the total calories respectively.

Analysis of diets. Samples were extracted with 20 vol. of chloroform–methanol–water (38:19:3, by vol.) and the residues obtained by filtration re-extracted with chloroform–methanol (2:1, v/v) according to the method of Folch, Lees & Sloane-Stanley (1957) after having stood overnight at room temperature with a small quantity of water. The combined extracts were washed with 0·2 vol. of 0·1M-NaCl. Methyl esters of the fatty acids released by alkaline hydrolysis were prepared and analysed by gas–liquid chromatography (Table 1).

Tissues. Usually four animals from each of the specialized dietary groups and two normals were killed by decapitation at 0, 4, 8, 12 and 34 weeks. Blood was collected and allowed to clot for approx. 30min. before centrifuging to separate the serum. Brains from which the cerebellum was rejected were stored at −10° for up to 2 days if not analysed immediately; brains for the preparation of myelin were not frozen.

Preparation of myelin. Myelin was prepared essentially as described by Adams, Davison & Gregson (1963). Brains were initially homogenized in the homogenizer described by Webster & Smith (1964). All sucrose solutions contained EDTA (1mM). The nuclear fraction obtained from the first centrifuging at $576g_{av.}$ (Measuring and Scientific Equipment Ltd. High Speed 13 refrigerated centrifuge, 8 × 50ml. angle head) was washed with 0·32M-sucrose and discarded, the washings being combined with the original supernatant. This was centrifuged at $10320g_{av.}$ for 15min. (8 × 50ml. angle head). The particulate fraction so obtained was washed with 0·32M-sucrose, resedimented and combined with a small portion of the particulate fraction recovered by centrifuging washings pooled with the original supernatant obtained at $10320g_{av.}$. The particulate fraction was suspended in approx. 5 vol. of 0·32M-sucrose and layered on to 0·8M-sucrose. After centrifuging at $15900g_{av.}$ for 55min. (8 × 5ml. swing-out head), myelin was collected from the interface and a so-called 'mitochondria-rich fraction' from the base of the tube. The myelin was diluted with 5–6 vol. of water and allowed to stand for 15min. After centrifuging at $15900g_{av.}$ (8 × 50ml. angle head) for 20min., the pellet was resuspended in 5 vol. of 0·32M-sucrose and relayered on to 0·8M-sucrose. Myelin was collected as before.

Extraction of tissues. Total lipid extracts of serum were made by the method of Sperry & Brand (1955). Lipids were extracted from brain, myelin and the mitochondria-rich fraction as described by Folch *et al.* (1957). This material was deproteinized by evaporating solutions in chloroform–methanol–water (64:32:4, by vol.) to dryness and filtering an extract in chloroform.

Gas–liquid chromatography. Lipids from 1ml. of serum or approx. 0·1g. wet wt. of brain were hydrolysed in 90%-ethanolic N-KOH (2·0ml.) at 37° for 30min. under N_2. Water (5ml.) and 2N-HCl (1·5ml.) were added and the fatty acids extracted into ether. This extract was washed with 0·1N-HCl and dried with anhydrous Na_2SO_4. Thin-layer chromatography revealed only one spot, corresponding to fatty acids plus cholesterol. A negligible trace of sphingolipid was seen at high loading; lysoplasmalogens were not detected. Since the alkaline hydrolysis does not disrupt amide linkages, the fatty acids measured in the present study were derived exclusively from ester groups.

The dried ethereal solution was evaporated to dryness and the fatty acid methyl esters were formed by reaction with 1·5% (v/v) H_2SO_4 in methanol (2ml.) at 60° for 30min. The methyl esters were recovered by extracting with ether after the addition of water (7·0ml.). The ether extract was washed with 0·1N-HCl and dried with anhydrous Na_2SO_4. No attempt was made to remove cholesterol, but no interference from this substance was detected in the gas–liquid-chromatographic procedure.

Methyl esters were separated on a Perkin–Elmer model 800 gas chromatograph at 190° (flame ionization detector). The columns were copper tubes (6ft. × 1mm.) packed with 80–100-mesh acid-washed Celite coated with 20% (w/w) diethylene glycol adipate polyester resin LAC–IR–296. The chromatograms were analysed by triangulation and the proportions of individual fatty acids expressed as percentages of the total. The major fatty acids were identified by markers, others by comparison of the carbon numbers (Woodford & van Gent, 1960) with those calculated from published retention data (Farquhar, Insull, Rosen, Stoffel & Ahrens, 1959) and also by log-plotting (James & Martin, 1956). Retentions were measured from the beginning of the ether peak, a point derived from a calculation involving the retentions of three successive members of a homologous series (R. W. R. Baker, personal communication).

Identification of the C_{22} polyenoic acids in particular is tentative. The major docosapentaenoic acid isomer of

Table 1. *Percentage composition of fatty acids from various diets*

Fatty acid	Percentage composition of fatty acids: Normal diet	Sunflower-seed-oil diet	Coconut-oil diet
8:0	0·0	0·0	2·6
10:0	0·0	0·0	4·9
12:0	0·2	0·2	42·8
14:0	1·2	0·4	21·1
15:0	0·1	0·0	0·0
16(br)	0·1	0·0	0·0
16:0	22·0	8·6	10·6
16:1	4·1	0·9	0·5
17:0	0·2	0·0	0·0
18:0	5·0	2·6	4·6
18:1	24·7	27·7	9·3
18:2	30·2	54·2	3·1
18:3	4·3	2·7	0·3
20:0	0·0	0·3	0·2
20:1*	1·8	0·3	0·0
20:5*	0·0	1·1	0·0
22:1*	2·6	0·0	0·0
22:5*	2·6	0·5	0·0
22:5*	0·0	0·4	0·0

* Tentative identification.

brain is designated $C_{22:5\omega6}$ since this has been shown to be the major isomer by Klenk & Montag (1958) and Mohrhauer & Holman (1963). Docosahexaenoic acid of brain is designated $C_{22:6\omega3}$ (Klenk & Montag, 1958).

Nomenclature. A shorthand designation of fatty acids similar to that suggested by Farquhar *et al.* (1959) is used throughout. To distinguish between acids of the linoleic acid and linolenic acid series, the notation used by Mohrhauer & Holman (1963) has been adopted. Thus $C_{22:5\omega6}$ indicates a docosapentaenoic acid with the first double bond on the sixth carbon atom counting from the terminal methyl group (linoleic acid family); $C_{22:6\omega3}$ indicates a docosahexaenoic acid with the first double bond on the third carbon atom (linolenic acid family).

Determination of total fatty acids. This was carried out by the method of Duncombe (1963).

RESULTS

Rats maintained on the sunflower-seed-oil diet grew initially slightly faster than the normal animals, although at the end of the experimental period there was little difference in the weights of the two groups. Rats given the coconut-oil diet grew at a similar rate to the normal animals for 2 months, but after this time there was a marked decrease in the growth rate. This decrease occurred at the time when the proportion of linoleic acid ($C_{18:2}$) in serum fatty acids became minimal (Table 2 and Fig. 1*a*).

Fatty acids of serum. The effects of the various diets on the fatty acid composition of serum are shown in Table 2. In addition to the acids listed, components tentatively designated $C_{15:0}$, $C_{17:0}$, $C_{18:3}$, $C_{19:1}$, $C_{20:1}$, $C_{20:_{un}}$, $C_{22:4}$ and two isomers of $C_{22:5}$ were found in serum, usually at levels of less than 1% of the total fatty acids. These collectively accounted for less than 5% of the total fatty acids and were not significantly affected by diet. An acid tentatively identified as an eicosadienoic acid ($C_{20:2}$) was present (0·6%) in rats maintained on the coconut-oil diet for 34 weeks.

When the effects of the sunflower-seed-oil and coconut-oil diets on the percentages of various fatty acids of serum are compared, linoleic acid exhibited the greatest change (Table 2 and Fig. 1*a*). The increase in the proportion of linoleic acid in the sera of rats on the sunflower-seed-oil diet occurred concurrently with decreases in the proportions of oleic acid ($C_{18:1}$), palmitic acid ($C_{16:0}$) and palmitoleic acid ($C_{16:1}$). A relatively small increase in the proportion of arachidonic acid ($C_{20:4}$) occurred (Fig. 2*a*), and there was a marginal increase in that of stearic acid. The proportion of docosapentaenoic acid ($C_{22:5\omega6}$) was slightly lower than in the group given the coconut-oil diet, but the proportions of docosahexaenoic acid ($C_{22:6\omega3}$) were similar in both

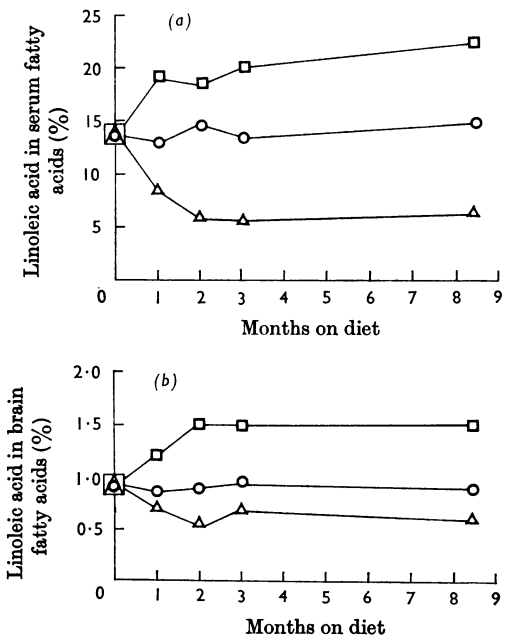

Fig. 1. Effect of diet on the percentage of linoleic acid in the fatty acids of (*a*) serum, (*b*) brain. ○, Normal diet; △, coconut-oil diet; □, sunflower-seed-oil diet. Values for serum at 8 weeks are approximate. For the remainder, the standard errors of the means are given in Table 2.

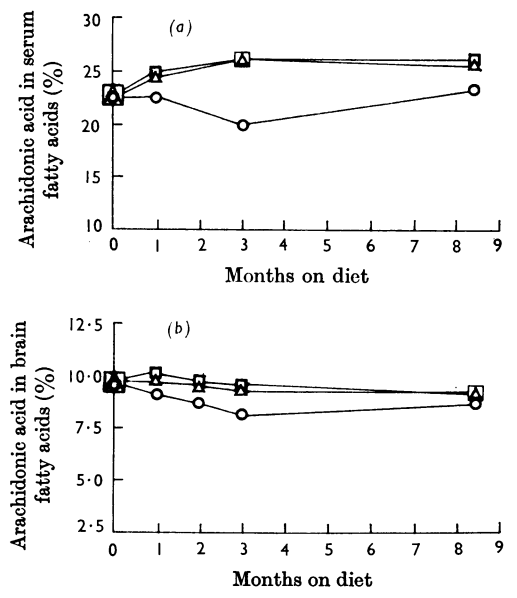

Fig. 2. Effect of diet on the percentage of arachidonic acid in the fatty acids of (*a*) serum, (*b*) brain. ○, Normal diet; △, coconut-oil diet; □, sunflower-seed-oil diet. Standard errors of the means are given in Table 3.

Table 2. *Effect of diet on the fatty acid composition of serum*

The results are expressed as means ± S.E.M.

Weeks on diet	Type of diet	No. of rats	Total fatty acids (μmoles/ml.)	Percentage composition of fatty acids										
				12:0	14:0	16:0	16:1	18:0	18:1	18:2	20:4	22:1*	22:5ω6*	22:6ω3*
0	Normal	4	7·1 ±0·6	0·15 ±0·03	0·55 ±0·03	18·2 ±0·3	4·2 ±0·1	16·0 ±0·4	15·3 ±0·6	13·8 ±0·6	22·5 ±0·8	1·9 ±0·1	1·0 ±0·0	3·9 ±0·2
4	Sunflower-seed oil	4	4·8 ±0·8	0·03 ±0·03	0·63 ±0·05	17·5 ±0·1	2·9 ±0·3	16·1 ±0·1	10·3 ±0·5	19·2 ±1·1	24·9 ±1·5	0·15 ±0·03	1·9 ±0·4	3·3 ±0·6
4	Coconut oil	4	3·9 ±0·5	2·4 ±0·8	2·4 ±0·3	18·4 ±0·6	4·5 ±0·1	17·2 ±0·7	15·0 ±0·5	8·6 ±0·6	24·4 ±1·2	0·22 ±0·09	2·8 ±1·3	2·9 ±0·5
4	Normal	2	6·4 ±0·0	0·05 ±0·05	0·60 ±0·00	16·4 ±1·0	3·7 ±0·1	17·4 ±1·4	15·9 ±0·2	13·1 ±1·1	22·7 ±0·3	2·2 ±0·5	1·1 ±0·2	4·5 ±0·4
12	Sunflower-seed oil	4	3·8 ±0·2	0·38 ±0·09	0·88 ±0·19	14·3 ±0·9	2·6 ±0·3	17·7 ±0·7	10·2 ±1·6	20·2 ±1·7	27·8 ±1·4	0·0	0·15 ±0·05	0·88 ±0·05
12	Coconut oil	4	4·0 ±0·1	3·0 ±0·1	3·1 ±0·1	17·1 ±0·5	4·4 ±0·1	16·5 ±1·0	17·8 ±1·1	5·7 ±0·4	25·6 ±0·6	0·0	0·40 ±0·14	1·3 ±0·1
12	Normal	2	4·0 ±0·2	0·4 ±0·0	0·9 ±0·0	19·0 ±0·3	4·2 ±0·7	17·7 ±0·8	15·7 ±0·6	13·6 ±0·4	19·7 ±1·7	2·4 ±0·0	0·2 ±0·0	2·1 ±0·0
34	Sunflower-seed oil	4	4·3 ±0·3	0·10 ±0·05	0·48 ±0·05	14·8 ±0·5	1·8 ±0·1	17·9 ±0·7	9·9 ±0·6	22·6 ±0·4	26·1 ±0 3	0·0	0·93 ±0·09	2·4 ±0·4
34	Coconut oil	3	4·5 ±0·4	2·3 ±0·4	3·0 ±0·3	18·2 ±0·7	3·5 ±0·3	15·3 ±0·2	17·2 ±0·5	6·6 ±0·6	25·6 ±1·3	0·0	2·1 ±0·5	2·7 ±0·4
34	Normal	4	4·6 ±0·9	0·0	0·40 ±0·06	17·9 ±0·8	2·9 ±0·1	18·3 ±1·0	11·7 ±0·4	15·1 ±0·5	23·3 ±0·8	2·4 ±0·1	0·60 ±0·12	3·7 ±0·4

* Tentative identification.

groups. The proportions of both acids, however, tended to fluctuate over the experimental period.

Increases in the proportions of lauric acid ($C_{12:0}$) and myristic acid ($C_{14:0}$) were observed in the sera of rats on the coconut-oil diet, but the final level was only approx. 3% in each case, although lauric acid and myristic acid comprised 42·8 and 21·1% of the dietary fat respectively. Palmitic acid, palmitoleic acid and oleic acid were maintained at higher, and stearic acid at slightly lower, proportions than in the group given the sunflower-seed-oil diet. The proportions of arachidonic acid were similar in both groups.

Although the normal diet differed from the two specialized diets in fatty acid composition and in the proportion of total calories contributed by fat, there was a similarity both in the general fatty acid pattern of serum and also in the absolute amount of total fatty acid/ml. for most of the experimental period. The proportion of linoleic acid in serum fatty acids lay between those of the groups given the coconut-oil and sunflower-seed-oil diets. The proportions of arachidonic acid and for most of the time docosapentaenoic acid were lower in this group than in the other two groups; the proportion of docosahexaenoic acid was higher. The serum of rats on the normal diet contained an acid comprising 1·3–2·4% of the total and tentatively identified as docosamonoenoic acid ($C_{22:1}$). This was present as a relatively minor constituent (2·6%) of the diet.

Fatty acids of brain. In addition to the acids shown in Table 3, the following acids were present in the whole brain: $C_{14:0}$, $C_{14:1}$, $C_{15:1}$, $C_{16(br)}$, and the tentatively identified $C_{20:un}$, $C_{20:5}$, $C_{22:3}$ and two isomers of $C_{22:5}$. These acids were present at less than 0·5% of the total, with the exception of $C_{14:0}$, $C_{14:1}$ and possibly $C_{14(br)}$, which were represented by a single chromatographic peak and which varied between 0·3 and 1·4%. None of these acids was significantly affected by diet.

Of all the brain fatty acids, linoleic acid showed the greatest variation in response to dietary change. Table 3 and Fig. 1(*b*) show that the changes reached a maximum at 2 months, when linoleic acid constituted 0·55, 0·90 and 1·5% of the total brain fatty acids in groups maintained respectively on coconut-oil, normal and sunflower-seed-oil diets. The proportion of arachidonic acid in brain fatty acids showed some response to dietary change (Table 3 and Fig. 2*b*). The proportions were higher in the groups given the sunflower-seed-oil and coconut-oil diets than in the normal animals. Brain docosahexaenoic acid showed a response in the reverse direction, the proportions in the brains of rats on the two specialized diets being lower than in the normal animals. Differences in the percentages of the tentatively identified docosatetraenoic acid and docosapentaenoic acid were small and no firm conclusions about the effect of diet may be drawn. As with serum, there was some fluctuation in the percentages of docosapentaenoic acid and docosahexaenoic acid over the experimental period. In view of the high degree of unsaturation of these acids, the possibility that these fluctuations are artifacts cannot be dismissed, although Kirschman & Coniglio (1961) have reported variations in the proportions of brain pentaenoic acids of male rats between 3 weeks and 6 months.

The palmitic acid and eicosamonoenoic acid ($C_{20:1}$) of brain fatty acids were maintained at relatively constant proportions in all groups; no distinct trends became apparent from a consideration of the percentages of palmitoleic acid and heptadecamonoenoic acid ($C_{17:1}$). The relative proportions of stearic acid and oleic acid appeared to change slightly in favour of oleic acid in the brains of rats given the coconut-oil and sunflower-seed-oil diets during the experiment.

Fatty acids of the mitochondria-rich fraction. In addition to the acids given in Table 4, gas–liquid chromatograms revealed a peak corresponding to $C_{14:0}$, $C_{14:1}$ and possibly $C_{14(br)}$ and others corresponding to $C_{15:0}$, $C_{20:un}$, $C_{22:3}$ and two isomers of $C_{22:5}$. These acids occurred at less than 0·8% of the total and were not significantly affected by diet. In addition, lauric acid was present at a level of 0·2% in this fraction prepared from rats maintained on the coconut-oil diet for 34 weeks. A tentatively identified eicosatrienoic acid ($C_{20:3}$) was present (0·4%) in normal rats at the start of the experiment. Two unidentified acids tentatively regarded as being unsaturated C_{20} acids were present at levels of less than 0·5%, except in normal rats initially when the combined levels reached 1·8%.

The proportions of the various fatty acids present in this fraction isolated from rats on the normal diet were similar to those in whole brain, with some exceptions (Table 4). In the mitochondria-rich fraction, the proportion of the tentatively identified eicosamonoenoic acid was about half that in whole brain; the percentage of arachidonic acid was slightly higher and the relative amount of docosapentaenoic acid ($C_{22:5\omega6}$) was more than doubled.

In the mitochondria-rich fraction from the brains of rats on the specialized diets, the changes in the proportions of linoleic acid, presumably in response to changes in the diet, were even more marked than in whole brain. The level of linoleic acid reached 2% in the fraction prepared from animals maintained on the sunflower-seed-oil diet and fell to 0·5% in the corresponding fraction from the animals given the diet containing coconut oil.

The relative proportion of arachidonic acid was higher with respect to that in the normal animals in the mitochondria-rich fraction prepared from rats on the sunflower-seed-oil diet, and there was a

Table 3. *Effect of diet on the fatty acid composition of rat brain*

The results are expressed as means ± S.E.M.

Weeks on diet	Type of diet	No. of rats	Percentage composition of fatty acids										
			16:0	16:1	17:1	18:0	18:1	18:2	20:1*	20:4	22:4*	22:5ω6*	22:6ω3 *
0	Normal	4	21·4 ±0·6	2·3 ±0·3	0·58 ±0·22	23·9 ±0·7	23·2 ±0·9	0·93 ±0·03	2·3 ±0·1	9·6 ±0·1	2·2 ±0·1	0·55 ±0·07	10·8 ±0·3
4	Sunflower-seed oil	4	20·1 ±0·2	1·5 ±0·1	1·2 ±0·1	27·1 ±0·6	18·8 ±0·5	1·2 ±0·0	2·1 ±0·0	10·1 ±0·1	3·3 ±0·1		12·1 ±0·2
4	Coconut oil	4	21·5 ±0·2	2·1 ±0·1	0·53 ±0·20	25·6 ±0·3	22·0 ±0·5	0·70 ±0·07	2·0 ±0·2	9·7 ±0·2	3·1 ±0·1		10·5 ±0·3
4	Normal	2	21·4 ±1·3	2·0 ±0·2	0·60 ±0·30	25·1 ±1·2	23·2 ±1·6	0·85 ±0·05	2·1 ±0·1	9·1 ±0·1	3·3 ±0·3		10·3 ±1·2
8	Sunflower-seed oil	2	23·7 ±0·6	2·0 ±0·2	0·50 ±0·00	25·0 ±1·0	23·1 ±1·0	1·5 ±0·0	2·2 ±0·3	9·7 ±0·1	2·6 ±0·0	1·4 ±0·0	7·4 ±0·1
8	Coconut oil	2	23·5 ±0·7	2·2 ±0·0	0·15 ±0·15	24·5 ±0·5	24·4 ±0·5	0·55 ±0·05	2·1 ±0·0	9·5 ±0·0	2·6 ±0·0	0·7 ±0·0	8·8 ±0·2
8	Normal	2	21·0 ±0·1	3·0 ±0·1	0·25 ±0·05	23·2 ±0·2	24·5 ±0·4	0·90 ±0·10	2·2 ±0·0	8·7 ±0·0	2·2 ±0·0	0·6 ±0·0	11·0 ±0·7
12	Sunflower-seed oil	4	22·6 ±0·8	2·5 ±0·1	0·52 ±0·40	23·3 ±0·8	26·2 ±0·4	1·5 ±0·1	2·0 ±0·4	9·5 ±0·4	2·4 ±0·1	0·40 ±0·04	6·6 ±0·4
12	Coconut oil	4	22·7 ±0·6	2·4 ±0·4	0·30 ±0·12	24·2 ±0·7	25·9 ±0·8	0·68 ±0·08	2·5 ±0·1	9·2 ±0·6	2·0 ±0·1	0·58 ±0·14	6·9 ±0·4
12	Normal	2	23·5 ±0·2	2·2 ±0·0	0·50 ±0·10	25·4 ±0·2	26·5 ±0·0	0·95 ±0·15	2·4 ±0·0	8·1 ±0·3	1·6 ±0·1	0·25 ±0·05	7·7 ±0·5
34	Sunflower-seed oil	4	20·3 ±0·7	2·0 ±0·2	0·68 ±0·09	23·0 ±0·8	26·6 ±0·3	1·5 ±0·1	2·3 ±0·1	9·2 ±0·2	2·6 ±0·1	1·2 ±0·1	8·6 ±0·5
34	Coconut oil	4	21·5 ±0·6	2·6 ±0·2	0·55 ±0·10	22·0 ±0·3	26·0 ±0·9	0·60 ±0·06	2·1 ±0·1	9·3 ±0·2	2·5 ±0·1	1·7 ±0·2	8·9 ±0·7
34	Normal	4	21·2 ±0·6	1·8 ±0·1	0·50 ±0·13	25·5 ±0·8	25·6 ±0·9	0·90 ±0·04	2·4 ±0·2	8·6 ±0·1	2·2 ±0·1	0·85 ±0·10	11·4 ±0·8

* Tentative identification.

Table 4. *Effect of diet on the ester-bound fatty acids of a mitochondria-rich fraction from pooled rat brains*

The brains from the four rats in each group were halved. One half-brain from each rat was pooled with a similar half-brain from a different animal for the preparation of the mitochondria-rich fraction.

Weeks on diet	Type of diet	No. of rats	Percentage composition of fatty acids										
			16:0	16:1	17:1	18:0	18:1	18:2	20:1*	20:4	22:4*	22:5ω6*	22:6ω3*
0	Previous diet normal	1+2	22·4	2·8	0·5	21·8	22·0	0·9	1·0	10·8	2·0	2·5	9·5
		3+4	22·8	2·7	0·5	23·1	19·5	0·9	1·5	11·0	2·2	2·0	10·6
		Mean.....	22·6	2·8	0·5	22·5	20·8	0·9	1·3	10·9	2·1	2·3	10·1
34	Sunflower-seed oil	1+2	20·0	2·2	0·5	24·6	21·2	1·8	1·0	11·6	2·7	2·6	10·3
		3+4	22·2	2·3	0·3	25·6	19·3	2·2	1·2	11·6	2·3	2·6	8·8
		Mean.....	21·1	2·3	0·4	25·1	20·3	2·0	1·1	11·6	2·5	2·6	9·6
34	Coconut oil	1+2	21·4	2·3	0·6	22·2	25·3	0·5	1·1	10·3	2·3	2·5	9·1
		3+4	21·9	1·9	1·1	24·5	21·1	0·5	1·1	10·9	2·4	2·6	9·1
		Mean.....	21·7	2·1	0·9	23·4	23·2	0·5	1·1	10·6	2·4	2·6	9·1
34	Normal	1+2	21·9	2·4	0·5	22·6	24·8	1·0	1·2	9·7	2·0	1·9	9·6
		3+4	22·4	1·8	1·1	23·6	19·3	1·1	1·6	10·1	2·1	2·1	11·9
		Mean.....	22·2	2·1	0·8	23·1	22·1	1·1	1·4	9·9	2·1	2·0	10·8

* Tentative identification.

Table 5. *Effect of diet on the ester-bound fatty acids of myelin from pooled rat brains*

The brains from the four rats in each group were halved. One half-brain from each rat was pooled with a similar half-brain from a different animal for the preparation of myelin.

Weeks on diet	Type of diet	No. of rats	Percentage composition of fatty acids											
			16:0	16:1	17:1	Unidentified	18:0	18:1	18:2	20:1*	20:4	22:4*	22:5ω6*	22:5*	22:6ω3*
0	Previous diet normal	1+2	13·7	2·2	1·4	1·2	20·0	33·1	1·0	3·9	5·1	1·6	5·9	0·6	4·2
		3+4	13·9	1·7	1·4	1·5	17·9	37·1	0·8	4·4	5·6	1·3	4·6	0·8	3·8
		Mean.....	13·8	2·0	1·4	1·4	19·0	35·1	0·9	4·2	5·4	1·5	5·3	0·7	4·0
34	Sunflower-seed oil	1+2	14·7	1·8	0·9	1·1	22·1	36·3	1·3	4·7	6·3	2·2	4·1	1·7	1·7
		3+4	14·7	2·1	1·0	1·2	22·1	36·4	1·2	4·7	6·5	1·6	5·7	0·8	1·4
		Mean.....	14·7	2·0	1·0	1·2	22·1	36·4	1·3	4·7	6·4	1·9	4·9	1·3	1·5
34	Coconut oil	1+2	14·9	2·8	0·8	0·7	21·6	37·5	0·4	4·6	5·6	1·5	4·1	0·8	1·7
		3+4	14·9	2·8	0·8	1·1	21·6	37·5	0·4	4·8	5·6	1·6	4·3	1·2	1·1
		Mean.....	14·9	2·8	0·8	0·9	21·6	37·5	0·4	4·7	5·6	1·6	4·2	1·0	1·4
34	Normal	1+2	15·7	2·4	1·0	1·2	21·2	37·7	0·8	4·7	5·8	1·3	3·4	0·7	1·9
		3+4	13·5	1·6	1·7	1·9	20·7	39·8	1·0	5·2	5·2	1·4	3·0	3·1	3·1
		Mean.....	14·7	2·0	1·4	1·6	20·5	38·6	0·9	4·9	5·5	1·4	3·2	1·4	2·2

* Tentative identification.

smaller increase in the fraction from the rats on the coconut-oil diet. This difference in the proportions of arachidonic acid in the mitochondria-rich fractions prepared from the two groups of rats on the specialized diets is noteworthy in view of the similarity of the proportions of arachidonic acid found in whole brain and serum of both groups at the end of the experimental period.

The proportions of docosapentaenoic acid ($C_{22:5\omega6}$) and possibly docosatetraenoic acid ($C_{22:4}$) were slightly raised in the fractions prepared from both the groups given the sunflower-seed-oil and coconut-oil diets when compared with the normal animals.

Fatty acids of rat-brain myelin. The ester-linked fatty acids of myelin are shown in Table 5. In addition to the acids listed, the following were also present: $C_{12:0}$, $C_{14:0}$, $C_{14:1}$, possibly $C_{14(br)}$, $C_{15:1}$, $C_{16(br)}$, $C_{20:0}$, two unidentified possibly C_{20} unsaturated acids, $C_{22:3}$ and one isomer of $C_{22:5}$. In normal rats initially $C_{20:2}$ was present at a level of 1·2%. Except where stated, these acids usually constituted less than 1% of the total and were not significantly affected by diet.

The proportions of arachidonic acid and particularly docosahexaenoic acid were much lower than those in whole brain; those of palmitic acid, stearic acid and docosatetraenoic acid were also lower. The relative proportions of eicosamonoenoic acid, docosapentaenoic acid ($C_{22:5\omega6}$) and heptadecamonoenoic acid were greater than in whole brain. The major ester-linked fatty acid of myelin was oleic acid, which accounted for approx. 35–39% of the total ester-linked acids. There was also an unidentified component with a retention similar to that of a branched octadecanoic acid ($C_{18(br)}$) or a heptadecadienoic acid ($C_{17:2}$). This was present in all samples of myelin analysed at a level of 0·7–1·9%. This component was poorly resolved from stearic acid under the chromatographic conditions used and was difficult to detect in methyl esters prepared from the fatty acids of whole brain extracts. It was readily detected in samples of fatty acids prepared from myelin, however, because of the relatively increased concentration. This acid appeared not to be present in the mitochondria-rich fraction, except for one sample containing 0·3% that might have been due to contamination of the mitochondria-rich fraction with myelin. A more extensive analysis of cell fractions would establish whether or not this acid is characteristic of the myelin sheath.

Table 5 shows that the fatty acids of the myelin sheath were affected by diet. The decrease in the proportion of linoleic acid of myelin in rats maintained on the coconut-oil diet was similar to that observed in whole brain and the mitochondria-rich fraction. The increase observed in rats on the sunflower-seed-oil diet was less.

The proportion of arachidonic acid in myelin appeared to be influenced by diet (Table 5). An increase was found in rats given the sunflower-seed-oil diet, but the difference between the proportions in myelin from rats on the coconut-oil-diet and the normal animals was negligible. As indicated for mitochondria, the difference between the proportions of arachidonic acid in myelin from the two groups of rats on the specialized diets is in contrast with the similarities of proportions in whole brain and sera of these groups (Tables 2 and 3).

There was possibly an increased proportion of docosapentaenoic acid ($C_{22:5\omega6}$) in myelin of rats from both specialized dietary groups and also a slight decrease in the proportion of docosahexaenoic acid. Palmitoleic acid appeared to be relatively more abundant in rats given the coconut-oil diet. More analytical data are necessary, however, to confirm these differences.

DISCUSSION

In view of the large differences between the proportions of some acids, notably linoleic acid, in serum and brain fatty acids, it is important to establish that these changes in brain fatty acid composition brought about by diet were not due to changes in the trapped blood. Assuming that blood represents at the most 5% of the fresh brain weight, a value given for the extracellular thiocyanate space by Streicher (1961), then, since the concentration of total ester-linked fatty acids of brain (approx. 100 μmoles/g. wet weight) is greater than that of serum by a factor approx. 20, it is possible to calculate that the change in the percentage of linoleic acid in brain fatty acids due to changes in the trapped blood represents only approx. 3·5% of the observed change. For this calculation, blood is assumed to have the same fatty acid composition as serum.

The remarkable similarity in the general fatty acid pattern of serum, with the exception of certain acids, in the various dietary groups is striking. After 34 weeks on the three diets, which differed markedly in fatty acid composition, the proportions of the individual serum fatty acids of the different groups differed by less than 3% of the total serum acids. Exceptions were linoleic acid (16·0%), oleic acid (7·3%) and palmitic acid (3·4%). Okey, Ostwald, Shannon & Tinoco (1962) have indicated the facility of the female rat for maintaining the concentration of plasma arachidonic acid when the supply of linoleic acid is limited. Other workers have described the relative changes of serum linoleic acid and oleic acid proportions in serum lipid fractions from various species in response to diet (Monsen, Okey & Lyman, 1962; Leat, 1963; Moore & Williams, 1964).

The variations in the composition of serum fatty acids under the influence of diet raise the question of the interrelationship of the fatty acids present in serum and brain. It appears from Figs. 1 and 2 and Tables 2 and 3 that there is an association between the proportions of linoleic acid, arachidonic acid and docosahexaenoic acid in serum and brain fatty acids. Most other acids do not exhibit a similar relationship. The proportion of oleic acid, for example, underwent wide variations in the serum, but that in the brain was relatively unaffected. This association between the relative proportions of certain acids in serum and in brain fatty acids emphasizes the possible importance of regulatory mechanisms governing the fatty acid composition in serum in determining the proportions of these acids in particular tissues.

It is noteworthy that the fatty acid composition of myelin may be altered by dietary means. The relationship between the proportion of linoleic acid in the myelin sheath and in serum at the end of the experimental period was linear (Fig. 3). Part of the change in the proportion of linoleic acid in the myelin sheath might have been due to accretion of new lipid with a different fatty acid composition. Alternatively, the changes might have been due to a metabolic turnover of the linoleic acid moiety of the myelin sheath, the final steady-state percentage being in equilibrium with that in the serum. Whatever the mechanism, however, it is clear that it is possible to produce populations of animals with different relative proportions of linoleic acid in the myelin sheath. The relative proportion of linoleic acid in myelin of the rats on the linoleic acid-rich diet differed from that of rats on the low-linoleic acid diet by a factor 3·1. There was no apparent difference in the neurological status of these groups.

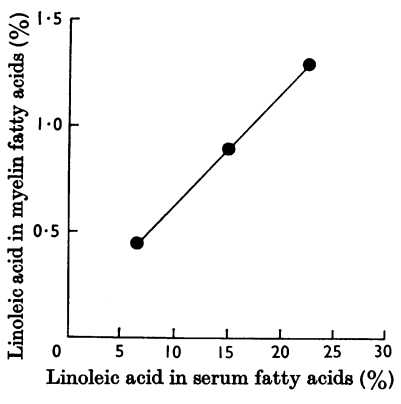

Fig. 3. Relationship between the percentages of linoleic acid in the fatty acids of serum and myelin at the end of the experimental period. The three points on the curve represent proportions of linoleic acid in the fatty acids of myelin and serum from the groups given the coconut-oil, normal and sunflower-seed-oil diets after 34 weeks on the various diets.

The demonstration that the fatty acid composition of myelin may be altered by dietary means in the rat raises the possibility of a similar phenomenon occurring in the human. This might have relevance to the various suggested roles of dietary fatty acids in the aetiology of multiple sclerosis discussed in the introduction and might in part account for the geographical distribution of the disease.

The author thanks Professor R. H. S. Thompson, who designed the dietary aspects of this work and supervised the various groups of animals during the period of the experiment. Thanks are also due to Mr P. M. Maroney and Miss J. M. Legg for technical assistance, to Mr K. G. Kilbourn for assistance with the animals and to Dr R. W. R. Baker for advice on gas–liquid chromatography. This work was carried out during the tenure of a grant from the Medical Research Council, the receipt of which is gratefully acknowledged.

REFERENCES

Aaes-Jørgensen, E. (1961). *Physiol. Rev.* **41**, 1.
Adams, C. W. M., Davison, A. N. & Gregson, N. A. (1963). *J. Neurochem.* **10**, 383.
Baker, R. W. R., Thompson, R. H. S. & Zilkha, K. J. (1964). *J. Neurol. Psychiat.* **27**, 408.
Duncombe, W. G. (1963). *Biochem. J.* **88**, 7.
Farquhar, J. W., Insull, W., Rosen, P., Stoffel, W. & Ahrens, E. H. (1959). *Nutr. Rev.* **17** (suppl.), 1.
Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
Hegsted, D. M., Mills, R. C., Elvehjem, C. A. & Hart, E. B. (1941). *J. biol. Chem.* **138**, 459.
Holman, R. T. (1960). *J. Nutr.* **70**, 405.
James, A. T. & Martin, A. J. P. (1956). *Biochem. J.* **63**, 144.
Kirschman, J. C. & Coniglio, J. G. (1961). *Arch. Biochem. Biophys.* **93**, 297.
Klenk, E. & Montag, W. (1958). *J. Neurochem.* **2**, 233.
Leat, W. M. F. (1963). *Biochem. J.* **89**, 44.
Mohrhauer, H. & Holman, R. T. (1963). *J. Neurochem.* **10**, 523.
Monsen, E. R., Okey, R. & Lyman, R. L. (1962). *Metabolism*, **11**, 1113.
Moore, J. H. & Williams, D. L. (1964). *Brit. J. Nutr.* **18**, 603.
Okey, R., Ostwald, R., Shannon, A. & Tinoco, J. (1962). *J. Nutr.* **76**, 353.
Peifer, J. J. & Holman, R. T. (1959). *J. Nutr.* **68**, 155.
Sinclair, H. M. (1956). *Lancet*, i, 381.
Sperry, W. M. & Brand, F. C. (1955). *J. biol. Chem.* **213**, 69.
Streicher, E. (1961). *Amer. J. Physiol.* **123**, 183.
Swank, R. L. (1950). *Amer. J. med. Sci.* **220**, 421.
Swank, R. L., Lerstad, O., Strøm, A. & Backer, J. (1952). *New Engl. J. Med.* **246**, 721.
Webster, G. R. & Smith, A. T. (1964). *Biochem. J.* **90**, 64.
Witting, L. A., Harvey, C. C., Century, B. & Horwitt, M. K. (1961). *J. Lipid Res.* **2**, 412.
Woodford, F. P. & van Gent, C. M. (1960). *J. Lipid Res.* **1**, 188.