Fatty Acid Structure and Nutritive Value[1,2]

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INFORMATION on the relationship of fatty acid structure to their essentiality in human nutrition is very meager. Our insufficient understanding of the role of fat in the human diet might very well be attributed to the inability to measure accurately and precisely the amount and distribution of both saturated and unsaturated fatty acids in blood and tissues and also to the difficulties coincident with long time feeding experiments of the human on low fat diets. As a consequence, much of the experimental knowledge of the nutritive role of fatty acids in the diet has been gained in studies on animals and certain microorganisms.

Since it is the purpose of this discussion to summarize our knowledge on the essentiality of certain fatty acids for the nutrition of microorganisms, experimental animals, and humans, it perhaps may be of advantage first to generalize on the need of fat in nutrition.

It is well known that fats play a part in the structure and function of tissues, especially of the brain and nerves. They help to retain the organs, blood vessels, and nerves in place, in addition to their function as an insulator. Further, fats are believed to assist the body in the conservation of thiamine and in the utilization of other elements (1). Although a few years ago fats were held in low esteem because of a reputed low digestibility, they are today considered a highly valued food. McLester (2) points out the necessary caloric value of the diet cannot easily be obtained without including fat, for this substance yields more than twice as many calories as the same quantity of protein or carbohydrate. As a matter of fact, many early investigators in the field of human nutrition believed that the only role of dietary fat was to serve as a rich source of calories. As pointed out by Burr and Barnes, (3) a sudden decrease in the fat content of human diets decreases working capacity and a sense of well-being. Fats have an important place in the dietary because they may serve as sources of the fat-soluble vitamins and because of their ability to impart flavor, to change texture, and to add attractiveness. They have the fortunate faculty of increasing our enjoyment of food (4). Fat has a high satiety value, which according to Sherman (5) is due to its ability to produce motor inhibition of gastric contractions, thus preventing hunger.

In 1929 Burr and Burr (6) discovered the essential nature of certain fatty acids in the nutrition of the rat. According to Hansen and Burr (7), "for the first time then a specific function could be attributed to dietary fat other than its energy-producing properties and its action as a vitamin carrier." Burr and Burr (6) showed that renal lesions and other evidence of disease appeared in rats regardless of vitamin intake when placed on a fat free diet. Burr (8) in a later report stated that careful exclusion of fat from the diet of the rat led to, 1. development of scaly skin and caudal necrosis, 2. marked retardation of growth, 3. kidney lesions and hematuria, and 4. early death. Macroscopically noticeable hemorrhage develops late and without great regularity. The percentage of animals showing this sign varies from group to group, reports ranging from 16% to over 90%. However, kidney lesions could be demonstrated in 100% of the cases at autopsy. Caudal necrosis is variable, but other more or less specific effects are histological changes in the ovaries and uterus, and other tissues, poor ovulation, reproduction and lactation in the female, male sterility, excessive water consumption, high respiratory quotients, and high metabolic rate. Similar findings have been described by a number of other investigators (6), (9-18).

Since inclusion in the diet of fats rich in unsaturated fatty acids alleviates the symptoms of the fatty acid deficiency syndrome in rats, a relatively large number of purified fatty acids have been tested for activity (Table I). Although linoleic, linolenic, and arachidonic acids have been studied the most extensively, it is only linoleic and arachidonic acids that give complete cures (7), i.e., if either linoleic or arachidonic acids are added to the diet, a complete cure of the deficiency syndrome occurs. However, since arachidonic acid is apparently synthesized in the animal body from linoleic acid, the latter acid should be considered as the primary factor.

TABLE I *

Purified Fatty Acids Tested for Activity in Curing Fat Deficiency in Small Animals

Positive Response	Negative Response
Linoleic acid	All saturated acids
Linoleyl alcohol	Oleic acid, elaidic acid
Linolenic acid	Erucic acid, ricenoleic acid
Arachidonic acid	Linoelaidic
Docosahexenoic acid	9,11-Linoleic acid
Hexahydroxystearic	α-Eleostearic acid
	Dioxidostearic acid
	Trihydroxystearic acid
	Tetrahydroxystearic acid
	Chaulmoogric acid
	Clupanodonic acid

* Taken from G. W. Burr, Fed. Proc., *1*, 224 (1942).

In addition to the fatty acids listed in Table I, Karrer and Koenig (19) tested 2-phytenic acid, 2,6-phytadienic acid, 10,13-nonadecadienoic acid, and 11,14-eicosadienoic acid and found that these acids had a negative effect, i.e., they were not able to cure rat acrodynia due to lack of fat in the diet. The rat apparently is unable to convert 11,14-eicosadienoic to linoleic by metabolic processes. Observations of the fatty acids giving positive responses in Table I, with the exception of docosahexenoic and hexahydroxystearic acid, would seem to indicate that the structural characteristics involved include a straight chain acid with at least two non-conjugated double bonds of *cis* configuration. Whether these characteristics are absolutely essential has not been demonstrated conclusively.

A relationship existing between pyridoxine and essential fatty acid metabolism in the rat has been observed by several investigators (20-27). More recently, Medes and Keller (28) have shown that relief from a deficiency of both linoleic acid and pyridoxine

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in rats could be obtained by either linoleic acid (ethyl linoleate) or pyridoxine although growth on pyridoxine alone was greater than on linoleic acid (ethyl linoleate). No conclusions were drawn, however, by Medes and Keller concerning the mechanism of action.

Although most of the work on the essential fatty acids has been done on the rat as the experimental animal, White and associates (29) have described unsaturated acid deficiency symptoms in other laboratory animals. These investigators showed that mice develop a scaly skin, disturbance in growth, and early death. The symptoms were cured by addition of lard to the diet. Other workers have studied the effect of low fat diet on the cow (30, 31), calf (32), White Leghorn chicks (33), and the hog (34-37). In the case of the cow and calf there was no clear evidence that a deficiency of linoleic acid was involved even though the iodine value of the blood lipids fell with the use of low fat diets. Although deficiency symptoms were not observed in White Leghorn chicks, the evidence that linoleic acid was involved was not conclusive. Although Ellis and co-workers (34-37) found that the linoleic acid content of the lard obtained from hogs fell to a low value, no fatty acid deficiency syndrome was observed.

The dog seems to lend itself as a suitable animal for observing the effects of a fat deficient diet upon the general health and condition of the skin of the animals as well as for studying the distribution of the fatty acids in the various lipid fractions of the blood. Hansen and Wiese (38) found that distinct alterations in the appearance of the skin occurred in puppies reared on a low fat diet. This skin change became evident at about three months of age with the gradual development of a generalized flaky desquamation together with a dryness and coarseness of the hair. The skin and the hair of the littermate puppies receiving 28% of their calories as lard in the diet remained clear and soft. Coincident with this phenomenon, marked differences in the degree of unsaturation of the fatty acids of the blood serum in the two groups of animals was demonstrated.

Comparatively few studies have been made with human subjects in which the effects of diets extremely low in fat have been observed over an extended period of time. Von Gröer in 1919 (39), Holt and co-workers (40) in 1935, Chwalibogowski in 1937 (41), and later Hansen and co-workers (42, 43), studied the effect of low fat diets on human subjects, particularly infants. However, on the basis of clinical observations made by these investigators on human subjects maintained on diets very low in fat, it is apparent that no specific clinical syndrome develops during a period of one to two years. In regards to infants Hansen and Burr (7) indicate that "the likelihood that a human infant would subsist for a prolonged period of time on a diet practically devoid of fat, yet complete as regards other known dietary essentials, is practically nil. Hence the recognition in infants and children of any characteristic clinical picture or type of malnutrition which may result from the specific lack of certain fatty acids in the diet has not been demonstrated conclusively."

Hansen (38, 44, 45), Wiese and co-workers (46) have found the degree of unsaturation of the serum lipids to be low in patients with eczema, and it was shown that the dietary use of fats rich in unsaturated fatty acids exerted a beneficial effect in certain of the patients (44, 45, 46, 47).

An investigation of the lipid extracts of the serum of eczematous patients was also made by Brown and Hansen (48). They found that the content of linoleic and arachidonic acids determined by the bromide precipitation method on pooled samples was less than in the control subjects (Table II).

TABLE II *

Arachidonic and Linoleic Acids in Patients with Eczema

	Arachidonic acid	Linoleic acid
Pooled samples	% T.F.A.	% T.F.A.
35 Control subjects	2.83	4.80
12 Control subjects	2.90	5.20
18 Young patients with eczema	1.34	3.20
8 Adult patients with eczema	1.60	4.20

* Taken from W. R. Brown and A. E. Hansen, Proc. Soc. Exptl. Biol. Med., *36*, 113 (1937).

Hansen and co-workers (49) have shown recently on clinical and blood lipid studies with 225 patients with eczema and 101 control subjects that when ingestion of unsaturated fatty acids was used as the sole form of therapy in 138 eczematous patients of various ages, a clinical response was produced which was judged to be good-to-excellent in 60, fair-to-good in 51. The remaining 37 patients showed slight or no benefit. Serum lipid studies on 171 of the eczematous patients disclosed that 80% of the infants under two years of age, 75% of children between two and 15 years, inclusive, and over 50% of adults had iodine values for the serum lipids below the mean values for the 101 control subjects. Periodic studies of the serum lipids for the patients with eczema showed that as the clinical condition improved with addition to the diet of fats rich in unsaturated acids, the iodine values of the serum fatty acids increased. Although on the basis of this work it is suggested that the role of the essential fatty acids appears to be that of restoring and maintaining the normal nutrition of the skin, the specific function of linoleic and arachidonic acids in maintaining a healthy skin is not known.

On the basis of the experimental evidence obtained thus far it would appear that fatty acids of a structure characteristic of linoleic and arachidonic acids are essential for adequate nutrition of the rat and certain other experimental animals, but on clinical observations with human subjects, aside from their apparent function in maintaining the integrity of the skin, there is no direct evidence to indicate that a lack of linoleic or arachidonic acids produces the same histopathological changes which have been shown to result from the lack of these acids in the diet of experimental animals.

It has been reported by Schantz, Elvehjem, and Hart (50) in 1940 that butterfat nutritionally is superior to vegetable oils in weanling rats, and in later publications by these investigators (51, 52) the growth promotional factor was believed to be a long chain fatty acid. There was no indication that the content of the factor varied in summer or winter butter. The growth promoting factor, if it exists, has not been isolated although Geyer, et al. (53) have recently reported a superior growth action for the rat of the liquid fraction of butter remaining after the separation of the solid portion from an acetone solution at −4°C.

In recent experimental investigations by Boer, Jansen, and Kentie (54), data were presented in sup-

port of the conclusion that summer butter possesses growth promoting activity for the rat other than vitamin A, which is not shared by winter butter or vegetable oils. Later, Boer, et al. (54) indicated that the factor in summer butter responsible for this effect was vaccenic acid, an 11,12-octadecenoic acid of *trans* configuration.

$$CH_3-(CH_2)_5-CH=CH-(CH_2)_9-COOH$$

Recently, Deuel, et al. (55) in an attempt to verify the observations of Boer, et al. (54), found no difference in the growth of male and female rats over a six-week period when the diet contained butterfat or cottonseed oil, nor did they find any stimulating effect on growth when vaccenic acid or hydrogenated tung oil added to rapeseed oil diet or cottonseed oil diet fortified with vaccenic acid was administered. Deuel, et al. have therefore concluded that vaccenic acid does not play a specific role in relation to growth of the rat. Although the vaccenic acid picture in relationship to a specific function in the dietary is not complete, it appears on the basis of the comprehensive study of Deuel that this monoethenoid acid possesses no unique or specific nutritional significance, at least in the rat.

The investigations of Schantz, et al. (50, 51) on the growth promotional factor for rats in butterfat prompted Harris and co-workers (56, 57) to study the nutritional significance of hydroxy fatty acids since these acids have been reported to be present in animal and vegetable fats. The authors fed a synthetic glyceride containing all of the fatty acids as dihydroxystearic acid in nutritionally complete diets to weanling rats at levels of 2.2% and 2.5% in replacement of equal weights of hydrogenated fat in control diets. Although a favorable effect on the growth and development of rats was observed, the effect was less favorable when trihydroxystearic acid was substituted for dihydroxystearic acid, probably due to less absorption because of the higher melting point of the trihydroxystearic acid.

Monodihydroxystearyl triglyceride when fed at levels of 0.5%, 1%, and 2% favorably affected the growth and development of rats in direct proportion to the amounts fed. Presumably because of greater solubility and digestibility of monodihydroxystearyl glyceride was more active than the tri-dihydroxystearyl glyceride. It is questionable whether any real significance can be attached to the growth performance of rats on these diets. Toxic manifestations (severe diarrhea and avitaminosis K) were exhibited when dihydroxystearic acid was included to the extent of 8% of the test diet in which the only source of fat was a glyceride containing 32% of dihydroxystearic acid and fatty acids of a hydrogenated vegetable oil (58). Apparently the dihydroxystearic acid interferes with the intestinal synthesis of vitamin K by blocking the biochemical system involved. It would be interesting and informational to determine the effect of diets rich in hydroxy fatty acids on other experimental animals.

Despite the general lack of understanding of the specific role of certain fatty acids in the nutrition of both animals and humans, considerable progress has been reported in the study of the significance of fatty acids in the nutritional requirements of microorganisms. Perhaps the increased activity in this field will lead to some significant results to further our knowledge on the synthesis of fatty acids and the interrelationships between the metabolism of fatty acids and the vitamins.

The stimulating effect of oleic acid on the growth of *lactobacilli* in the presence of suboptimal amounts of riboflavin and pantothenic acid has been demonstrated by Strong and Carpenter (59) and Bauernfeind, et al. (60), respectively. A particular strain of lactobacillus of cecal origin isolated from rats was found by Whitehill, Oleson, and SubbaRow (61) to require oleic acid as an essential factor in its nutrition. These investigators found that the lactobacillus could be transferred repeatedly on synthetic media only in the presence of oleic acid. Mueller and coworkers (62, 63) have demonstrated that *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively, require oleic acid for growth. Water-soluble esters of oleic acid such as the polyoxyethylene ester of oleic acid and the polyoxyethylene derivative of sorbitan monooleate (Tween 80) were found by Dubos (64, 65) to cause more rapid and abundant growth of avian strains of *tubercle bacillus*; as little as 0.1 to 1 γ of oleic acid per ml. of synthetic medium, however, is sufficient to cause retardation or inhibition of growth. Williams and Fieger (66) have demonstrated that oleic acid had a pronounced stimulating effect in biotin assays, and later (67) found that *Lactobacillus casei* could be grown in an essentially biotin-free medium, providing oleic acid was present. Snell and co-workers (68) observed the same effect on a number of related bacteria.

Recently, Hofmann and Axelrod (69), et al. (70) have shown that the biotin activity of the fat-soluble fraction of human blood plasma could be explained in terms of known fatty acids. They found that the liquid acid fraction (containing oleic, linoleic, and arachidonic acids) obtained from a lead soap separation of the plasma fat possessed the greater growth activity for *lactobacilli* although the solid acid fraction had pronounced synergistic growth activity. Of the liquid acid fraction the biotin-like activity of various lipids for *Lactobacillus arabinosus, Lactobacillus casei, Saccharomyces cerevisiae, and Streptococcus haemolyticus* was also determined by these workers. The nutritional activity of various lipids on these organisms are summarized in Table III. It

TABLE III *

Biotin-Like Activity of Various Lipids

The values are expressed as millimicrograms of biotin per mg. of substance.

Lipid	*L. arabinosus*	*L. casei* pH 6.8 [a]	*L. casei* pH 5.5 [a]	*S. cerevisiae*	*S. haemolyticus*
Oleic acid	6.0	5.9	11.5	0.60	<0.05
Linoleic acid	4.0	0.58	1.4	<0.05	<0.05
Elaidic acid	1.0	10.5	15.2	<0.05	<0.05
Methyl oleate	3.2	0.75	1.3	<0.05	0.16
Stearic acid	<0.05	<0.05	<0.05	<0.05	<0.05
Tween 80	0.58	1.4	2.9	0.32	0.55
Cholesterol	<0.05	<0.05	<0.05		<0.05
Vaccenic acid	1.2	6.0	5.3	<0.05	
Fraction I [b]	1.6	3.3	6.6	<0.05	<0.05
Fraction II [c]	<0.15	<0.05	<0.05	<0.05	<0.05
Fraction III [d]	5.8	2.2	10.5	<0.05	<0.05

* Taken from A. E. Axelrod, M. Mitz, and K. Hofmann, J. Biol. Chem., *175*, 265 (1948).
[a] pH of the medium before autoclaving.
[b] Ether-soluble fraction from human blood plasma.
[c] Non-saponifiable portion of fraction I.
[d] Saponifiable portion of fraction I.

is to be noted from the data in the table that the lipids with but few exceptions were unable to substitute for biotin in the nutrition of *S. cerevisiae* and *S. haemolyticus*.

Since oleic acid possessed considerable biotin activity, a significant portion of the work of Axelrod and Hofmann (70) was the investigation of the biological activity of oleic acid derivatives using *L. arabinosus* as the test organism. The compounds tested and their biological activities are given in Table IV.

TABLE IV *

Biological Activity of Oleic Acid Derivatives

	Compound	Activity [a]
I	Oleic acid	6.0
II	Methyl oleate	3.2
III	Oleic acid amide	6.3
IV	Oleyl alcohol	<0.05
V	Linoleic acid	4.0
VI	Linolenic acid	1.7
VII	Elaidic acid	1.0
VIII	Vaccenic acid	1.2
IX	Stearic acid	<0.05
X	Dihydroxystearic acid (m.p. 94°)	<0.05
XI	Dihydroxystearic acid (m.p. 130.7°)	<0.05
XII	Azelaic acid [b]	<0.05
XIII	Pelargonic acid	<0.05

* Taken from A. E. Axelrod, M. Mitz, and K. Hofman, J. Biol. Chem., *175*, 265 (1948).

[a] *L. arabinosus* was employed as the test organism. Values are expressed as millimicrograms of biotin per mg. of substance.

[b] Aqueous solution employed for assay.

It will be observed from the data in Table IV that hydroxylation and hydrogenation of the oleic acid led to complete inactivation and that the configurational and positional isomers of oleic acid have relatively little effect. It is also apparent that increased unsaturation resulted in decreased activity.

It is pertinent to indicate, however, that although the investigators cited have demonstrated oleic acid to be a growth stimulant for *lactobacilli* with suboptimal amounts of riboflavin, pantothenic acid, and biotin, maximum growth could be obtained in the absence of oleic acid. The strain of *lactobacilli* isolated from the cecum of the rat definitely required oleic acid in addition to acetate and biotin for optimal growth.

Although the relationship between the function of biotin and lipids in the nutrition of microorganisms is little understood, it has been suggested that both biotin and oleic acid serve as cell permeability factors (67) or that biotin is essential for the synthesis of oleic acid (68). Perhaps a study of the effects of oleic acid and other fatty acids in biotin-deficient higher animals will help to clarify the situation. Trager (71) recently reported that intramuscular injection of oleic acid did not have the same effect in reducing the severity of chick dermatitis in chicks fed a diet high in egg white as did biotin or the fat-soluble material from plasma.

The story of the relation of fatty acid structure to nutritive value has yet to be revealed; however, several things stand out in sharp focus. 1. Linoleic and/or arachidonic acids are essential for adequate nutrition of the rat and are apparently essential factors in maintaining the integrity of the skin of the dog and the human. 2. A definite relationship seems to exist between the metabolism of the essential fatty acids and pyridoxine in the rat. 3. Since unsaturated fatty acids can substitute for biotin in the nutrition of certain organisms, it seems that a definite relationship exists in their biosynthesis.

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