Speculations Concerning the Functions of Serum Cholesterol

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"Lernen wir traümen, dann finden wir vielleicht die Wahrheit."
KEKULÉ

No lipid parameter is measured more frequently or discussed as endlessly as is serum cholesterol. However, the possible functions of serum cholesterol are seldom touched upon. I would like to indulge in a few speculations in this area.

Serum cholesterol is present in both the free and esterified form, and the ratio between the free and esterified cholesterol is constant with the exception of certain liver diseases and lipidoses.[1] In dogs, an equilibrium between the whole blood free cholesterol and the plasma ester cholesterol has been demonstrated.[2] Isotope experiments have shown, however, that free and ester cholesterol do not appear in the serum at the same rate. In human subjects who have been fed radioactive cholesterol[3] the peak serum radioactivity occurs two to three days after feeding, with more of the radioactivity present initially in the free cholesterol. In human subjects biosynthesizing cholesterol from labeled acetate[4] the peak serum radioactivity is seen within eight hours, but the initial difference between specific activities of the free and ester cholesterol still holds. Eventually the specific activity of the ester cholesterol becomes higher than that of the free cholesterol, and remains so. The inability of hepatectomized rats[5] to replace lost cholesterol ester and the absence of cholesterol-C^{14} in the plasma sterol ester[6] of hepatectomized dogs fed acetate-C^{14} demonstrates that the liver is responsible for the appearance of cholesterol ester. The observation of Portman and Sinisterra[7] may be relevant to the following discussion. They found that in monkeys given cholesterol-C^{14} intravenously, the biological half-life of serum cholesterol was 8.8 days, while orally administered labeled cholesterol showed a die-away curve of serum cholesterol with two distinct components exhibiting half-life of 2 to 6.6 days and 17 to 34 days, respectively.

Assume that the free and ester cholesterol in the serum had distinct and separate functions. I do not mean to imply two distinct and unrelated pools, but rather that when cholesterol is present in the serum in the free state it is available to perform certain metabolic functions, and when it is present as esterified cholesterol it may be available to perform others. Any given cholesterol molecule, according to this supposition, may go from the free form to the ester form. In doing so the specific metabolic functions change. What could be assigned as the metabolic role of the free cholesterol?

FREE CHOLESTEROL

First, there is the rapid exchange with the red blood cell cholesterol,[8] an exchange not noted with ester cholesterol. Thus, only serum free cholesterol is required for maintenance of the integrity of the red blood cell structure. The constancy of the red blood cell cholesterol

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content has been demonstrated in man over a wide range of clinical conditions.[8] Eightfold elevations of the serum cholesterol resulted in no increase in the red blood cell cholesterol[9] in rabbits fed large doses of cholesterol.

Cholesterol is a precursor of the bile acids, adrenocortical hormones and sex hormones.[11] Landon and Greenberg[12] have suggested that the circulating cholesterol is the precursor of the cholesterol found in the testes, kidneys, adrenals, spleen and lungs. Assume this cholesterol, the hormonal precursor, is derived from the plasma free cholesterol. LeRoy[13] has estimated that 0.5 to 1 per cent of the plasma free cholesterol which is utilized daily is transformed to corticosteroids. Estimates of cholesterol conversion to bile acids are also available,[14,15] but what portion of this comes from the circulating cholesterol is not known.

Some of the carbon atoms biologically removed from the cholesterol molecule may be used for synthesis of fatty acids[16,17] and glycogen[17] as has been shown in experiments using randomly labeled sterol. Assume that these, too, are contributions of the free cholesterol.

Another important function of the circulating cholesterol may be in the "replacement" or turnover of tissue cholesterol. Chevallier[18,19] estimates that almost half of the tissue cholesterol of the rat is "replaceable," the amount being turned over varying from zero for the brain cholesterol to almost 100 per cent for tissues such as the liver, adrenals and intestines. Suppose, then, that the major function of the free cholesterol is that of an integral metabolite, serving as a substrate for steroid synthesis and for the renewal of red blood cells and tissues.

ESTER CHOLESTEROL

On the basis of his own esterification studies Favarger[20] concluded that the ester cholesterol was a means for fatty acid transport. Popjak[21] fed cholesterol to rabbits on a fat-free diet and showed a progressive increase of all plasma lipids and a concomitant depletion of body fat depots. The ester:free cholesterol ratio in the plasma rose as feeding continued, the major, if not only, source of fatty acid being body pools. He therefore inferred a role for cholesterol in fat mobilization.

The type of fatty acid which is esterified with cholesterol is also significant. Popjak[21] found that as cholesterol feeding progressed there was a progressive drop in the iodine number of the phospholipid fatty acid and an increase in the non-phospholipid fatty acid. Serum cholesterol is preferentially esterified with unsaturated fatty acids[22,23] and *in vitro* enzymic esterification of cholesterol proceeds more rapidly with unsaturated fatty acids than with saturated fatty acids.[24] It has also been shown that the iodine number of total serum fatty acids of rats is correlated with the unsaturation of the dietary fat.[25] A similar correlation has been observed with respect to the iodine number of the fatty acids of the combined cholesterol ester and neutral fat fractions in dogs.[26]

In man it has been shown that the iodine number of the cholesterol ester fatty acid is a function of the unsaturation of the dietary fat.[27] The cholesterol ester fatty acids are generally derived from the dietary fat and are the most unsaturated in the serum.

Studies on the synthesis of fatty acids in man indicate that practically no linoleic acid is synthesized,[28] yet most of the fatty acids of the sterol ester are unsaturated. Feeding of labeled fatty acids (as their methyl esters) to rats shows a preferentially rapid incorporation of linoleate (over oleate and stearate) into the sterol esters.[29] In their thorough review of the transport of fatty acids, Fredrickson and Gordon[30] conclude that there is no evidence that sterol esters play a significant role in the net transport of fatty acids, but they do not exclude the possibility that cholesterol ester may be a means of transport within the serum. However, Miller, Roheim and Spitzer[31] have shown that cholesterol ester may be a means of transport of fatty acid to the heart.

Bates[32] has found that the half-life of plasma cholesterol ester fatty acids in the dog (7.8 ± 3.4 hours) is much shorter than the half-life of plasma cholesterol itself in either the rat or monkey, and she suggests that one plasma cholesterol molecule might be esterified suc-

cessively with a series of fatty acid molecules while in the plasma. Seven hours has been found to be the half-time for the whole blood free cholesterol–plasma ester cholesterol system in the dog,[2] suggesting a renewal of the entire sterol ester molecule. The serum sterol ester originates in the liver. Louédec has reported that the liver cholesterol esters are not transferred to the serum as such;[33] thus the liver may be the site of transesterification between sterol ester and exogenous neutral fat, as well as the site of cholesterol ester synthesis by other mechanisms. The sterol ester then may function as one of several transport mechanisms for fatty acids and perhaps is the mechanism of choice for the transport of highly unsaturated or essential fatty acids. Kinsell and his associates[27] have speculated that the cholesterol ester functions as a donor of essential fatty acid for phospholipid synthesis.

Both dietary polyunsaturated fatty acids[34] and cholesterol[17] pass through the placenta. This may be used as another piece of evidence for the transport function of cholesterol esters. This may be one source of the incompletely explained hypercholesteremia of pregnancy.

LIPOPROTEINS

One other possible role of serum cholesterol, free and ester, is as an integral structural unit of lipoproteins. One explanation of the fact that cholesterol is distributed in all the lipoprotein fractions is that its function may be structural. The lipoproteins contain most of the other lipid entities, however, and their delicately balanced physical state in the blood probably demands a specific structural make-up for stability. Lindgren et al.[35] have suggested a series of structures for lipoproteins, taking into consideration their size and lipid content. The absolute cholesterol content of various lipoproteins has been studied during fat and cholesterol absorption;[36] only the cholesterol content of the chylomicron-containing fraction is elevated. In man the specific activity of the S_f 3–7, S_f 10–13, S_f 17 and S_f 20+ lipoprotein cholesterol is the same as that of the total serum cholesterol twenty-four hours after administration of cholesterol-H^3,[3] suggesting rapid equilibration of cholesterol between lipoprotein classes.

It is hard to imagine any one segment of the lipoprotein being an inert building block. The dietary alteration of the cholesterol content of various lipoproteins, even in the face of unchanged serum cholesterol levels,[37] also argues against this point. It does suggest the intimate inter-relationship between serum lipids, as affected by diet, and structure and composition of lipoprotein. The rather constant free:ester cholesterol ratio also points towards an attempt at maintenance of physical integrity, but again this may also be a physiological mechanism for keeping cholesterol in solution until it reaches its site of utilization.

I have speculated that the free cholesterol of the serum plays an integral metabolic role, whereas the ester cholesterol is partly involved in fatty acid transport, although it must be recognized that an equilibrium between these two types of cholesterol exists. The real question is how to go about proving some of these hypotheses. The work on free cholesterol might be aided by double-labeling experiments involving carbon- and hydrogen-labeled cholesterol. The labeling of body depots and the observation of their exchange with newly synthesized or ingested sterol may be an answer. The most obvious immediate problem would be in the detection of small amounts of radioactivity. Chevallier[18] has carried out some experiments of this type.

Cholesterol ester as a means of fatty acid transport depends on the availability of labeled unsaturated fatty acids. Carboxyl-labeled oleic, linoleic and linolenic acids have been synthesized by Drs. Howton and Nevenzel,[38–40] but are generally hard to obtain. I should like to suggest a biosynthetic source of labeled unsaturated fatty acids—the chicken egg. The normal egg yolk consists of the following fatty acids: Oleic acid (50%), palmitic acid (27%), linoleic acid (11%), stearic acid (6%), palmitoleic and linolenic acids (6%). It has been shown that the chicken egg is a good source of fat which can be labeled biosynthetically so as to attain fairly respectable levels of specific activity.[42,43] Since the actual composition of the fatty acids in the egg yolk

can be altered by variations in the diet,[44,45] a combination of specific diet plus administration of labeled precursor should yield labeled fatty acids of the desired kind. Obviously, the fed fats cannot be labeled, but the amount of any desired fatty acid can be raised so as to insure good recovery. With enough radioactive precursor, fairly high specific activity should still be attainable. Double-label experiments involving cholesterol and labeled fatty acids may help to resolve some of these problems.

SUMMARY

Speculations concerning the metabolic functions of the free and ester cholesterol of the serum are presented. The discussion is based on the supposition that serum free cholesterol serves a structural purpose and as a precursor of other steroids whereas serum ester cholesterol is part of the fatty acid transport mechanism. The literature bearing on these points is reviewed.

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