The Homeostatic Control of Cholesterol Synthesis in Liver

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DESPITE wide fluctuations of cholesterol in their diets, most animals are able to maintain tissue and plasma concentrations of cholesterol at fairly constant levels. It was shown almost ten years ago by Gould[1] and subsequently confirmed by both Tomkins et al.[2] and Frantz et al.[3] that this constancy of cholesterol concentration could be explained, at least in part, by the fact that an increase in ingested cholesterol results in a marked decrease in the rate at which cholesterol is synthesized by the liver.

These earlier observations could be readily confirmed in that we have been able to demonstrate repeatedly that feeding rats a 2.5 or a 5 per cent cholesterol diet for from twelve hours to as long as one month will result in a ten to 300 fold depression in cholesterol synthesis by the livers of such animals. As indicated in Figure 1, these observations indicate that the body must normally possess a sensitive feedback mechanism by which exogenous cholesterol, and presumably endogenous cholesterol, serve to block one or more of the reactions involved in the conversion of acetate to cholesterol. As a result, cholesterol synthesis is decreased and a homeostatic control of the cholesterol concentration of the body is thereby maintained.

The mechanism of this negative feedback reaction is not known. As an initial approach to a study of this process, attempts have been made to localize the site or sites on the pathway of cholesterol synthesis at which this physiologic regulation of cholesterogenesis occurs.[4-6] Approximately twenty-six separate reactions are believed to be involved in the biochemical conversion of acetate to cholesterol. These can be briefly summarized as follows:

$$\text{acetate} \xrightarrow{4\ \text{steps}} \text{mevalonic acid} \xrightarrow{7\ \text{steps}} \text{squalene} \xrightarrow{15\ \text{steps}} \text{cholesterol.}$$

The approximate location of the cholesterol-induced block of cholesterol synthesis was examined by determining the effect of feeding a 2.5 or 5 per cent cholesterol diet on the ability of rat liver slices to carry out these major steps of cholesterol synthesis. Carbon^{14}-labeled acetate, mevalonate or squalene were the labeled substrates employed to measure the rates of reaction. The results of a typical experiment are illustrated in Figure 2. As shown in experiment 1, the feeding of a 2.5 per cent cholesterol diet for eleven days depressed the over-all conversion of acetate to cholesterol by a factor of thirty-four (Fig. 2). The influence of cholesterol feeding on the reactions involved in the conversion of squalene-C^{14} to cholesterol is shown in experiment 2, Figure 2. This phase of cholesterol synthesis was found to be either unaffected or, as in this example, slightly increased by the feeding of cholesterol. The major site of the cholesterol block is not, therefore, in one of the fifteen reactions involved in the conversion of squalene to cholesterol. Of the eleven reactions required for the incorporation of acetate into squalene, seven can be examined by following the effect of cholesterol feeding on the conversion of mevalonate-C^{14} to squalene. As shown in experiment 3, Figure 2, dietary

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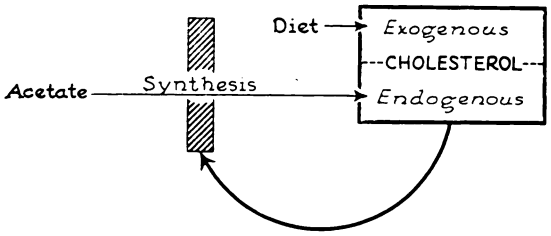

FIG. 1. Cholesterol inhibition of cholesterogenesis.

cholesterol does not inhibit these reactions, and in fact, has consistently produced a slight increase in the incorporation of mevalonate into squalene. This series of experiments suggest that the major site of the cholesterol block cannot be located in the reactions involved in the conversion of mevalonate to cholesterol.

This conclusion is further supported by the finding shown in experiment 4, Figure 2. Cholesterol feeding caused less than a 50 per cent depression in the conversion of mevalonate to cholesterol, a decrease which is not sufficient to account for the thirty-four fold depression which is seen in the over-all conversion of acetate to cholesterol. Both Gould and Popják[7] and Bucher et al.,[8,9] using similar approaches in liver homogenates, have also demonstrated that the reactions between mevalonate and cholesterol are not greatly influenced by cholesterol feeding.

It is apparent, therefore, that the site of the feedback block must be located in one of the four reactions of cholesterol synthesis located prior to the production of mevalonate.

These four reactions are believed to occur as follows:

acetate $\xrightarrow{1}$ acetyl CoA $\xrightarrow{2}$ acetoacetyl CoA $\xrightarrow{3}$ β-hydroxy-β-methylglutaryl CoA $\xrightarrow{4}$ mevalonic acid.

The site of the cholesterol block could be localized to one of these four reactions by utilizing the fact that acetoacetyl CoA serves as an important intermediate for the synthesis of long-chain fatty acids as well as for the synthesis of cholesterol, while β-hydroxy-β-methylglutaryl CoA, as has been shown by Lynen et al.,[10] is a precursor of the ketone bodies, acetoacetic acid and β-hydroxybutyric acid. A block at the site of reactions 1 or 2 should therefore lead to a depression in the conversion of acetate-C^{14} to fatty acids as

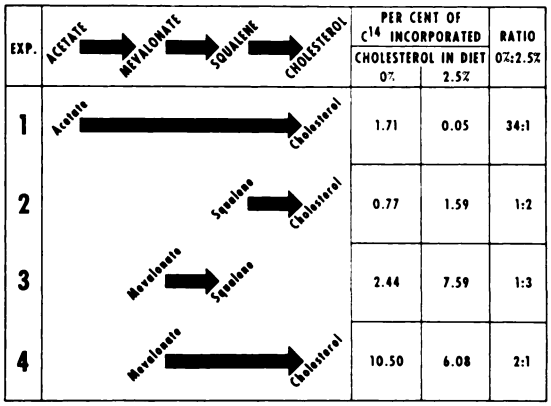

FIG. 2. Initial localization of cholesterol block.

well as to cholesterol. Similarly, a cholesterol-induced block at the site of reaction 3 would be expected to produce an inhibition in the conversion of acetate-C^{14} to ketone bodies. Studies on the effect of dietary cholesterol on these reactions were carried out, and it was found that the conversion of acetate-C^{14} to long-chain fatty acids and to ketone bodies is not inhibited significantly by cholesterol feeding. It follows, therefore, that of the four reactions involved in the conversion of acetate to mevalonate, reactions 1, 2 and 3 cannot be the site of the block in cholesterol synthesis.

As summarized in Figure 3, these findings have led to the conclusion that reaction 4 involving the conversion of β-hydroxy-β-methylglutaryl CoA to mevalonic acid is the major site at which cholesterol feeding inhibits the synthesis of cholesterol.[4,5]

The location of the cholesterol feedback mechanism at a site just beyond the last branch of the synthetic pathway would appear to be ideally suited to regulate this over-all sequence of reactions. Feedback control to a reaction beyond mevalonic acid (Fig. 3) would result in the unnecessary synthesis and perhaps the accumulation of useless intermediates whenever cholesterol was present in the diet. On the other hand, a feedback block located prior to β-hydroxy-β-methylglutaryl CoA might lead to the incidental depression of fatty acid or ketone body synthesis whenever cholesterol was fed.

Feedback mechanism, involving product inhibition of a specific early reaction site, has

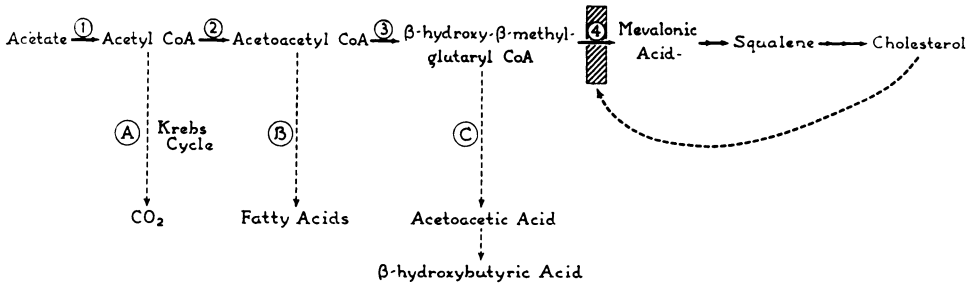

FIG. 3. Proposed site of feedback control of cholesterogenesis.

been shown to regulate synthetic processes in numerous bacterial systems[11–13]; however, as far as we are aware, such a mechanism has not been demonstrated previously in animal tissues. The finding that cholesterol synthesis is controlled by a mechanism similar to that found in bacteria suggests that this means of controlling biologic syntheses may be more widespread than has hitherto been assumed. It should be emphasized that there is already evidence to suggest that in higher animals the synthesis of fatty acids,[14] pyrimidines[15] and purine[16] may be controlled by a type of negative feedback reaction, though the location of the site or sites of control in these cases have not been determined as yet.

The exact mechanism by which cholesterol feeding inhibits the conversion of β-hydroxy-β-methylglutaryl CoA to mevalonic acid is not known; however, cholesterol itself is probably not directly responsible for the inhibition of this reaction. Feeding amounts of cholesterol sufficient to depress hepatic cholesterogenesis need not cause any elevation in total liver cholesterol.[6] Furthermore, as shown in Table I, the addition of cholesterol to normal liver slices has only a relatively small inhibitory effect on cholesterol synthesis from acetate-C^{14}. Several fatty acid esters of cholesterol have been prepared and although the addition of large amounts of these compounds caused some inhibition of cholesterol synthesis, fatty acid synthesis was likewise inhibited. This depression of cholesterogenesis appears, therefore, to be non-specific and never approaches

TABLE I

Influence on Cholesterol Synthesis of Cholesterol and Cholesterol Esters Added *in Vitro* to Normal Rat Liver Slices

Addition	Per cent Added C^{14} Incorporated Into	
	Cholesterol	Fatty Acids
Albumin, 1%	1.3	1.11
Cholesterol, 10 mg.	0.58	0.69
Cholesteryl palmitate, 10 mg.	1.23	0.86
Cholesteryl linoleate, 10 mg.	0.26	0.25
Cholesteryl arachidonate, 10 mg.	0.79	0.28

NOTE: Each flask contained one of the above cholesterol derivatives dissolved in 1% albumin plus 5 ml. Krebs-Bicarbonate buffer, 5 ml., 500 mg. liver slices and 1 μM acetate-2-C^{14} (0.5 μC). The flasks were incubated two hours at 37°c.

TABLE II

Influence on Cholesterol Synthesis of Bile Acids Added *in Vitro* to Normal Rat Liver Slices

Experiment	Addition	Per cent Acetate-1-C^{14} Converted to		
		Cholesterol	Fatty Acids	CO_2
1	Nothing	0.23	1.51	32.5
2	Taurocholic acid 10^{-5}M	0.26	1.88	36.2
3	Taurocholic acid 10^{-4}M	0.30	1.56	35.1
4	Taurocholic acid 10^{-3}M	0.32	2.46	37.2
5	Taurocholic acid 10^{-2}M	0.25	1.39	35.6
6	Cholic acid 10^{-4}M	0.28	1.30	35.0
7	Cholic acid 10^{-3}M	0.05	0.10	11.3

NOTE: Each flask contained the concentration of bile acid noted above plus 500 mg. liver slices and 10 μM acetate-1-C^{14} (1 μC) in 5 ml. Krebs-Bicarbonate buffer. The flasks were incubated for two hours at 37°c.

TABLE III
Effect of Cholesterol Synthesis of Feeding Taurocholic Acid for Twenty-three Hours

Experiment	Taurocholic Acid in Diet	Per cent Acetate-1-C^{14} Converted to		
		Cholesterol	Fatty Acids	CO_2
1	0	0.59	2.46	43
2	0	1.33	3.41	39
3	0	0.74	1.08	40
4	0	0.38	1.66	39
5	2.5%	0.63	0.83	35
6	2.5%	0.32	0.39	39
7	2.5%	0.35	0.74	37
8	2.5%	0.22	1.29	34

NOTE: Each flask contained 500 mg. of liver slices in 5 ml. Krebs-Bicarbonate Buffer plus 10 μM acetate-1-C^{14}. The flasks were incubated for two hours at 37°c.

the marked inhibition produced by cholesterol feeding.

Since the major biochemical route by which the cholesterol is metabolized in the rat is via taurocholic acid,[17,18] attempts have been made to determine whether this breakdown product of cholesterol might be the direct mediator of the inhibition of cholesterol synthesis. As is shown in Table II, taurocholic acid, when added to liver slices even at a concentration of 10^{-2}M, had no effect on cholesterol synthesis. Cholic acid at a level of 10^{-3}M depressed cholesterol synthesis somewhat, but this effect is not specific since a definite depression in lipogenesis was produced also at this bile acid concentration. Similarly, the feeding of taurocholic acid at a concentration of 2.5 per cent for a period of twenty-three hours produced only a small depression of cholesterogenesis (Table III). Administration of cholesterol at this level for only twelve hours will cause at least a tenfold depression in cholesterol synthesis.* The exact mechanism by which cholesterol feeding blocks the conversion of β-hydroxy-β–methylglutaryl CoA to mevalonic acid and thereby inhibits cholesterogenesis is still to be worked out; further studies of this problem are currently in progress.

* Behers et al.[19-21] have found that feeding rats or mice an 0.5 per cent cholic acid diet for from twelve hours to three weeks causes some depression in cholesterol synthesis; however, this effect is not great, and, as suggested by these authors,[20] is probably secondary to the elevation of hepatic cholesterol which results from feeding such diets.

SUMMARY

The feedback mechanism by which cholesterol synthesis is regulated in liver has been studied. Evidence has been presented which indicates that exogenous cholesterol inhibits cholesterol synthesis primarily by blocking the conversion of β-hydroxy-β–methylglutaryl CoA to mevalonic acid. This reaction would appear to represent the major biochemical site of normal homeostatic control of cholesterol synthesis in the liver. This inhibition of cholesterol synthesis does not appear to be directly mediated by cholesterol itself, the common cholesterol esters, or taurocholic acid. The exact mechanism by which cholesterol feeding inhibits the conversion of β-hydroxy-β–methylglutaryl CoA to mevalonate remains to be elucidated.

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DISCUSSION

DR. O'CONNELL (*The Upjohn Company*): I believe this step is the same one that Nancy Bucher found to be inhibited in the starvation of animals. They seem to lose their ability to synthesize cholesterol. If this is so, this seems to be a particularly sensitive step in two directions, both in feeding and in starvation.

Do we know enough yet about the kinetics of the overall process to know if this is a rate-determining step in the whole process?

DR. SIPERSTEIN: The only additional evidence that I can cite to support the generalization that this is the important step in the control of cholesterol in a variety of circumstances, is by Fletcher and Myant who showed that thyroxin may act at a step between acetate and mevalonic acid, and I proffer a guess that the HMG-CoA reductase will be the actual site, when localized, for the action of thyroxin on cholesterol synthesis.

The action of glucose in controlling cholesterol synthesis may also be at this site. Glucose has been shown to affect cholesterol synthesis by the production of TPNH. And, as you will note, reduced triphosphopyridine nucleotide (TPNH) is involved in β-hydroxy-β–methylglutaryl CoA reduction to mevalonic acid.

I should state categorically, that however, the cholesterol feedback mechanism, which I just described, does not involve TPNH since added TPNH will not reverse the feedback in broken-cell preparations. The glucose mechanism of control, however, does involve TPNH, rather than a direct enzymatic effect.

DR. JAMES SALTER (*Toronto, Canada*): Is it the cholesterol *per se* that actually suppresses its synthesis, or some product of cholesterol catabolism, if there is such a thing, or something involved in the excretion of cholesterol by the bile salts?

It is not often that a product inhibits by itself. With urea synthesis, inhibition is brought about by the automatic formation of pyruvic acid under some circumstances.

DR. SIPERSTEIN: In bacterial systems, it is usually the product which feeds back to inhibit the reaction. It can inhibit in one of several ways. The product could inhibit the enzyme reaction directly by blocking the enzyme, competitively or noncompetitively. Such a mechanism has been shown to operate in several bacterial systems, including the one described by Yates and Pardee for pyrimidine synthesis. It is probably true for adenine synthesis, as well.

Likewise, the feedback mechanism might operate by blocking the synthesis of the enzyme, and this mechanism has been shown in bacterial systems also.

The addition of cholesterol *in vitro* has had no effect on this reaction, so that we do not feel that this is a direct effect of cholesterol on the enzyme, the β-hydroxy-β–methylglutaryl CoA reductase.

We have synthesized cholesterol palmitate and cholesterol oleate, linoleate and arachidonate, and none of these, added *in vitro*, have an inhibiting effect on the synthesis of cholesterol.

The addition of the common bile acids or their amide complexes will not inhibit the reaction in physiologic amounts.

Finally, I would speculate that since it takes at least twelve to sixteen hours of cholesterol feeding to produce the inhibition, it would seem likely that inhibition of enzyme synthesis might be involved.

DR. RACHMIEL LEVINE (*Chicago, Illinois*): Dr. Siperstein, your objection to the idea that cholesterol would act by mass action was your demonstration that the cholesterol level does not increase. How does the enzyme know that you are feeding cholesterol?

DR. SIPERSTEIN: The other evidence that cholesterol does not act by mass action is, of course, the demonstration that the last sequence of reactions is not involved.

In order to explain such a process, which apparently comes up in designing electric feedback mechanisms, an amplifier is needed to detect minute amounts of air in the feedback mechanism.

One could picture such an amplifying system operating by using the concept which Monod has advocated for enzyme induction or Vogel has advocated for enzyme repression. Small amounts of cholesterol could get into the RNA template which is responsible for the synthesis of β-hydroxy-β-methylglutaryl CoA reductase and plug the template so that many molecules of enzyme cannot then be manufactured.

DR. LILLIAN RECANT (*St. Louis, Missouri*): If you took an animal that had nephrosis or hypothyroidism, in which you have elevated levels of cholesterol, would this feeding mechanism inhibit the reaction?

DR. SIPERSTEIN: We have not done that.

The first experiment along these lines which we have wanted to do is in human beings; namely, to study the intactness of the feedback mechanism in familial hypercholesteremic states by simply doing liver biopsies and seeing whether this reaction is inhibited.

We have now been studying the isolated reaction. We want to see whether an inhibitor is present in normal liver which is not present in familial hypercholesteremia. It is quite reasonable that a breakdown in either the sensitivity of the enzyme to the feedback mechanism or in the feedback mechanism itself might be responsible for these conditions of hypercholesteremia.

Dr. F. D. W. LUKENS (*Philadelphia, Pennsylvania*): Do you think that a difference in the effectiveness of this system accounts for the fact that hypercholesteremia can be produced in the rabbit by feeding but not in the normal dog?

DR. SIPERSTEIN: It is difficult to quantitate the sensitivity of the reaction so far as we have been carrying it out. The rabbit does possess this mechanism for feedback inhibition, but by the time we can detect it, the rabbit has already built up fairly significant amounts of cholesterol in his liver.

The reaction is probably less sensitive, but I cannot state so categorically until we really have an inhibitor and can show that *in vitro* this enzyme is more or less inhibited.

DR. RECANT: You mentioned at the beginning of your presentation that the liver was the only organ that showed this effect. Did you actually study adrenal tissue?

DR. SIPERSTEIN: We have not studied adrenal tissue.

DR. RECANT: I am interested in the possibility that in a situation such as myxedema, in which we have a diminished formation of steroid and secretion, we may be getting into some of these feedback mechanisms there, and perhaps not in the liver.

DR. SIPERSTEIN: The tissues which we have studied are: skin, which is not inhibited; and intestine, which, under the influence of cholesterol feeding, will synthesize more cholesterol than the liver because of the depression of the liver and not of the intestine.

We have not studied the endocrine organs. We have studied various sites in the intestinal tract and, for no good reason, the spleen.