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THE FEEDBACK CONTROL OF CHOLESTEROL BIOSYNTHESIS*

W. T. BEHER**, G. D. BAKER**, W. L. ANTHONY**, AND M. E. BEHER**

The 29 carbon alicyclic alcohol, cholesterol, participates in the metabolic reactions of a wide spectrum of cells. It is a precursor of the steroid hormones synthesized by ovarian, testicular, placental, and adrenal cortical tissues[1]. It is rapidly metabolized by liver cells to bile acids, which in turn have important influences on intestinal absorption[2,3]. Cholesterol has been implicated in the transport of unsaturated fatty acids in the blood and in the regulation of the transfer of fatty acids across the cell membrane[4]; its role in the structure and metabolism of the nervous system has received at least preliminary investigation[5]. Although these functions are obviously vital to the metabolic and structural integrity of the living organism, it is the abnormally high blood and tissue cholesterol levels found in the disease, atherosclerosis, that is responsible for the present vigorous research interest in cholesterol metabolism.

Since we are concerned with the etiology and reduction of abnormally elevated tissue cholesterol levels in atherosclerosis, it would seem that basic research on the mechanisms by which various tissues maintain normal cholesterol levels should be of prime importance. Such studies necessarily involve factors influencing and controlling the relative rates of cholesterol anabolism, catabolism and distribution in many tissues. As an example, consider a very simple case: the ability of a given tissue to maintain a static cholesterol level in an isolated system such as a tissue slice or a homogenate. In such a system, the rate of synthesis simply must balance the rate of degradation; i. e. if the rate of degradation is increased, the rate of synthesis must increase equally or a net decrease in cholesterol level will take place. In the intact animal such a simple system does not exist. Here we must consider not only relative rates of anabolism and catabolism but also such factors as level of dietary cholesterol, rate of exchange of tissue and organ cholesterol with the blood, rate of enterohepatic recirculation, intestinal absorption, availability of cholesterol precursors, availability of certain coenzymes, and possibly the existence of active and inactive forms of cholesterol[6]. Since various hormones can cause alterations in the relative contributions of these and other parameters, we can see the enormous complexity of the problem. Obviously in any systematic study of these phenomena, one aspect at a time should be investigated while attempting to maintain a steady-state with respect to other variables. The following discussion will be limited to a consideration of the control of cholesterol biosynthesis by cholesterol itself and by the bile acids.

CHOLESTEROL FEEDBACK INHIBITION

Since enzymatic reactions may be retarded by (a) specific reaction product inhibitions or (b) reversal by accumulated reaction products, one would expect that cholesterol formation would be controlled by cholesterol or intermediates in cholesterol synthesis. A number of papers have appeared which show that, in various species,

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increases in liver cholesterol levels brought about by dietary cholesterol supplements, lead to striking decreases in the ability of the liver to synthesize cholesterol[7-17]. Conversely, decreasing hepatic cholesterol levels by bile duct cannulation[18], triton[19,20], lymph duct cannulation[21,22], x-radiation[20,23], or hyodeoxycholic acid[24] results in many fold increases in hepatic cholesterol synthesis rates, despite the strikingly different mechanisms involved. In studies on the effects of some of these procedures[12,24,25] in various tissues, it appeared that, with the exception of the adrenal, they had little, if any, effect on extrahepatic cholesterol synthesis rates. This suggests that these rates are controlled by factors other than those controlling liver cholesterol synthesis. In mice or rats fed cholesterol or hyodeoxycholic acid[26], we detected little change in cholesterol levels in tissues other than liver and blood. Recently, however, Morris and Chaikoff[27], employing a different approach to this aspect of the problem, concluded that dietary cholesterol does have an effect on extrahepatic cholesterol synthesis in all tissues studied except testes. Further experiments will be necessary to clarify this problem, as the details of cholesterol synthesis in extrahepatic tissues may differ significantly from those encountered in liver, and few studies have been made of the sequence of reactions in these tissues.

Frantz *et al*[28] showed that there is an inverse relationship between the logarithm of the rate of cholesterol biosynthesis and the total cholesterol concentration in rat liver slices. Gould[29] refined this work and found that the rate of hepatic cholesterol synthesis is more directly related to free cholesterol concentration than to total cholesterol. Neither of these approaches however explains the rapid changes in cholesterol synthesis rates obtained during the initial phases of cholesterol administration[30] or lymph duct cannulation[21,22], which are unattended by measurable alterations in hepatic cholesterol concentration. It might be suggested that the changes in cholesterol synthesis are probably more related to the cholesterol concentration in or at the surface of specific cellular particulates than to the concentration of ester, free, or total cholesterol in a given liver.

Recently Siperstein[31] has shown that the addition of emulsions of cholesterol or cholesterol esters to rat tissue slices, synthesizing cholesterol from acetate-1-C^{14}, has little if any effect on the synthesis rate as long as the cholesterol concentrations are held within physiological limits. The validity of these *in vitro* experiments may be questioned on the grounds that there is some doubt whether the cholesterol or cholesterol ester in the artificial emulsions used in this study were able to penetrate or permeate cell or particulate membranes. Studies using either lipoproteins rich in cholesterol or a series of different types of emulsions would increase the value of these observations.

SITE OF CHOLESTEROL FEEDBACK INHIBITION

Since the sequence of reactions involved in the synthesis of cholesterol from acetate (with the exception of those reactions between lanosterol and cholesterol) are rather well defined (Fig. I)[32,33], and since several C^{14}-labeled compounds in the sequence are available, attempts have been made to pinpoint the reaction inhibited by cholesterol. In an *in vitro* investigation, Gould and Popjak[34] found that homogenates prepared from livers of cholesterol-fed rats, incorporated acetate-1-C^{14} into

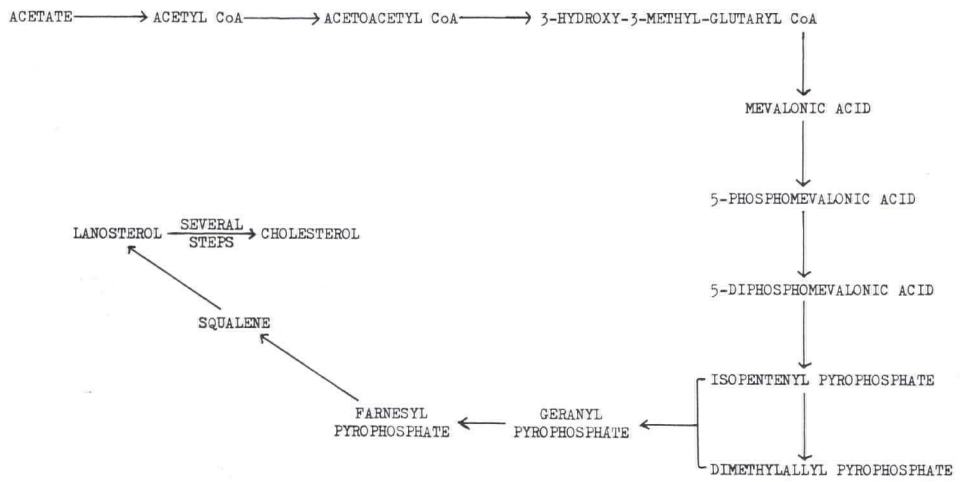


Figure 1
Pathway of cholesterol biosynthesis from acetate.

cholesterol much more slowly than homogenates from normal rats, while the rate of incorporation of mevalonic acid-2-C^{14} was not affected. On the other hand inhibition between mevalonic acid and cholesterol has been detected in other investigations. Siperstein and co-workers[30,35,36] found an inhibition of 30 to 40% in liver slice experiments; while Bucher *et al*[20] detected somewhat more inhibition in homogenates. The latter observations, however, represent only two tests. Bucher and co-workers also investigated the effect of cholesterol on the conversion of squalene to cholesterol, and detected some inhibition. This observation is not in agreement with Siperstein's experiments[31]. Study of cholesterol synthesis rates in microsomes and supernatant fractions isolated from homogenates derived from livers of normal and cholesterol-fed rats led to a series of interesting observations[20]: (a) cholesterol synthesis from acetate or mevalonic acid depends on the presence of both microsomes and the supernatant fraction; (b) combination of the supernatant and microsomes from liver homogenates derived from cholesterol-treated rats results in very little cholesterol synthesis from acetate; (c) combination of liver microsomes from cholesterol-treated rats with liver supernatant from normals results in very little cholesterol synthesis; (d) combination of liver microsomes from normal rats with liver supernatants derived from cholesterol-treated rats results in a near normal rate of incorporation of acetate into cholesterol. Thus, the effect of cholesterol on cholesterol synthesis influences some parameter carried in the microsomes. This may be a series of enzyme reactions, a single reaction, or a biophysical change in microsomal structures and/or permeabilities.

In an effort to study some of these effects in *in vivo* systems, our group investigated the influence of cholesterol feeding on the incorporation of acetate-1-C^{14} and mevalonic acid-2-C^{14} into mouse liver cholesterol. The results, shown in Table I, indicate that while cholesterol synthesis from acetate-1-C^{14} is depressed 95%, mevalonic acid-2-C^{14} incorporation is depressed 50% in the several groups. Purification of the digitonide precipitate fraction via the dibromide[37], which removes digitonin

Table I

Influence of Diets Supplemented with 1% Cholesterol on Mouse Liver Cholesterol Levels and Synthesis Rates as a Time Function

Diet Interval	Liver Cholesterol Level	Acetate-1-C^{14} Incorporation	Mevalonic Acid-2-C^{14} Incorporation	
			Before Dibromide Purification	After Dibromide Purification
Days	mg/g	Cholesterol counts per minute per gram tissue		
0	4.22 ± 0.9	2,496	22,729	19,774
4	8.81 ± 1.87	125	11,106	5,886
14	12.3 ± 2.85	135	8,703	4,265
21	15.2 ± 4.14	129	10,496	5,248

precipitable cholesterol precursors, results in a further loss of activity, bringing the inhibition of mevalonic acid-2-C^{14} incorporation to about 75%. This still may not be a maximal figure, for the dibromide purification is not quantitative[37]. Moreover, the control group of animals, with which the cholesterol-fed group was compared, may have totally exhausted the small amount of injected mevalonic acid-2-C^{14} by conversion to cholesterol during the one-hour incorporation. While further investigations will be necessary to establish its full extent, obviously the inhibition of mevalonic acid-2-C^{14} incorporation, while not as large as that of acetate-1-C^{14}, is substantial. The inhibition must lie in the steps from lanosterol to cholesterol, since dibromide purification, which removes digitonin precipitable 3β-hydroxysterols from lanosterol through desmosterol, results in a loss of C^{14} activity. Therefore the metabolism of some cholesterol precursor between squalene and cholesterol is being inhibited. Gould[29] demonstrated inhibition of the conversion of mevalonic acid to cholesterol by cholesterol *in vivo* in rats, but did not purify the isolated cholesterol digitonide.

The effects of some of the possible intermediates between mevalonic acid and cholesterol on biosynthesis of cholesterol from acetate have also been investigated. Feeding squalene, lathosterol, or 7-dehydrocholesterol inhibits cholesterol synthesis[11,13], while lanosterol and farnesol are ineffective. Farnesoic and geranoic acids, which are probably products of an alternate pathway of squalene metabolism, inhibit the conversion of mevalonic acid and farnesyl pyrophosphate to sterols[38]. Farnesoic acid inhibits the conversion of mevalonic acid to 5-phosphomevalonic acid[39]. Therefore the formation of these acids has been suggested as a possible control mechanism in sterol synthesis[32].

The foregoing observations have been interpreted in two ways: (a) Since the inhibition of cholesterol synthesis by feedback is much greater from acetate than from mevalonic acid or squalene, the main inhibition must lie prior to mevalonic acid; and further since acetyl Co A, acetoacetyl Co A, and hydroxymethylglutaryl Co A are all involved in vital reactions other than cholesterol syntheis (Fig. 2), which proceed uninhibited during the feedback, the only reaction where inhibition is likely is the conversion of hydroxymethylglutaryl Co A to mevalonic acid, a relatively nonreversible reaction. Lynen and coworkers[40] showed that this reaction was inhibited under feedback

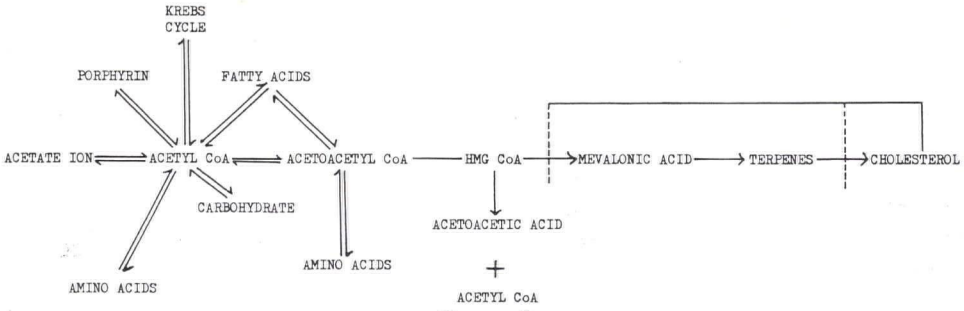


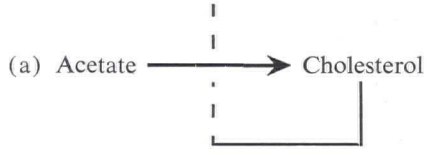
Figure 2

Metabolic interrelationships in cholesterol feedback inhibition.

conditions. (b) It is possible to regard the smaller feedback inhibition, mevalonic acid —/—/→ cholesterol, as very important. The reaction sequence acetate ——→ mevalonic acid ——→ cholesterol, can be divided as follows: The metabolites prior to mevalonic acid are readily interconvertible (Fig. 2), and in two cases, viz. acetyl Co A and acetoacetyl Co A, are among the substrates most in demand for a variety of major synthesis pathways. In contrast, mevalonic acid seems to have little future save incorporation into isoprenoid compounds and subsequently into the steroid nucleus. That which can not be utilized is excreted unchanged.[41] Therefore during feedback inhibition, a uniform feedback all along the sequence of reactions would *appear* much greater if studied with acetate-1-C^{14} than it would *appear* if studied with mevalonic acid-2-C^{14} because the acetate-1-C^{14} would disappear more rapidly into other major pathways while the mevalonic acid-2-C^{14} would be rapidly converted to cholesterol*.

BILE ACID FEEDBACK INHIBITION

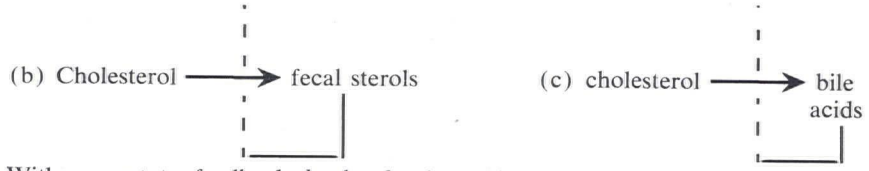
The feedback reaction we have been considering up to this point may be indicated in the following way:



i.e., cholesterol prevents its own synthesis by inhibiting a reaction or reactions between acetate and cholesterol.

Cholesterol is eliminated from an organism in a number of ways: (a) excretion in the fecal sterol fraction, (b) conversion to bile acids followed by fecal elimination, (c) loss by exfoliation of tissues containing the sterol, (d) metabolism to the steroid hormones with subsequent excretion, and (e) excretion in the sebum. Pathways (a) and (b) account for most of the elimination. If we once again turn to the concepts of product inhibition and reaction reversal, it might be expected that the fecal sterols and bile acids would limit the conversion of cholesterol to these endproducts by feedback reactions, such as the following:

*Only one isomer of dl-mevalonic acid is utilized for steroid synthesis. Therefore a maximum of 50% dl-mevalonic acid could be converted to cholesterol.



With respect to feedback *b*, the fecal sterols have an extrahepatic origin. They may be synthesized in several extrahepatic tissues, but the small intestine is the probable point of origin of the bulk of these substances. Recently Danielsson[42] has suggested that the synthesis of these sterols may be controlled by a feedback reaction similar to *b*. Reaction *c* has been investigated at least preliminarily. Eriksson[43] found that the normal rat produces around 5 to 6 mg per day of bile acid. If a tube is placed in the bileduct of a normal rat and led to the outside so that the bile acids which normally enter the enterohepatic recirculation are eliminated (bile duct fistula), the rate of bile acid production increases 10 fold. This is indirect evidence inasmuch as an animal under these conditions is abnormal. Bergström and Danielsson[44] more recently have shown that if one administers taurochenodeoxycholic acid (one of the two main bile acid conjugates of rat bile) to bile fistula rats, the bile acid excretion is lowered from around 60 mg per day to between 5 and 10 mg per day.

We have studied this feedback reaction in intact mice with elevated hepatic cholesterol levels[45]. Cholesterol-4-C^{14} was administered orally until the specific activity of the liver cholesterol pool was constant and liver cholesterol levels elevated. Feces were collected and the C^{14} activity of the bile acid and sterol fractions determined as a function of time. Cholic acid (Fig. 3) retarded the excretion of bile acids at all time intervals. There was little or no effect on the fecal sterol excretion. While the animals used in this study were intact, they did have elevated hepatic cholesterol levels and there is some doubt that this pool is representative of the pool in normal animals because of the presence of a high concentration of cholesterol esters[46]. We therefore investigated the effect of cholic acid on the fecal bile acid output in normal animals receiving a single injection of mevalonic acid-2-C^{14}. The results showed that cholic acid inhibits bile acid production by about 50% in the normal mouse[47]. Whitehouse and Staple[48] studied the inhibition of cholesterol degradation *in vitro*, and found that $C^{14}O_2$ production from cholesterol-26-C^{14} metabolized by a liver mitochondrial suspension, is retarded by the taurine and glycine conjugates of cholic acid. Danielsson[49] has recently pointed out that many reactions occurring during the conversion of cholesterol to bile acids in mitochondrial suspensions *in vitro* are due to auto-oxidation, are non-enzymatic, and do not correspond to *in vivo* reactions. Therefore observations on the production of $C^{14}O_2$ from cholesterol-26-C^{14} are suspect when applied to rate studies.

Scant attention has been given to the site of bile acid inhibition of reactions in sequence *c* from cholesterol to bile acids. Whitehouse and Staple[48] found that the metabolism of 3*a*, 7*a*, 12*a*-trihydroxycoprostane-27-C^{14} (an intermediate between cholesterol and the bile acids) measured by $C^{14}O_2$ production, was inhibited less by tauro- and glyco-cholic acids than the metabolism of cholesterol-26-C^{14}. However, as mentioned above, doubt can be cast on the validity of using $C^{14}O_2$ as an indicator of bile acid formation.

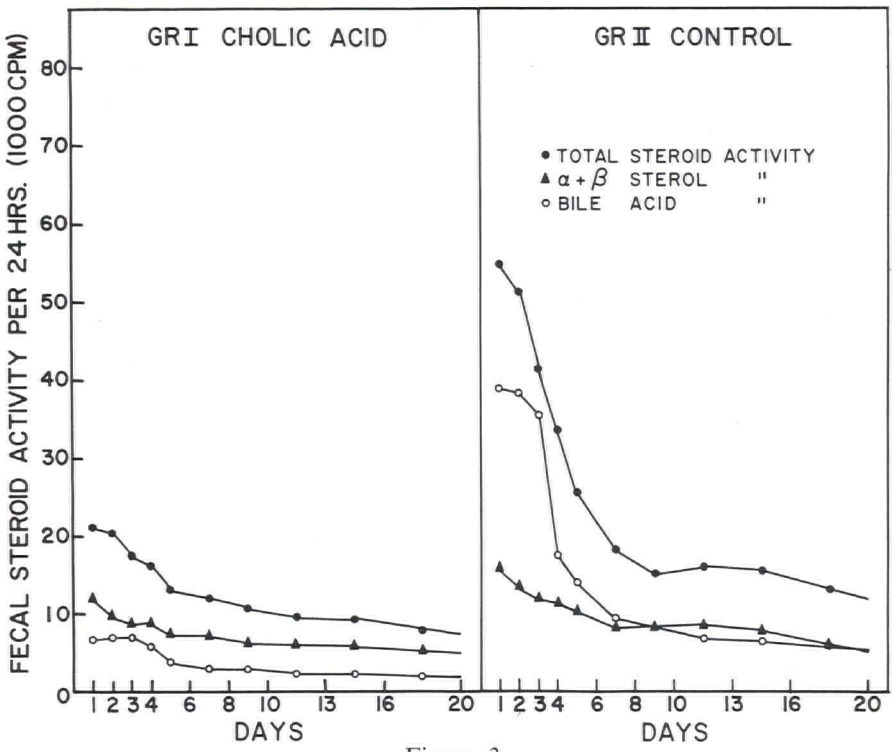
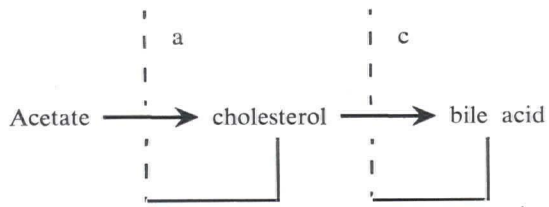


Figure 3
Fecal excretion of C^{14}-labeled steroids in normal and cholic acid treated mice with elevated tissue cholesterol-4-C^{14}

The use of free bile acids in metabolic studies has been questioned by Bergström[50]. He points out that since free bile acids are rapidly conjugated with taurine or glycine in the liver, their use may exhaust the supply of sulfur-containing amino acids, glycine, and serine, and thus upset normal hepatic metabolic patterns. While this is undoubtedly true, recent *in vivo* experiments[51] (Fig. 4) indicate very little difference between the effects of free and conjugated bile acids on cholesterol metabolism when administered at the 0.5% level.

THE DOUBLE FEEDBACK INHIBITION OF CHOLESTEROL BIOSYNTHESIS

Thus far we have been considering feedback reactions involving cholesterol or the bile acids as separate entities. However, if these two reactions are coupled a double feedback reaction (shown below), which may be important in controlling cholesterol metabolism, can be visualized.



Here an increase in bile acid concentration would retard reaction sequence *c* leading to an increase in the cholesterol level, which would in turn inhibit reaction sequence *a*.

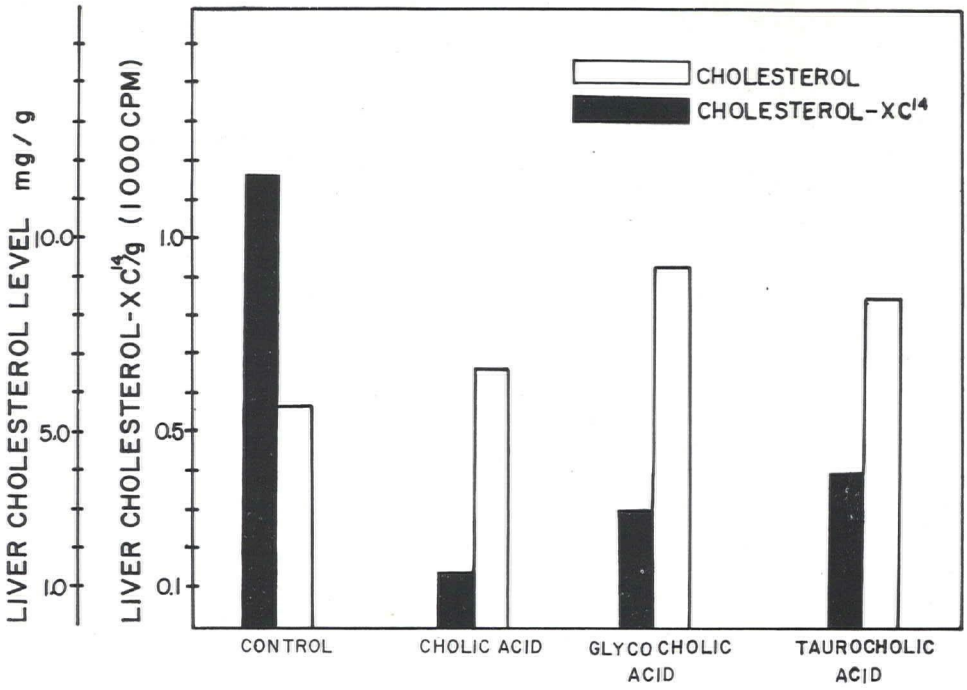


Figure 4
The effects of free and conjugated cholic acid on mouse liver cholesterol levels and synthesis rates (cholesterol-x-C^{14} levels).

Such a feedback system presumably would be effective only in the liver, since cholesterol oxidation to bile acids is only significant in hepatic cells[50] and feedback reaction *a* appears to occur only in the liver and possibly the adrenals.

The following experimental evidence supports the double feedback hypothesis. Swell *et al*[18] noted that 24 hours after cannulation of the bile duct of rats, cholesterol synthesis was increased 10 fold. Inasmuch as there is about a 10 fold increase in bile acid production at the same time[43], this is at least indirect evidence in favor of the double feedback reaction. Work done by the authors[24,45, 52-55] has provided considerable evidence in favor of this sequence. The previously discussed experiments, illustrated in Figure 3, show that cholic acid inhibits reaction sequence *c*, the conversion of cholesterol to the bile acids. If the double feedback hypothesis is correct, cholic acid administration should lead to increases in hepatic cholesterol levels and decreases in liver cholesterol synthesis rates. To investigate this point, mice or rats were fed basal diets supplemented with cholic acid, taurocholic acid or glycocholic acid. The animals received intraperitoneal injections of acetate-1-C^{14}, were sacrificed and liver cholesterol and cholesterol-x-C^{14} levels determined. The results (Fig. 4) show that cholic acid and its glycine and taurine conjugates increase liver cholesterol levels and decrease the rate of cholesterol synthesis (inhibition *a*). That the decrease in cholesterol synthesis was due to the increased level of hepatic cholesterol was shown by a further experiment (Fig. 5). When liver cholesterol levels, synthesis rates and bile acid levels were determined as a function of time, increases in bile

acid and cholesterol levels were in phase and correlated well with decreases in hepatic cholesterol synthesis rates (cholesterol-x-C^{14} levels).

If the double feedback reaction is valid, cholic acid or its conjugates should not inhibit directly at *a*. This point was investigated by Siperstein[31] who demonstrated that *in vitro* cholesterol synthesis in rat liver slices is not inhibited by cholic acid or its conjugates *per se*. Taken as a whole, there is considerable evidence in favor of the double feedback reaction.

APPLICATIONS OF THE FEEDBACK INHIBITIONS

Various attempts have been made to apply these basic findings to the problem of control of tissue cholesterol levels. It has been demonstrated, for example, that

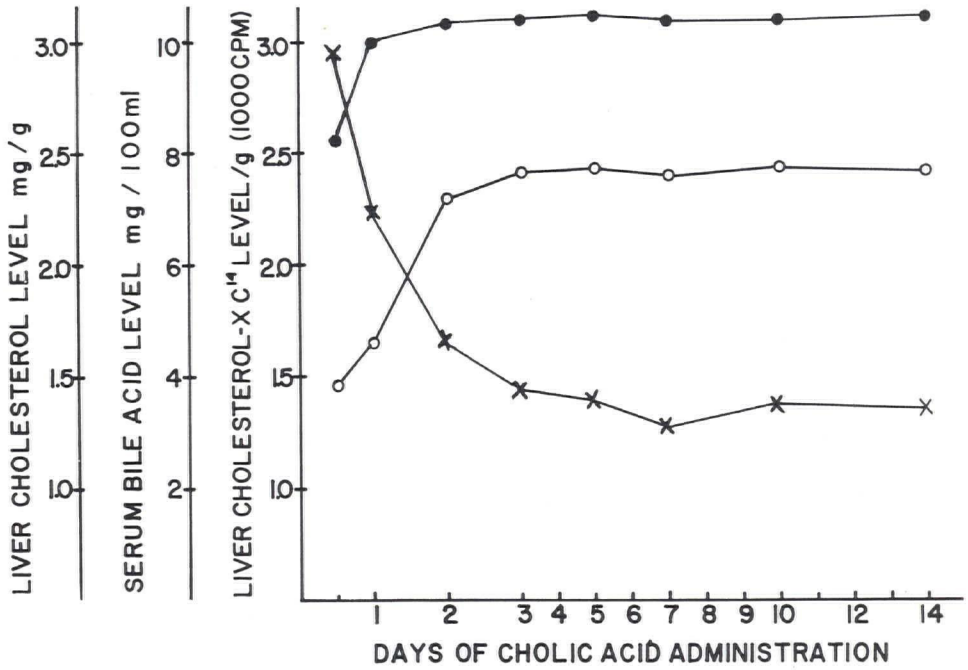


Figure 5

The effect of cholic acid on cholesterol, cholesterol-x-C^{14}, and serum bile acid levels as a function of time.

● Liver cholesterol. O Serum bile acid. χ Liver cholesterol-x-C^{14}

intestinal micro-organisms have important effects on bile acid turnover rates. Gustafsson and co-workers[56,57] found that in germ free rats the normal bile acid turnover rate of 5 to 6 days is doubled. Under germ free conditions, rat blood cholesterol is elevated[58]. Various antibiotics, which alter and decrease intestinal flora concentrations, increase serum cholesterol levels[50, 59-63], presumably by decreasing the conversion of cholesterol to bile acids.

Attempts have been made to lower blood cholesterol levels by increasing bile acid excretion rates, and in turn increasing cholesterol catabolism. Siperstein *et al*[64,65] observed that diets containing 3% ferric chloride (which precipitates bile acids *in vitro*)

limited the rise in blood cholesterol and the severity of atherosclerosis in cholesterol-fed cockerels. More recent experiments[66-68] show that ferric ions are ineffective in preventing cholesterol absorption or increasing the rate of conversion of cholesterol to bile acids in mice and rats. Setty and Ivy[68] studied the effect of aluminum phosphate gel in rats and found no significant increase in bile acid excretion. Rodbard[69] had earlier reported a decrease in blood cholesterol and atherosclerosis in chickens fed similar gels. In any case, the use of metal ions is limited by toxicity. Effects of anion exchange resins, which sequester bile acids *in vitro* systems, have been investigated *in vivo*. Tennent *et al*[70] showed that MK-325 and MK-135, (polymeric organic bases) inhibit blood cholesterol increases and aortic plaque formation in cholesterol-fed cockerels. These resins also lower blood cholesterol levels and increase fecal sterol and bile acid excretion in dogs. Feeding MK-135 to rats did not lower blood cholesterol, possibly because of the relatively greater ability of the rat to synthesize hepatic cholesterol[71]. Setty and Ivy[68] investigated several compounds of this type; most increased bile acid excretion to some extent. MK-135 has been found to induce steatorrhea in human subjects[72].

Hyodeoxycholic acid is another interesting substance. This compound is unique in that it effectively lowers blood and liver cholesterol levels[24,73-75] in mice by increasing the excretion of fecal sterols[45]. In this case part *c* of the double feedback reaction is bypassed. In normal mice, hyodeoxycholic acid or its glycine conjugate increases fecal sterol output about 3 fold[47]. Hyodeoxycholic acid has no demonstrable effect on rat hepatic cholesterol synthesis rates[47], while it increases this parameter in mice 3 to 6 fold[51]. A species difference appears to exist.

Little is known concerning the role of defects in the feedback reactions in various diseases which affect cholesterol levels. Recently Dubach and co-workers[76] investigated this problem in experimentally nephrotic rats, and found that the feedback mechanism responds to dietary cholesterol in an essentially normal fashion in these animals.

CONCLUSIONS

1. It has been shown by various approaches that in hepatic tissue the rate of cholesterol biosynthesis is controlled by the cholesterol level. There is conflicting evidence concerning the effect of cholesterol on cholesterol synthesis rates in extra-hepatic tissues.

2. The feedback inhibition of cholesterol synthesis may take place at several steps in the cholesterol synthesis sequence. It appears that the major point of inhibition is at the conversion of hydroxymethylglutaryl Co A to mevalonic acid; however, strong inhibitions are also effected between mevalonic acid and cholesterol.

3. The rate of conversion of cholesterol to bile acid is also controlled by feedback inhibitions, but few studies have been reported in connection with the site of inhibition in this sequence of reactions.

4. The cholesterol feedback reaction and bile acid feedback reaction are coupled in *in vivo* systems, resulting in a double feedback reaction sequence. Study of factors affecting this double feedback reaction may help to explain abnormal cholesterol

levels in various metabolic disturbances, and offers a promising approach to the problem of controlling tissue cholesterol levels.

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