The Intermediary Metabolism of Cholesterol

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Animal tissues synthesize cholesterol from metabolites of small molecular size, principally cholesterol. This synthetic process is known to take place in the liver but may also occur in other organs. In all tissues, with the exception of brain and nerve, cholesterol is continually regenerated. Cholesterol is the parent substance which the animal organism uses to produce bile acids, progesterone and possibly also other steroid hormones. The catabolism of cholesterol leads to the formation of several saturated, metabolically inert sterols, cholesterone presumably being the common intermediate.

It is widely believed that arterial disease, specifically atherosclerosis, is associated with derangements in the metabolism of cholesterol. This view is largely based on two findings: the fact that atherosclerotic aortae contain abnormally large amounts of cholesterol, and the demonstration that by feeding of cholesterol a condition resembling human atherosclerosis can be induced experimentally in rabbits. To correlate these phenomena with specific events in the metabolism of cholesterol has so far not been possible. Wide gaps exist in our present knowledge of the intermediary metabolism of cholesterol even under normal conditions and it is likely that the elucidation of the role of cholesterol in arterial disease must await the solution of some of the basic problems in cholesterol metabolism.

In the following report no attempt will be made to review the literature which deals with the relationship of cholesterol metabolism to atherosclerosis. Instead, some aspects of the metabolism of cholesterol in normal animals will be discussed in the hope that this may stimulate investigations which are more specifically concerned with the behavior of this compound in various pathologic states.

Cholesterol is derived from the hydrocarbon cyclopentanoperhydrophenanthrene, a structure which occurs widely and in numerous modifications in living cells. Although sterols are found in plants and in certain microorganisms, cholesterol itself is specifically a constituent of animal cells. In view of the ubiquitous distribution of sterols it has been speculated that these compounds are essential components of the cell, an attractive hypothesis but for the fact that none of the known steroids are essential for the functioning of the bacterial cell. Therefore, the role of steroids in cellular metabolism appears to be more specialized than this hypothesis would indicate.

In animal tissues cholesterol is found in all body cells and as far as is known the content of cholesterol is remarkably constant in various nutritional states excepting those which involve excessive feeding of cholesterol itself.

Cholesterol is not required as an essential dietary constituent since synthetic, sterol-free diets are adequate for growth and maintenance of most animals. In this respect the larvae of certain insects and some parasites form a notable exception. For these organisms, sterols are indispensable dietary components. It can be stated, however, that the large majority of animals is capable of synthesizing the steroid structure from generally available food elements. Until recently, sterols of plant origin were considered to be supplementary or even exclusive sources of cellular cholesterol. The studies dealing with sterol absorption, which have been carried out by Schoenheimer and Page, have made it appear likely that sterols of other than animal origin are not significantly absorbed from the gastrointestinal tract and consequently that they cannot serve as adequate sources of tissue cholesterol. It is interesting to note that in the rabbit, which is highly susceptible to the experimental atherosclerosis by cholesterol feeding, even excessive amounts of plant sterols such as sitosterol show
no harmful effect and this indicates that in omnivorous and herbivorous animals alike the conversion of plant to animal sterols is not significant. The findings relating to the lack of absorption of plant sterols call attention to the unique selectivity which characterizes the intestinal absorption process with regard to sterols. Slight alterations in the structure of the molecule interfere with the passage through the intestinal wall. Those few cholesterol derivatives which are adsorbed are also known to play a role in the intermediary metabolism of cholesterol, notably cholestenone and 7-dehydrocholesterol. Further studies are needed to ascertain whether intestinal absorption is restricted to those sterols and sterol derivatives which are products of the intermediary metabolism.

Since it has been recognized that animals obtain sterols from endogenous syntheses, the nature of the processes leading to the formation of cholesterol and other steroids has become a challenging problem. The ability of animal cells to carry out the total synthesis of the steroid structure has been demonstrated in recent years by both direct and indirect experiments. Balance experiments with animals on diets of known composition have shown that the deposition of cholesterol in the tissues during growth is greatly in excess of the uptake of sterols from the diet. Therefore, the extra cholesterol in these experiments must have been of endogenous origin. These balance experiments, which have been carried out with various animal species, have been important in establishing the occurrence of the over-all process of steroid synthesis but have failed to disclose details of the mechanism. On the other hand, somewhat more concrete information on various aspects of the biosynthesis of cholesterol has been obtained with the aid of the tracer technic which has more recently been applied to this problem. The utility of the tracer technic in the study of steroid synthesis as well as for biochemical syntheses in general derives from the fact that it permits the detection of processes which occur in the so-called steady state. Steady-state reactions cannot be followed by ordinary analytic procedures because they do not result in a net change of the concentration of reaction products and reactants respectively, synthesis and degradation of the metabolite taking place concurrently. Since steroid synthesis has so far been observed only in systems containing intact cells, i.e., under steady-state conditions, it is clear that the tracer technic is uniquely suited to the study of this process. The first instructive experiment with tracers was performed in 1937 by Rittenberg and Schoenheimer who administered heavy water to mice and studied the rate at which isotopic hydrogen was incorporated into the tissues' lipids. The incorporation of deuterium into cholesterol from a heavy-water medium is the result of chemical reactions and the speed at which it occurs provides a measure of the rate at which the cholesterol molecules present at the beginning of the experiment are replaced by newly synthesized cholesterol. This rate is moderately rapid for the cholesterol of most tissues and one can calculate that, for instance, the cholesterol in the liver of the rat is regenerated or rejuvenated at a rate corresponding to a half-life of less than ten days. The results obtained by Rittenberg and Schoenheimer suggested that cholesterol is formed by a process of total synthesis which involves the condensation of numerous small-sized molecules rather than by modification of a preformed steroid structure such as those of plant sterols. We have extended these findings and have tested the isotopically labeled forms of a number of small molecular compounds with respect to their ability to yield labeled cholesterol in rats and have found acetic acid to be the most efficient precursor. There are other compounds which like acetic acid give rise to isotopic cholesterol but they are probably not precursors or intermediates in the sense that they are specifically utilized. They merely serve as sources of acetic acid which in turn provides the basic building blocks. Cholesterol which is biologically synthesized from labeled acetic acid has been degraded chemically by various procedures to ascertain whether the entire sterol structure or only a limited portion of the molecule is derived from these small units. So far, acetic acid carbon has been encountered in all fractions which have been investigated,
and the available evidence suggests that the majority if not all carbon atoms of the steroid structure are contributed by acetic acid. No theory can be offered at present to explain the synthetic mechanism by which these small units are joined to yield the complex steroid structure. Intermediates which by their chemical nature might provide clues to the reaction mechanism have not been isolated to date. Moreover, since complex compounds such as steroids require for their synthesis intact cellular systems a study of cholesterol synthesis in simple systems of known compositions is not feasible at the present time.

Synthesis of cholesterol can be demonstrated under in vitro conditions as well as in the intact animal. The reaction takes place readily in slices of surviving liver but comes to a complete standstill when the organization of the liver tissue is destroyed by grinding or homogenizing of the cells. By using test substances labeled by C, which permits detection of synthetic activities of a very small order, it can be shown that in a liver preparation which contains no intact cells cholesterol synthesis proceeds at a rate less than one ten-thousandth of the rate which is observed in slices. It appears, therefore, that the synthesis of cholesterol requires the cooperation of various enzymes which are suitably organized in space. This condition is fulfilled only in the intact cell. We have not been successful in demonstrating the occurrence of cholesterol synthesis in testes, spleen, intestine, kidney and heart, but it would be unwarranted to state at this time that cholesterol synthesis is confined to the liver. The brain is the only organ which may be excluded as a site of cholesterol synthesis. Waelch, Sperry, and Stoyanoff have maintained growing rats on a heavy-water regimen and have shown that the appearance of newly synthesized cholesterol in the brain is arrested when myelination is complete. The metabolic inertia of brain cholesterol can also be demonstrated by feeding of labeled acetate to rats. The isotope concentrations in cholesterol formed from acetate vary from organ to organ and follow roughly the order of metabolic activities which have been observed for other tissue constituents such as fatty acids and proteins. It appears that the newly formed cholesterol is rapidly distributed from the site of synthesis throughout the body. The brain and central nervous system, however, do not participate in this distribution process, nor does the brain appear to be capable of synthesizing cholesterol. The singular behavior of brain cholesterol is also illustrated by an experiment in which a dog was injected intravenously with labeled cholesterol. The labeled material was subsequently encountered in every organ with the exception of brain and spinal cord. Thus, the relatively large masses of cholesterol in the central nervous system are metabolically inert and here the function of cholesterol does not seem to be associated with any chemical reactions but evidently must reside in some physical or structural properties of the molecule.

The factors responsible for regulating the rate of cholesterol synthesis have not been explored, but there is reason to believe that in normal animals this rate is little affected by variations in dietary composition. A rather striking gradation of rates is observed when cholesterol synthesis is investigated in slices of surviving rat liver. In this system the isotope concentration in cholesterol depends very critically on the age or weight of the animal from which the liver is taken. The isotopic values in cholesterol for the two extreme age or weight groups were found to differ by a factor of more than 10. An extension of these experiments in the direction of both younger and older animals will be necessary before the significance of these observations can be evaluated.

All attempts to accelerate cholesterol synthesis in vitro beyond the rate which is observed by simply placing liver slices in a buffer medium in an atmosphere of oxygen have so far been unsuccessful. Apparently, the liver tissue contains endogenous substrate in amounts which are adequate to allow cholesterol synthesis to proceed at optimal rates for several hours. This is in contrast to the synthesis of other lipid material such as fatty acids which are also synthesized from acetic acid units as the principle precursor. In a salt-buffer medium, fatty acid synthesis proceeds
slowly but can be stimulated by the additions of substrates, such as glucose and pyruvate, which serve as energy sources. The addition of pyruvate markedly stimulates fatty acid synthesis from acetate, but affects cholesterol synthesis adversely. It is evident from our data that under certain conditions the isotope concentration in cholesterol can greatly exceed that in the fatty acids showing that the higher fatty acids are not intermediates in the conversion of acetate to cholesterol. Another example may serve to illustrate the differences in the mechanism responsible for fatty acid and cholesterol synthesis respectively. In the absence of calcium ion, fatty acid synthesis in surviving liver is strongly inhibited while cholesterol synthesis is much less affected.

It should be emphasized that in the in vitro experiments which have been cited the rate of cholesterol synthesis is calculated from the extent of isotope incorporation without consideration of any change in total quantity of cholesterol which might occur. The rate of synthesis which is observed, although actually of the same order as it occurs in the intact animal, corresponds to a few per cent only of the cholesterol originally present in the tissue. A change of this magnitude could not be detected by the methods available for quantitative cholesterol analysis. Indeed, it is quite conceivable that the synthesis which we observe is not associated with a net change but that the quantity of sterol remains constant during the experimental period. In this case we would deal with a dynamic equilibrium, synthesis being balanced by simultaneous destruction of cholesterol in vitro as well as in vivo.

While the first portion of this discussion has been concerned with the total synthesis of the steroid molecule, I would like to consider now a number of anabolic and catabolic reactions which represent modifications of the cholesterol structure and which yield the excretion products and the various steroids of specialized function. Under conditions of nutritional equilibrium the quantity of cholesterol in animal tissues is constant and products of cholesterol catabolism must be formed at the same rate at which cholesterol is newly synthesized. The daily excretion of fecal sterols corresponds roughly to the quantity of cholesterol synthesized during the same time interval. Hence, in animals which are kept on a sterol-free diet, the sterols excreted in the feces, which consist mainly of coprosterol, account for the bulk of the catabolic products. A different situation may arise when the exogenous supply of cholesterol is increased. As shown by Page and Menschick and later by Schoenheimer and Breusch, in balance experiments, destruction of cholesterol takes place in the tissues when the quantity of cholesterol in the diet becomes abnormally high. It appears that the mechanisms which are responsible for the conversion of cholesterol to fecal sterols become saturated and that excess exogenous cholesterol enters an alternative metabolic path. The so-called cholesterol destruction which takes place under such conditions has not been defined chemically and may not, therefore, be taken too literally. It merely indicates that products are formed which are no longer precipitable by digitonin but which may still have an intact steroid nucleus.

The pathways leading to the end products of cholesterol metabolism have been investigated extensively. There is evidence from several sources that the first step is a dehydrogenation to cholestenone and that this unsaturated ketone is the common precursor of coprosterol, dihydrocholesterol, and probably also of epicoprosterol. Page and Menschick have presented evidence for the occurrence of cholestenone by showing that the sterols from atheromatous lesions contain a fraction which has the same spectral absorption maximum as cholestenone. Later, Rosenheim and Webster succeeded in isolating cholestenone in considerable quantities from rat and dog feces. Schoenheimer, Rittenberg and Graff by using partially labeled cholestenone have demonstrated that in the dog and the human cholestenone is converted to coprosterol. In our laboratory, additional evidence for the occurrence of this unsaturated ketone in cholesterol metabolism has been obtained. After the administration of deuteriocholestenone to rats, a high isotope concentration was found not only in the fecal sterols but also in the sterols
from the tissues. The deuterium concentration was particularly high in the fraction containing the saturated sterols which consist essentially of dihydrocholesterol. Cholestenone can therefore be converted into both dihydrocholesterol and coprosterol. On the other hand, the cholesterol isolated from the same experiment contained only a small excess of isotope showing that the transformation of cholesterol to cholestenone is not readily reversible. These experiments also demonstrate that cholestenone in contrast to most derivatives of cholesterol is absorbed from the gastrointestinal tract. One of the aspects of cholesterol catabolism which has not been settled satisfactorily concerns the site at which these conversions take place. Coprosterol has not been found in the tissues proper and it is therefore believed that bacterial action in the gut is responsible for its formation.\textsuperscript{25} On the other hand, it is probable that at least the initial step in the conversion, namely, that of cholesterol to cholestenone, occurs in the tissues themselves. Whether intestinal bacteria can reduce cholestenone to coprosterol has not been tested. A second problem which deserves further attention is the effect of dietary variations on the composition of fecal sterols. The relative proportions of unsaturated and saturated sterols in the excreta are known to vary widely and, as Rosenheim and Webster\textsuperscript{26} have shown, the degree of saturation is controlled by dietary factors. A clarification of these relationships is desirable because derangements in cholesterol metabolism which lead to an accumulation of cholesterol in the tissues may well reflect an insufficiency of the mechanisms which effect the conversion of cholesterol to excretory products.

The only specific role in metabolism which can at present be assigned to cholesterol is that of a precursor of steroids of specialized function. It has long been suspected that cholesterol and the structurally related steroids are also interrelated biochemically. Direct experimental evidence for the existence of such relationships has recently been furnished in two specific instances. Cholesterol has been identified as the principal precursor for cholic acid\textsuperscript{27} and for progesterone.\textsuperscript{27} In both cases the proof for these transformations is based on the demonstration that the administration of isotopically labeled cholesterol results in the formation of labeled bile acid and steroid hormone respectively. The data indicate that these conversions are direct without disruption of the basic steroid ring structure. The total synthesis of the steroid skeleton from small building blocks such as acetic acid therefore appears to lead to cholesterol as the principal product, while bile acids and steroid hormones are formed secondarily by a modification of the pre-existing steroid structure of cholesterol. An origin from cholesterol is also strongly indicated for the steroid hormones of the adrenal cortex. The work of Long and his associates has shown that adrenotropic hormone causes a marked decline of the cholesterol in the adrenal cortex suggesting that this cholesterol deficit is due to the formation of cortical steroids.\textsuperscript{28} Similar evidence points to cholesterol as the parent substance of the estrogenic hormones. Ovarian activity and therefore an increased output of estrogens has been shown to be accompanied by a decrease of cholesterol in this organ.\textsuperscript{29} Experimental technics which permit a direct study of the biologic interrelationships of the divers steroids are now available and it should be possible, therefore, to establish whether cholesterol is indeed the primary source of all compounds having the steroid structure.

The quantities of cholesterol which are available in various tissues of the animal and which are continuously replenished by endogenous synthesis are far in excess of the quantities required for the production of steroids of specialized function. It is therefore unlikely that the metabolic reactions of cholesterol are confined to processes which involve chemical alterations of the cholesterol molecule of the type mentioned. For example, since cholesterol occurs in tissues both in the free form and esterified with fatty acids it is likely that cholesterol plays some role in the metabolism of fatty acids. In any case there is sufficient evidence to indicate that cholesterol possesses a variety of functions in intermediary metabolism, and it is probably correct to say that its chief functions are still unknown.

The investigations which have been dis-
cussed here have served to emphasize the necessity of considering intermediary metabolism in terms of the rates of synthesis and destruction of a given tissue constituent. Clearly, the evaluation of metabolic events on the basis of concentration changes alone is inadequate. In the specific case of cholesterol it may be asked whether a condition associated with an increased sterol level in a given tissue or body fluid is the result of an accelerated synthesis or of a decline in the rate of catabolic and anabolic conversions. A change of the level of cholesterol which is observed in a pathologic condition may then acquire a different meaning and might be indicative of the site and the nature of the metabolic derangement. Only scant attention has been paid to these aspects of metabolism, largely because traditional technics did not lend themselves to this type of analysis. The tracer technic opens an approach to these problems and it may be hoped that it will be of aid in the understanding of metabolic disorders.

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