Effect of Dietary Fat Intake and Age on Polyunsaturated Fatty Acids in Human-Blood Serum

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During recent years many observers (Sinclair, 1956; Page, Stare, Corcoran, Pollack & Wilkinson, 1957; Mayer, Connell, DeWolf & Beveridge, 1954; Hardinge & Stare, 1954) have shown that animal and vegetable fats differ in their effect on lipids in blood serum of human beings. It has been suggested (Ahrens *et al.* 1957; Keys, Anderson & Grande, 1957; Malmros & Wigand, 1957; Bronte-Stewart, Antonis, Eales & Brock, 1956; Hegsted, Gotsis & Stare, 1957) that the effect of dietary fats upon lipids in serum depends upon the relative proportion of saturated to polyunsaturated fatty acids. Moreover, the concentration of polyunsaturated fatty acids in serum may have a bearing on the development of atherosclerosis (Hammond & Lundberg, 1955; Schrade, Biegler & Böhle, 1958; Antonis, 1958).

It is not yet certain whether linoleic acid and higher polyunsaturated fatty acids in serum decrease with age. In view of the important role played by diet as well as by age in the aetiology of atherosclerosis, an investigation of the nature of polyunsaturated fatty acids in human serum, especially among different age groups and groups receiving different diets and having different incidence of the diseases, will be of major importance. The present work was therefore undertaken to study (a) the effect of dietary fat intake on the polyunsaturated fatty acids in serum of normal human beings and (b) the concentration of polyunsaturated fatty acids in serum of human beings of age groups between 30 and 60 years and in atherosclerotic patients.

EXPERIMENTAL

The monthly food consumption of different families was recorded and from this the calorific and fat intakes/day/man were calculated (Patwardhan & Ranganathan, 1956). From the percentages of linoleic acid in hydrogenated fats (Patil & Magar, 1956), groundnut oil and sesame oil (Hilditch, Ichaporia & Jasperson, 1938) and in cereals, pulses, vegetables and foods of animal origin (Hilditch, 1956), the consumption of linoleic acid/day/man was calculated.

Clinical examination of the 'normal' subjects failed to show any disease. Atherosclerosis was diagnosed by clinical and electrocardiographic findings.

Analysis of polyunsaturated acids in human serum. About

3 ml. of serum from fasting human beings was analysed for total fatty acids according to the method of Wiese & Hansen (1953). Polyunsaturated fatty acids were estimated by alkali-isomerization-spectrophotometric methods (Herb & Riemenschneider, 1953). Calculations for the percentage of the individual polyunsaturated fatty acids were made according to Herb & Riemenschneider (1953), as modified by Hammond & Lundberg (1953), for the inclusion of hexaenoic acid. It was assumed that the pentaenoic acids consisted of 50% of docosapentaenoic acid and 50% of eicosapentaenoic acid. Values of *t* were calculated by Mather's (1949) method.

RESULTS AND DISCUSSION

Fat in the diet. The quantity of fat derived from cereals, vegetables and fruits consumed/day/man varied from 4.0 to 7.2 g., whereas that derived from fish, meat and eggs was 10–25 g. Hydrogenated vegetable fat and fat from milk consumed were 4–12 and 5–9 g. respectively, and about 21–25 g. of groundnut oil was consumed. About 36–42 g. of sesame oil was consumed by one vegetarian group.

Effect of linoleic acid on polyunsaturated fatty acids in human serum. Total fatty acids in serum of non-vegetarians of age groups 30–40, 40–50 and 50–60 years were significantly ($P \leq 0.05$) higher than those of vegetarians on sesame oil (Tables 1–3). Total fatty acids in serum of vegetarians of age group 30–40 years were significantly lower than those of non-vegetarians (Table 1). Concentrations of tetraenoic acid in serum of vegetarians for age groups 30–40 and 50–60 years were also significantly high compared with those in non-vegetarians (Tables 1 and 3).

The concentrations of dienoic acid and tetraenoic acid in the serum of vegetarians on sesame oil in all age groups were significantly higher than those of the corresponding vegetarians and non-vegetarians (Tables 1–3). This may be due to the high intakes of linoleic acid (17.2–19.3 g.) in vegetarians who consumed sesame oil as compared with the intakes of linoleic acid in vegetarians (9.0–11.9 g.) and in non-vegetarians (8.4–9.5 g.). The concentration of trienoic acid in the serum of non-vegetarians was higher than that of the vegetarians on sesame oil for age group 30–40 years (Table 1). The value of pentaenoic acid for vegetarians on sesame oil was

Table 4. *Polyunsaturated fatty acids in human-blood serum*Results are given $\pm$ S.D.

Age group	No. of subjects	Total fatty acids (mg./100 ml.)	Polyenoic acids as percentage of total fatty acids				
			Dienoic	Trienoic	Tetraenoic	Pentaenoic	Hexaenoic
30-40	24	311 ± 18.2	23.2 ± 2.04	2.15 ± 0.31	6.65 ± 0.88	1.17 ± 0.22	2.74 ± 0.36
40-50	21	332 ± 34.1	22.4 ± 1.45	2.05 ± 0.23	6.21 ± 0.44	1.04 ± 0.15	2.51 ± 0.29
50-60	21	358 ± 30.2	21.7 ± 1.51	2.27 ± 0.20	5.66 ± 0.20	0.91 ± 0.11	2.30 ± 0.44
Atherosclerosis (50-60)	15	705 ± 85.5	13.8 ± 2.06	1.48 ± 0.32	3.75 ± 0.45	0.89 ± 0.25	2.00 ± 0.29
<i>t</i> values for differences between groups							
30-40 and 40-50	—	2.56*	1.46	1.24	2.03*	2.23*	2.23*
40-50 and 50-60	—	2.55*	1.50	3.22*	5.00*	3.16*	1.77
50-60 and 30-40	—	6.23*	2.72*	1.48	4.99*	4.8*	3.54*
50-60 and atherosclerosis (50-60)	—	16.7*	12.9*	9.32*	16.7*	0.32	2.51*

* Values significant at P 0.05.

significantly higher than that for non-vegetarians for the age group 50-60 years (Table 3).

Effect of age on polyunsaturated fatty acids in human serum. As the age increased from 30 to 60 years the total fatty acids in the serum increased significantly and the concentrations of tetraenoic acid, pentaenoic acid and hexaenoic acid usually decreased significantly (Table 4).

The percentages of trienoic acid, pentaenoic acid and hexaenoic acid in serum of Indians (Table 4) were higher than those of blood-bank donors (1.59, 0.76 and 1.3 % respectively) reported by Hammond & Lundberg (1955). Total fatty acids (311-358 mg./100 ml.) in serum of Indians were low compared with those of Europeans (464 mg./100 ml.), reported by Antonis (1958), and of Guatemalans (394 mg./100 ml.) reported by Hammond & Lundberg (1955). However, the percentages of dienoic acid and tetraenoic acid (21.7-23.2 and 5.6-6.0 % respectively) in serum of Indians were similar to those (20.5-22.0 and 4.8-6.0 % respectively) reported by Antonis (1958) and Hammond & Lundberg (1955).

Polyunsaturated fatty acids in serum of atherosclerotic patients. The total of fatty acids in serum of atherosclerotic patients was significantly higher and the concentrations of dienoic acid, trienoic acid, tetraenoic acid and hexaenoic acid in serum were significantly lower than those of normal subjects of age group 50-60 years (Table 4). The results reported here are supported by the findings of Hammond & Lundberg (1955), Antonis (1958) and Schrade *et al.* (1958) in atherosclerotic patients. Hammond & Lundberg (1955) found 492 ± 38 mg. of total fatty acids/100 ml. of serum in atherosclerotic patients compared with the results (705 ± 85 mg./100 ml.) obtained in this experiment.

SUMMARY

1. The relative concentrations of dienoic acid and tetraenoic acid in the serum of Indians were proportional to the intake of linoleic acid from the diet.

2. Total fatty acids in serum increased, and the relative concentrations of dienoic acid, tetraenoic acid, pentaenoic acid and hexaenoic acid decreased, with age.

3. In atherosclerosis, total fatty acids in serum were prominently increased and the percentages of dienoic acid, trienoic acid, tetraenoic acid and hexaenoic acid were significantly lowered compared with those of normal subjects of the same age group.

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Serum-Cholinesterase Activity in Health and in Liver Disease

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It has frequently been reported that abnormally low values of serum cholinesterase are found in the sera of patients suffering from liver disease. Although there is a considerable variation in the cholinesterase activity in normal sera (Callaway, Davies & Rutland, 1951), as assayed by the manometric method of Ammon (1933), abnormally low activities have been found in liver disease which are statistically significant (McArdle, 1940; Wilson, Calvert & Geoghegan, 1952). McArdle (1940) used acetylcholine as the substrate for the enzyme estimation and Wilson *et al.* (1952) used benzoylcholine.

Human-serum cholinesterase is now a fairly well described enzyme although its physiological role is not understood. The optimum pH for the hydrolysis of acetylcholine by the enzyme was found by Glick (1937) to lie between pH 8.4 and 8.5 at 25°. The substrate-specificity pattern of the enzyme has been extensively studied. Augustinsson (1949) found that butyrylcholine was the most rapidly hydrolysed substrate when a series of acyl choline esters were compared. Adams & Whittaker (1949) examined a series of alkyl esters and found that the maximum rates of hydrolysis were obtained with the esters of 3:3-dimethylbutanol, the carbon analogue of choline. Electrophoretic and fractionation studies reported by Surgenor *et al.* (1949); Surgenor & Ellis (1954); Kekwick, Mackay & Martin (1953) and Kekwick (1955) have shown that the enzyme is probably an α -globulin.

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In view of this fairly extensive knowledge of the nature of the enzyme in normal serum, and because the activity of the enzyme was known to be decreased in liver disease, it was thought that a comparison of the properties of the enzyme found in the serum of persons having liver disease with those of the enzyme of normal serum might reveal some differences.

MATERIALS AND METHODS

Enzyme assay. The enzyme assays were carried out in the Warburg apparatus at 37° in a medium containing 0.023 M-sodium bicarbonate and 0.15 M-sodium chloride at pH 7.4, according to the method of Ammon (1933). The gas phase was $N_2 + CO_2$ (95:5). The substrates used were acetylcholine chloride and benzoylcholine chloride, both supplied by British Drug Houses Ltd., and the perchlorates of acetylcholine, propionylcholine and butyrylcholine, which were synthesized by the method of C. A. Lewis (personal communication). The concentration of acetylcholine, butyrylcholine and propionylcholine used was 0.06 M and that of benzoylcholine was 0.12 M. The concentrations were twice those found necessary by Mendel, Mundell & Rudney (1943) to give a maximal rate of hydrolysis of the substrate by the enzyme. These concentrations were also used in the study of the effect of temperature on the enzyme, in order to compensate for any change in the Michaelis constant with temperature. Estimations of enzyme activities at temperatures higher than 30° were obtained from measurements made during the first 30 min. after mixing serum and substrate; below this temperature it was necessary to prolong the period over which measurements were made owing to the lower reaction rates involved. The reactions proceeded linearly with time over the period of measurement in all cases, indicating that the enzyme was saturated with sub-