THE JEREMIAH METZGER LECTURE

THE RELATIONSHIP OF CHOLESTEROL BIOSYNTHESIS TO CANCER

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The first unequivocal evidence that animals can synthesize cholesterol from smaller molecules[1, 2] was followed within a few years by a series of studies demonstrating that the rate of cholesterogenesis is subject to a number of control processes. The studies of Gould,[3] Tomkins, et al,[4] and Frantz, et al,[5] demonstrated independently that the feeding of cholesterol leads to a striking inhibition of *de novo* cholesterol synthesis. As shown in Table 1, the administration of even relatively small amounts of cholesterol to rats causes a significant depression in the rate of cholesterogenesis in liver slices from such animals; there is, moreover, now good evidence that even on a cholesterol free diet, hepatic cholesterol synthesis is normally depressed as a result of endogenous cholesterol acting upon cholesterogenesis as a feedback inhibitor.[6] At high levels of exogenous cholesterol, hepatic cholesterogenesis can be markedly depressed to less than 3 % of control levels (Table 1).

A series of studies from our laboratory over the past decade has attempted to elucidate the biochemical site and mechanism of this cholesterol feedback reaction. As shown in Fig. 1, cholesterol is known to be synthesized from acetyl CoA through the intermediate, β-hydroxy-β-methylglutaryl CoA, which also serves as an obligatory precursor of all ketone bodies. On the pathway to cholesterol, β-hydroxy-β-methylglutaryl CoA contributes its carbons to mevalonic acid, which through a series of reactions forms squalene and finally cholesterol itself. In 1960,[7] on the basis of indirect measurements of various steps in cholesterol biosynthesis, we first suggested that the specific reaction responsible for the feedback control of cholesterol synthesis is located at the point of conversion of β-hydroxy-β-methylglutarate to mevalonate. A few years later we were able to develop methods for measuring directly the synthesis of mevalonic acid,[8] and in 1968[9] it was

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TABLE 1

Effect of Varying Amounts of Dietary Cholesterol on Cholesterol Synthesis

Cholesterol in Diet %	Cholesterol Synthesis (nmoles of added acetate-2-^{14}C)
0	67
0.1	36
0.25	28
0.5	1.8
1.0	2.4
2.5	1.8

Tissue slices, 500 mg, incubated with 1 μc acetate-1-^{14}C for 2 hours in 5 ml of Krebs-Ringer buffer at 37° C.

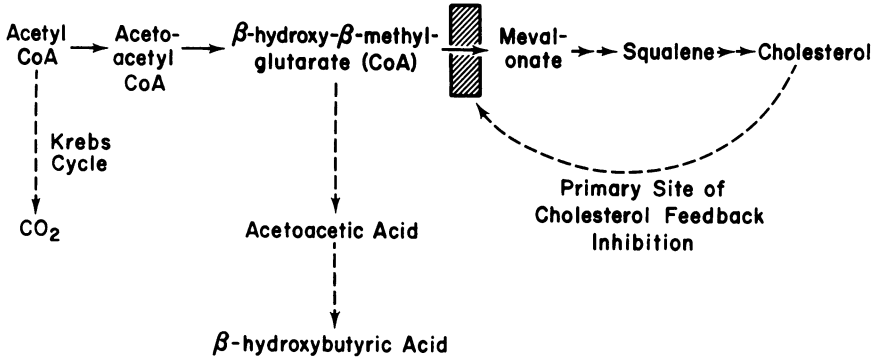

FIG. 1. Biochemical site of the cholesterol negative feedback regulation.

possible to show unequivocally that the feedback regulation of cholesterogenesis depends upon a specific control mechanism located at the enzyme responsible for mevalonate synthesis, i.e., β-hydroxy-β-methylglutaryl CoA reductase. This conclusion has subsequently been confirmed by several laboratories[10, 11, 12, 13, 14]; and while it is clear that the prolonged feeding of cholesterol may lead to secondary inhibition of reactions beyond mevalonate,[7, 15] there is no question that the reaction responsible for the synthesis of mevalonic acid represents the primary site of cholesterol feedback control.

From the anatomical standpoint, it is of interest that the intracellular site of this feedback control mechanism clearly lies in the membranous structure that makes up the endoplasmic reticulum of the cell. Mevalonic acid synthesis does not take place to a significant extent in the mitochondria. On the other hand, the microsome is responsible for over 90% of mevalonic acid produced, with the cytosol accounting for the remaining 10%. Finally, when the microsome is subfractionated into its three major

components, the ribosome, a soluble interior and the lipoprotein outer membrane, mevalonic acid synthesis, and therefore the control of cholesterol synthesis, is found to be localized almost exclusively on this lipid membrane.[9]

It has been known for many years that the synthesis of cholesterol can take place in many extrahepatic tissues of the body, notably in the intestine[16, 17]; however, it has generally been held that the cholesterol feedback control mechanism is localized only to the liver. Recently, studies by Dr. John Dietschy[18, 19, 20] have demonstrated that in the rat, intestinal cholesterogenesis is also under feedback control, but in contrast to the case in liver, bile acids rather than cholesterol are responsible for activating this feedback control mechanism. Furthermore, it is now clear that there are striking species differences in the tissue localization of this feedback mechanism. The recent studies of Swann, et al[21] have indicated that in contrast to the rat, every tissue in the guinea pig so far studied possesses a *cholesterol* sensitive cholesterol feedback system. Most surprisingly, the lung of the guinea pig can synthesize cholesterol at a rate as rapid as that of liver, and in this tissue, too, cholesterol synthesis is under feedback control. The physiologic significance of pulmonary cholesterogenesis remains unknown; moreover, there are at present no data to indicate whether the tissues of man behave like those of the guinea pig with its ubiquitous cholesterol feedback system or like the rat, in which feedback control of cholesterol synthesis appears to be localized to the liver, and in a modified form to the intestine.

The relationship of the cholesterol feedback control mechanism to hypercholesterolemia and atherosclerosis remains unknown. However, there is to date only suggestive evidence that a damping of this feedback system may be present in familial hypercholesterolemia.[22] By contrast, over the past few years there has developed a striking and consistent association between a complete absence of the cholesterol feedback system and cancer. The first suggestion that there might theoretically be a relationship between loss of a feedback control mechanism and malignancy was published by Potter.[23, 24] This investigator raised the possibility that deletion of a specific feedback mechanism could lead to the overproduction of a critical cellular intermediate, which in turn might be responsible for uncontrolled cell growth, and hence produce a malignant change within the afflicted cell. The first, and to date the only, example of the consistent loss of such a feedback system has proven to be that controlling cholesterol synthesis. This relationship was first shown in the case of a mouse hepatoma, BW 7756.[25] In striking contrast to normal mouse liver, this spontaneous hepatoma undergoes no inhibition of cholesterol synthesis on adding cholesterol to the diet. Most significantly, the cholesterol feedback control

mechanism has been shown to be absent in every one of a series of minimal deviation rat hepatomas studied to date.[26, 27] Many of these tumors, as their name implies, show only insignificant morphologic differences from normal liver, and are so highly differentiated that they possess almost all of the biochemical properties that characterize liver. Such tumors have for these reasons been widely employed as a means of detecting chemical alterations that might be characteristic of cancer *per se* rather than the results of the non-specific dedifferentiation that usually accompanies malignant change. The consistent loss of the cholesterol feedback mechanism in such minimal deviation hepatomas, therefore, provides at least suggestive evidence that this lesion may be a characteristic of cancer. Loss of the feedback control of cholesterol synthesis is not restricted to hepatic cancers, as evidenced by the presence of a similar lesion in leukemic cells, as reported at these meetings two years ago.[28] Absence of this control mechanism has, moreover, been shown in two cases of human hepatoma.[26] While the exact mechanism by which tumors lose their ability to respond to exogenous cholesterol is unknown, we have recently been able to demonstrate that the defect responsible for the loss of the cholesterol feedback system in tumors is specifically located at β-hydroxy-β-methylglutaryl CoA reductase.[29]

The consistent loss of the cholesterol feedback mechanism in all tumors, coupled with the fact that this lesion remains the only example of such a biochemical defect in malignant tissues, indicated that conceivably there might be a causal relationship between the loss of this control mechanism and carcinogenesis. Perhaps the most suggestive evidence for such a relationship is the observation, initially made by our laboratory,[30, 31] and subsequently confirmed by Sabine,[32] that various carcinogens, specifically aflatoxin-B[30] and N-2-fluorenylacetamide,[32] result in a loss of the cholesterol feedback system in precancerous liver many months before overt malignancy appears.

As indicated in Table 2, the feeding of aflatoxin to either the rainbow trout or the laboratory rat causes a relatively prompt blunting or loss of cholesterol feedback control, whereas this carcinogen produces malignancy only many months after administration. It should be emphasized, however, that the mechanism by which an alteration in mevalonic acid synthesis might lead to a malignant change in the cell remains completely unknown.

The finding that hepatomas are not subject to the same biochemical restraint in sterol synthesis that is present in liver raises the question of the physiologic impact of such a tumor on the overall metabolism of cholesterol in the intact animal. It was possible that such hepatomas would retain newly synthesized sterols for use in building new cell membranes, or alternately they could release cholesterol into the circulation to contribute to

TABLE 2

Effect of Aflatoxin on Cholesterol Feedback Control

Animal	Treatment	Diet	Cholesterol Synthesis (nmoles acetate-^{14}C)
Trout	None	Normal	50
		Cholesterol	15
	Aflatoxin 300 μg/Kg IP once 5 days before study	Normal	32
		Cholesterol	36
Rat	None	Normal	4.8
		Cholesterol	0.2
	Aflatoxin 1 mg/day on Days 1 & 2*	Normal	32
		Cholesterol	28

* Study carried out on Day 5.

the circulating cholesterol pool. Dr. Lee A. Bricker has approached this problem employing a cholesterol blocking agent, Triparanol (or MER/29) as an aid in evaluating the endogenous synthesis and release of sterols by such tumors.[33, 34, 35] Triparanol blocks the conversion of the cholesterol precursor, desmosterol, to cholesterol; and as a result, desmosterol in the blood replaces and can serve as a marker of endogenous cholesterol production. Since desmosterol can readily be detected in minute quantities by means of gas liquid chromatography, it was possible to determine the effect of cholesterol feeding upon endogenous sterol production and release into the bloodstream simply by measuring serum desmosterol concentrations in triparanol-treated animals. As shown by the gas liquid chromatogram in Fig. 2B, triparanol administration causes an accumulation of desmosterol in the blood; however, the feeding of cholesterol to a normal animal results in the complete disappearance of this desmosterol from the circulation. This experiment in normal rats clearly demonstrates that the cholesterol feedback system is operative in the intact animal, and by implication suggests that in the normal rat, endogenous cholesterol in the blood is probably derived primarily from the liver, in which cholesterol synthesis is suppressable by exogenous cholesterol.

As shown in Fig. 2D, E, and F, when the comparable experiment is carried out in a tumor bearing rat, the results are strikingly different. Triparanol, as shown in Fig. 2E, again leads to the appearance of a relatively large desmosterol accumulation in the blood; however, in contrast to the normal animal, when cholesterol is fed to the hepatoma bearing rat, Fig. 2F, the circulating desmosterol is not significantly affected.

These results strongly suggest that in the tumor-bearing animal, plasma sterol is derived almost exclusively from the hepatoma rather than from

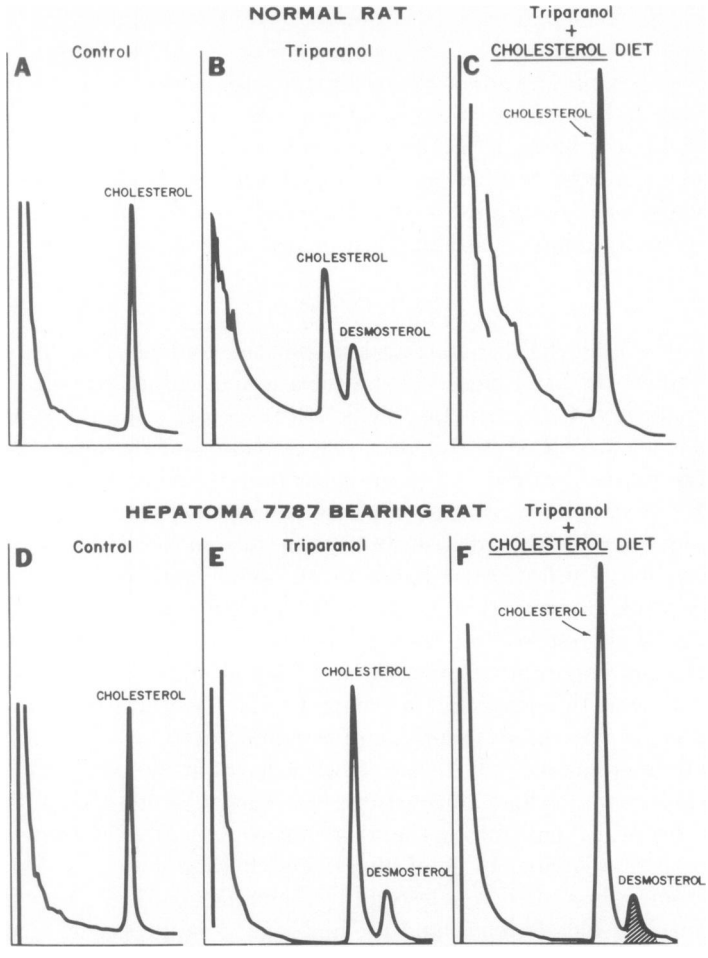

FIG. 2. GLC demonstration of feedback control of sterol synthesis in normal rats (A, B, C); and loss of the cholesterol feedback system in rats bearing hepatoma 7787 (D, E, F).

the liver, and as a result, complete suppression of sterol synthesis in the liver by the feeding of cholesterol has no influence upon the circulating endogenous sterol concentration. These studies therefore demonstrate that the hepatoma readily releases newly synthesized cholesterol into the bloodstream and in fact it can be calculated that in the case of the tumor under study, Morris hepatoma 7787, only 2% of the newly synthesized cholesterol is retained by the tumor, with the remaining 98% being contributed to the circulation, and hence to the overall sterol pool of the rat.

Aside from the theoretical implications of such a loss of cholesterol feed-

back control in hepatomas, it is apparent that the failure of exogenous cholesterol to suppress the endogenous cholesterol in the plasma provides a relatively simple means of detecting the presence of hepatomas in the intact animal. In fact, by measuring the desmosterol concentration of such cholesterol-fed animals, it has been possible to detect hepatomas no larger than 100 mg in size. Studies are currently underway to determine whether this procedure can be applied to other types of tumors, and more important, whether the technique is applicable to man.

Summary

1) These studies have demonstrated that the control of cholesterol synthesis is mediated by a single enzymatic reaction, catalyzed by β-hydroxy-β-methylglutaryl CoA reductase, which is responsible for the synthesis of mevalonic acid. At least in the liver, this enzyme, and therefore the control of cholesterol, is localized to the endoplasmic reticulum of the cell.

2) This feedback system has recently been demonstrated to have a far more widespread tissue distribution than has hitherto been suspected, operating in the intestine probably in all higher animals and, at least in the guinea pig, in every tissue examined, including the lung and the brain.

3) Loss of the cholesterol feedback control mechanism appears to be a consistent accompaniment of malignant change in all cancers studied to date; moreover, the defect in this control system can precede the development of cancer by many months and perhaps years.

4) Finally, it has recently been demonstrated that the cancer-induced loss of the cholesterol feedback mechanism can readily be detected in the intact animal simply by gas chromatographic measurement of desmosterol concentrations of a small sample of blood. This finding indicates that at least in hepatomas, loss of the cholesterol feedback system leads to a release of sterols into the bloodstream; and the inability to suppress such endogenous sterol production and secretion by cancer cells may provide a relatively simple means of detecting the presence of such tumors in the intact organism.

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