The Lipids in Relation to Atherosclerosis

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The usual definition of atherosclerosis suggests that the disease is a fatty degeneration of the arterial wall. It is becoming increasingly apparent that the disease accompanies abnormalities in the metabolism of various lipids, and the number of lipids involved in the process seems to be growing as investigators delve into the matter. At this stage of our knowledge, it cannot be stated dogmatically that abnormalities of lipid metabolism are the cause of the disease. Such may be the case, however, for imbalances induced in lipid metabolism have led to lesions resembling atherosclerosis in many respects.

The number of lipid substances in nature is legion, and most of these are not well characterized. The variety of structures is great, and their single common property is that they occur together because of similar characteristics of solubility. The state of affairs in lipid chemistry is rather parallel to that of protein chemistry. We know the structures of many of the simple units which together make up the complex lipids, and we are continually finding new units previously unsuspected within the lipid family. The compositions of the more complex lipids are known only empirically, and the detailed structures have yet to be worked out for many compounds with molecular weights of less than 1,000. The structures of proteolipids and lipoproteins are not known, and for the present we must be content with knowing the approximate composition of these substances.

Even the determination of composition of many of the mixtures or concentrates that biochemists isolate presents a difficult problem. Thus far, there is no good method for determining the triglyceride content of a lipid, and until recently the common fatty acid, oleic acid, had never been measured directly—only by difference. We have only a few chemical or physical methods which allow us to determine specific lipid components. We are accustomed to group analyses such as saponification number, iodine value, Lieberman-Burchard reaction, spectrophotometric analyses for polyunsaturated fatty acids and lipid-soluble phosphorus.

Although this condition persists in our field of work, some progress is being made. Lipid substances differ not so much by possession of different specific structures as by being homologs, isomers or vinyllogs of each other. To distinguish such minor differences, physical methods have proved to be more effective than chemical methods. Through partition, displacement and paper chromatography we are now able to separate fatty acids differing by as little as one carbon atom, a double bond or by isomerism of several types. Countercurrent distribution and fractional distillation are used for determination of composition and isolation of significant quantities of highly pure substances, but none of these more precise methods has become common practice.

Progress in biochemistry can be made only in proportion to the precision of its methodology. Thus, the rather primitive state of lipid analyses precludes a highly detailed knowledge of the role lipids play in atherosclerosis. Nevertheless, information gained by epidemiologic, biologic, nutritional and crude chemical studies indicts the lipids as being involved in atherogenesis. If we wish to approach the ultimate answer by another magnitude of detail, we must adopt stricter criteria of purity and we must develop and use modern methods.

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which have another magnitude of precision. To state this another way, "we must not pick fleas with boxing gloves."

The following will survey the lipid substances briefly and point out known or suspected connections which specific lipids may have to the problem of atherosclerosis. This will necessitate elimination of entire areas of the lipid field, and emphasis will rest on specific substances which are implicated more than others.

### THE STEROIDS

This class of lipids has been implicated in atherogenesis largely because of the presence of cholesterol in the fatty plaques. Its importance in the problem of atherosclerosis may be exaggerated by the happenstance that it is the only serum lipid which can be easily determined by a color reaction. Cholesterol, its precursor squalene, and its chief metabolic product, cholic acid, are shown in Figure 1.

For a long time it has been known that a relationship exists between the level of plasma cholesterol and the incidence of atherosclerosis and coronary heart disease. It is not known whether hypercholesterolemia is the cause of the atherosclerotic lesion or whether it merely accompanies the lesion, but, the plasma cholesterol content is a useful index of susceptibility to circulatory accidents. Moreover, feeding cholesterol to either rabbits or chickens induces lesions of their aortas which resemble atherosclerosis. This experimentally-induced atherosclerosis is the starting point for many of the tests of therapeutic value of substances.

Cholic acid, a metabolic product of cholesterol, is excreted in the bile. If the cholic acid content of the plasma is raised, it is accompanied by an increase in plasma cholesterol. Feeding cholic acid thus increases cholesterol transport. If cholic acid is fed with an otherwise fat-free diet, essential fatty acid deficiency rapidly develops (vide infra). Hypercholesterolemia, induced by cholic acid, may lead to atherosclerosis as experiments of Fillios et al. suggest.

Plant sterols fed to animals and man have been reported to lower plasma cholesterol. The therapeutic effect is not great, the plasma cholesterol levels being diminished 5 to 25 per cent by the treatment. This effect has been presumed to be the result of decreased absorption of cholesterol although this explanation has been questioned.

The estrogens have been found to prevent experimental atherosclerosis. Estradiol (Fig. 2) and a variety of other substances possessing estrogenic activity, have been found by Katz and other workers to reduce the atherosclerotic lesions in the aorta of chickens fed an atherogenic diet containing cholesterol. Thus, hormone balance may help explain the sex difference in susceptibility of man to atherosclerosis. However, the mechanism of this action is not known.

Vitamins D, one of which is shown in the formula in Figure 2, are derived from or related to the steroids. The steroid nucleus, ruptured in ring 2, carbon 19, becomes a methylene group. Vitamins D are not obviously involved in lipid metabolism, but their function in regulating calcium metabolism suggests that these lipids may play a role in the calcification which often accompanies the development of atherosclerosis.

Vitamins A, E and K have a superficial structural resemblance in that they all have an aro-
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Vitamin K is known to promote the formation of prothrombin, and inasmuch as it is involved in the coagulation of blood, it plays a minor role in atherogenesis.

The involvement of vitamins A and E is the prevention of atherogenesis is suggested by the work of many laboratories. Vitamin E (tocopherol) is known to be antioxidant in vitro, preventing the development of rancidity of the unsaturated fats. There is also reason to believe that tocopherol acts as a biological antioxidant. The experiments performed in Dam's laboratory have shown that when animals are fed a tocopherol-free diet containing easily oxidized fat, the depot fats contain peroxidized unsaturated fatty acids. Glavind et al. found liperoxides in atherosclerotic aorta but not in normal aorta. This suggests that the reserve of biological antioxidants of the atherosclerotic subject is low. Weitzel et al. found that tocopherol had a weak antiatherosclerotic effect on hens at a dose level of 50 mg./day. The administration of vitamin A, 7 mg./day, notably improved the atherosclerotic lesions. The effect was even greater when the two vitamins were given together. Recently Hill and Larson, at our institute, found that in six of eight young swine which were fed a tocopherol-free diet for fifty days or more sclerotic lesions developed in the aorta. Histologic examinations of the plaques by Dr. Jesse Edwards of the Mayo Foundation revealed the lesion to be medial calcification. All this evidence points to the involvement of tocopherol, and perhaps vitamin A, in the complicated pattern of abnormality which results in atherosclerosis.

THE PHOSPHOLIPIDS

Phospholipids also exist in great variety. They differ in the fatty acids and bases found in their structures. In Figure 4, a lecithin molecule is pictured. The lecithins contain choline esterified with a phosphatidic acid which has an unsaturated fatty acid esterified at the α' position of the glycerol and a saturated fatty acid at the β position. The phosphatidyl ethanolamines (cephalins) are analogous to the lecithins except that their base is ethanolamine. In the phosphatidyl serines, the amino acid serine replaces the nitrogen base. Phosphoinositides are known in which inositol replaces the nitrogen base. Sphingomyelins are complexes in which the base sphingosine is esterified with one fatty acid and phosphoryl choline. In the acetal phosphatides the fatty acids of phosphatidyl ethanolamine are replaced by one fatty acid, aldehyde. Most of these substances have not figured in the discussions of atherosclerosis, probably because they are not well understood.
known, the methods for their determination being primitive. Thromboplastin, the coagulation factor of tissue and platelets, contains cephalin as a necessary component. Thus the phosphatidyl ethanolamines may be involved in the mechanism of atherogenesis.

The phospholipids are known to be universal components of tissue structure and circulating lipoprotein. Alterations of the content of the essential fatty acid components have been shown to be induced by changing the dietary fat. Such changes in fatty acid composition, or changes in the amount of phospholipid, can alter the physicochemical properties of the circulating lipid leading to abnormal transport or utilization of fat. Generally speaking, when lipemia occurs in an animal, the phospholipids, cholesterol esters and neutral fat increase in proportion. Therefore, if the reserve of one component of any of these lipids is low in the animal it may be depleted by a lipemia induced by ingestion or synthesis of another component, and the composition of the lipoprotein may become abnormal.

THE GLYCERIDES

The most important of the lipid groups from the point of view of abundance are the glycerides, which comprise more than 95 per cent of the common vegetable oils and animal depot fats. These substances can exist in great variety because of isomerism and the larger number of fatty acids which are present in these molecules. The natural fats and oils are mixtures of mixed triglycerides. Triglycerides cannot be determined easily, either as a group or as individual substances. Thus far, the glyceride composition of no natural fat or oil has been determined farther than to state the approximate content of the groups of saturated and unsaturated glycerides. Only a few pure glycerides of known structure have been isolated from natural sources. Obviously our knowledge of this group of rather simple substances is extremely primitive, and this is a field which needs new methodology.

The triglycerides are non-polar substances, insoluble in water. The triglyceride molecule shown in Figure 5 represents one containing three of the more common fatty acids, stearic, oleic and linoleic acids. If one or two of these fatty acids are removed by hydrolysis, the resulting di- and monoglycerides have properties much different from the triglyceride. The inherent polar groups of the triglyceride are masked by the long hydrocarbon chains, but in the di- and monoglycerides the polar hydroxyl groups are exposed. This gives these substances great surface activity, and they become excellent emulsifying agents which facilitate the digestion and absorption of fat. Classwise, the triglycerides, and the di- and monoglycerides derived from them, have important nutritional functions but no specific

Fig. 5

Fig. 6. Relationship between death rate from coronary heart disease and fat content of the diet. From: Keys, A. Atherosclerosis: a problem in newer public health. J. Mt. Sinai Hosp., 20: 118, 1953.
Fatty acids

Fatty acids occur as components of almost all lipid structures, and the wide range of properties of the fatty acids contribute to the wide range of properties of lipids. The fatty acids themselves differ in respect to chain length, number and position of double bonds, branching of the chain and other minor variations. The state of development of analysis for individual fatty acids is far in advance of that for other classes of lipids. Displacement chromatography, paper chromatography, gas phase chromatography and countercurrent distribution can be used for the sharp separation and measurement of closely related fatty acids. An entire new range of biochemical problems can now be attacked by the newer methods.

Formulas for a group of fatty acids is shown in Figure 7.

Dietary fatty acids bear some relationship to the blood-clotting process. Lipemic blood is found to be more coagulable than non-lipemic blood and is conducive to fat embolism. Lipemia caused by saturated fat reduces the activity of fibrinolysin but lipemia caused by unsaturated fats does not. Poole found that free fatty acids added to plasma reduced the clotting times, and his experiments suggest some differences in effect dependent upon the fatty acid added. Davis has found that gellation time of serum albumin is influenced by the type of free fatty acid added. Saturated acids reduce the gellation time more than do the unsaturated acids. Thus, prolonged or continual lipemia is a hazard which increases susceptibility to circulatory accidents. This phenomenon should be studied more closely, attention being paid to the effects of different pure fatty acids.

Bronte-Stewart measured the extent and duration of lipemia following a fatty test meal given to persons who had had myocardial infarction. For one week thereafter the patients were given corn oil in addition to their regular diet. The fat tolerance curve was measured again and the lipemia was not as severe nor did it remain as long as in the previous test. It is therefore clear that the lipemia following a fatty meal can be moderated by the type of fatty acid ingested.

In our laboratory it has been found that the requirement of essential fatty acids is increased

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Fig. 7. Formulas for some of the common fatty acids. (1) butyric acid, (2) caprylic acid, (3) caprylic acid, (4) capric acid, (5) lauric acid, (6) myristic acid, (7) palmitic acid, (8) stearic acid, (9) elaidic acid (trans isomer of oleic acid), (10) oleic acid, (11) one isomer of linoleic acid, (12) one isomer of linolenic acid, (13) one isomer of arachidonic acid, (14) one isomer of eleostearic acid, a conjugated unsaturated acid.
as the content of saturated fat is increased in the diet.\(^{19}\) This suggests that the proportion of essential fatty acids to other fatty acids in the diet may be as important as the absolute amount of essential fatty acids present. The high fat intake of Americans is predominantly of the saturated type of fat, whereas the fat content of the low fat diet of the Japanese or the Bantu is proportionately more unsaturated. Hence the problem of total fat intake may be a problem of the type of fat eaten.

Most of the fatty acids have no known specific function. However, some of the unsaturated acids with two or more \textit{cis} methylene-interrupted double bonds have been shown to be required by animals as dietary nutrients. The discovery of the essentiality of these fatty acids was made by Burr and Burr\(^{19}\) in 1929, and in succeeding years these substances have been the subject of much investigation.\(^{20}\) The “essential fatty acids” are shown in formula in Figure 8. Linoleic and arachidonic acids, which have identical hydrocarbon ends on their molecules, provide both restoration of growth and recovery from skin symptoms in animals made deficient of essential fatty acids by feeding them a fat-free diet. Linolenic acid and presumably those acids which have identical hydrocarbon ends on their molecules provide only the growth function. Many polyunsaturated acids existing in nature have not yet been isolated in pure form or tested for essential fatty acid activity.

The methylene group flanked by two double
bonds is reactive and it is this structure which provides special chemical properties and the physiologic activity of these substances. The non-conjugated double bonds can be isomerized by alkali at high temperatures to yield conjugated forms which can be identified specifically by their ultraviolet spectrums. This is the basis of the best current method for the analysis of the polyunsaturated fatty acids.\textsuperscript{21}

The active methylene group is also easily attacked by oxygen and it is this oxidation that is the cause of rancidity in fats and oils. Autoxidation can presumably take place in vivo, for lipoperoxides have been found in tissues of vitamin E-deficient animals.\textsuperscript{4} Glavind et al.\textsuperscript{5} found that lipoperoxides exist in the atherosclerotic aorta but not in the normal aorta, and that the severity of the lesions parallels the peroxide value of the aorta lipid.

The polyunsaturated acids occur in a large proportion of the cholesteryl esters of plasma. Thus, they are circumbiausely involved in cholesterol transport and metabolism. Cholesteryl esters accumulate in the livers of rats deficient in essential fatty acids and the esters become more saturated. The relationship of essential fatty acids to cholesterol transport also has been inadvertently pointed out by some experiments from our laboratory.

In an effort to accelerate essential fatty acid deficiency, means were sought to increase lipid turnover. Our first experiments\textsuperscript{22} tested the effect of diabetes upon the onset of essential fatty acid deficiency. Alloxan diabetes in rats fed an essential fatty acid-free diet induced severe symptoms of deficiency within one month rather than the usual three months required on a fat-free diet alone. Figure 9 shows that the more severe the diabetes, the more severe the symptoms of essential fatty acid deficiency at the end of one month. Rats which received linoleate were only partially protected from the essential fatty acid deficiency.

Because of the known association of essential fatty acids and cholesterol in the circulating cholesteryl esters, it was suspected that the accelerated deficiency was caused by the hypercholesterolemia which accompanies diabetes. Therefore, rats were fed a fat-free diet containing 1 per cent cholesterol, and a similar acceleration of essential fatty acids deficiency was observed. This is shown in Figure 10 in which the onset and cure of cholesterol-accelerated deficiencies are traced.

Hyperthyroidism, which also is accompanied by hypercholesterolemia, was induced in rats fed a fat-free diet.\textsuperscript{18} Again the deficiency appeared within a month. The accelerated deficiency was so severe that it could not be prevented fully by doses of linoleate usually adequate to prevent essential fatty acid deficiency. This is illustrated in Figure 11. Recently we have found that the aminonucleoside derived from puromycin, when injected into rats fed a fat-free diet, induces early deficiency of essential fatty acids. This drug induced a hypercholesterolemia of 450 mg./100 ml. and deficiency symptoms within two to three weeks. These results suggest that (1) hypercholesterolemia depletes the reserve of essential fatty acids in the animal faster than a fat-free diet.
and (2) that essential fatty acids are required for the transport of cholesterol.

The essential fatty acids have been shown by Kramar and Levine\textsuperscript{23} to maintain capillary resistance. These investigators showed that the capillaries of rats deficient in essential fatty acids ruptured at much smaller negative pressures than did the capillaries of normal rats or rats fed linoleate. Thus, if atherosclerotic lesions are initiated by hemorrhages, a relative essential fatty acid deficiency could increase susceptibility to the disease.

In some recent experiments at our institute, Hill et al.\textsuperscript{24} found that in three of six young swine fed an essential fatty acid-free diet lesions of the aorta developed within fifty days. In two swine fed linoleate, and on an otherwise
similar regimen, the lesions did not develop. Histologic examination of the aorta by Dr. Jesse Edwards of the Mayo Foundation revealed the lesions to be medial calcification. The lesions which developed in the first case observed are shown in Figures 12 and 13. Although the lesion is not an intimal lesion, and was not found in all experimental animals, this limited experiment also indicates that essential fatty acids function in the maintenance of a normal arterial wall.

CONCLUSION

This review, representing studies of various lipids and methods of attack, shows that many lipids are involved in atherosclerosis. Essential fatty acids are in some way related to most lipids and the evidence presented seems to indicate that these acids might be at the heart of the problem. It may be trite to say so, but perhaps restoration of lipid homeostasis in man is the problem we should attempt to solve in combating the disease. The problem of atherosclerosis involves the proper balance between dietary essential fatty acids, nonessential fat, fat-soluble vitamins and other nutrients. It is hoped that this hypothesis is provocative enough to stimulate more precise investigation of the relationships of lipids to atherosclerosis using newer, more incisive methodology.

REFERENCES

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