



Lipids of Biologic Importance

Peroxidation Products and Inclusion Compounds of Lipids

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THIS paper is devoted to a brief consideration of two special classes of lipid materials which may not have received the attention they deserve in connection with metabolic problems in the lipid field.

One class consists of the various autoxidation or peroxidation products of lipid materials, particularly the initial peroxidation products of fatty acid esters. The second class consists of so-called lipid inclusion compounds, the existence of which has been recognized vaguely for many years but about which little definite information has been available until recently.

These two classes of materials are not related in any direct sense. From a biologic standpoint, peroxidation products usually are regarded as abnormal lipids. The inclusion compounds, on the other hand, are regarded as biologically normal substances, but their biologic importance has been little recognized. In another sense, however, there is a relationship between these two classes, in that the existence of the second prevents formation of compounds of the first. This will become more apparent as the discussion progresses.

PEROXIDATION PRODUCTS OF NORMAL LIPIDS

{A} Nature of Peroxidation Reactions

The products of these reactions are formed by the interaction of free or loosely bound molecular oxygen with lipid materials, at or in

the vicinity of unsaturated centers. Most unsaturated fatty acid esters react directly with oxygen of the atmosphere, initially forming peroxides, most of which are hydroperoxides; hence the term "peroxidation." One of the best known features of the *in vitro* reaction is its autocatalytic nature; hence the term "autoxidation." It has been established that the autoxidation reaction is a free radical chain reaction, and that the hydroperoxides formed in the initial phases are themselves the catalysts that are responsible for acceleration of the oxidation as the reaction progresses.

In general, the oxidizability or the rate of autoxidation increases with increasing unsaturation, and esters of polyunsaturated fatty acids, such as arachidonic or hexaenoic acid, oxidize many times more rapidly than esters of oleic acid under the same conditions.

The rate of oxidation also is accelerated by various other catalytic influences. Thus, various forms of light energy, such as visible light, ultraviolet light, x-rays, and nuclear radiations accelerate autoxidation. Light photons of low energy do this by accelerating decomposition of peroxides to give chain-initiating free radicals; with radiation of higher energies, the production of free radicals from unoxidized lipid molecules also is involved.

The oxidation of polyunsaturated fatty acid compounds that contain *cis*, *cis*-1,4-pentadiene groups is accelerated by lipoxidases. These enzymes are found in various leguminous plants, but as yet their presence in animal tissue is problematical. The enzymes catalyze the production of hydroperoxides very similar to those formed in ordinary autoxidation, except that the peroxides are optically active.

The formation of lipid hydroperoxides is catalyzed also by various metallic ions, in some

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cases at very low concentrations. Copper or iron at a concentration of 1 p.p.m., or less, markedly accelerates the autoxidation of lipid materials in general. Certain iron-containing biologic substances are very effective catalysts, particularly hematin compounds such as cytochrome C and hemoglobin.

Because of the chain nature of the autoxidation reaction, it also may be inhibited by very small amounts of certain substances. Among biologic substances, the various forms of vitamin E, i.e., the tocopherols, are the best known inhibitors of lipid oxidations. Vitamin E is found in small amounts in almost all natural fats and oils. In general, various polyphenolic substances are effective as antioxidants or inhibitors of autoxidation because of their ability to interrupt the propagation of autoxidation reaction chains. However, vitamin E is considered to be a biologic antioxidant, also, and, largely on the basis of circumstantial evidence, may conceivably be involved in preventing *in vivo* oxidations by some other mechanisms.

The autoxidation of lipid materials does not end with the formation of peroxides. As already indicated, peroxides or hydroperoxides decompose, and this decomposition is more rapid with hydroperoxides of more unsaturated lipids. At the same time, further oxidation, and, also, polymerization, may take place. Thus, as oxidation progresses a host of secondary oxidation products is formed from fatty acid esters, including various aldehydes, ketones, acids, and other substances.

(B) Biologic Importance of Autoxidized Lipids

Autoxidized or peroxidized lipid materials are important nutritionally and in other biologic ways. The autoxidation of fats is responsible for rancidity in fat-containing food products. The prevalence of oxidative rancidity in foods is far greater than is suspected generally. Frequently, the rancid flavor and odor of oxidized fats are hidden by other flavor components of food products so that, although the over-all flavor of a product may be regarded as inferior, the presence of fat rancidity is not recognized.

Until recent years, rancidity was considered

objectionable primarily from the standpoint of aesthetics. Now, however, it is recognized that rancid fats in food products are objectionable because of nutritionally harmful qualities, although the extent of their harmfulness has not yet been assessed adequately. It is known that oxidized fat contributes to a rapid destruction of autoxidizable vitamins, such as the fat-soluble vitamins A, D, and E, and that it contributes to the destruction of various other nutrients, also. There are increasing evidences, also, that fat peroxides and other autoxidation products may have certain direct toxic effects.

Another area in which lipid peroxidation products may be important concerns the effects of high-energy radiation on tissue. It has been found that, when animal tissues are irradiated with high-energy radiations, peroxides are formed, including peroxides of lipid materials. These peroxides have been implicated in carcinogenic and other toxicologic effects of high-energy radiations. However, much remains to be done to elucidate the role of the lipid peroxides in this field.

It has been shown that, with some animals, there is an accumulation of fat peroxides and other lipid oxidation products in vitamin E deficiency. The peroxides accumulate in adipose tissues, as well as in the fatty components of various other tissues. In vitamin E-deficient rats, brown pigment forms in muscle tissue, which is believed to be a high polymer formed from oxidized fat.

It has been reported, also, that peroxides are present in the lipids of atheromatous plaques. Their role and importance in relation to atherosclerosis and heart disease, however, remain to be determined.

In summary, it may be stated that products formed by the reaction of free or loosely bound oxygen with lipid materials do have importance in biologic systems, although, in general, they are to be regarded as abnormal biologic substances.

LIPID INCLUSION COMPOUNDS

It long has been known that soybeans and various other oil-containing seeds may be stored for relatively long periods without any demonstrable oxidation of their lipid content.



This cannot be explained satisfactorily on the basis of impermeability of the seeds to oxygen.

Again, although blood contains hemoglobin and oxyhemoglobin, which are powerful agents for promoting lipid oxidations *in vitro*, normally there appears to be no appreciable peroxidation of any of the lipid materials in blood.

Vitamin E is known to be present to an appreciable extent in soybeans and, to a lesser extent, in normal blood. Nevertheless, the amounts present are quite insufficient to account for the inhibition of lipid oxidation, at least on the basis of any antioxidant mechanism that has been observed *in vitro*. These observations provide a point of departure for consideration of another unusual class of lipid materials, namely, lipid inclusion compounds.

Although, for many years, complexes of lipids with various materials had been known to exist, it was not until 1941 that a clear insight into the nature of some of the simpler inclusion compounds was obtained. It was discovered that straight chain aliphatic substances, such as fatty acids and their esters, would form crystalline complexes with urea, in which molecules of urea were arranged in a spiral structure around the aliphatic carbon chain. The urea was regarded as a "host" substance, and the straight chain lipid material, a "guest" substance.

It has since been discovered that various other relatively simple molecules can serve as host molecules for lipid materials, including thiourea, desoxycholic acid, and Schardinger dextrans. In more complex inclusion compounds, molecules of high molecular weight may serve as host molecules, and the inclusion compounds themselves, although having highly

organized structures, are frequently non-crystalline.

At ordinary room temperatures, linoleic acid is a colorless liquid. However, it readily forms an inclusion compound with urea, in the form of a crystalline solid. One of the most remarkable features of this complex, and a feature of lipid inclusion compounds in general, is that it is completely stable to autoxidation. The urea-linoleic acid complex may be exposed to the atmosphere for many months without showing any measurable uptake of oxygen.

Linoleic acid has come to be known as one of the "essential" fatty acids. In purified form, it is also quite susceptible to oxidation. In order to eliminate the problem of the destruction of linoleic acid by autoxidation in experimental animal diets, it now is the practice, in some laboratories, to add linoleic acid to the diet, in the form of its urea complex. The essential fatty acid activity of the complex is comparable to that of an equivalent quantity of pure linoleic acid. The complex evidently is broken down completely in the gastrointestinal tract.

More recently, it has been found possible to stabilize vitamin A as an inclusion complex in cornstarch. A variety of other lipid inclusion compounds have been observed and characterized in the past several years.

It is highly probable that these lipid inclusion compounds are of great importance in nature, and, that, in many instances, the existence of inclusion compounds is responsible for the stability of those lipid materials which, in their isolated state, are highly unstable to atmospheric oxygen.

