Mode of Action of Cholesterol-Lowering Agents

A Critique of Facts and Theories

The implication of hypercholesteremia in atherogenesis has stimulated a continued search for cholesterol-lowering agents within the last decades. In addition to various dietary regimens ranging from a glass of orange juice to special formula diets, a great number of drugs ranging from household remedies as aspirin (acetylsalicylic acid) to new sophisticated compounds have been reported to possess hypcholesteremic activity. Their efficacy, side-effects, and clinical usefulness will be discussed elsewhere.

The present review is confined to the mode of action of unsaturated fats, sitosterol, nicotinic acid, thyroid hormones and analogues, and triparanol. They are selected for discussion because they have been extensively studied clinically and with respect to their mode of action. Since, as will be delineated, these agents act at different sites of the metabolic pathways of cholesterol, several avenues are thus opened for further investigation of cholesterol metabolism and atherogenesis.

Unsaturated Fats

It has long been known that the shift from the regular mixed diet to a rice diet, which contains no fat, leads to a drastic fall in serum cholesterol level. For some time in the past, the influence of dietary fat on serum cholesterol was believed to be dependent solely upon its amount in the diet, regardless of its vegetable or animal origin.2-5

Since the reports of Kinsell et al.6 and Groen et al.7 in 1952, convincing evidence has been accumulated8-24 which indicates that there is indeed a difference in the effect on serum cholesterol between relatively unsaturated fats (e.g., corn, safflower, cottonseed, peanut, olive, or marine oil) and relatively saturated fats (e.g., butter, lard, coconut oil, or hydrogenated vegetable fats).

It was found that the change of a regular mixed diet to a formula diet high in unsaturated fat caused a significant reduction in serum cholesterol. When the unsaturated fat of the formula diet was replaced with calorically equivalent amounts of a saturated fat, the serum cholesterol concentration rose; it fell again when the substitution was reversed. In order to obtain a significant lowering of serum cholesterol level, at least 50% of the saturated fats in the diet must be replaced by unsaturated fats.

The mechanism by which the substitution of unsaturated for saturated fats in the diet reduces serum cholesterol is still the subject of controversy. The different schools of thought may be summarized as follows:

1. Unsaturation.—The "unsaturation" hypothesis has been advanced by Ahrens et al.11-15 They demonstrated in 1957 that the major factor responsible for the depression of serum cholesterol concentration by unsaturated fats resided in the glyceride portion and that the effect was directly related to the net unsaturation of the glyceride fatty acids as measured by the iodine value.
of dietary fat.\textsuperscript{12} In 1959, the investigators presented additional evidence to support the "unsaturation" hypothesis.\textsuperscript{15} They found that in two patients in whom the serum cholesterol was reduced by the corn-oil-formula diet, there was a further depression of serum cholesterol when corn oil was replaced with isocaloric amount of menhaden oil, a marine oil which, as compared with corn oil, is higher in unsaturation, lower in "essential" fatty acids, and free of sitosterol (vide infra).

To explore the mechanism of action, these investigators carried out in collaboration with Hellman and Rosenfeld two experiments.\textsuperscript{13,14} In the first, the effect of dietary fats on the rate of cholesterol synthesis was evaluated by measuring the incorporation of labeled acetate into free and esterified cholesterol. The results indicated that there was no significant difference between saturated and unsaturated fats in their effect on cholesterol synthesis. In the second experiment, radioactive cholesterol was given intravenously to a patient, and the fecal excretion of labeled cholesterol and its end-products were measured. When the patient was on the corn-oil-formula diet, the fall in serum cholesterol level was accompanied by an increase in the fecal excretion of labeled materials. On the other hand, when the serum cholesterol level rose following substitution of butter for corn oil in the formula diet, the fecal excretion of labeled materials decreased. It was concluded that either increased excretion of cholesterol into the intestine or decreased reabsorption of cholesterol from the intestine may be the mechanism by which corn oil reduces serum cholesterol concentration. In accord with this hypothesis is the report by Lewis in 1958 that oral or intravenous administration of highly unsaturated fats to patients with complete bile fistulae caused an increase in the rate of cholic acid excretion which preceded the reduction of serum cholesterol.\textsuperscript{25} In 1959 Goldsmith et al. also observed in their clinical study a 20\% to 25\% increase in fecal excretion of bile acids when the diet was changed from saturated to unsaturated fat.\textsuperscript{26}

2. Essential Fatty Acids.—Kinsell and his associates suggested that the cholesterol-lowering effect of unsaturated fats is due to their content of "essential" fatty acids (linoleic, linolenic, and arachidonic acid).\textsuperscript{6,9} They found that linoleic acid given as purified ethyl ester was more effective in reducing serum cholesterol than natural unsaturated fat (safflower oil) or purified ethyl ester of oleic acid, an unsaturated but "nonessential" fatty acid.

As an explanation for the effect of "essential" fatty acids, these investigators proposed the following working hypothesis (vintage 1957-1958). It is based on the concept that (1) the glyceride fraction of the lipids in the plasma functions as the donor of saturated fatty acids which are used as "fuel" in the tissues; (2) phospholipid is the most metabolically active fuel-fatty-acid "transporter"; (3) cholesterol ester is a donor of essential fatty acids for new phospholipid synthesis; (4) essential fatty acids are a most important part of the phospholipid molecule, perhaps in terms of "key-in-lock" enzymatic specificity. "If essential fatty acid is in short supply, there is an increased rate of cholesterol and phospholipid formation, an increased level of both materials in the plasma in an effort to make up in quantity what is lacking in quality."\textsuperscript{9} In other words, more cholesterol and phospholipid are produced to meet the need for the transport of fuel-fatty-acid.

According to this hypothesis, unsaturated fats lower serum cholesterol concentration by supplying essential fatty acids, particularly linoleic, which lead to decreased cholesterol synthesis. The report of Wood and Migicovsky\textsuperscript{27} that unsaturated fatty acids inhibited the cholesterol synthesis in rats was interpreted by Kinsell et al. to be in accord with their hypothesis.

3. Sitosterol Content.—According to Beveridge et al., the reduction of serum cholesterol concentration by exchange of dietary fats is the net result of both the inclusion of cholesterol-lowering substance(s) present in
unsaturated fats and the exclusion of cholesterol-elevating substance(s) present in saturated fats. In numerous dietary experiments in man, various fractions (obtained by vacuum distillation) of corn oil and butter were separately studied with respect to their effect on the serum cholesterol concentration. It was found that a large part of hypocholesteremic activity of corn oil was due to its content of sitosterol, which, as will be discussed, prevents the absorption of cholesterol from the intestine. On the other hand, the cholesterol-elevation substances were found to be saturated fatty acids of short-chain length and, contrary to previous reports by others, dietary cholesterol. The responsiveness to dietary cholesterol was largely influenced by the nature of the accompanying fat, and it was enhanced by triglyceride having certain types of fatty acids attached at the alpha, beta, or alpha\(_1\) positions on the glycerol moiety.

There is still vigorous debate centered around the three hypotheses described above. Actually, all of them are valid conclusions from the respective studies, but none of them provides full explanation for findings obtained from all studies. Clarification will be gained (1) from further experiments with synthetic individual components of fats instead of natural fats which contain two or more variables (2) with the use of methods developed in recent years, such as was reported by Hirsch and Ahrens, which permit accurate analysis of fatty-acid components of cholesterol esters and other lipids in the blood and other body tissues, and (3) from further studies of the ultimate mechanisms by which unsaturated fats reduce serum cholesterol: increased excretion or decreased absorption or decreased biosynthesis of cholesterol, alone or combined.

**Sitosterol**

Chemically, sitosterol differs from cholesterol merely in having an ethyl group at Position 24. Several isomers of sitosterol have been identified: the predominant member of the group is \(\beta\)-sitosterol (Fig. 1). It is interesting that, although there is only a minor difference in chemical structure, sitosterol differs strikingly from cholesterol in metabolic behavior, and it possesses cholesterol-lowering activity as has been demonstrated in animal experiments and clinical studies. In contrast to cholesterol, sitosterol has been found to be very poorly absorbed across the wall of the intestine. The amounts absorbed are so small as to be detectable only by the most sensitive radioactive tracer techniques, but not by the standard chemical methods. The absorbed sitosterol has no effect on the biosynthesis of cholesterol. It is not converted to cholesterol, not accumulated in the lymph, blood, or liver, but excreted through the bile and feces. It would thus appear that the site of action of sitosterol must be confined to the intestine.

Hernandez et al. administered orally to rats 4 mg. of radioactive-labeled cholesterol, alone and with sitosterol. It was found that the addition of an equal amount of sitosterol prevented more than 66% of the radioactive cholesterol from being absorbed. When the amount of sitosterol was increased to 25 mg,
the fraction of cholesterol absorbed dropped to one-sixteenth. Similar results in rabbits were obtained by Pollak.\(^{35}\) He further showed that when the proportion of sitosterol to cholesterol was increased up to 7:1, none of the cholesterol appeared to have been absorbed. From these findings, it seems beyond doubt that, as was suggested by Peterson\(^ {81}\) and by Pollak,\(^ {35}\) the cholesterol-lowering effect of sitosterol is due to interference with the absorption of cholesterol from the intestine.

In 1954, Best et al. suggested that sitosterol interferes also with the reabsorption of endogenous cholesterol which is formed in the liver and excreted through the bile to be partly reabsorbed from the intestine (enterohepatic circulation).\(^ {46}\) The suggestion is sound (and may perhaps be extended to apply to cholesterol formed by the intestinal mucosa), since the endogenous cholesterol is in the same form (the free form) and subjected to the same absorptive process in the intestine as is dietary cholesterol. That sitosterol in fact acts upon endogenous cholesterol present in the intestine seems evident from the animal studies by Wells and Alfin-Slater and by Diller et al.\(^ {69}\) A reduction of serum and liver cholesterol was obtained with the addition of sitosterol to diets low in cholesterol. Beveridge et al. showed that in individuals in whom the serum cholesterol was reduced by a diet free of fat and cholesterol the administration of sitosterol caused a further lowering of serum cholesterol.\(^ {19}\) Similar observations were made by Joyner and Kuo,\(^ {49}\) Breslaw,\(^ {60}\) Shipley,\(^ {54}\) and Chiu\(^ {70}\) in patients on diets low in fat and cholesterol.

The following mechanisms for the interference of sitosterol with the absorption of cholesterol from the intestine have been proposed:

1. **Mixed Crystal.**—The formation of a nonabsorbable 1:1 mixed crystal of sitosterol and cholesterol in the intestine was postulated by Pollak\(^ {35}\) in 1953, based on the observation that such a mixed crystal was obtained from a solution of sitosterol and cholesterol in ethanol or in acetone and ethyl alcohol; neither sitosterol nor cholesterol could be recovered from the solution by recrystallization or by partition chromatography. Pollak's observation was confirmed by Davis\(^ {71}\) in 1955, who showed that when equal amounts of sitosterol and cholesterol were mixed in methyl alcohol or were melted together, a mixed crystal was formed, which had an x-ray powder diffraction pattern different from that of either sitosterol or cholesterol. Since these experimental conditions (alcohol and heat for melting) apparently do not exist in the intestine, the formation of this crystal remained a speculative hypothesis until 1959, when Hudson et al. obtained such mixed crystals from (1) aqueous suspensions of sitosterol, cholesterol, and sodium desoxycholate at 38°C, and (2) intestinal contents or villi washings of rabbits fed cholesterol and sitosterol.\(^ {72}\) It was also demonstrated that the mixed crystal was only one-third as dispersible as cholesterol in the aqueous suspension of sodium oleate or sodium desoxycholate. Since colloidal dispersion of cholesterol is regarded as essential for absorption, the conversion of cholesterol in the intestine to a less dispersible form by the formation of a mixed crystal with sitosterol seems to be one of the mechanisms by which cholesterol absorption is reduced.

2. **Esterification.**—The interference with the esterification of cholesterol in its passage from the lumen of the intestine to lymph was proposed by Hernandez et al. in 1953 as the mechanism by which sitosterol inhibits the absorption of cholesterol.\(^ {83}\) The investigators found that when sitosterol was added to a test meal containing \(^{14}\)C-cholesterol fed to rats, the total \(^{14}\)C-cholesterol recovered in the thoracic duct lymph was significantly reduced, and less of the lymph's \(^{14}\)C-cholesterol was esterified as compared with the values obtained from rats receiving the test meal without sitosterol. As an explanation for these findings, Swell et al. suggested in 1954 that soybean sterol (sitosterol) competes with cholesterol for esterification, since both sterols are esterified under the same condition in vitro and presumably in vivo.\(^ {73}\) However, this hypothesis appears hard to
defend, for the finding of a decrease in the percentage of lymph’s C¹⁴-cholesterol in esterified form was not confirmed in a subsequent study by Daskalakis and Chaikoff.⁷⁴ Apparently, the lower value obtained in the previous study was due to incomplete extraction of esterified cholesterol from the lymph. Blomstrand and Ahrens also showed that sitosterol did not alter the degree of cholesterol esterification.⁶⁸ In 1958, Peterson⁷⁵ in his review of the mechanism of the effect of plant sterols pointed out that cholesterol ester, as compared with free cholesterol, is less readily absorbed and less effective in causing elevation of plasma and liver cholesterol in the chicken⁷⁶ and in the rat.⁷⁷,⁷⁸ Since plant sterols are more slowly esterified by pancreatic cholesterol esterase than cholesterol itself,⁷³,⁷⁹ it was suggested that plant sterols may possibly in some manner favor the esterification of cholesterol in the intestinal tract and decrease the absorption of cholesterol. At the present it is a moot question whether and, if so, how plant sterols may “favor” the esterification of cholesterol.

3. Acceptor Sites.—To explain the inhibition of cholesterol absorption by phytosterols (including sitosterol), Glover and co-workers in 1957 advanced the theory that phytosterols compete with cholesterol for the active adsorption centers on the acceptor lipoproteins, either within the lumen of the intestine or on the cell membrane, or in both places.⁸⁰,⁸¹ The theory presupposes that the absorption of cholesterol occurs at the molecular level and that cholesterol must be adsorbed on lipoprotein to be transferred across the mucosal cells. Accordingly, in the presence of phytosterols, the adsorption centers on the acceptor lipoprotein become partially blocked; thus the capacity for the transfer of cholesterol is reduced. As regards the poor absorption of phytosterols, because of slight stereochemical difference the phytosterols do not perfectly fit on to the acceptor lipoprotein; hence they are rejected by the intestinal mucosa. This interesting theory warrants further investigation.

![Nicotinic acid and nicotinamide](image)

**Nicotinic Acid**

Nicotinic acid or niacin (Fig. 2), one of the B vitamins, is essential in human nutrition. The daily requirements range from 12 mg. to 18 mg. per day, and the therapeutic dosage for pellagra, a clinical syndrome chiefly due to deficiency in nicotinic acid, is usually 50 mg. 10 times a day. Nicotinamide is equally effective as nicotinic acid in the prevention and treatment of pellagra. Since Altschul’s report⁸² in 1955, nicotinic acid, when given by mouth in doses of 3 to 6 gm. per day, has been found by several investigators to reduce hypercholesteremia.⁷⁰,⁸³,⁸⁴,⁸⁵ Nicotinamide given at equal dosage levels has no effect.

The mechanism by which nicotinic acid reduces serum cholesterol has not been established. Many hypotheses have been proposed; they are discussed in the following paragraphs.

1. “Oxycholesterols.”—Altschul et al. suggested that nicotinic acid may promote the oxidative processes in the body and thereby the formation of “oxycholesterols” which are presumed to be more readily excreted or less readily reabsorbed from the intestine than cholesterol.⁸² Kritchevsky et al. found that nicotinic acid enhanced the oxidation of cholesterol-26-C¹⁴ by rat liver mitochondrial preparations.⁸²a However, no experimental data are available on the isolation of “oxycholesterols” from feces of individuals receiving large doses of nicotinic acid. On the other hand, there is evidence that “oxycholesterols” are probably artifacts formed by aerial oxydation of cholesterol during the working-up process if air is not completely excluded.⁸⁵

2. “Stress.”—These investigators also conjectured that nicotinic acid may lower serum cholesterol by a “stress mechanism,” for the severe flushing and burning sensa-
tion in the skin caused by nicotinic acid may be considered as a manifestation of "stress." It seems difficult to reconcile this hypothesis with the following facts: (1) the cutaneous reactions occur most often in patients receiving a single dose of 100 mg. of nicotinic acid, which is far below the daily dosage of 3 gm. required for reduction of serum cholesterol; (2) the reactions subside rapidly after the first few days of administration of 3 gm. or more of nicotinic acid per day, whereas the reduction of serum cholesterol is maintained by continued medication; (3) the reactions can be avoided altogether when the dosage of nicotinic acid is small (20 mg. several times a day) at the start and gradually increased to levels effective in lowering serum cholesterol.

3. Homeostatic Effect.—O'Reilly et al. noted in their patients that nicotinic acid depressed elevated serum cholesterol levels but raised normal serum cholesterol concentrations. Therefore, it was suggested that nicotinic acid has a homeostatic effect on serum cholesterol. In supporting this theory, Gaylor et al. showed that nicotinic acid decreased the serum cholesterol level in the chick, which normally has a high serum cholesterol level, and increased the level in the rat, which normally has a low one.

It is true that nicotinic acid is more effective when the initial serum cholesterol concentration is very high. However, an increase in the "normal" level has not been observed in other studies.

4. Heparin.—O'Reilly also postulated that the effect of nicotinic acid may be due to release of heparin or due to its direct action on the lipoprotein lipase. The hypothesis was based on their observation that nicotinic acid exhibited anticoagulant activities. However, anticoagulant effect of nicotinic acid was not reported by other investigators, and hyperlipemia was observed by Chiu to persist in some patients treated with large doses of nicotinic acid. Moreover, heparin was found by Kraupp et al. to increase serum concentration of acetone-ethanol-extractable cholesterol.

5. Anorexia.—Duncan and Best concluded from their extensive studies that in the rat the reduction of serum cholesterol by nicotinic acid was due at least in large part to its anorectic effect. They found that nicotinic acid, when given at the level of 1% of the diet for 42 days, consistently exerted an inhibitory effect on the appetite and weight gain. When the food intake of the rats of the control group was restricted so that the rate of weight gain became the same in both the treated and control groups (pair feeding), there was no significant difference in serum cholesterol levels. Chiu observed, in long-term toxicity studies of nicotinic acid in dogs, that a gradual loss in body weight from 12.57 kg. to 10.1 kg. occurred in one dog receiving nicotinic acid 100 mg. per kg. per day for 32 months. Another dog refused to eat, had loose stools, and lost 6.6 kg. of weight at the end of 8 months of experiment.

Notwithstanding the results obtained in experimental animals, anorexia and weight loss do not seem to account for the cholesterol-lowering effect of nicotinic acid in man. For, as was reported by Achor and Berge and by Parsons and Flinn, anorexia, nausea, and loose stools were experienced by only a small percentage of patients, and no significant reduction in body weight was noted by a great number of patients treated with nicotinic acid for many years.

6. Elevated Metabolism.—Altschul and Hoffer in 1958 reported an increase in the basal metabolic rate in patients treated with nicotinic acid. This suggests the possibility that nicotinic acid may lower serum cholesterol by stimulating thyroid function. However, no symptoms or signs of hypermetabolism were reported by other investigators in their long-term and large-scale clinical studies of nicotinic acid. Furthermore, Duncan and Best found no significant difference in the mean oxygen consumption between the rats fed nicotinic acid and their controls.

7. Depletion of Methyl Groups.—It has long been known that methylation is involved
in the catabolism of nicotinic acid and that toxic doses of the acid deprive the body of the methyl groups and cause fatty infiltration of the liver in experimental animals.\textsuperscript{101,102} Since reduction of serum cholesterol has been observed in hepatocellular damage due to a variety of drugs,\textsuperscript{103} it is possible that the depletion of methyl groups and liver damage may account for the cholesterol-lowering effect of large doses of nicotinic acid in man. This possibility seems very remote, however, since it was demonstrated by me\textsuperscript{70} in 1957 and independently by Miller et al.\textsuperscript{104} in 1960 that the addition of methionine (a "methyl donor") had no influence on the cholesterol-lowering effect of nicotinic acid. Moreover, in my study the abnormalities in liver function observed in some patients during nicotinic acid therapy were not abolished by the addition of methionine.

8. Nicotinuric Acid.—In 1953, Reddi and Kodicek reported that nicotinuric acid (Fig. 3) was not present in the urine of man and the rat on a normal diet.\textsuperscript{105} However, nicotinuric acid became the principal metabolite excreted in the urine following the administration of 100 mg. nicotinic acid, but not nicotinamide. Similar observations were made in patients with hypercholesteremia by Miller et al.\textsuperscript{104} in 1960. These findings have led to further experiments and hypotheses which are discussed in the following paragraph and under Sections 9 and 10.

Miller et al. found that the administration of 3 gm. of nicotinic acid 3 times a day caused a reduction of serum cholesterol comparable to that obtained with nicotinic acid given in doses of 1 gm. 3 times a day.\textsuperscript{104} From comparison of blood concentrations of both the acids, it was concluded that nicotinuric acid was responsible for the cholesterol-lowering effect of nicotinic acid. This interesting hypothesis warrants further study for confirmation, even though the effectiveness of nicotinuric acid was not observed by Berge et al. in their preliminary studies.\textsuperscript{90}

9. Depletion of Glycine.—The conversion of nicotinic acid to nicotinuric acid may be depicted in Equation 1 below.

Since glycine is needed for the conversion, it has been speculated that a depletion of body glycine-pool may result from the administration of large doses of nicotinic acid (vide supra) and may be responsible for the cholesterol-lowering effect. This theory has been tested in an indirect way by reasoning that if the theory is correct, benzoic acid should possess hypcholes teremic activity because glycine also is needed for the biosynthesis of hippuric acid from benzoic acid (see Equation 2 below).

However, Gaylor et al. demonstrated in 1960 that the serum cholesterol of rats and chicks on the basal diet was not changed by benzoic acid but was significantly reduced by nicotinic acid.\textsuperscript{97} Both agents were given at 1\% dietary level.

10. Taurine Conjugation.—Failey et al. studied the effect of nicotinic acid on conjugation pattern of bile acids in 3 patients.\textsuperscript{108} The bile was obtained by duodenal intubation, first while they were taking 2 gm. of nicotinic acid per day and then 1 to 3 days after cessation of medication. It was found that the ratio of the taurine-conjugated bile acids to the glycine-conjugated bile acids was reduced following cessation of nicotinic acid therapy. The explanation may be that during administration of nicotinic acid, which requires glycine for its conversion to

\begin{equation}
\text{nicotinic acid} + \text{glycine} \xrightarrow{ATP, \text{CoA}} \text{nicotinuric acid (nicotinoylglycine)}
\end{equation}

\begin{equation}
\text{benzoic acid} + \text{glycine} \xrightarrow{ATP, \text{CoA}} \text{hippuric acid (benzoylglycine)}
\end{equation}
nicotinuric acid, there is a reduction in the amount of glycine available for other purposes, and consequently there is an increase in taurine conjugation of the bile acids. It is known that animals, in which the bile acids are predominantly conjugated with taurine rather than glycine, are in general relatively resistant to experimentally induced hypercholesteremia and atherosclerosis. This led the investigators to suggest that taurine conjugation induced by nicotinic acid may represent a more efficient means of excretion of cholesterol.

This hypothesis finds no support in the report of Miller et al. who found no increase in fecal excretion of bile acids and sterols in patients treated with large doses of nicotinic acid.¹⁰⁴

11. Decreased Biosynthesis.—Schade and Saltman found a decrease in the rate of cholesterol biosynthesis by liver slices from rabbits fed nicotinic acid.¹⁰⁷ The total liver cholesterol was also reduced. The investigators postulated that nicotinic acid lowers serum cholesterol by inhibiting biosynthesis of the sterol. The mechanism was presumed to be a competition of nicotinic acid for coenzyme A which is required in the conjugation of nicotinic acid as well as in the biosynthesis of cholesterol from acetate. Perry reported in 1960 that incorporation of C¹⁴-acetate into cholesterol by rat liver slices was reduced when the slices were incubated in a medium containing high concentration of nicotinic acid.¹⁰⁸

On the other hand, Merrill observed an increase in the biosynthesis of cholesterol by liver slices from rats treated with nicotinic acid.¹⁰⁹ Contrary to the findings of these two studies, Duncan and Best, who used intact rats for study, demonstrated that nicotinic acid had no significant effect on the rate of incorporation of acetate-¹⁴C into cholesterol of the liver and the serum.¹⁰⁰ Furthermore, in rats fed a regular diet containing 1% nicotinic acid, there was a decrease in serum cholesterol, an increase in liver cholesterol, but no change in total carcass cholesterol. This suggests reduction of serum cholesterol may be merely due to a shift of cholesterol from plasma to liver rather than any net change in cholesterol balance.¹⁰⁰ It would be of interest to determine whether the contradictory results of these studies are due to different experimental designs or due to species difference.

**Thyroid Hormones and Analogues**

As is well known, the administration of the thyroid hormones, thyroxine and triiodothyronine (Fig. 4) leads to an increase in the basal metabolic rate (BMR) and a reduction of serum cholesterol. Despite the consistent association, there is circumstantial yet strong evidence that the cholesterol-lowering effect of the hormones is not the result of an increased BMR. Cutting et al. demonstrated in hypothyroid and euthyroid patients that 2,4-dinitrophenol caused a significant rise in BMR but little or no fall in serum cholesterol.¹¹⁰ Similar observations were made by Stamler et al. in the chick.¹¹¹ On the other hand, reduction of serum cholesterol without increase in BMR was observed with the use of small doses of tetraiodothyroacetic acid or other thyroxin analogues (vide infra).

The effect of the thyroid hormones on the absorption, distribution, biosynthesis, and...
catabolism of cholesterol has been extensively studied by Rosenman et al., Marx et al., and Dayton et al. in experimental animals, and by Gould et al., Kurland et al., and Lipsky et al. in man. It was found that the administration of thyroid preparations to rats did not cause a decreased absorption of cholesterol or a shift of cholesterol from the serum to the fixed tissues. On the other hand, both the biosynthesis and the elimination (degradation and excretion) of cholesterol were decreased in hypothyroid state, but were increased in the hyperthyroid state or following the administration of thyroid preparations.

Therefore, the most plausible explanation for the reduction of serum cholesterol by the use of the thyroid hormones seems to be, as was suggested by Rosenman et al., that the elimination is increased to a greater degree than the biosynthesis of cholesterol.

In recent years, a great number of derivatives and isomers of the thyroid hormones have been compared, using L-thyroxine as standard, in their physiological activities. Some of them (Table) were studied clinically for hypocholesteremic effect and were found to produce, when given at low dosage levels, reduction of serum cholesterol with little or no increase in the BMR. However, anginal attacks occurred in some patients with coronary heart disease, even though the BMR was not elevated. This suggests that these thyroxine analogues may have caused an increase in myocardial oxygen consumption which could not be detected by the gross measurement of the BMR but which was too great to be tolerated by the damaged heart. It was also observed that the BMR became elevated when these thyroxine analogues were given at high dosage levels. It would appear that none of them shows a complete dissociation of cholesterol-lowering activity and general metabolic effect. There is perhaps only a difference in threshold dosage between these analogues and the thyroid hormones. The mode of action of the thyroxine analogues has not been established but is presumably the same as that of the thyroid hormones.

**Triparanol**

Since the reports of Blohm and co-workers in 1959, triparanol, a new compound synthesized by Palopoli et al., has been found to reduce cholesterol concentration in the plasma, liver, and other tissues of experimental animals and to lower serum cholesterol in man.

It seems well established that the hypocholesteremic effect of triparanol is due to inhibition of cholesterol biosynthesis, the important steps of which are depicted in the following simplified scheme:

\[
\text{acacetate} \rightarrow \text{acetyl-CoA} \rightarrow \text{acetoacetyl-CoA} \rightarrow \text{branched chain acids} \rightarrow \text{mevalonic acid} \rightarrow \text{squalene} \rightarrow \text{lansosterol} \rightarrow \text{zymosterol} \rightarrow \text{desmossterol} \rightarrow \text{cholesterol.}
\]

Bloom et al. demonstrated that in rats treated with triparanol the incorporation of

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**Thyroxine Analogues**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Daily Dosage</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>3, 5, 3'-Triiodothyroacetic acid (Trlac)</td>
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<td>121-124, 135, 136</td>
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<td>3, 5, 3', 5'-Tetraiodothyroacetic acid (Tetrac)</td>
<td>8-12 mg.</td>
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</tr>
<tr>
<td>3, 5, 3', 5'-Tetraiodothyroformic acid (TFA)</td>
<td>200-500 mg.</td>
<td>126, 129, 135, 136</td>
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<tr>
<td>3, 5, 3', 5'-Tetralodo-D-Thyronine (DTD)</td>
<td>2-16 mg.</td>
<td>130, 135, 136</td>
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<td>3, 5, 3'-Triodo-D-Thyronine (DT)</td>
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<td>3, 5-Diiodo-D-Thyronine (DT)</td>
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<td>3, 5-Diiodo-L-Thyronine (LT)</td>
<td>10-15 mg.</td>
<td>135, 136</td>
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</tbody>
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Fig. 5.—Triparanol.
labeled acetate into cholesterol in the liver and intestine was greatly reduced.\textsuperscript{137,138} However, there was increased persistence of digitonin-precipitable "higher counting companions" (HCC).\textsuperscript{137} As has been identified by Schwenk and co-workers, HCC are non-cholesterol sterols, which are rapidly converted to cholesterol and, hence, precursors of cholesterol.\textsuperscript{160-163} Blohm et al. concluded that triparanol inhibits cholesterol biosynthesis at a step after the formation of the sterol nucleus.\textsuperscript{137,138} This conclusion finds support in the observations of Gould et al. that the synthesis of cholesterol from labeled mevalonic acid in rat liver homogenates was reduced when the rats were fed triparanol or when the agent was added to the homogenates in vitro.\textsuperscript{140} The exact site of the inhibition was investigated by the following investigators. Frantz et al.\textsuperscript{141} found that in rats triparanol failed to convert zymosterol to cholesterol but caused accumulation in the liver of large amounts of sterol which appears to be desmosterol. Significant concentrations of desmosterol also appeared in the serum of rats and human beings treated with triparanol. Schroer also found no conversion of zymosterol to cholesterol in livers of rats fed triparanol.\textsuperscript{142} Avigan et al. isolated the sterol from the livers of rats treated with triparanol and found it to be identical with an authentic sample of desmosterol in respect to melting point and infrared spectrum.\textsuperscript{142} Desmosterol was present in significant amounts not only in the liver but also in the serum of animals and patients receiving triparanol.\textsuperscript{142} From these studies it seems well established that triparanol blocks the conversion of desmosterol to cholesterol.

The elucidation of the site of triparanol-induced blockage has contributed to our knowledge that desmosterol, which in the past was isolated only from chicken embryos, rat skin,\textsuperscript{164} and sea barnacles,\textsuperscript{165} and was presumed to be a precursor of cholesterol, is in fact the immediate precursor of cholesterol in animals and in man. Now, a new field is open for further investigation to provide answers to the following questions. First, what is the elimination route of desmosterol when its conversion to cholesterol is blocked by triparanol? The report of Blohm et al.\textsuperscript{166} that desmosterol may be more readily converted to bile acids than cholesterol is of interest because this, if confirmed, would indicate that cholesterol may not be the obligatory precursor of bile acids. The second question is whether or not desmosterol, which is closely related to cholesterol in chemical structure, is atherogenic.\textsuperscript{141,148} This question stems from the observation that dihyd rochol esterol, which is also chemically related to cholesterol, is, in contrast to sitosterol, as atherogenic as cholesterol.\textsuperscript{149} At the present, there is no direct evidence obtained from experimental animals to prove or disprove the atherogenicity of desmosterol.

**Summary**

The mode of action of a variety of cholesterol-lowering agents introduced in recent years for clinical use has been discussed. The sites of the metabolic pathways of cholesterol at which these agents act may be summarized as follows:

- **Decreased Supply.**—1. Decreased absorption of dietary cholesterol and reabsorption of endogenous cholesterol from the intestine: (a) sitosterol; (b) replacement of saturated fats with vegetable oils high in sitosterol.

- 2. Decreased dietary intake of both cholesterol and certain triglycerides which enhance cholesterol absorption: (a) substitution of vegetable (unsaturated) for animal (saturated) fats in the diet.

- 3. Decreased biosynthesis: (a) triparanol; (b) unsaturated fat (?); (c) nicotinic acid (?).

- **Shift of Cholesterol from the Plasma to the Liver.**—Nicotinic acid (?).

- **Increased Elimination.**—1. Increased excretion of cholesterol: unsaturated fats (?).

- 2. Increased degradation plus increased excretion of cholesterol (cholesterol biosynthesis also increased but to a lesser degree): (a) thyroid hormones; (b) thyroxine analogues (?).
Apparently, many aspects of the mode of action of these agents, particularly unsaturated fats and nicotinic acid, remain to be elucidated. Investigational pursuit in this field is not merely to satisfy our academic curiosity about the agents themselves but also to gain insight into cholesterol metabolism and, by deduction, into the factors responsible for hypercholesteremia and possibly atherogenesis.


REFERENCES

CHOLESTEROL-LOWERING AGENTS


83. Achor, W., Jr.: Effect of Nicotinic Acid upon Serum Cholesterol and upon Basal Metabolic Rate of Young Normal Adults, Arch. Biochem. 73:420, 1958.


120. Money, W. L.; Kumaoka, S., and Rawson, R. W.: Comparative Effects of Thyroxine Ana...
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134. Forsham, P. H.; Kent, J. R., and Stenberg, E.: Clinical Trials of 3:5-Diodothyroacetic Acid as a Cholesterol Lowering Agent, in Derivatives and Isomers of the Thyroid Hormones: A Survey of Some Basic Concepts and of Current Clinical Investigations, summaries of papers presented at a conference of the School of Medicine, University of Pennsylvania, Feb. 5 and 6, 1960, p. 65.

135. Oliver, M. F., and Boyd, G. S.: Comparative Effects of Thyroxine Analogue in Patients with Coronary Heart Disease, in Derivatives and Isomers of the Thyroid Hormones: A Survey of Some Basic Concepts and of Current Clinical Investigations, summaries of papers presented at a conference of the School of Medicine, University of Pennsylvania, Feb. 5 and 6, 1960, p. 71.


