Essential Fatty Acids as Food Supplements

E. AAES-JORGENSEN, Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen, Denmark

One of a series of papers from the San Francisco ACS meeting on the intentional use of additives to improve nutritional status of foods

THE CLASSICAL EXPERIMENTS of Burr and Burr (*13, 14*) with fat-free diets for rats led, some 30 years ago, to discovery of the essential fatty acids (EFA). Lack of these acids in the diet causes development of the EFA-syndrome, which so far is best described in rats.

The EFA deficiency in weanling rats is characterized by cessation of growth in about three months. Skin signs are scaliness of the tail and feet, dandruff, failure of yellow-brown pigment to develop on the back, rough fur, and in severe cases development of caudal necrosis, or death of tail tissue. Histological studies of skin have revealed a distinct increase in the cell layers, and degeneration of the oil-secreting glands (*17, 32*); this latter finding is probably related to the lack of pigment on the backs of EFA-deficient animals.

Other characteristic indications of EFA deficiency are pronounced increases in calorie and drinking fluid consumption, and in evaporation through the skin, but a distinct decrease in urine output; in some cases blood appears in the urine. Severe degeneration can be found in the papillary tissue of the kidneys, which may also develop calculi, or stones, especially in the cortico-medullary section. The presence of calculi, however, cannot be considered decisively characteristic of changes caused by a fat-free diet (*4*).

Another interesting symptom in EFA deficiency is the degeneration of the spermatogenic tissue layer of the testis, which in severe cases results in tubules containing only a few Sertoli cells, and epididymes which are either empty or filled to some extent with cell debris or degenerated cells. In the ovaries, in the interstitial cells, is seen a shrinkage of the nucleus and condensation of the chromatin, resulting in "wheel nuclei" often with a thorn apple appearance. Pregnant rats fed an EFA-deficient diet do not deliver normal offspring, and lactation is impaired also.

So far the EFA deficiency has been handled almost entirely based on scoring of skin signs and growth rate; the first is a subjective grading and the latter is more an over-all than a specific indicator that something is abnormal. Furthermore, neither measure can give quantitative information about the EFA content in the animal—the EFA status.

The development of a micromethod for analysis of polyunsaturated acids in less than a gram of tissue (*22*) has made analysis of biopsy material feasible. Recently a method has been proposed for *in vivo* determination of the EFA status by analysis of heart tissue and by orchectomy followed by analysis and histological examination (*6*). However, spectrophotometric methods do not allow distinction of differences in chain length, or detection of biological activity of the polyenoic, or polyunsaturated, fatty acids.

Several studies have been made to determine the content and composition of the polyenoic acids in various organs as well as of the carcasses of normal and deficient animals (*34, 36*). Studies of this type have also shown that certain transformations of the unsaturated fatty acids take place *in vivo*. A characteristic progressive decrease of dienoic acid is to be expected in EFA-deficient animals, and has been demonstrated several times, e.g. in mice (*19*) and in rats (*15*). Nunn and Smedley-Maclean (*30*) demonstrated in 1938 an increase in the trienoic acid content of tissue lipides from EFA-deficient animals. These findings have been confirmed and extended recently (*6*). In Tables 1 and 2 are shown analytical data from lipides of heart and testis tissue of rats fed an EFA-deficient diet or a diet with EFA for 12 weeks. The greatest changes are found in the content of dienoic and trienoic acids; the former is low, the latter very high in the EFA-deficient animals, whereas

Table 1. Effect of EFA-Free Diet and of EFA Supplementation on the Polyenoic Acid Pattern of Heart Tissue in Rats

	Initial Controls	5% Safflower Oil (18 Weeks)	EFA-Free (18 Weeks)
Diene	286	722	69
Triene	42	9	455
Tetraene	346	267	134
Pentaene	124	49	21
Hexaene	114	6	11
Total	912	1053	690

Table 2. Effect of EFA-Free Diet and of EFA Supplementation on the Polyenoic Acid Pattern of Testis Tissue in Rats

	Initial Controls	5% Safflower Oil (18 Weeks)	EFA-Free (18 Weeks)
Diene	62	55	32
Triene	29	42	109
Tetraene	161	227	146
Pentaene	109	197	171
Hexaene	37	8	18

Table 3. EFA Activity of Certain Polyenoic Fatty Acids and Alcohols

	No. of C Atoms	Position of Double Bonds (Counted from the $-CH_3$ Group)	Activity: Growth	Activity: Skin Cure
Linoleic acid	C_{18}	6, 9	+	+
Linoleyl alcohol	C_{18}	6, 9	+	+
Arachidonic acid	C_{20}	6, 9, 12, 15	+	+
γ-Linolenic acid	C_{18}	6, 9, 12	+	?
Eicosadienoic acid	C_{20}	6, 9	+	?
Linolenic acid	C_{18}	3, 6, 9	+	-
Linolenyl alcohol	C_{18}	3, 6, 9	+	-
Docosahexaenoic acid	C_{20}	3, 6, 9, 12, 15, 18	+	-

the reverse is true in the animals given essential fatty acids. Changes in content of the other polyenoic acids are less pronounced.

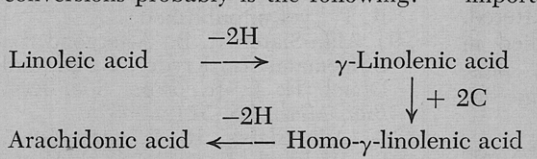
Supplementation of EFA-deficient animals' diets with linoleic acid and linolenic acid indicates that *in vivo* the dienes can be converted to tetraene (arachidonic acid), while linolenic acid is converted primarily to pentaene and hexaene (*33, 38, 41*).

Some of the unsaturated fatty acids and alcohols with activity as essential fatty acids are listed in Table 3.

Thomasson (*40*) and Klenk (*25*) have postulated that the polyunsaturated fatty acids can be divided into two different classes depending upon the structure of the hydrocarbon tail of the carbon chain, namely the linoleic and linolenic acid families. Recently a third family has been added to the discussion by Mead *et al.* (*28*). This is the oleate family, which contains oleic acid and the C_{20}-trienoic acid; the latter piles up in the tissue of EFA-deficient animals. Oleic acid can be synthesized in the body, whereas linoleic and linolenic acids, for instance, cannot, or at least not in quantities large enough to meet the requirements of young rats. Apparently, neither extension nor dehydrogenation of the tail end, i.e., between the methyl group and the first double bond, of these polyenoic acids can take place metabolically.

How conversions within the three families of unsaturated fatty acids take place is not yet known in detail; certain possible routes have been proposed (*19*), as shown in Figures 1, 2, and 3. It will be seen that linoleate, for example, cannot be converted to a C_{20} pentaene, while the acids of the linolenate family can give rise to a C_{20} pentaene and a C_{22} hexaene. On the other hand, the acids of the oleic acid family can be converted to a C_{20} triene or a C_{22} tetraene, but not to a C_{20} tetraene.

Arachidonic acid can be formed *in vivo* by addition of the two carbon atoms of acetate to the carboxyl group of linoleate and removal of four hydrogen atoms to form two additional double bonds, according to Steinberg *et al.* (*37*), and Mead *et al.* (*29*). Recently Mead and Howton (*27*) have shown that the scheme for these conversions probably is the following:



Hydrogenated oils are used in various food industries such as those producing oleomargarine and baking products, for example. The nutritive value of hydrogenated fats has been studied in several laboratories, often with rather conflicting interpretations of the results. With different types of hydrogenated oils, given as the sole dietary fat, we have found depressed growth and accentuation of the essential fatty acid deficiency symptoms in young rats, as compared to rats reared on a fat-free diet. The symptoms appeared especially severe with hydrogenated marine oils. These oils are known to contain large amounts of highly unsaturated fatty acids (*1, 3, 5*). Linoleate supplementation of the diet of rats fed the hydrogenated oils prevents or removes the symptoms (*17*).

It is well known that partial hydrogenation of an oil causes formation of positional as well as geometric isomers of the polyenoic acids. The possibility that the presence of such isomers in the hydrogenated fat is more or less responsible for the intensification of EFA deficiency, when this type of fat is used in the diet, has been suggested, but a definite answer to this question has not yet been reached. Christensen *et al.* (*15*) found an accumulation of conjugated fatty acids in the depot fat of rats fed a high amount of hydrogenated peanut oil. Experiments with feeding conjugated *cis, trans* and *trans, trans* dienoic acids, and *cis, trans, trans* or *trans, trans, trans* trienoic acids to rats showed no significant accentuation of the EFA deficiency, but all of them markedly increased the diene conjugation of fatty tissue (*2*).

A recovery experiment with EFA-depleted animals revealed a worsening of the symptoms through supplementation with the geometrical isomers of linoleate, whereas no effect of the *trans* monoenoic acid was observed (*21*). Also Alfin-Slater *et al.* (*9*) found no growth retardation in rats fed hydrogenated triolein containing 33% *trans* monoenoic acid. In this connection it is interesting that Johnston *et al.* (*23*) found that no transfer of *trans* fatty acids takes place from mother to young. When the young were allowed to suckle the maternal milk, the amount of *trans* fatty acids in the carcass fat of the young increased markedly.

Age of animals appears to be an important factor in EFA deficiency. As a matter of fact, inducing EFA deficiency in adult rats is rather difficult. Barki *et al.* (*11*) produced EFA deficiency in adult rats only after keeping them on a restricted food intake until the animals were reduced to half their starting weight. When the animals were then fed the fat-free diet *ad lib.*, the skin signs appeared. We have developed skin signs in adult rats by adding cholesterol or cholic acid or both to an EFA-free diet or by feeding hydrogenated whale oil as the sole dietary fat (*7, 8*). Analyses of heart and testis tissue from these animals showed a pronounced increase in the content of trienoic acids, and a decrease of the dienoic acids. The difficulties in provoking EFA deficiency in adult animals are probably related to EFA reserves in the body, and to the fact that actual growth has ceased.

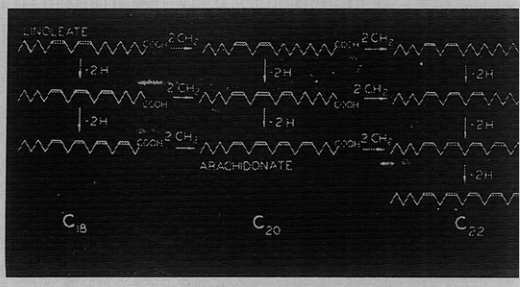


Figure 1

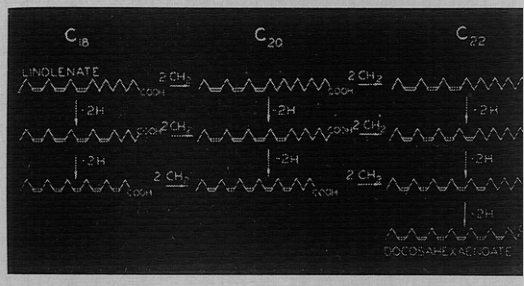


Figure 2

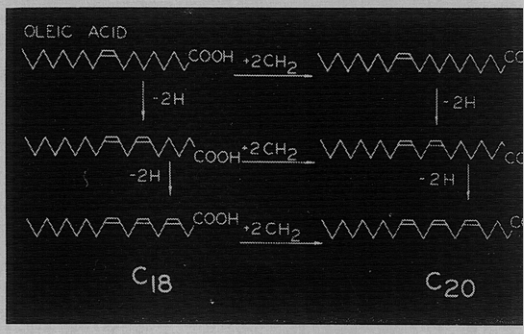


Figure 3

The reasons why a few of the polyunsaturated fatty acids are essential are not too well understood as yet. Polyunsaturated fatty acids occur very generally in all types of tissues, in mitochondria, enzyme preparations, and cell membranes. This widespread distribution in "key compounds" in the body could indicate that they are of special importance, but how? So far, efforts to clarify these problems have concentrated mainly on two topics, the importance of polyenoic acids in the transport of other lipide compounds, e.g. cholesterol, and the fact that their molecule has one or more "active centers," namely the methylene-interrupted double bond system.

Linoleic acid is transported in the lymph largely as triglycerides, according to Blomstrand (*12*). Studies with carboxyl-labeled linoleic acid by Mead *et al.* (*26*) showed that more than half of the ingested linoleate appeared as phospholipides half an hour later. This amount decreased only slightly later on; at the same time, a decrease in initial triglycerides and an increase in sterol ester was observed. This rapid conversion of linoleate to phospholipides, and at a somewhat slower rate, to cholesterol esters, supposedly takes place in the liver.

Alfin-Slater *et al.* (*10*) found in the liver of EFA-deficient rats an accumulation of cholesterol esters containing primarily fatty acids, suggesting that polyenoic acids are of importance in the transport of cholesterol out of the liver. Similar results were obtained by Dam *et al.* (*16*) in rats fed a fat-free diet. The serum lipoprotein complex contains free and esterified cholesterol, phospholipides, and glycerides of saturated and unsaturated fatty acids; much of the cholesterol is present in the form of esters of polyenoic acids.

On this background Holman (*19*) suggested that cholesterol esters of EFA are required as a part of the serum lipoprotein lipides to maintain the optimum physico-chemical properties of the mixture; therefore, these unsaturated esters must be present as a rather constant proportion of the normal lipide in transport, i.e., ingestion of or mobilization from the *in vivo* fat depots of large amounts of saturated fat requires large amounts of unsaturated cholesterol ester.

This assumption is in accord with recent experiments with rats, in which Peifer and Holman (*31*) showed that inclusion of cholesterol in an EFA-free diet hastens the appearance of the skin signs. Similar effects have been obtained with other compounds or treatment known to cause hypercholesterolemia, such as dietary cholic acid, alloxan diabetes, thiouracil, Triton (a nonionic detergent), and tetramethyl-benzidine. On the other hand, several observations have repeatedly indicated that ingestion of fats high in polyunsaturated fatty acids will cause a decrease in serum cholesterol in patients or animals with above normal blood fat and cholesterol levels.

The methylene groups between the double bonds in the polyunsaturated fatty acids are very reactive. Oils and fats containing these fatty acids are very easily autoxidized. It seems quite possible that these active methylene groups may function *in vivo* in oxidation-reduction systems, either in connection with, or as part of enzyme systems. Such a function has also been postulated by Tappel *et al.* (*39*) in the coupled oxidation of antioxidants and other substances by lipoxidase, under conditions in which linoleate itself is not oxidized by the enzyme.

The presence of polyenoic fatty acids in enzyme preparations has also been indicated. Crude preparations of cytochrome oxidase have been found to contain 20–30% of the fatty acids as polyunsaturated acids. Recently, Widmer and Holman (*42*) studied the unsaturated fatty acid content of a number of enzyme preparations primarily from beef heart mitochondria. They found appreciable amounts of polyenoic fatty acids present. Typical analyses are shown in Table 4. EFA deficiency has also been shown to change the activity of several enzymes, e.g. of liver cytochrome oxidase, succinic, glutamic, and butyric dehydrogenase.

Table 4. Polyunsaturated Fatty Acid Content of Enzymatically Active Subcellular Particles

Acid Type	Beef Heart Mitochondria	Electron Transport Particles
	(% of total fatty acids)	
Diene	28.6	33.4
Triene	5.1	5.5
Tetraene	11.8	15.4
Pentaene	2.9	3.5
Hexaene	0.03	0.02
Total	48.4	57.8
Total (% of protein)	9.0	15.2

The fact that EFA deficiency causes a drastic increase in the permeability of the skin (*32*) may indicate that polyenoic acids are also of importance in connection with cell and membrane permeability. All in all, we must admit that our knowledge of the function of the essential fatty acids is as yet very limited.

This discussion has been concentrated upon studies with rats, because that is the animal in which the vast majority of experiments in this field have been carried out. However, the literature indicates that these particular unsaturated fatty acids are essential for other species, also, although information is rather scattered. EFA deficiency has been studied in dogs, mice, chickens, calves, pigs, guinea pigs, rabbits, insects, and humans.

So far, experiences with EFA deficiency in adult humans are scarce, and the characteristic EFA symptoms are not readily observed. Nutritional studies in infants, especially by Hansen and coworkers (*18*)°, have shown that essential fatty acids are required by man. Another indication that polyenoic fatty acids are important nutrients for man is the large number of medical reports on a relationship between the amount and the kind of dietary fat and atherosclerosis. However, the findings reported so far are rather controversial in regard to the effect of saturated, unsaturated, and essential fatty acids, respectively, upon hypercholesterolemia and atherosclerosis. It seems impossible, therefore, to give any final answer to these problems today.

Dietary fatty acids have also been found to influence the activity of fibrinolysin (*35*), and to increase the coagulability of blood (*24*).

There can be no doubt that polyenoic fatty acids are necessary dietary components for man, although it is not yet possible to give any figures for the minimum daily requirements. Furthermore, it might well be that the requirements for EFA depend upon age and not least upon the diet's content of other nutrients such as saturated fatty acids, unsaturated fatty acids with no EFA activity, or cholesterol. It is significant that the essential fatty acids occur in abundant amounts in most vegetable oils. Therefore, a reasonable variation in the diet most likely will supply man with the necessary amounts of essential fatty acids.

° Since this paper was prepared, three papers from Hansen's group (*J. Nutrition*, 66, 345, 555, and 565 (1958)), on essential fatty acids in infant nutrition have indicated optimum levels of linoleic acid to be about 4 to 5% of the caloric intake.

References

(1) Aaes-Jørgensen, E., The Role of Fat in the Diet of Rats. 6. Influence on Growth of Various Fats in Ordinary Refined State and after Hydrogenation or Polymerization, (Thesis), St. Nordiske Videnskabsboghandel, Copenhagen, 1954.
(2) Aaes-Jørgensen, E., *J. Nutrition*, **66**, 465 (1958).
(3) Aaes-Jørgensen, E., and Dam, H., *Brit. J. Nutrition*, **8**, 285 (1954).
(4) Aaes-Jørgensen, E., Funch, J. P., Engel, P. F., and Dam, H., *Ibid.*, **10**, 32 (1956).
(5) *Ibid.*, **10**, 292 (1956).
(6) Aaes-Jørgensen, E., and Holman, R. T., *J. Nutrition*, **65**, 633 (1958).
(7) *Ibid.*, **66**, 245 (1958).
(8) Aaes-Jørgensen, E., and Holman, R. T. (yet unpublished).
(9) Alfin-Slater, R. B., Aftergood, L., Bingemann, L., Kryder, G. D., and Deuel, H. J., Jr., *Proc. Soc. Exp. Biol. Med.*, **95**, 521 (1957).
(10) Alfin-Slater, R. B., Aftergood, L., Wells, A. F., and Deuel, H. J., Jr., *Arch. Biochem. Biophys.*, **52**, 180 (1954).
(11) Barki, V. H., Nath, H., Hart, E. B., and Elvehjem, C. A., *Proc.*

Soc. Exp. Biol. Med., **66**, 474 (1947).
(12) Blomstrand, R., *Acta Physiol. Scand.*, **32**, 99 (1954).
(13) Burr, G. O., and Burr, M., *J. Biol. Chem.*, **82**, 345 (1929).
(14) *Ibid.*, **86**, 587 (1930).
(15) Christensen, F., Dam, H., and Engel, P. F., *Acta Physiol. Scand.*, **38**, 373 (1957).
(16) Dam, H., Prange, I., and Søndergaard, E., *Ibid.*, **34**, 141 (1955).
(17) Funch, J. P., Aaes-Jørgensen, E., and Dam, H., *Brit. J. Nutrition*, 11, 426 (1957).
(18) Hansen, A. E., Adam, D. J. D., Boelsche, A. N., Haggard, M. E., Wiese, H. F., Pratt, E. L., and Hunter, V., *Federation Proc.*, **16**, 387 (1957).
(19) Holman, R. T., *Svensk Kem. Tidskr.*, **68**, 282 (1956).
(20) Holman, R. T. (in press).
(21) Holman, R. T., and Aaes-Jørgensen, E., *Proc. Soc. Exp. Biol. Med.*, **93**, 175 (1956).
(22) Holman, R. T., and Hayes, H., *Anal. Chem.*, **30**, 1422 (1958).
(23) Johnston, P. V., Johnson, O. C., and Kummerow, F. A., *Proc. Soc. Exp. Biol. Med.*, **96**, 760 (1951).
(24) Keys, A., Buzina, R., Grande, F., and Anderson, J. T., *Circulation*, **15**, 274 (1957).
(25) Klenk, E., II Internat. Conf. Biol. Problems of Lipids, Ghent, 1955, Abstracts, p. 24.
(26) Mead, J. F., and Fillerup, D. L., *J. Biol. Chem.*, **227**, 1009 (1957).
(27) Mead, J. F., and Howton, D. R., *Ibid.*, **229**, 575 (1957).
(28) Mead, J. F., and Slaton, W. H., *Ibid.*, **219**, 705 (1956).
(29) Mead, J. F., Steinberg, G., and Howton, D. R., *Ibid.*, **205**, 683 (1953).
(30) Nunn, L. C. A., and Smedley-MacLean, I., *Biochem. J.*, **32**, 2178 (1938).
(31) Peifer, J. J., and Holman, R. T., *Arch. Biochem. Biophys.*, **57**, 520 (1955).
(32) Ramalingaswami, V., and Sinclair, H. M., *Brit. J. Dermatol.*, **65**, 1 (1953).
(33) Reiser, R., *J. Nutrition*, **42**, 325 (1950).
(34) Rieckehoff, I. G., Holman, R. T., and Burr, G. O., *Arch. Biochem.*, **20**, 331 (1949).
(35) Scott, R. P., and Thomas, W. A., *Proc. Soc. Exp. Biol. Med.*, **96**, 24 (1957).
(36) Smedley-MacLean, I., "The Metabolism of Fat," Methuen & Co., London, 1943.
(37) Steinberg, G., Slaton, W. H., Jr., Howton, D. R., and Mead, J. F., *J. Biol. Chem.*, **220**, 257 (1956).
(38) *Ibid.*, **224**, 841 (1957).
(39) Tappel, A. L., Boyer, P. O., and Lundberg, W. O., *J. Biol. Chem.*, **199**, 267 (1952).
(40) Thomasson, H. J., *Intern. Z. Vitaminforsch.*, **25**, 62 (1953).
(41) Widmer, C., and Holman, R. T., *Arch. Biochem.*, **25**, 1 (1950).
(42) Widmer, C., and Holman, R. T. (to be published).

JOURNAL OF Agricultural and Food Chemistry

TECHNICAL SECTION

APRIL 1959 Volume 7, Number 4

PESTICIDES

NUTRITION/PLANT NUTRIENTS AND REGULATORS

PLANT REGULATORS

FOOD PROCESSING