of water but smaller amounts of sodium hydroxide would be required to obtain the same results. If the two oils had the same initial free fatty acid content and were as a consequence refined in the same manner, say, using 3 lbs. of water per 100 lbs. of oil, the degummed oil would be expected to be lower in quality. The resulting "over-refining" would have reduced its residual phosphorus to 0.5 p.p.m.

A study of the data for the light absorption at 465, 550, 620, and 670 mμ indicates that the higher strengths should remove more of some pigments than of others, but it is possible, as in this case, that the net effect on the official spectrophotometric color could not be observed. This is expected to be true especially with "green" oils which show high initial absorption at 670 mμ. The higher strengths would also remove more tocopherol, slightly reducing the stability.

Summary

Restricted tests in pilot-plant equipment have indicated that, starting with good quality crude soybean oil, the phosphorus level of the deodorized oil is closely associated with its color and oxidative stability but that the refined or bleached color is not a good criterion for predicting the quality of the finished oil. Phosphorus is removed by water in the presence of adequate concentrations of alkali, and optimum oil quality is achieved at phosphorus levels in the deodorized oil of no less than about 2 and no greater than about 20 p.p.m. Over-refining to a lower phosphorus content, by the use of too great an excess of caustic (and therefore of water) was harmful to oxidative stability in every instance and generally increased the color of the deodorized oil. The ash analysis of the deodorized oil is a fair indication of its residual phosphorus content at levels above 20 p.p.m.

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Fat, Cholesterol, and Atherosclerosis[1]

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THE TRUTH ABOUT FATS in the diet is of tremendous importance to the edible fat and oil industry of the United States. Considerable publicity has been directed to a potential danger from fats in human food which relates to cholesterol content, to meat fats *versus* vegetable oils, and to various and sundry other allegations. It is our purpose in this paper to review and discuss known and published facts about fats in metabolism and nutrition. We believe that present research in the field of fat metabolism, particularly as it relates to such abnormalities as atherosclerosis, is not only quite inadequate but wholly contradictory and confusing. Much more research is vitally needed before the truth can be definitely established. A large part of this research is now being sponsored and financed by the food industry. What are some of the facts concerning fat and cholesterol in relation to atherosclerosis?

Cholesterol

Cholesterol is a solid, fat-like monoatomic alcohol with a cyclic structure containing the cyclopentanophenanthrene ring. The word cholesterol is derived from the Greek and means "solid bile," referring to its physical character and its source. Cholesterol is found in all animal cells and is particularly abundant in fat, brain, spinal cord, bile, milk, egg yolk, liver, kidney, and adrenal. Since it is a universal constituent of all animal cells, cholesterol occurs in all foods of animal origin.

The cholesterol present in the blood is distributed between the plasma and the cells in about equal amounts. Normally the serum cholesterol level in man is between 130 and 220 mg. per cent. This level is not altered appreciably by feeding 2 to 10 g. of cholesterol, either in crystalline form or as cholesterol-rich food (4, 65, 67, 68, 69, 70). The blood cholesterol level is fairly constant for continued cholesterol intakes up to 700 mg. per day but can be increased with diets very rich in fats and in such conditions as pregnancy, alcoholism, icterus, nephritis, and diabetes. In 1950 London and Rittenberg (1) studied the regeneration rate of cholesterol in man with the aid of deuterium-labeled cholesterol and found it to be 50% in eight days. It has been estimated therefore that cholesterol synthesis in man is at the rate of 1.5 to 2 g. per day (2).

Cholesterol and phospholipid circulate in blood plasma as combination products with protein. These lipoproteins account for 90% of the total plasma lipid and consist of free cholesterol, cholesterol esters, neutral fat, phospholipids, and protein. There are two types of lipoproteins, α and β, depending on whether the conjugated protein is α- or β-globulin.

The recent work by Gofman and others (3) has shown that these plasma lipoproteins differ in size and density. They have different sedimentation and flotation rates and may therefore be separated into classes according to the speed of the analytical ultracentrifuge that is required for their migration. By using solvents of high density, the less dense β-lipoproteins can be made to float in the centrifugal field. Thus, at a specified density of solvent (usually 1.063

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with sodium chloride), this "upward sedimentation" can be characterized by a flotation constant. The values are recorded in S units after T. Svedborg, the father of the ultracentrifuge. By way of illustration, a molecule that undergoes flotation at a rate of 10×10^{-13} cm. per second per dyne per gram has an S_f value of 10 or is a molecule of the S_f 10 class.

In a summary of their findings Gofman *et al.* (3) have stated that the lipoprotein molecules in the serum of man and the cholesterol-fed rabbit are associated with atherosclerosis, that the molecules do not represent any part of the acute alimentary lipemia, that dietary restriction of fat and cholesterol in man results in a gradual decrease in the serum level of such molecules over a period of weeks to months, and that more of these molecules are present in patients who have survived a myocardial infarction. In Gofman's words, "the basic premise of this research was that there might be a defect in the molecules which transport fats and cholesterol that could be more intimately related to the pathogenesis of atherosclerosis than are the total analytical levels of the various lipids themselves, *e.g.*, serum cholesterol levels."

Keys has questioned these conclusions. He analyzed Gofman's data statistically and concluded that the measurement of the giant molecules is not superior to and may well be inferior to the measurement of total cholesterol in serum. Keys further stated that neither measurement (giant molecules or total cholesterol) is a good discriminator between persons with coronary disease and clinically healthy persons and that there is no evidence that a level of giant molecules in the blood may be of practical value as an indicator of the probability of present or impending coronary disease (4). Kroehler and Hill have gone further and have raised the question as to whether or not cholesterol forms definite lipoprotein molecules. They believe that cholesterol becomes adsorbed to the protein molecules without forming a definite compound. These same authors also postulate from electrophoretic mobility studies that no large serum lipoprotein complexes are present in arteriosclerosis (5).

The function of cholesterol in the animal body is still somewhat of a puzzle. As a universal tissue constituent it is involved in the organization and permeability of cell membranes and is therefore of considerable importance in general physiology. Cholesterol is the mother substance of bile acid (6, 7, 8) and of the sex hormone, pregnanediol (9). Cholesterol is the precursor of 7-dehydrocholesterol, which is a provitamin D, and is also believed to be the precursor of other sex hormones and adrenal cortical hormones. The unusually high cholesterol content of nervous tissue is suggestive of a functional significance, possibly beyond that of being an insulator by virtue of its low conductivity. A recent paper by Del Veccio, Keys, and Anderson (10) suggests that most of the cholesterol in muscle has a definite metabolic or structural function.

Despite its physiological importance, cholesterol is not an essential dietary constituent for it can be synthesized within the animal body (11, 12, 13, 14, 15, 16). That vegetable fats can bring about cholesterol production in the body has been reported by many investigators (4, 17, 18, 19, 20, 21, 22, 23, 24). By means of isotope studies it has been demonstrated that acetate is the precursor of endogenous cholesterol (2, 25, 26). Such a simple starting material as acetate can be obtained from carbohydrate, fat, or protein, from either animal or vegetable sources.

The daily intake of cholesterol in the mixed diet of an adult varies from 200 to 360 mg. (27). The presence of two-thirds of the cholesterol in the body as cholesterol esters indicates a possible role in the transport and metabolism of fatty acids. Cholesterol protects the body against the hemolytic action of bacterial toxins, snake venoms, phospholipids, soaps, and salts. Cholesterol from food is absorbed *via* the lymphatics but seems to require the presence of fat (28). The work of Schoenheimer and others showed that mammals do not absorb sterols of vegetable origin (29). Why cholesterol could and vegetable sterols could not be absorbed from the gastro-intestinal tract has indeed been a physiological mystery. In 1953, however, Hanahan and Wakil (30) completed some studies with C^{14}-labeled vegetable sterols and were able to show that ergosterol and possibly 22-dihydrocholesterol are absorbed to a slight extent from the gut of the rat. Recently Swell *et al.* (30a) reported that plant sterols are absorbed through the same mechanism as cholesterol in the rat and that plant sterols may be converted to cholic acid, cholesterol, or a cholesterol intermediate in the intestine or liver.

It has been accepted for some time that cholesterol is converted to dihydrocholesterol and coprosterol (31, 32, 33) and that coprosterol is the chief end-product of cholesterol metabolism (34, 35, 36). In September 1955 Siperstein and Chaikoff (37), using cholesterol labeled with carbon-14 in either of two positions, reported that the chief pathway of cholesterol catabolism involves its oxidation to bile acids. They stated that cholesterol is eliminated in the bile and in the feces, primarily as taurocholic acid in the rat and as glycocholic acid in man. The pathways are not clearly understood at present, and considerable work remains to be done before the fate of cholesterol in the body is definitely established.

We do know however that cholesterol is destroyed in the body, the amount varying with the intake. We likewise know that part of the cholesterol in the body is converted to cholic acid and is excreted *via* the bile but may possibly be reabsorbed in the upper intestine. We also know that part of the cholesterol is deposited in the tissues. It remains to be determined whether bile acid or coprosterol is the chief end-product of cholesterol metabolism.

Atherosclerosis

Atherosclerosis is the term coined by Marchand (38) in 1904 to denote a particular type of arteriosclerosis characterized by fibrous and fatty degenerative changes in the intimal layer of the blood vessels of the arterial system. Atherosclerosis is the most common form of arteriosclerosis, and one of the many confusing items is the frequent use of the two terms interchangeably. As Boyd has pointed out, if arteriosclerosis is the same as atherosclerosis, then either one or the other term should be dropped. It seems better for the present to use arteriosclerosis in a broad sense to include a variety of non-inflammatory forms of arterial disease, which may or may not have a common etiology (39).

The earliest changes in the blood vessels in atherosclerosis consist of fatty streaks in the intima. These lipid-laden deposits increase in size, alter the lumen,

ulcerate, and may even become hemorrhagic and necrotic. In time scar formation and calcification may ensue. It is not a question of presence or absence of atherosclerosis, but rather to what degree, for atherosclerosis has been found in young as well as old individuals. In 1914 Stumpf studied the aortas of children and found microscopic deposits of lipid during the third and fourth years of life (40). Yater *et al.* found instances of coronary artery disease in 866 men in the military service of the U. S. during World War II between the ages of 18 to 39 years, 450 of which were confirmed at autopsy (41). Enos *et al.*, in a study of coronary disease among U. S. soldiers killed in action in Korea, found coronary disease present in 77.3% of the 300 soldiers whose average age was 22.1 years (42). McGill, Peck, and Holman, in an analysis of aortic involvement in 300 consecutive autopsies, found atheromata of the aorta in every patient over 7 years of age (43).

Atherosclerosis is the most common form of arterial disease. An analysis of 2,030 autopsied cases of sudden and unexpected natural death shows that coronary artery disease accounted for 30.4% of all these cases, a figure higher than that for any group of diseases listed. Since atherosclerosis accounts for 99% of all coronary artery disease (44), it is the number-one killer today. Katz and Stamler (45) have estimated that atherosclerosis is the leading cause of death in the United States, claiming 200,000 per year or one-seventh of those dying from all causes.

Just what causes atherosclerosis is still unknown, but many possible etiological factors have been proposed through the years.

1. Inflammation
2. Imbibition of lipides from the blood stream
3. Chemical factors
   a. Bacterial toxins
   b. Hypertonic agents—nicotine, adrenalin
   c. Lead, alcohol, and tobacco
4. Effects of mechanical strain
   a. Hypertension and strain on arterial wall
   b. Loosening of connective tissue ground substance of intima
   c. Churning effect
   d. Hemorrhage and exudations into vascular wall
5. Metabolic factors
   a. Anoxemia, interfering with oxidative metabolism of vascular wall
   b. Lipemia and hypertension
   c. Gout, obesity, diabetes
6. Neurogenic factors—the stress and strain of daily living
7. Senescence—degenerative changes in vessel wall increasing with age
8. Hormones—adrenal cortex and medulla, thyroid, and sex hormones
9. Excess cholesterol, cholesterol esters, or cholesterol-containing lipoproteins, due to excessive cholesterol intake or deranged lipid metabolism affecting the synthesis or utilization of cholesterol

In any discussion of the etiology of atherosclerosis it becomes increasingly obvious not only that the cause is unknown but that no one of the causes suggested will suffice to explain the many factors involved. However two very different processes are inevitably mentioned in detail: the progressive morphological changes in arterial tissue and cholesterol metabolism. Are these two related? How do they come about? Which comes first, the deposition of lipid or the fibrous tissue changes in the blood vessels? Or do they go together? One of the pitfalls of experimental atherosclerosis is the absence of common ground as to what constitutes successful production of the condition in the first place. Some have been content with infiltration of lipid into the arterial wall whereas others have insisted on a definite thickening, some even to the extent that the lesion be grossly apparent.

For many years investigators had been attempting to produce lesions in the arterial system by means of vasotonic agents, bacterial toxins, by mechanical manipulation including cautery and ligation, and by dietary procedures. In 1908 Ignatowski (46) and Saltykow (47), working independently, were successful in producing atherosclerosis in rabbits by feeding meat, milk, or eggs. In 1913 Anitschkow (48), also Wacker and Hueck (49), produced atherosclerotic lesions in rabbits by administering pure cholesterol in oil. In 1930 Uchiyama produced intimal changes in the aortas of chicks fed cholesterol, which were more advanced than the spontaneous lesions noted in the controls (50). In 1946 Steiner *et al.* (51) were able to produce atherosclerosis in dogs fed cholesterol plus thiouracil. The purpose of the latter was to depress thyroid function.

Let us analyze these successful experimental productions of atherosclerosis. Cholesterol in the diet is foreign to the vegetarian rabbit, and the high levels fed cannot be handled readily by this animal. Duff (52) has questioned this experimental atherosclerosis in the rabbit because the atheroma is not similar to the spontaneous arterial lesion in the rabbit. In the chick Tamura found spontaneous fatty lesions in the aorta in 70% of apparently normal birds (53), and others (52, 54) have questioned whether cholesterol feeding in the chick affects the arteries over and above the lesions normally found in untreated controls. Dauber and Katz (55) produced intimal changes in the arteries of chicks, but it required a total of 48 g. of cholesterol over a 42-day period to produce microscopic changes and a total of 55 g. of cholesterol over a period of 49 days before the first gross lesion appeared. In the dog the required use of thiouracil to depress thyroid function before cholesterol feeding caused vascular changes after one year raises the question as to whether cholesterol alone can be considered the causative agent. The hazards of drawing too close analogies between the mechanism of experimental atherosclerosis and clinical atherosclerosis have been pointed out by Duff and McMillan (56).

Knowledge as to diagnosis and treatment of atherosclerosis is very meager. Practically all adults have atheromatous lesions, and individuals react differently to comparable degrees of involvement. Thus the treatment of atherosclerosis, as in all conditions where the cause is unknown and diagnosis is impossible, has been limited to empirical procedures. Because of the unusual emphasis on cholesterol in atherosclerosis, treatment has been directed toward lowering the blood cholesterol level. The use of thyroid hormone to decrease production of cholesterol is based on the observation that antithyroid compounds favor production of hypercholesterolemia (2, 51).

Reduction of blood cholesterol levels by dietary means has been attempted by many who have advocated feeding a host of materials including eggplant (57), sitosterol (58, 59), brain extract (60), and a low, cholesterol-low fat diet (61). It is practically impossible to remove cholesterol from the diet and still retain nutritional adequacy and palatability. Moreover marked reduction of cholesterol intake in

the treatment of atherosclerosis has been questioned because cholesterol can be synthesized in the body from fat, carbohydrate, or protein, either animal or vegetable; because increased blood cholesterol levels have not been established as the cause of atherosclerosis; and because such limitations of cholesterol intake would deprive man of such highly important foodstuffs as meat, milk, fish, and eggs. Several groups of investigators have shown that very low to very high levels of cholesterol in the diet have no marked effect on the cholesterol level in the blood (4, 62, 63). There have been numerous reports that vegetable fat increases cholesterol production in the body (4, 17, 18, 19, 20, 21, 22, 23, 24).

The idea of feeding eggplant to decrease blood cholesterol (57) could be carried still farther. But even if one were willing to substitute cowpeas for meat, milk weed for milk, pickerel weed for fish, and eggplant for eggs and to eat nothing else, the body in its wisdom would still synthesize its needed cholesterol from vegetable fat, from vegetable protein, and from carbohydrate.

The low-fat, low-cholesterol diets recommended by Morrison (61) were based on such intangibles as improved morale and increased sense of well-being in the patients studied. Morrison did not conclude that the pathologic process had been altered and agreed that his data were not amenable to statistical analysis. According to Robinson (23) and Mirone (64), the value of this low-fat, low-cholesterol diet in vascular disease has not been established. Gertler, Garn, and White (65) have raised the question as to the wisdom of removing cholesterol from the diet of individuals with coronary artery disease and have stated that there is no advantage to be gained from imposing a low-cholesterol diet on these patients. Keys *et al.* (4) have stated that unhappily for the proponents of dietary prophylaxis against atherosclerosis an effectively low level of cholesterol in the diet is not easily achieved, and Davidson (66) has concluded that further work is essential before the merit of low-fat, low-cholesterol diets for prophylaxis and therapy of human atherosclerosis can be fully ascertained.

There is more to nutrition than merely eating food. But until a causal relationship between nutrition and atherosclerosis has been established, we shall be confused by opinions, conjectures, and a plethora of controversial data. Much work has been published, but obviously still more needs to be done before the problems in atherosclerosis are solved.

Summary

Cholesterol is a universal constituent of all animal cells and therefore occurs in all foods of animal origin. Cholesterol is the mother substance of bile acid and of sex hormones and has other important physiological roles. Cholesterol is synthesized in the body from acetate obtainable from ingested carbohydrate, fat, or protein, of animal or vegetable origin.

The fate of cholesterol in the body has not been definitely established. The cause of atherosclerosis is unknown. Atherosclerosis occurs in young as well as old individuals. Atherosclerosis is the number-one killer today.

Diagnosis of atherosclerosis in the healthy individual is not possible. Treatment of atherosclerosis is empirical. Reduction of blood cholesterol levels by dietary means is difficult under conditions consonant with good nutrition. A well-balanced intake of all available foods on a modified total caloric basis appears to be the practical approach to the problem of fat, cholesterol, and atherosclerosis.

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