Cholesterol Content of Foods Versus Cholesterol Content of Animal Tissues

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LANGE (1) in a recent issue of this Journal published a paper under the title of "Cholesterol, Phytosterol, and Tocopherol Content of Food Products and Animal Tissues." Essentially the paper is a compilation from the literature of analytical data for, as the author states, "convenient consultation." The paper is preceded by an editorial note stating that, "It is offered as a help to technologists, dieticians, nutritionists, and physicians who need information on the cholesterol and vitamin E content of foods, animal tissues, and other materials." While such information is useful to the research worker, the implication should not be drawn from the editorial note that the cholesterol and vitamin E content of foods is of much use to the practising physician.

First, let us dispose of vitamin E or the tocopherols. These are interesting antioxidants, and food technologists have made use of them in a variety of ways. They play a role in experimental nutrition. They have no known function in human health or disease; hence let us not imply (at least until facts are available) that the dietician, nutritionist, and physician should further complicate practical human nutrition with a consideration of the vitamin E content of the diet.

Second, what about dietary cholesterol and its relationship to blood cholesterol and the type of arteriosclerosis known as atherosclerosis which is commonly associated with coronary heart disease? A number of studies, particularly the recent ones of Keys, *et al.* (2, 3), have shown quite conclusively that the blood level of cholesterol is not influenced by the dietary intake of cholesterol within wide variation of the dietary intake. As stated by Keys (2), "The serum cholesterol level of 'normal' men as represented here is not significantly related to differences in the habitual cholesterol intake over a range of something like 250-800 mg. per day." A level of 200-250 mg. cholesterol is that range of intake one obtains by avoiding cholesterol-rich foods such as eggs, whole milk, ice cream, butter, fat meats, etc. A level of 800 mg., or thereabouts, is obtained when these foods are included in the diet in the usual amounts. Hence why consider so-called "low cholesterol diets" at the risk of jeopardizing over-all good nutrition by limiting the intake of foods known to be superior from a nutritional viewpoint?

Can one decrease blood cholesterol by a diet devoid or practically devoid of cholesterol? Yes, if one has a diet devoid of fat, and that means vegetable fat as well as animal fat. This fact has been shown by Keys (2), also in animals by Abelin (4), and in unpublished studies with experimental animals from this laboratory. After blood cholesterol is decreased by means of a cholesterol free-fat free diet, and fat is then added to the diet, *either vegetable or animal,* there is a prompt increase in blood cholesterol even though the diet contains *no* cholesterol. Why then talk about limiting dietary cholesterol intake unless one is also going to remove *all* fat, both vegetable and animal from the diet?

It might also be pointed out that the actual feeding of large amounts of cholesterol to man (assuming the absence of hypothyroidism and any of the essential xanthomatoses) does not result in any increase in serum cholesterol (5). Unpublished studies from this laboratory with rats, dogs, and monkeys show that the addition of cholesterol, even at such high levels as 5 grams per kilo of body weight per day, does not result in an increase in serum cholesterol or deposition of cholesterol in body tissues including the blood vessels.

The classic studies of Rittenberg and co-workers (6) have shown that cholesterol can be synthesized in the body from acetate, a substance formed in the body from fat, *either vegetable or animal,* and also from glucose and protein, though most likely not preferentially, and much less rapidly from these latter two substrates.

Too often when the subject of dietary cholesterol and the level of blood cholesterol is discussed, only one side of the story is told. Thus a recently published book (7) on low cholesterol-low fat diets fails to make any mention of the fact that cholesterol is itself synthesized by the body (endogenous cholesterol as compared with exogenous or dietary cholesterol) (8), that fat, *either vegetable or animal,* appears to be most important in accelerating this synthesis, and that actually dietary cholesterol itself has no influence on the level of blood cholesterol within wide variations of the dietary intake of cholesterol. Furthermore the desirability of maintaining a low serum cholesterol is based upon the still unestablished assumption that atherosclerosis is related to the level of serum cholesterol. While it seems clear that extremely high serum cholesterol levels, i.e., over 400 mgm. per cent, do accelerate the development of atherosclerosis, these instances are not the common clinical occurrences. More often the disease is seen in subjects with serum cholesterol levels within the accepted normal range.

In February of 1950 an interesting publication appeared from the University of California at Berkeley by John Gofman and colleagues (9), calling attention to a blood lipoprotein-cholesterol complex which appears to correlate with the presence of atherosclerosis. This is certainly a brilliant discovery and seems to be the most promising lead that has developed in research in atherosclerosis for many years. A number of other laboratories are now pursuing similar work, and the results will be watched with extreme interest. What does diet—protein, fat, and cholesterol—have to do with the formation of this newly discovered lipoprotein-cholesterol complex? Will vegetable fats favor the synthesis of this lipoprotein equally with animal fat as is known to be the case with the total blood cholesterol? Will the body synthesize this lipoprotein-cholesterol complex more or less independently of dietary intake—e.g., some have it and some don't?

Facts to answer these questions have not yet been published, and it will probably take several years to get sufficient evidence, confirmed by several laboratories, to answer these and similar questions accurately. In the meantime it seems unfortunate to scare the public away from the common foods that make up the bulwark of present day "good nutrition" and attempt to turn us all into California vegetarians living on wheat germ, blackstrap molasses, and a jigger of yogurt on the side.

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A Modified Method for the Determination of Anionic Surface Active Compounds

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OF the many methods proposed for the determination of anionic surface active compounds, probably the simplest and most readily carried out are those methods involving the direct titration of the anionic agent with a solution of a cationic agent, using a two-phase system. The end-point is detected by the transfer of a colored complex from an aqueous phase to an organic solvent phase, or vice versa. For the most part the relationship between the anionic and cationic agents is not claimed to be stoichiometric, the cationic solution being used merely as an intermediary and the results being expressed in terms of known weights of the same or a similar anionic compound. One method (2) however is based on stoichiometry of the reaction between the anionic and cationic agents, the cationic solution being standardized by an independent method against potassium dichromate. It is the purpose of this paper to point out certain discrepancies in data obtained by this method and to propose a simple modification which apparently leads to more precise results.

Experimental

Sodium alkyl sulphates were prepared from Eastman lauryl, myristyl, cetyl, and stearyl alcohols by the method described by Lottermoser and Stoll (3). The crude products were recrystallized repeatedly from absolute alcohol and finally dried to constant weight in vacuo at 56°C. The melting points (uncorrected) of the purified compounds were as follows:

Sodium lauryl sulphate	179°C.
Sodium myristyl sulphate	182°C.
Sodium cetyl sulphate	184°C.
Sodium stearyl sulphate	170°C.

The analytical method used was essentially that proposed by Epton (2), except that Cetavlon (alkyl trimethyl ammonium chloride, supplied by Imperial Chemical Industries) was substituted for Fixanol C (cetyl pyridinium bromide) as the cationic titrant. Details of the procedure follow.

The required aliquot of anionic solution was pipetted into a 250-ml. glass-stoppered iodine flask and the volume was made up to 50 ml. with distilled water. To this was added 25 ml. of solution containing 0.03 gm. Methylene Blue, 12 gm. conc. sulphuric acid, and 50 gm. anhydrous sodium sulphate per liter, followed by 15 ml. of chloroform. The mixture was titrated with standard Cetavlon solution (approximately 5 mM.), the flask being shaken vigorously[1] after each addition. The end-point was taken when the transfer of color from the chloroform layer to the aqueous layer was such that both layers appeared the same color when viewed by reflected light. The flask was allowed to stand for about 1 min. after each shaking before making the color comparison. The Cetavlon solution was standardized against pure potassium dichromate by the method given by Epton (2) for the standardization of Fixanol C solutions.

Solutions containing 0.05% by weight of each of the purified sodium alkyl sulphates were prepared, and 10, 20, and 40 ml. aliquots of each solution were titrated with the Cetavlon solution by the method given above, the concentration of the original solution in millimoles/liter being calculated in each case. The results are given in Table I.

Discussion

It may be noted that the experimental values for the molar concentrations of all of the solutions are lower than the corresponding values calculated from the known weight concentrations of the solutions and the theoretical molecular weights of the compounds, and furthermore the experimental values appear to depend on the size of the aliquot taken for analysis, the larger aliquots giving the higher values.

These findings suggest that the observed titration should be adjusted by the addition of a blank, and consideration of the analytical procedure confirms this conclusion. On shaking together the reagents, but substituting water for the anionic solution, all of the color remains in the aqueous phase and the chloroform phase is colorless. When anionic solution is added dropwise, with shaking after each addition,

[1] In the Epton method it is recommended that "the bottle is shaken with just sufficient force to ensure that the phases mix thoroughly." In the present work however it was found that the data obtained were more consistent and more reproducible when the flask was shaken vigorously after each addition of titrant.