The non-eicosanoid functions of the essential fatty acids

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Coincident with the discovery of the essential fatty acids (EFA), the symptoms of their deficiency were thoroughly delineated. The mechanisms by which these symptoms were produced, however, and the intimate functions of these substances remained obscure for many years. Smedley-MacLean and Hume (1) considered that the EFA are involved in the formation of new cells, and a careful consideration of the deficiency symptoms commonly described in EFA deficiency reveals that most of them are related in some way to a failure of membrane synthesis or maintenance.

Essential fatty acid deficiency symptoms

These symptoms were first described by the Burrs (2, 3) but have been confirmed by many studies since that time and additional symptoms have been noted. Although it is obviously difficult to extrapolate from gross deficiency symptoms to the intimate biochemical mechanisms underlying them, a brief review of the more prominent signs of deficiency at least hints at directions in which attention might be rewarding (4).

The most obvious sign of deficiency is decreased growth rate, particularly in male animals. It is also significant that in the absence of growth, either general or local, the other symptoms are milder or absent.

Dermatitis, including lesions of the skin in exposed areas, hair loss and, in some animals, tail necrosis, is a typical symptom of EFA deficiency as it is of other deficiencies. The use of this family of symptoms is complicated by the fact that they may not appear in areas of moderate to high humidity but are very apparent in dry locations.

Many organs undergo changes of various sorts but all are not consistent or revealing. Some of the more prominent signs are as follows.

Fatty livers may result from an inability to form the lipoproteins responsible for transporting cholesterol and triacylglycerols out of the liver.

Kidney damage appears to result as a consequence of the fatty and ultimately necrotic liver (5).

Impaired reproduction includes fetal resorption in females and testicular degeneration in males and it has been noted by most investigators that deficiency symptoms in the young are more severe if the fat-free diet is started during pregnancy in the mother.

Perhaps the most revealing symptom is the increased permeability of the skin to water. This is shown not only in the increased intake of drinking water without loss in the urine but also by increased absorption of water by rats immersed in warm water for short periods (6). This permeability of the skin has its counterpart in increased permeability and fragility of cellular membranes revealed, in part, by increased swelling and fragility of mitochondria and probably providing the basic mechanism of the typical increased metabolic rate typical of the deficiency state.

While this is not a complete list, it leads to the suspicion that a common underlying cause may be the reduced ability to form and maintain a wide variety of cell membranes, since in the absence of EFA, cell growth and membrane integrity appear to be compromised.

Assuming, for the moment, that this is the case, we can now inquire into the mechanisms by which such a defect in membrane structure might be brought about.

Structure of the membrane lipid bilayer

Without going into detail on membrane structure, which is the subject of many recent discussions (7–11), it is important for our purpose to note that, in general, the lipid bilayer tends to exist at the transition point between gel and liquid crystal and that, with a change in environmental temperature, the lipid composition and structure are varied to maintain the existence of both phases. A consequence of this partition of phases is that, in addition to the sidedness of families of lipids, both fluid and solid-like domains exist with different fatty acid compositions. Between these domains, boundary defects exist and these interfacial areas may correspond to binding sites for enzymes or transport proteins or may even accommodate pockets of water (10). The

lipid bilayers are thus not homogenous mixtures containing a cross-section of the total fatty acids and the influence of different types of fatty acids is, in consequence, much greater than if their properties were simply averaged. The size of the fluid domains is, of course, in large part dependent on the proportion and nature of the unsaturated fatty acid components of the membrane lipids.

Lipid-protein interactions in membranes

A great many physical techniques have been employed to investigate lipid-protein interaction (9–12). In sum they reveal that the principle forces binding integral proteins to the lipid bilayer are hydrophobic, although ionic forces may have been involved in the initial steps of membrane assembly and are certainly a major force in the binding of peripheral proteins to the head-group region of the bilayer.

In the interior of the bilayer, both the lipids and integral proteins have profound effects on the physical properties of the other component. The alpha helix component of the protein is generally increased by contact with phospholipid at the transition temperature. In a fluid bilayer, the proteins undergo conformational changes and are in a "fluid-like" state in which accessability to other molecules within the bilayer is increased (13). Increased viscosity decreases the conformational freedom of the proteins and their ability to respond to external effectors.

The lipid in the vicinity of the integral protein is also profoundly changed. Complexes appear to be formed between the proteins and a region of the lipid bilayer in its immediate vicinity with the result that this "boundary" lipid represents a non-melting or gel-like area in distinction to the bulk of the meltable lipid (13, 14). This boundary lipid has been thought to consist of about 30 molecules of lipid to one cylindrical protein with radius about 30 Å. Whether the relationship between bilayer lipids and integral proteins can be considered in terms of such a formed complex or not is problematical; but, in any event, it is evident that in the presence of the protein, the adjacent acyl chains of the bilayer lipids will be constrained in a gel-like arrangement in which lipid-protein interactions are stronger than lipid-lipid interactions (15, 16).

Effect of lipid environment on membrane enzyme activity

Having discussed, somewhat superficially, the nature of membranes and the relationships of their components, it is important to consider the effects exerted by the membrane lipids on an important property of the integral proteins, namely, their activity as enzymes. There seems to be no fixed pattern to this effect, at least insofar as present means of measurement can ascertain. In general, rates of membrane-bound enzyme activity are increased with low microviscosity and decreased with formation of a gel-like environment, but this is not true for all cases. For example, many enzymes function optimally at the transition point. An example is phospholipase A_2, which requires a gel-like area for an enzyme-substrate organizational step, but then shows maximal activity in a more fluid domain (17). Heron and coworkers (18, 19) have considered that a decreased membrane fluidity brought about by increased cholesterol/phospholipid or sphingomyelin/phosphatidylcholine or increased saturation of the acyl chains, causes increased lipid-lipid interaction and decreased lipid-protein interaction. This may result in overexposure of the enzyme to the aqueous phase and increased vulnerability to enzymatic degradation or susceptibility to being released from the membrane entirely. Under these conditions, the capacity of the enzymes for modulation is decreased. Increased fluidity, on the other hand, may result in vertical displacement of the protein receptors into the membrane. In this case, activity is either lost or markedly altered. This same effect is shown in the effect of fluidity on the transfer of intrinsic proteins between membranes. Such transfer is facilitated when the recipient membrane is more fluid than the donor (20).

Activity of most membrane-bound enzymes, however, increases with increased membrane lipid unsaturation and hence, fluidity (21, 22). This is true for cytochromes c, c_1, a_3, and cytochrome c oxidase, adenylate cyclase (norepinephrine-stimulated) (23), and 5′nucleotidase. On the other hand, ($Na^+ + K^+ + Mg^{2+}$) ATPase had the reverse behavior (24, 25) indicating a possible inactivation of the enzyme by polyunsaturated fatty acids (PUFA). In general, the effects of PUFA appear to depend on their effect on membrane fluidity, which is a function of unsaturation in general rather than of the structures of any specific families of fatty acids. However, in a few cases, some fatty acid specificity is seen. Thus, the membrane-bound 5′-mononucleotidase of brain decreases in animals on a fat-free diet and increases with dietary linolenic, but not linoleic acid, thus hinting at some more specific function for the former (25).

The polar head groups of the membrane lipids have been shown to have a definite influence on enzyme activity (26, 27). Since these studies were usually done with pure phospholipids, it is interesting to speculate on the consequences of the usual membrane bilayer structure in which different phospholipids tend to concentrate on different sides of the bilayer. Sandermann (28) discusses the "viscotropic regulation" of enzyme activity by the fatty acid chains and the "interfacial regulation" by the polar groups of the phospholipids. He also proposes that phospholipids bind to membrane proteins

in two or more "clumps" of about 25 molecules each. Enzyme activity appears with the first lipid shell (or boundary lipid) bound to identical binding sites on the protein and increases cooperatively as the remaining lipid is bound.

Effect of membrane lipid environment on transport

A related effect of membrane lipids on membrane proteins is the regulation of transmembrane transport processes. Disregarding, in the present context, ion transport dependent on $(Na^+ + K^+)$ATPase or $(Na^+ + K^+ + Mg^{2+})$ATPase, which have already been considered briefly, it appears that water and many small cations, anions, and non-electrolytes are transported across membranes through specific pores or channels (29–31). These channels are generally formed by a dimer or higher oligomer of a transmembrane protein with a largely hydrophilic inner surface making up the channel and a hydrophobic outer surface in contact with the lipid bilayer. The type of substance transported is regulated by the electrostatic or hydrogen-bonding groups lining the pore surface and by the size of the pore (29). Gating of the channel or quantitative control of the substances transported may be a function of the nature of the lipid bilayer, first, in its ability to change between liquid crystal and gel forms and second, in the specific nature of the lipids and, possibly, in their acyl chains (31, 32). Such regulation may come about from the known ability of such changes in the lipid bilayer to change the conformation of the transport proteins and thus their ability to permit or reject access to the channels. Such a conformational change may not be dependent on alteration of the bulk bilayer lipids, but may be related to the much more dramatic change in the relatively few molecules making up the boundary lipid associated with the transport proteins (32). It is possible that this type of change may be more specific than a simple response to bilayer fluidity alterations and may, indeed, depend in some cases on specific acyl groups associated with the lipids associated in this way with the transport proteins (33).

Effect of topical application of prostaglandins on EFA deficiency symptoms

Following the discovery of the prostaglandins and other eicosanoids, it was tempting to assume that some, if not all, of the symptoms of EFA deficiency were actually caused by a deficiency of these substances and that the disease might be cured by administration of prostaglandins or related substances. Of course, the route of administration was of utmost importance since many eicosanoids have very short chemical or biological half-lives. It had been shown in several studies (34, 35) that a deficiency of EFA results in decreased production of prostaglandins, at least in some tissues. Ziboh and his coworkers (36, 37) have reported that topical application of PGE_2 clears the typical scaly skin of EFA-deficient rats and that the major cause for the low production of prostaglandins is the inhibition of cyclooxygenase by 20:3 (n-9) rather than the lack of substrate 20:4 (n-6). Kupiecki, on the other hand, had previously reported that infusion of PGE_1, PGE_2, or $PGF_{2\alpha}$ failed to correct the dermal signs of EFA deficiency in rats (38).

The evidence from columbinic acid

Probably the most definitive evidence distinguishing the eicosanoid and non-eicosanoid functions of the EFA stems from the discovery and study, by Houtsmuller and coworkers (39), of a fatty acid unrelated biosynthetically to arachidonic acid but possessing the nutritional properties of an essential fatty acid. This acid, called columbinic, from its occurrence in the seeds of the columbine, has the structure: *t*5,*c*9,*c*12-octadecatrienoic acid. In preventive and curative studies with rats, it is about as effective as arachidonate in promoting growth and in preventing or curing skin symptoms such as the dermatitis of EFA deficiency and the water loss and other symptoms of loss of skin integrity. It was also found to replace 20:4 in the mitochondrial lipids and to promote oxidative phosphorylation. Columbinic acid is not converted to a prostaglandin-like substance and, indeed, inhibits cyclooxygenase synthetic activity. Its bishomo derivative, *t*7,*c*11,*c*13-eicosatrienoic acid, is inactive as an essential fatty acid and actually exacerbates the EFA deficiency symptoms.

However, columbinic acid does not correct the typical haematuria of EFA deficiency and, although it improved fertility in EFA-deficient females somewhat, most mothers died during parturition, probably because lack of prostaglandins prevented adequate uterine contractions. Columbinic acid also prevented the inflammatory reaction and depressed platelet aggregation, again probably because of its inhibition of cyclooxygenase activity. Of particular interest was the finding that heart cells could not be cultured in the absence of serum but with columbinic acid, because of inability to adhere to the glass.

This information thus distinguishes some of the structural functions of the essential fatty acids from their action as prostaglandin precursors. It again serves to emphasize the direct EFA function in maintenance of epidermal integrity and function.

Conclusion

It seems likely, from the results discussed above and from the many studies on the subject (some of which are included in the discussion), that the mechanisms of

action of the essential fatty acids fall into three major categories.

I. As the major source of mammalian polyunsaturated fatty acids, they are involved in the homeoviscous control of the membrane bilayers of most cells. In this capacity, they also exert control over the intrinsic enzymes of the membranes and on those integral proteins with other functions for which conformation is dependent on the nature of the surrounding membrane lipids.

II. They are the necessary precursors of the eicosanoids, the prostaglandins, leukotrienes, and related substances that have a profound influence on many of the cellular reactions.

III. They have a controlling influence on many transport processes by which small molecules are translocated in either direction across cell membranes. In this capacity, they may exert a quantitative control over a vital process by regulating the amounts of these small molecules that can be transported through a transmembrane protein channel or pore. This function may be accomplished by control of the conformation of the transmembrane proteins that form the channel.

While the first two mechanisms have been well-documented and thoroughly studied, the third is still unclear and much research remains to be done before it can be thoroughly understood.

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