J. Nutr. Dietet. (1964), 1, 201

ESSENTIAL FATTY ACIDS - THEIR ROLE IN NUTRITION

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(Received 1 May 1964)

1. Introduction

Until recently, the importance of fat in the nutrition of animals and man was not adequately recognised. The essential nature of fat in the nutrition of the albino rat was first discovered in 1929 by Burr and Burr[1] who observed that rats fed on a fat-free diet for 70 to 90 days did not grow and developed a deficiency syndrome characterised by scaliness of the skin and necrosis of the tail. Similar results were reported by other workers also.[2] This condition was cured by administration of linoleic acid.[3] The term 'essential fatty acid' was introduced by Burr and Burr[3] in 1930 for linoleic acid. They also suggested that linolenic acid might have a similar effect. In a later study, Burr *et al*,[4] reported that linolenic acid was effective in curing fat deficiency syndrome in rats. In 1938, Turpeinen[5] found methyl arachidonate to be highly effective in promoting growth in rats fed on a fat-free diet. During recent years, a large volume of data has been obtained by various workers on the biochemical and nutritional importance of essential fatty acids. In the present review, a brief account of the available information is given.

2. Chemistry

The chemical formula of the three essential fatty acids namely linoleic acid, γ-linolenic acid and arachidonic acid are given in figure 1; data regarding the location of the double bonds in these fatty acids are given in Table I. The location of the double bonds decides the biological potency of the fatty acids in curing fat deficiency syndrome. Thus linolenic acid which differs from γ-linolenic acid in the location of the double bonds possesses only 9% of the activity of γ-linolenic acid.

In 1953 Thomasson [6,7] proposed a numbering of the C-atoms from the terminal hydrocarbon-end of fatty acid molecules instead of from the carboxyl-end which has been the general practice. This view suggests that the presence of double bonds at the 6, 7 and 9, 10 positions counting from the terminal methyl group is fundamental for the biological activity of essential fatty acids (Table I).

Some of the important chemical properties of the essential fatty acids are as follows: (i) All of them react with halogen and this is the basis of the method for the determination of total unsaturation i.e. iodine value, (ii) Thiocyanogen reacts with the double bonds as follows: linoleic acid, one molecule of thiocyanogen; linolenic acid, two molecules of thiocyanogen; and arachidonic acid, three molecules of thiocyanogen, [8,9] (iii) The unsaturated linkages in the essential fatty acids are readily hydrogenated, (iv) Treatment with alkali at high temperatures causes the double bonds to shift, yielding conjugated isomers,[10] (v) The essential fatty acids are highly susceptible to oxidative rancidity when exposed to air, (vi) After a variable

$$CH_3-(CH_2)_4-CH=CH-CH_2-CH=CH-(CH_2)_7-COOH$$

Linoleic acid

$$CH_3-(CH_2)_4-CH=CH-CH_2-CH=CH-CH_2-CH=CH-(CH_2)_4-COOH$$

γ-Linolenic acid

$$CH_3-(CH_2)_4-CH=CH-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH-(CH_2)_3-COOH$$

Arachidonic acid

$$CH_3-(CH_2)_4-CH=CH-CH_2-CH=CH-(CH_2)_8-COOH$$

11,14-Eicosadienoic acid

Fig. 1. *Chemical formula of essential fatty acids*

Table I. *Biological activity and location of double bonds in essential fatty acids*

No. of C—atoms	Location of double bonds from :		Biological activity (growth)
	Carboxyl group	Terminal CH_3—group	
Linoleic acid 18	9: 10; 12: 13	6: 7; 9: 10	100
Linolenic acid 18	9: 10; 12: 13; 15: 16	3: 4; 6: 7; 9: 10	9
γ—Linolenic acid 18	5: 6; 8: 9; 11; 12	6: 7; 9: 10; 12: 13	100
Arachidonic acid 20	5: 6; 8: 9; 11: 12; 14: 15	6: 7; 9: 10; 12: 13; 15: 16	131
11,14-Eicosadienoic acid 20	11: 12; 14: 15	6: 7; 9: 10	43

induction period the oxygen absorption increases autocatalytically; the rates of oxidation being roughly 1:40:100:200 for oleate, linoleate, linolenate and arachidonate respectively[11] and (vii) Prolonged heat as for example during the frying of foods in oil, causes the destruction of a part of the essential fatty acids depending on the duration and severity of heat treatment.[12]

3. Methods of Assay

The methods available for the assay of essential fatty acids may be grouped as follows: (i) Chemical methods, (ii) Spectrophotometric methods, (iii) Chromatographic methods (iv) Enzymatic methods and (v) Biological assay.

3.1 Chemical methods: The methods available are (i) thiocyanogen number and (ii) polybromide method.

3. 1. 2 Thiocyanogen number: This method, evolved by Kaufmann [13] has been adopted as the official method of the American Oil Chemists Society.[14] By the determination of the thiocyanogen number and the iodine value of the fat, it is possible to calculate the oleic and linoleic acid contents of many common vegetable oils with fair accuracy. The method is, however, not applicable to fish oils containing acids of higher unsaturation.

3.1.3 *Polybromide method:* The relative solubilities of the bromides of the unsaturated fatty acids were employed by some workers for the determination of linoleic and linolenic acid content of certain fats.[15,16] This method is tedious and yields less satisfactory results than the thiocyanogen method.

3.2 *Spectrophotometric method*: Moore [17] observed that methylene interrupted double bonds in polyunsaturated fatty acids become partially conjugated upon prolonged treatment with hot alkali. Since conjugated double bonds absorb ultraviolet light of specific wavelengths in contrast to methylene-interrupted double bonds which do not possess this property the above observation has been used as the basis of a quantitative method of

assay of linolenic and linoleic acids. The method has been accepted as a tentative method by the American Oil Chemists Society.[18] This appears to be an accurate and sensitive method for the routine assay of these fatty acids. This method, however, cannot be applied to fish oils containing highly unsaturated fatty acids.

3.3 Chromatographic methods: The development of paper chromatographic method which can be used for lipid constituents has added an important technique for the separation, identification and determination of essential fatty acids.[19,20] Other methods of separation of essential fatty acids are counter-current distribution,[21,22] reverse phase chromatography[23,24] and column chromatography.[25,26] One of the newer developments in the field of fatty acid analysis is gas-liquid chromatography.[27,28] Another promising technique in lipid analysis is thin-layer chromatography.[29,30] The above techniques require special and costly equipment and trained personnel and hence not suited for routine use.

3.4. Enzymatic method: Soyabean lipoxidase has been shown to be specific in attacking only linoleic acid. Saturated fatty acids partially inhibit the enzyme, thus limiting its use to natural fats.

3.5 Biological methods

3.5.1 Growth method of Greenberg and coworkers:[31] The rationale of the growth method is similar to that suggested for the assay of vitamin A using albino rats. The rats when fed on fat deficient diet for several weeks cease to grow. After the animals attain a constant weight, if EFA or fats containing EFA are included in the required amounts in basal diet, the animals start growing. Within certain limits, the gain in body weight is proportional to the EFA added to the diet. Greenberg and associates[31] reported that when the log dose of linoleate fed was plotted against the gain in weight of male rats for doses of linoleate of 5, 10, 20 or 50 mg. per day a straight line relationship was observed at 3 weeks and continued for as long as 12 weeks. By comparing the gain in weight with the unknown oil at different levels fed and with that noted when standardised doses of linoleate are used, the linoleate equivalent of the unknown oil can be calculated. The only disadvantage of the method is that it takes 20 weeks for the depletion and assay of a sample.

3.5.2 Growth method of Thomasson[32] *based on restricted water intake* : This method proposed by Thomasson[32] is based on the disturbed water metabolism which occurs in EFA deficiency. Burr and Burr[3] made the first observation that rats on a fat-free diet drink more water than normal animals do. By restricting the intake of water to 14 ml. per day, Thomasson[32] reduced the depletion period to 5 weeks and the assay period to 4 weeks, thus making a total assay period of 9 weeks, as compared with 20 weeks required in the original assay.

4. Relative Biopotency of the Essential Fatty Acids

Linoleic, linolenic and arachidonic acids have been recognised as the chief polyunsaturated fatty acids which can cure and prevent fat deficiency symptoms in animals. Data regarding the relative biopotency of some polyunsaturated fatty acids (taking the value of linoleic acid as 100) are given in Table I. Burr *et al*[4] reported that linoleic and linolenic acids possessed about equal potency in rats in preventing or curing the fat deficiency syndrome. On the other hand, widely varying results regarding the biopotency of linolenic acid were reported by some other workers.[31,33,34] Thomasson[32] using the bioassay technique, reported a new finding which may possibly explain the divergent results of earlier workers regarding the biopotency of linolenic acid. He observed that ordinary linolenic acid (9:12:15-Octadecatrienoic acid) possessed only about 9% of the biological activity of linoleic acid while γ-linolenic acid (isolinolenic acid which is chemically 6:9:12 -Octadecatrienoic acid) has 100% of the biopotency of linoleic acid. In the original report of Burr *et al*,[4] methyl arachidonate was listed as being somewhat inferior to linoleate in its biopotency. Later workers assigned to arachidonic acid, potencies two or three times that of linoleic acid[5,35,36]. More recently Thomasson[32] reported the activity of arachidonic acid

as 131% that of linoleic acid which is now generally accepted by all workers Thomasson[32], determined the biological activity of several other polyunsaturated fatty acids and found that 11,14 -eicosadi -enoic acid possessed about 43% of the activity of linoleic acid.

5. Occurrence in Fats and Oils

The most important sources of EFA are certain vegetable fats. Animal fats in general (except fish oils) are poor sources. The EFA content of common oils and fats and foodstuffs are given in Tables II-V.

5.1 Vegetable fats and oils: Among vegetable fats and oils the rich sources of EFA are safflower seed oil, niger seed oil, soyabean oil, linseed oil, sunflower seed oil, cottonseed oil, sesame seed oil and corn oil; good sources are groundnut oil, olive oil, rapeseed oil, and poor sources are coconut oil and palmkernel oil.

5.2 Animal fats: Among animal fats the fat of egg yolk, hen and pig are good sources, fish fats are moderate sources, while ox and sheep fats are poor sources of EFA.

5.3 Hydrogenated fats : When fats are completely hydrogenated, they obviously lose all the EFA present. But partially hydrogenated fats and blends of fully hydrogenated fats and refined oils may contain appreciable amounts of EFA. A significant fraction of the polyunsaturated fatty acids in partially hydrogenated fats may be present in the form of conjugated and trans forms which are biologically inactive. Hence chemical methods may give apparently higher values for the EFA content of partially hydrogenated fats than that are actually present. The EFA values of some samples of hydrogenated fats reported in the literature are given in Table IV.

5.4 Milk fat : The EFA content of the fats of the milk of buffalo, cow and goat are low (Table V). Human milk fat contains somewhat larger amounts of EFA than that present in other milk fats.

6. Signs and Symptoms of Deficiency in Animals and in Infants

The signs and symptoms of essential fatty acid deficiency have been studied in great detail in the albino rat. Observations have also been made by some workers in other animals and in infants.

6.1 *Albino Rat* : The signs and symptoms may be described under two heads: (a) the gross symptoms directly observable on inspection of the experimental animals and (b) symptoms recognizable by means of histological examination of various tissues.

(a) *Gross symptoms* : The rats fed on a fat-free diet cease to grow after about 2 months.[1] When the feeding is continued for another 2 months, scaliness of the skin develops. The tip of the tail may become heavily scaled and rigid. Haemorrhagic spots are often seen throughout the entire length of the tail. The distal part of the tail may become swollen and may finally necrotize. Scales also develop on the dorsal and plantar surface of the feet and around the ears. Hair is lost from the back and around the face. Blood may appear in the urine.[1,3,37]

(b) *Histological changes in certain tissues*: A detailed histological study of the abdominal skin of EFA deficient rats was carried out by Ramalingaswamy and Sinclair.[38,39] They found a marked increase in the thickness of the surface epithelium, both in the stratum Malpighi and granulosum. The stratum corneum was also grossly increased in thickness. The hyperplasia of surface epithelium was especially pronounced in the regions of openings of hair follicles. This occlusion stops the outflow of sebum and results in the dry appearance of the skin. The sebaceous glands remain intact and are slightly hypertropic. Over a longer period of time, the glands may degenerate. Histological changes in the skin of the paws and ears were similar to those described above. Histological changes have also been observed in the kidneys and the primary sex organs by certain groups of workers.[2,40-41]

6.2 *Other animals* :

Mice : Young mice reared on a fat-free diet developed signs and symptoms like the rat.[42]

Chicken : The signs and symptoms of EFA deficiency in chicken include depigmentation of feathers and scaliness of the skin. In a few cases subcutaneous edema was seen.[43]

Table II. *The relative amounts of essential fatty acids in vegetable fats as determined by analysis and by bioassay**

Vegetable fat or oil	Values by analysis		Total essential fatty acids by bioassay %
	Linoleic acid %	Linolenic acid %	
Almond	19.9	0	—
Avocado	10.3	0	—
Beechnut	38.0	2.9	30.1
Castor oil	3.6	0	3.8
Cocoa butter	21.1	0	2.2
Coconut	2.6	0	1.1
Corn	39.1	0	—
Cottonseed	50.4	0	48.5, 48.0
Hempseed	68.8	24.3	—
Kapokseed	31.3	0	26.9
Linseed	46.7	60.9	11.9, 25.6
Niger	53.5	—	—
Olive	15.0	0	15.8
Palm	10.9	0	10.6
Palm kernel	0.7	0	1.5
Peanut	27.4	0	31.1
Poppyseed	62.2	—	—
Rapeseed	29.0	3.5	17.3
Safflower	78.0	0	78.8
Sesame	40.4	0	28.2
Soybean	58.8	8.1	62.4
Sunflower	68.0	0	55-65
Walnut	75.5	10.0	—
Wheat germ	—	—	52.9

* Source : Lipids, Vol. HI, p. 827 by H. J. Deuel, Jr., Interscience publishers, Inc., New York, 1957

Table III. *The relative amounts of essential fatty acids in animal and fish fats as determined by analysis and by bioassay**

Animal and fish fat or oil	Values by analysis			Total essential fatty acids by bioassay %
	Linoleic acid %	Linolenic acid %	Arachidonic acid %	
Animal fats				
Egg-yolk (hen)				
Hempseed oil diet	41.9	10.0	0	—
Linseed oil diet	24.9	17.4	0	—
Various diets	21.7	2.9	2.3	—
Phosphatides	8.9	0	0	—
Goose fat (body)	19.3	0	0	—
Hen fat (body)	21.3	0	0.6	—
Human fat	11.0	0	1.0	—
Ox depot fat	5.3	0	0.5	1.5
Pig depot fat	15.6	0	2.1	6.9
,, Soyabean oil diet	38.9	0.5	—	—
,, Peanut diet	19.7	—	—	—
,, Cottonseed oil diet (12%)	26.8	—	—	—
,, Cottonseed oil diet (8%)	18.2	—	—	—
,, Cottonseed oil diet (4%)	13.3	—	—	—
Sheep depot fat	5.0	—	—	—
Fish fats				
Cod-liver oil	9.5	0	16.5	3.9
Herring oil	10.6	0	26.9	7.9
Menhaden oil	13.2	0	19.2	4.4
Whale oil	11.0	0	33.4	6.4

* Source : Lipids, Vol. III, p. 829 by H. J. Deuel, Jr., Interscience Publishers, Inc., New York, 1957

Table IV. *The relative maximum amounts of essential fatty acids in hydrogenated fat and milk fats as determined by analysis and by bioassay**

Hydrogenated fat or milk fat	Values by analysis			Total essential fatty acids by bioassay %
	Linoleic acid %	Linolenic acid %	Arachidonic acid %	
Hydrogenated fats				
Coconut oil	0	0	0	0
Vanaspati (Groundnut oil)	4.9	—	—	—
Margarine oil I	4.8	0	0	4.17
Shortening (non-selective)	6.8	0	0	13.24
Milk fat				
Milk fat (cow)	5.8	0	0.4	—
Milk fat (goat)	1.5	0	0	—
Milk fat (human)	7.9	5.4	0	—
Milk fat (buffalo)	1.8	0	0	—

* Source: Lipids, Vol. III, p. 831 by H. J. Deuel, Jr., Interscience Publishers, Inc., New York, 1957

Guinea pigs : The deficiency symptoms which appeared after 4 to 5 weeks on the fat-free diet, were characterised by retardation of growth, dermatitis and marked dryness of the inside of the ear. In some animals, ulcers, loss of fur and tendency to swollen and somewhat cyanotic condition of the feet were observed.[44]

Hamsters: When weanling hamsters were reared on an EFA deficient diet the growth rate was low; severe scaliness developed all over the body and a pronounced loss of hair was observed. The hamsters also developed gall-stones with high cholesterol content.[45]

Dogs: Young dogs fed on an EFA deficient diet developed within 3 to 5 months, dryness of the skin and hair with desquamation, swelling and redness of the paws and emaciation.[45a]

Pigs: Leat[46] fed early weaned (17 days) piglets on a fat-free diet. After 13 weeks on the diet, the pigs showed dry flaking skin on the back and particularly over the shoulders.

6.3 *Infants* : The study of EFA deficiency in the human is difficult for obvious reasons. Only the results of a few recent studies will be mentioned here. Extensive studies of EFA deficiency in infants have been carried out by Hansen and his coworkers.[47-49] Hansen and Wiese[47] described observations in three infants kept on an almost fat-free diet for 2½ to 6 months. The respiratory quotient increased and there was a tendency for the development of a mild dermatitis. In more recent studies, Hansen *et al*,[48,49] reported that infants fed on an EFA deficient diet developed perianal irritation and changes in the skin within a few weeks. The skin changes appeared as dryness, thickening and desquamation with oozing in the intertriginous folds. Supplementation of the diet with linoleic acid restored the skin to normal condition within 2 weeks.

7. Physiological and Biochemical Functions

The fact that EFA deficiency affects growth and results in the development of pathological changes in the skin and other vital organs indicates that EFA play an important role in cellular metabolism. EFA are present in all tissues and within the cell. EFA have been recognised also in mitochondria, microsomes and cell membranes.

7.1 *Growth* : Animals fed on a EFA deficient diet cease to grow, thus indicating that some of the vital functions are affected.

7.2 *Reproduction and lactation* : Evans *et al*,[50,51] were the first to show that animals fed on EFA deficient diet do not reproduce normally. This observation has been confirmed by other workers.[6,52] Loosli *et al*.,[53] observed improved lactation performance of rats fed diets containing corn oil as compared with animals fed fat-free diet or a diet containing hydrogenated fat. In recent studies of Deuel *et al*,[54] high mortality of young ones was observed

Table V. *The polyunsaturated and saturated fatty acid contents and the P/S ratio of certain common food fats and oils**
(% of total fatty acids)

Food	Poly-unsaturated fatty acids %	Saturated fatty acids %	Mono-unsaturated fatty acids %	P/S ratio
I. *Eggs, meat, fish, nuts*				
Eggs	14	34	52	0.41
Beef	3	43	54	0.07
Lamb	3	59	38	0.05
Poultry : Chicken & turkey	22	30	48	0.73
duck	31	26	43	1.19
Pork, Ham, Bacon, Frankfurters	10	39	51	0.26
Luncheon *meat*				
Liver	41	36	23	1.14
Fish & sea food	75	25	0	3.00
Nuts, unspecified	32	21	47	1 52
Peanuts	31	23	46	1.35
Walnuts	70	8	22	8.75
Pecan	20	6	74	3 33
Almond	27	7	66	3.86
Filbert	10	12	78	0.62
Cashew	8	18	74	0.44
Coconut	2	92	6	0.02
II. Milk and milk products	3	70	27	0.04
III. Fruits	11	30	59	0.37
V. Fats and oils				
Fats : Butter	3	60	37	0.05
Lard	12	40	48	0.30
Oils : Coconut	2	92	6	0.02
Corn	55	12	33	4.58
Cottonseed	52	26	22	2.00
Herring	79	21		3.76
Linseed	25	11	64	2.27
Niger	54	9	37	6.00
Olive	5	9	86	0.55
Palm kernel	1	85	14	0.01
Peanut	31	19	50	1.63
Safflower	75	7	18	10.71
Sesame	43	14	43	3.07
Soyabean	64	18	18	3.56
Sunflower	53	12	35	4.42
Whale	81	19		4.26

* Source : Jolliffe, N. (1961), *Metabolism*, 10, 497

before weaning when the fat-free diet of the mothers was supplemented only with small amounts of cottonseed oil (10mg) or linoleic acid (10mg). However, when the quantity of cottonseed oil was increased to 100 or 200 mg. or of linoleic acid to 80 mg. there was no mortality and the weaning weights of rats were normal, thus indicating that lactation of the mothers was normal when the diet contained adequate amounts of EFA.

7.3 *Integrity of cell membrones and cells*: The fact that EFA deficiency causes a drastic increase in the permeability of the skin of animals may indicate that EFA are of importance for the structural integrity of cell membranes. The EFA occur as esters of cholesterol in a number of phospholipids and as part of a number of lipoproteins in cell and mitochondrial

membranes.[55] Thus the increased permeability of skin to water and the increased fragility and permeability of the capillaries, are probably due to structural changes in the cells. Several groups of workers have reported that the mitochondria of EFA deficient rats are much more fragile than that of normal rats.[56-58]

7.4 *EFA and tissue enzyme systems*: Swanson and Artom[59] and Kunkel and Williams[60] investigated the effect of fat deficiency upon the activity of certain oxidative enzyme systems. A marked decrease in the endogenous respiration and a marked increase in liver cytochrome oxidase activity were observed. Tulpule and Patwardhan[61] observed a significant decrease of the activity of succinic, glutamic and butyric dehydrogenases of liver of fat deficient rats. The possibility of uncoupling of oxidative phosphorylation in EFA deficiency was suggested.[62] The main site of action of EFA appears to be in the phosphate esterification system, coupled with the oxidation of reduced cytochrome C. Recently, Holman and Widmar[63] examined the EFA in beef heart mitochondria and derived enzymatically active lipoprotein fractions. The mitochondria and the fractions obtained from them contained considerable amounts of lipids and the EFA content of the fatty acids isolated from the various fractions ranged from one to two thirds of the total amounts of fatty acids. Although no relationship could be seen between a specific enzyme activity and EFA, the data suggest that EFA are constituents of particulate enzyme systems.

7.5 *Transport of cholesterol and other lipids*: A number of reports have indicated that EFA may be required for the normal transport of lipids *in vivo*. According to Blomstrand[64] and Blomstrand *et al*,[65] linoleic acid is transported in the lymph largely as triglycerides. Using labelled linoleate, Mead and Fillerup[66] showed that more than half of the ingested linoleate appeared in the blood plasma as phospholipids half an hour later. This level decreased only slightly later on. The rapid conversion of linoleate to phospholipids and to cholesterol esters evidently takes place in the liver. Accumulation of cholesterol esters in liver has been reported to occur in EFA deficient animals.[67-69] The serum lipoprotein complex contains free and esterifed cholesterol phospholipids and triglycerides and much of the chlolesterol is present as esters of EFA.[70] Holman[71,72] suggested that cholesterol esters of EFA[70] are required as part of the serum lipoprotein lipids in a constant proportion. Thus any condition causing increased transport of cholesterol or neutral fat will require a corresponding increase in EFA from food fat or release of EFA from the tissues.[72] The appearance of EFA deficiency symptoms in rats by supplementing a fat-free diet with 2% cholesterol and 3% hydrogenated fat has been reported.[3,73,74] The role of EFA in the regulation of blood cholesterol is dealt within the next section (Section 8).

7.6 *EFA and water balance*: There is considerable increase in the water consumption of EFA deficient animals.[3,5,7,38] This is probably due to the increased loss of water through skin due to the increased permeability of the skin to water in EFA deficiency.[75,76]

7.7 *Protection against X-ray irradiation*: Cheng *et al*,[77] reported that incorporation of cottonseed oil in a fat-free diet even at 2% level affords protection against multiple sublethal doses of X-ray. Cheng and Deuel[78] proved that hydrogenated coconut oil gave no protection. In subsequent studies of Deuel *et al*,[79] it was demonstrated that the protective effect of cottonseed oil was due to the EFA present in it. More recently Cheng *et al*,[30] established that the optimal daily protective dose of linoleate for male rats against X-ray irradiation injury is about 100 mg.

8. Role in Cholesterol Metabolism

In 1952, Groen *et al*,[81] showed that purely vegetarian diets are associated with a low serum cholesterol level. In the same year, Kinsell *et al*,[82] showed that the ingestion of certain vegetable oils in place of the customary animal fats was followed by a major fall in serum cholesterol and phospholipid levels. These findings of Kinsell *et al*.,[82] were soon confirmed by several laboratories[83-88] so that it is now recognized that the substitution of

vegetable, fish and marine mammal oils rich in polyunsaturated fatty acids for fats rich in saturated fatty acids leads in most subjects, to a major fall in the serum cholesterol and other lipid levels. Various investigators have attributed the change in serum lipid levels either to (i) the reduction in saturated fatty acids in the diet, or to the increased amount of the polyunsaturated fatty acids, (ii) the change in net unsaturation as measured by the iodine number, or (iii) the change in the ratio between polyunsaturated fatty acids (P) and saturated fatty acids (S) i.e. to the P/S ratio. The P/S ratio of pure fats and fats in certain common foods is given in Table V. It should be pointed out here that in the calculation of the P/S ratio, only linoleic acid or its biological equivalents can be included as a polyunsaturated fatty acid. This means that iso-acids, conjugates and trans isomers should not be included even though polyunsaturated. This does not involve any problem with the edible oils and fats unless they have been partially hydrogenated. This is discussed later under net unsaturation.

8.1 *Net unsaturation*: Ahrens *et al*,[89] have proposed that the effect of the dietary fats on serum cholesterol levels is a function of their net unsaturation as measured by their iodine number. Fats and oils with iodine numbers of 85 to 144 tended to lower the serum cholesterol, while fats and oils with iodine numbers from 70 down to 10 tended to raise it. His data, however, showed little difference between the effects of butter fat with an iodine number of 40 and coconut oil with an iodine number of 10; or between corn oil with an iodine number of 126, safflower oil with an iodine number of 144, or in a later study, with menhaden oil with an iodine number of 179. In this later study, Ahrens *et al*[90] substituted menhaden oil with an iodine number of 179 isocalorically in the feeding formula for corn oil with an iodine number of 126. The serum cholesterol level remained the same in one subject, and in the other, fell from 253mg per cent after feeding corn oil to 158 mg per cent on the fish oil and rose to 197 mg per cent on resubstitution of corn oil for menhaden oil. The original study of Ahrens *et al*,[90] which included coconut oil, cocoa butter, butter beef, four samples of lard, palm, chicken, olive, peanut, cottonseed, corn and safflower oils and which showed an inverse relation between serum cholesterol and the iodine number would fit equally well if expressed in terms of the P/S ratio. The use of the P/S ratio should be restricted in dietary calculation to those instances in which the conjugated and trans-acid forms of the polyunsaturated fatty acids (such as are contained in conventional partially hydrogenated shortenings and margarines) are absent or are present only in negligible amounts. This is because these forms increase both iodine number and the P/S ratio with, as yet, no demonstration of a concurrent cholesterol lowering activity. Controlled studies are needed in this area. The effects on the serum cholesterol of the various types of polyunsaturated fatty acids are not necessarily related to their greatly varying iodine values and P/S ratios or to their EFA activity. This pertains especially to the fatty acids with 3 or more double bonds. It is obvious that much basic research remains to be done on the effects of oils with very high iodine values (over 145) and P/S ratio (over 9.0) on cholesterol metabolism. For example, it will be necessary to explain why an oil with extremely high iodine number such as menhaden with 179 has about the same effectiveness in lowering serum cholesterol as corn oil with 126 and safflower oil with 144. Important exceptions to the relationship between the P/S ratio and hypocholesterolemic effect are rare in naturally occurring edible food fats. Tung oil, which does not lower serum cholesterol in the rat despite an iodine value of 250 and a P/S ratio of about 20, is not an exception since this oil is extremely rich in a conjugated trienoic acid, eleostearic. This conjugated polyunsaturated fatty acid does not lower the serum cholesterol.

In the menhaden oil study by Ahrens *et al*,[90] however, the essential fatty acids, as determined by biossay, was 4 units when compared to linoleic acid which yielded 100 units. Gas chromatography indicated that the menhaden oil contained 2.0% linoleic acid, 1.3% linolenic acid and 0.6% ara-

chidonic acid, while corn oil contained 53.5% linoleic acid and no linolenic or arachidonic acids. Thus the highly polyunsaturated menhaden oil, extremely poor in linoleic acid or its biological equivalent in the form of linolenic or arachidonic acids, but rich in polyunsaturated acids, gives as good or better results as corn oil rich in linoleic acid. At the other end of the spectrum, a synthetic mixture of medium-chain fatty acids (6–12 carbon atoms) with an iodine number less than 1, distilled from coconut oil will lower the serum cholesterol [91] when substituted for an equal amount of butter fat with an iodine number of 40. The exact mechanism of action of medium chain saturated fatty acids (6-12 carbon atoms) in lowering cholesterol level has not yet been understood.

9. Role in the Prevention of Atherosclerosis

A considerable volume of evidence has been built up correlating difference in the EFA content and amount of fat in the diet with the incidence of atherosclerosis.[92,93] Rosenthal's[94] review of literature upto 1934 indicated a positive correlation between the intakes of large amounts of dietary cholesterol and animal fat and the occurrence of atherosclerosis and a very low incidence of the disease in groups consuming diets low in fat and cholesterol. Data collected by Malmros[95] on dietary pattern in several European countries during world War II indicated that population groups having major dietary restriction in fat and fatty foods had a definite decrease in deaths due to atherosclerosis. The studies of Bronte-Stewart and his associates[96,97] in South Africa emphasised the striking difference in blood lipid levels and occurrence of atherosclerosis which can occur in a single political unit in population groups with outstandingly different dietary habits in the nature and quantities of fat consumed. The incidence of atherosclerosis is rare in the Bantu, is moderately high in the coloured people of mixed Malay and European stock and very high among the Europeans. The serum cholesterol levels and the intake of animal fats roughly paralleled the incidence of atherosclerosis in the above groups. Further convincing proof of this is seen in comparisons of Japanese who have remained in Japan with those who have migrated to Hawaii or U.S.A. The incidence of atherosclerosis in the latter two groups is very much higher than those in the former.[98,99]

Preliminary results of practical application of the knowledge in patients suffering from atherosclerosis have been published.[90,99a] In one study 280 patients with cardiac infarction were followed up for a period of 4 years. During this period, the incidence of deaths were four times greater in those consuming the usual high fat diet than in those consuming a low-fat diet of approximately 50g fat per day.[99a] In another study in a metabolic ward,[90] considerable subjective improvement and a lowering of serum cholesterol were recorded as a result of incorporating vegetable oils with a high EFA content. There is no doubt that many more studies on the beneficial effects of consuming oils rich in EFA in lowering the serum cholesterol level and in decreasing the severity of atherosclerosis will be published in the near future.

10. Requirements

Deuel[100] concluded on the basis of linoleic acid required for maximum growth, that the daily requirements of male rats is about 200 mg while that of female rats is 20 to 50 mg. Wiese, Hansen and Adam[101] from studies with infants found that EFA should provide about 4% of the daily calories. On this basis, an adult consuming 3000 calories will need about 13g of EFA. This quantity can readily be provided by 20 to 50g of common vegetable oils depending on their EFA contents. In view of the important role of EFA in maintaining the blood cholesterol content at a normal level, it will be essential to ensure that the adult diet should contain at least 13g of EFA in the form of vegetable oils.

11. Conclusion

It is evident from the present review that the essential fatty acids play an important role in animal and human nutrition. The most important of the functions of EFA are (i) maintenance of the integrity of cells of various tissues

and (ii) maintenance of the cholesterol content in the blood at normal levels. A deficiency of EFA in the diet may lead to several disorders such as skin changes, increased capillary fragility and increased lipid and cholesterol levels in the blood. The last condition may be one of the contributory causes towards the development of atherosclerosis. In view of the fact that several common vegetable oils are rich sources of EFA, it should be possible to ensure an adequate intake of EFA in average human diets.

REFERENCES

1. Burr, G. O. and Burr, M. M. (1929), *J. Biol. Chem.*, **82**, 345.
2. McAmis, A. J., Anderson, W.E. and Mendel, L.B. (1929), *J. Biol. Chem.*, **92**, 247.
3. Burr, G. O. and Burr, M. M. (1930), *J. Biol. Chem.*, **86**, 587.
4. Burr. G. O., Burr, M. M. and Miller, E. S. (1932), *J. Biol. Chem.*, **86**, 589.
5. Turpeinen, O. (1938), *J. Nutr.*, **15**,351.
6. Thomasson, H. J. (1956), *In 'Intern. Conf. Biochem. Problems Lipids,'* Ed. by G. Popjak and E. LeBreton, Butterworth, London.
7. Thomasson, H. J. (1953), In *'Intern. Conf. Biochem. Problems Lipids'*, by R. Ruyssen, Brussels, Paleis Der Academien.
8. Lambou, M. G. and Dollear, F. G. (1945), *Oil and Soap*, **22**, 226.
9. Lambou, M. G. and Dollear, F. G. (1946), *Oil & Soap*, **23**, 97.
10. Mitchell, J. H., Jr., Kraybill, H. R. and Zscheile, F. P. (1943) *Indust. Eng. Chem. Anal Ed.*, **15**, 1.
11. Holman, R.T. and Elmer, O. (1947), *J. Amer. Oil Chemists Soc.*, **24**, 127.
12. Crampton, E. W., Common, R. H., Farmer, F. A., Wells, A.F. and Crawford, D. (1953), *J. Nutr.*, **49**, 333.
13. Kaufman, H.P. (1926), *Z. untersuch Lebensm.*, **51**,15.
14. AOCS (1946), *Official and Tentative Methods*, Sect. C. Cd., **2**-38, pp. 1-6.
15. Ault, W. C. and Brown, J. B. (1934), *J. Biol. Chem.*, **107**, 615.
16. White, M F. and Brown, J. B. (1949), *J. Amer. Oil Chemists Soc.*, **26**, 385.
17. Moore, T. (1937), *Biochem. J.*, **31**, 138.
18. Stillman, R C. (1949), *J. Amer Oil Chemists* Soc., **26**,389.
19. Inouye, I., Noda, M. and Hiroyama, J. (1955), *J. Amer. Oil Chemists Soc.*,**32**, 132
20. Schlenk, H., Gellerman, J.L., Tillotson, J.A., and Mangold, H. K. (1957), *J. Amer. Oil Chemists Soc.*, **34**, 377.
21. Ahrens, E. H. Jr. (1955), In *'Intern. Conf. Biochem. Problems Lipids,'* Ed., by G. Popjak and E. LeBreton, Butterworth, London.
22. Dutton, H.J. (1954), In *'Progress in Chemistry of Fats and Lipids,'* Ed., by R. T. Holman, W. O. Lundberg and T. Malkin, Pergaman Press, London.
23. Howard, G. A. and Martin, A. J. P. (1950), *Biochem. J.* **46**, 532.
24. Mead, J.F. (1957), *J. Biol. Chem.*, **227**, 1025.
25. Hirsch, J. and Ahrens, E. H , Jr. (1958), *J. Biol. Chem.*, **233**, 311.
26. Klein, P.D. and Janssen, E. T. (1959), *J. Biol. Chem.*, **234**, 1417.
27. Farquhar, J.W., Insull, J. W., Jr., Rosen, P., Stoffel, W. and Ahrens, E. H., Jr. (1959), *Nutr. Revs.*, **17** (Supple. II)
28. Stoffel, W., Insull, W., Jr. and Ahrens, E. H. Jr. (1958), *Proc. Soc. Exptl. Biol. Med.*, **99**, 238.
29. Jatzkewitz,H. and Mehl,E. (1960), *Z. Physiol. Chem.*, **320**, 251.
30. Mangold, H. K. and Malins, D. C. (1950), *J. Amer. Oil Chemists Soc.*, **37**, 383.
31. Greenberg,S.M., Calbert, C.E., Savage, E.E. and Deuel,H. J.,Jr. (1950), *J. Nutr.*, **41**, 473.
32. Thomasson,H. J.(1953), *Inter. Rev. Vit. Res.*, **25**, 62.
33. Tange,U. (1932), *Sci. Papers Inst. Phys. Chem. Res.* (Tokyo), **20**, 13.
34. Deuel, H. J., Jr., Martin, C. R. and Alfin-Slater, R. B. (1955), *J. Nutr.*, **57**,297.
35. Hume, E. M., Nunn, L. C. A., Maclean, I.S. and Smith, H.H. (1940),*Biochem. J.*, **34**,879.
36. Greenberg,S.M., Calbert, C.E., Deuel, H. J., Jr. and Brown, J. B. (1951), *J. Nutr.*, **45**, 521.
37. Burr, G. O. (1942), *Fed. Proc.*, **1**, 224.
38. Ramalingaswamy, V. and Sinclair, H. M. (1951), *Brit. J. Nutr.*, **5**, x.
39. Ramalingaswamy, V. and Sinclair, H. M. (1953), *Brit. J. Dermatol.* **65**, 1.
40. Evans, H. M., Lepkovsky, S. and Murphy, E. A. (1934), *J. Biol. Chem.*, **106** 445.
41. Panos, T.C. and Finerty, J.C. (1953), *J. Nutr.* **49**, 397.
42. White, E. A., Foy, J. R. and Cercedo, L. R. (1943), *Proc. Soc. Exptl. Biol. Med.*, **54**, 301.
43. Bieri, J. G., Briggs, G. M., Fox, M.R.S., Pollard C. J. and Ortiz, L. O. (1956), *Proc. Soc. Exptl. Biol. Med.*, **93**, 237.
44. Reid, M. E. (1954), *Proc. Soc. Exptl. Biol. Med.*, **86**, 708.
45. Dam, H. and Christensen. F., (1952), *Acta. Pathol. Microbiol. Scand.*, **30**, 236.

45a. Hansen, A. E. and Wiese, H. F. (1951), *Texas Repts. Biol. and Med.*, **9**, 555.

46. Leat, W.M.F. (1959), *Proc. Nutr. Soc.*, **18**, xxxi.
47. Hansen, A. E. and Wiese, H. F. (1954), In *'The Vitamins'*, Ed. by W. H. Sebrell, Jr. and R. S. Harris, New York Academy Press, U. S. A.
48. Hansen, A. E., Adam, D. J. D., Wiese, H.F., Boelsche, A. N. and Haggard, M. E. (1958), In *'Essential Fatty Acids,'* Ed., H. M. Sinclair, Butterworth, London.

49. Hansen, A. E., Haggard, M. E., Boelsche, A. N., Adam, D. J. D. and Wiese, H. F. (1958), *J. Nutr.*, **66**, 565.
50. Evans, H. M., Lepkovsky, S. and Murphy, E. A. (1934), *J. Biol. Chem.*, **106**, 431.
51. Evans, H. M., Lepkovsky, S. and Murphy, E. A. (1934), *J. Biol. Chem.*, **106**, 443.
52. Quackenhush, F. W., Kummerow, F. A. and Steenhock, H. (1942), *J. Nutr.*, **24**,213.
53. Loosli, J. K., Lingenfelter, J. F., Thomas, J. W. and Maynard, L. A. (1944), *J. Nutr.* **28**, 81.
54. Deuel, H. J. Jr., Martin, C. R. and Alfin-Slater, R. B (1954), *J. Nutr.*, **54**, 193.
55. McMillan, A. N. and Sinclair, H. M. (1958) In *'Essential Fatty Acids'*, Ed. by H. M. Sinclair, Butterworth, London
56. Klein, P. D. and Johnson, R. M. (1954), *J. Biol. Chem.*,**211**, 103.
57. Levin, E., Johnson, R. M and Albert, S. (1957), *J. Biol. Chem.*, **228**, 15.
58. Hayashida, T. and Portman, O. W. (1960), *Fed. Proc.*, **19**, 324.
59. Swanson, M. A. and Artom, C. (1950), *J. Biol. Chem.*, **187**, 281.
60. Kunkel, H.O. and Williams, J.N., Jr. (1951), *J. Biol. Chem.*, **189**, 755.
61. Tulpule, P. G. and Patwardhan, V. N (1952), *Arch. Biochem. Biophys.*, **39**,450.
62. Tulpule, P.G. and Williams, J.N., Jr. (1955), *J. Biol. Chem.*, **217**, 229.
63. Holman, R. T. and Widmer, C., Jr. (1959), *J. Biol. Chem.*, **234**, 2269.
64. Blomstrand, R. (1954), *Acta Physiol. Scand.*, **32**, 99.
65. Blomstrand, R., Dahlback, O. and Linder, E. (1959), *Proc. Soc. Exptl. Biol. Med.* **100**, 769.
66. Mead, J.F. and Fillerup, D.L. (1957), *J. Biol. Chem.*, **227**, 1009.
67. Beveridge, J. M. R. and Lucas, C. C. (1945), *J. Biol Chem.*, **157**, 311.
68. Alfin-Slater, R.B., Aftergood, L., Wells, A.F. and Deuel, H. J., Jr. (1954), *Arch. Biochem. Biophys.*, **52**, 180.
69. Dam, H., Prange, I. and Sondergaard, E. (1955), *Acta. Physiol. Scand.*, **34**, 141.
70. Kelsey, F. E. and Longenecker, H. E. (1941), *J. Biol. Chem.*, **139**, 727.
71. Holman, R. T. (1958), *Sevensk. Kem. Tidskr.*, **68**, 282.
72. Holman, R.T. (1958), *Nutr. Revs.*, **16**, 33.
72a. Holman, R.T., Hayes, H., Malmros, H. and Wigand, G. (1957), *Proc. Soc. Exptl. Biol. Med.*, **96**, 705.
73. Peifer, J. J. and Holman, R. T. (1955), *Arch. Biochem. Biophys.*, **57**,520.
74. Holman, R.T. and Peifer, J. J. (1960), *J. Nutr.* **70**, 411.
75. Kramar, J. and Levine, V. E. (1953), *J. Nutr.* **50**, 149.
76. Basnayake, V. and Sinclair, H. M. (1954), *J. Physiol.*, **126**, 55.
77. Cheng, A. L. S., Kryder, G. D., Bergquist, L. and Deuel, H.J., Jr. (1952), *J. Nutr.*, **48**, 161.
78. Cheng, A. L. S. and Deuel, H. J., Jr. (1953), *Fed. Proc.*, **12**, 410.
79. Deuel, H. J., Jr., Cheng, A. L. S., Kryder, G. D. and Bingemann, M.E. (1953), *Science*, **117**, 254.
80. Cheng, A. L.S., Ryan, M., Alfin-Slater, R. B. and Deuel, H.J., Jr. (1954), *J. Nutr.*, **52**,537.
81. Groen, J., Tjiong, B. K., Kaminga, C. E. and Willerbrand, A. F. (1952), *Voeding*, **13**, 556.
82. Kinsell, L. W., Partridge, J., Boling, L., Margen, S. and Michaels, G. (1952), *J. Clin. Endocrinol.*, **12**, 909.
83. Ahrens, E. H., Tsaltas, T. T., Hirsch, J. and Insull, W. (1955), *J. Clin. Invest.*, **34**, 918.
84. Beveridge, J., Connell, W. F., Mayer, G. A., Firstbrook, J.B. and DeWolfe, M. S. (1955), *J. Nutr.*, **56**, 311.
85. Beveridge, J., Connell, W. F., Mayer, G. A., Firstbrook, J. B. and DeWolfe, M. S. (1956), *Can. J. Biochem. & Physiol.*, **34**,441.
86. Jolliffe, N., Rinzler, S. and Areher, M. (1959), *Amer. J. Clin. Nutr.*, **7**, 451.
87. Malmros, H. and Wigand, G. (1955), *Minn. Med.*, **38**, 864.
88. Malmros, H. and Wigand, G. (1957), *Lancet*, **2**, 1.
89. Ahrens, E. H., Hirsch, J., Insull, W., Tsaltas T.T., Blomstrand, R. and Peterson, M. L. (1957), *Lancet*, **1**, 943.
90. Ahrens, E. H., Insull, W., Hirsch, J., Stoffel, W., Peterson, N. L., Farquhar, J.W., Miller T. and Thomasson, H. J. (1959), *Lancet*, **1**, 115.
91. Hashim, S. A., Arteoga, A. and Van Itallie, T. B. (1960), *Lancet*, **1**, 1105.
92. Kinsell, L. W. (1963), In *'The Progress In the Chemistry of Fats and other Lipids,'* Vol. 6, Eds. R. T. Holman, W. O. Lundberg and T. Malkin, Pergaman Press, Oxford.
93. Bronte-Stewart, B. (1962), In *'Clinical Nutrition'*, Ed. by N. Jolliffe, Second Ed., Harper and Brothers, Publishers, New York.
94. Rosenthal, S. R. (1934), *Arch. Path., 18*, 473, 660.
95. Malmros, H. (1950), *Acta. Med. Scand.*,Suppl. **246**, 137.
96. Brock, J. F. and Bronte-Stewart, B. (1955), *Minn. Med.*, **38**, 852.
97. Bronte-Stewart,B., Keys, A. and Brock, J. F. (1955), *Lancet*, **2**, 1103.
98. Keys, A. (1958), *Brit. Med. J.*, **2**, 98.
99. Larsen, N. P. (1955), *Am. Pract.*, **6**, 1681.
99a. Lyon, T. P., Yankley, A., Gofman, J. W. and Strisower, B. (1956), *Calif. Med.*, **84**,325.
100. Deuel, H. J., Jr. and Reiser, R. (1955), In *'Vitamins and Hormones,'* Vol. 13, Ed. by R. S. Harris, G. F. Marrian and K. V. Thimann, Academy Press, New York.
101. Wiese, H. F., Hansen, A. E. and Adam, O. J. D. (1958), *J. Nutr.*, **66**, 345