Drugs Affecting Lipid Synthesis

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Abstract

The apparent direct casual relationship of elevated blood lipids to the pathogenesis of atherosclerosis has resulted in many lines of investigation directed toward the control of lipids, particularly cholesterol, in blood and tissues. Much of this work during the past decade has been concerned with the regulation of endogenous synthesis of cholesterol. No attempt has been made herein to discuss the many cholesterol synthesis inhibitors which have been reported, but rather the salient features of two compounds, triparanol and nicotinic acid, both of which have been extensively used in the clinic, have been reviewed with a major emphasis on their mechanism of action. In addition, preliminary studies with two classes of azasteroids, a group of 3-(β-dialkylaminoethoxy)-substituted steroids, and a substituted alphatic acid, ethyl-α-p-chlorophenoxyisobutyrate, have been discussed. Brief mention is made of the effect of another class of hypocholesterolemic drugs, D- and L-triiodothyronine, on the synthesis of chenodeoxycholate and cholate from cholesterol.

Introduction

MANY LINES of investigation have implicated elevated blood lipids as an important factor in the pathogenesis of one of the major diseases of the modern world, namely, atherosclerosis. As a consequence, a considerable amount of research directed toward the chemotherapy of this disease has centered around attempts to control blood and tissue lipids.

Despite the intense interest in all classes of lipids, by far the greatest attention has been focused on attempts to reduce the concn of cholesterol in blood and tissues. Much of this work, particularly during the past decade, has been concerned with the regulation of endogenous synthesis of this sterol and/or its degradation to bile acids. It is the purpose of this paper to summarize briefly some of the more recent studies in this field. The reader is referred to several excellent reviews (1–5) for a complete discussion of earlier work.

To date, it appears that relatively few attempts have been made to interfere specifically with the endogenous synthesis of either triglycerides or phospholipids. A number of drugs have been observed to affect the synthesis of these lipids *in vitro* or *in vivo;* therefore, when applicable, brief mention will be made of these agents during the ensuing discussion.

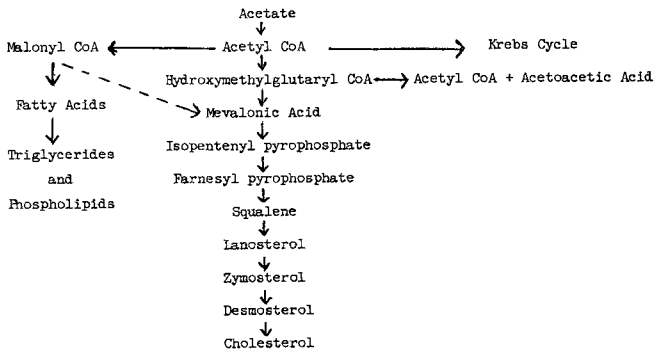


FIG. 1. Scheme showing some of the metabolic functions of acetate and its conversion to cholesterol.

Pathway of Cholesterol Biosynthesis

Since this paper is concerned primarily with inhibitors of cholesterol biosynthesis, it appears desirable to comment briefly on the possible consequences of interfering with the synthesis of this sterol. Some of the metabolic functions of acetate together with the complex pathway involved in its conversion to cholesterol are presented in abbreviated form in Figure 1. Complete details concerning the reactions involved in cholesterol biosynthesis are available in the excellent reviews of Cornforth (6) and Popják and Cornforth (7). More recently Brodie et al., (8) have established a new pathway for the biosynthesis of mevalonic acid from malonyl-CoA. Since the importance of this particular pathway in the synthesis of sterols is still unknown it has been denoted in Figure 1 by means of a broken arrow.

It is evident from the scheme shown in Figure 1 that cholesterol can be synthesized from any compound metabolized to acetyl-CoA. Clearly, any attempt to interfere with the utilization of this key intermediate for sterol synthesis might produce an undesirable effect on other important metabolic reactions. Siperstein and Guest (9) and Bucher (10) have suggested that the site of physiological regulation of cholesterol synthesis involves the reductive transformation of β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) to mevalonic acid. This particular reaction, therefore, would seem to offer an ideal site at which to attempt a specific inhibition of cholesterol synthesis. One possible drawback to a block at this level might be an accumulation of ketone bodies produced by the HMG-CoA cycle described by Lynen et al. (11).

L-Mevalonic acid appears to be converted almost quantitatively to sterols *in vitro* (12) and *in vivo* (13). Further, it is known to be involved in only one other process in animals, i.e., the synthesis of coenzyme Q. Popják and his associates (14) have demonstrated the presence of a catabolic pathway in liver for the disposal of the polyprenol pyrophosphate precursors of squalene. It seems highly unlikely, therefore, that these intermediates would accumulate in the presence of an inhibitor acting at this level. Thus, it would appears that another desirable site to attempt a specific inhibition of cholesterol synthesis is one of the reactions between mevalonate and squalene. At least six intermediate sterols are believed to be involved in the conversion of squalene to cholesterol. Theoretically, therefore, inhibition at any step after the cyclization of squalene would be undesirable because of the possibility of the accumulation of a potentially atherogenic sterol precursor of cholesterol.

Triparanol (MER-29)

Triparanol, 1-[(4-diethylaminoethoxy)phenyl]-1-(p-tolyl)-2-(p-chlorophenyl)ethanol is probably the most extensively studied, at both the basic and clinical level, of all the cholesterol synthesis inhibitors. This agent was shown by Blohm and Mackenzie (15) in 1959 to effectively lower the serum and tissue levels

of cholesterol in rats and monkeys. These workers further showed that triparanol caused an accumulation in rat liver and intestine of a large amount of β-hydroxysterol which did not form an insoluble dibromide, indicating that cholesterol biosynthesis was blocked at some point beyond the formation of lanosterol.

Avigan et al. (16,17) confirmed these findings in rats, and localized the site of inhibition at the very last step on the biosynthetic pathway, i.e., the reduction of 24-dehydrocholesterol (desmosterol) to cholesterol. Subsequent investigations (18–20) revealed a marked accumulation of desmosterol in the serum of triparanol-treated patients, indicating that, as in animals, the main site of action of this drug in man lies at the desmosterol level.

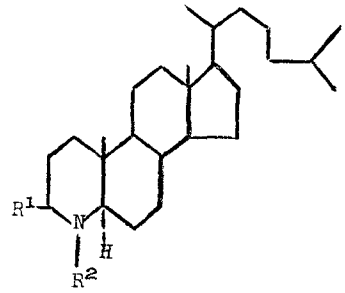
Evidence has been presented which suggests that triparanol also inhibits other stages of cholesterol synthesis. Holmes and DiTullio (21) showed that this compound blocked the synthesis of cholesterol *in vitro* at two stages, one at the alcohol pyrophosphate level and the other at a site between lanosterol and zymosterol. A similar but somewhat lesser degree of inhibition at these sites was observed *in vivo* when these workers studied the synthesis of cholesterol from intraperitoneally administered mevalonate-2-C^{14} in the liver of mice pretreated with triparanol. Hollander et al., (22) and Chobanion and Hollander (23) presented evidence that this drug blocks the conversion of acetate-1-C^{14} to cholesterol in man at or prior to the cyclization of squalene. Frantz and his associate (20) reported that incubation of acetate-1-C^{14} with rat liver slices from triparanol-treated animals resulted in the accumulation of several labeled nonsaponifiable substances which were more polar than cholesterol. During the course of gas chromatographic investigations of the nonsaponifiable fraction of several tissues from triparanol-treated animals Holmes (24) observed the accumulation of small amounts of squalene in some of the tissues. Also, as is seen in Figure 2, appreciable amounts of a third sterol, zymosterol, were occasionally found in some of the tissues. There seems to be little doubt that in addition to its major effect of blocking the conversion of desmosterol to cholesterol, triparanol also interferes, but to a lesser extent, with several other reactions involved in cholesterol synthesis.

Unfortunately, a number of serious adverse effects of this drug in a small percentage of patients have made it necessary to withdraw triparanol from clinical use. Despite this, it should be emphasized that extensive investigations with this agent, at both the basic and clinical level, have provided a wealth of information concerning several aspects of sterol metabolism. In addition, the locus of action of triparanol has provided a partial answer to the question of the atherogenicity of sterol precursors of cholesterol. Avigan and Steinberg (25) and Herndon and Siperstein (26) have found that desmosterol is deposited in experimental atheromatous lesions in rabbits. The latter workers also reported the deposition of desmosterol in atheroma of triparanol-treated patients. Jose and Peak (27) made similar observations in one patient; in a second patient they found large amounts of desmosterol in xanthoma which had developed entirely during the period of triparanol therapy. Thus, it appears that desmosterol behaves no differently than cholesterol in regard to atheroma and xanthoma formation.

Steroids

4-Azasteroids

Three 4-azasteroids represented by the following structures have been tested for their ability to interfere with cholesterol synthesis by rat liver homogenates, and for hypocholesterolemic activity in mice (Table I). All three compounds effectively blocked the conversion of mevalonate-2-C^{14} to digitonide precipitable sterol, but had little effect on the conversion of this substrate to total nonsaponifiable lipid, indicating that these compounds interfere *in vitro* with one of the reactions lying between lanosterol and zymosterol on the biosynthetic pathway. When administered orally to mice, SK&F 11252 had no effect on plasma cholesterol but did cause a reduction in the concn of this sterol in liver. In contrast, the other two compounds effected a marked lowering of cholesterol in both liver and plasma. GLC examination of the total nonsaponifiable fraction of the livers from the treated animals revealed the presence of appreciable amounts of desmosterol. Similarly, these compounds caused a marked accumulation of desmosterol in plasma and liver of rats. A typical example is shown in Figure 3.



SK&F No.	R^1	R^2
11252	C_2H_5	CH_3
12621	CH_3	CH_3
12622	$C_6H_5CH_2$	CH_3

22,25-diazasteroids

Another series of azasterols, the most active of which was 22,25-diazacholestanol, have been reported by Ranney and Counsell (29) to inhibit cholesterol bio-

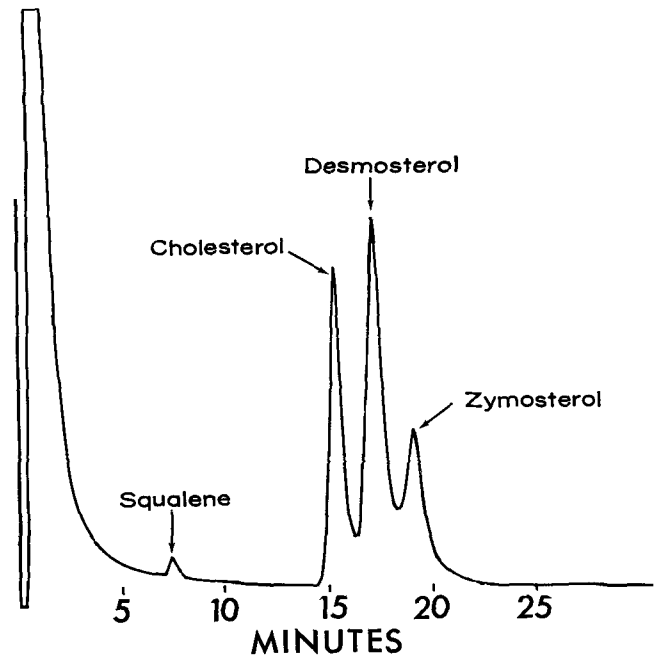
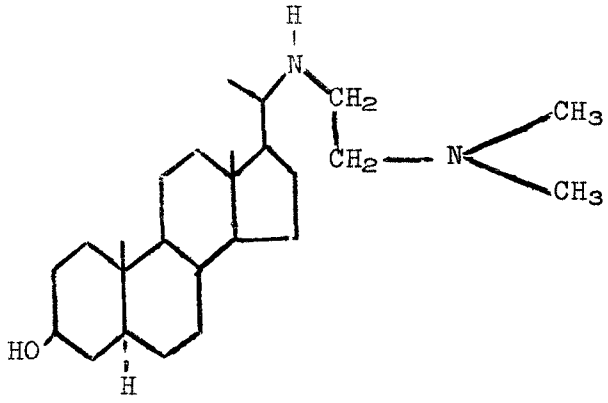


FIG. 2. Gas chromatogram of the nonsaponifiable fraction of liver from a rat treated for 14 days with triparanol. The drug was administered orally, twice daily, at a dose of 25 mg/kg. Methods of tissue work up and gas chromatography previously described (24).

synthesis primarily by inhibiting the activity of β-hydroxy-β-methylglutaryl-CoA reductase. In subse-

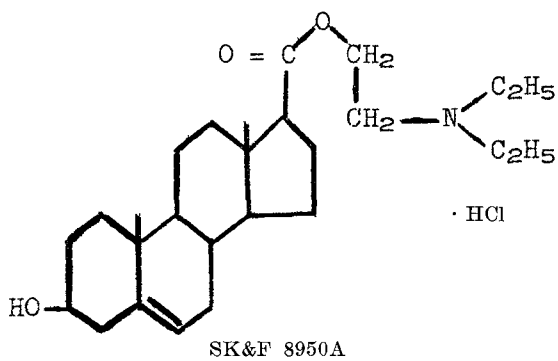


22,25-diazacholestanol

quent investigations these workers (30) showed that this compound effectively lowered the plasma cholesterol of normal and hypercholesterolemic rats. It was presumed that the site of activity *in vivo* was the same as *in vitro* and, therefore, it was suggested that diazacholestanol was exerting a "cholesterolmimetic" effect on cholesterol synthesis, i.e., blocking synthesis by the feedback mechanism in a manner similar to that described for cholesterol by Siperstein and Guest (9).

A hypocholesterolemic response by this mechanism would be expected to affect only the concn of cholesterol in tissues. Studies by Sachs and Wolfman (31) of patients treated with this compound revealed a marked accumulation of desmosterol in their plasma; as much as 28% of the total sterol was desmosterol. This finding was confirmed in another species by Dvornik and Kraml (32) who found that desmosterol comprised more than 70% of the total sterol of plasma, liver and adrenal glands of rats treated with relatively high doses of 22,25-diazacholestanol for seven days. Examination of the data of both groups of workers suggests that 22,25-diazacholestanol had some tendency to lower total sterols. It would appear, therefore, that the major activity of 22,25-diazacholestanol *in vivo* involves the inhibition of the conversion of desmosterol to cholesterol, and that it may also interfere with other reactions involved in cholesterol synthesis but to a much lesser extent.

Another compound, SK&F 8950A (2-diethlamino-ethyl-3β-hydroxyetiochol-5-enate hydrochloride) although not strictly an azasterol will be mentioned



SK&F 8950A

briefly because of some structural similarity to the 22,25-diazacompounds. Holmes and DiTullio (33) found this compound, at a concn of 10^{-4}M, to cause a 50% inhibition of the conversion of mevalonate-2-C^{14} to nonsaponifiable lipid by rat liver homogenates. In this respect, it is similar to some other esters of diethylaminoethanol such as SK&F 525-A which inhibit cholesterol synthesis *in vitro* and *in vivo* at a presqualene stage (28). When administered orally to mice at a daily dose of 50 mg/kg for 11 days, the plasma cholesterol was decreased 26%, but the concn of this sterol in liver was increased some 20%. Examination of the nonsaponifiable fraction of liver showed that SK&F 8950 caused an appreciable accumulation of desmosterol. Its activity *in vivo*, therefore, appears to be more like that of triparanol or 22,25- diazacholestanol.

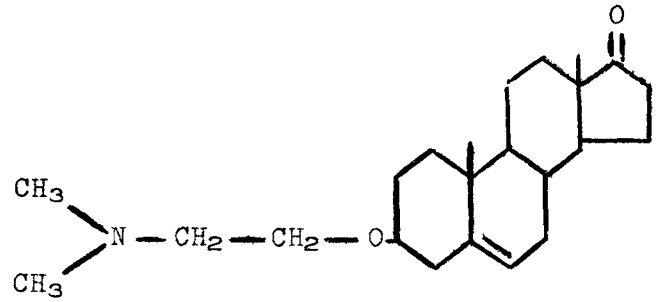
TABLE I
Biological Activity of Some 4-Azasteroids

SK&F No	% Inhibition of mevalonate Incorporation *in vitro* [a]		Cholesterol lowering in mice [b] % Change from control	
	Total NS [c] lipid	Digitonin prec.[d] lipid	Plasma	Liver
11252	6.6	49.6	+14.2	−18.6
12621	4.3	85.3	−59.7	−68.2
12622	1.7	29.4	−38.8	−63 7

[a] All data reported obtained with final inhibitor concn of 10^{-4}M. Details of test method previously described (25).
[b] All compounds administered orally twice daily for 11 days at a dose of 25 mg/kg to eucholesterolemic mice.
[c] NS = Nonsaponifiable.
[d] Digitonide precipitable.

3-(β-Dialkylaminoethoxy)-Substituted Steroids

Gordon and associates (34) reported on the hypocholesterolemic activity in rats of an interesting new steroid 3-β(β-diethylaminoethoxy)-androst-5-en-17-one.



A dose response effect was noted over the range of 0.75–15 mg/kg/day, and some toxicity was suggested by a reduction of food intake and growth at a dose of 45 mg/kg. Essentially similar results were obtained with U-18666A, the diethylamino analog of this compound, by Phillips and Avigan (35).

The mechanism of action of these derivatives of dehydroisoandrosterone, which itself is not hypocholesterolemic, proved to be most interesting. Gas chromatography of the sterols from liver of animals treated with these compounds showed them to be comprised of 40–98% desmosterol depending on dose and length of treatment (34,35). Similar studies carried out in our laboratory on the nonsaponifiable fraction of several tissues (Fig. 4) from animals treated with 3-β(β-dimethylaminoethoxy)-androst-5-en-17-one showed the presence of only two sterols, desmosterol and cholesterol. Thus, the main site of action of these compounds, like that of triparanol and the azasteroids, appears to be at the desmosterol level.

More recently the Lederle group (36) extended their studies to include a series of 14 3-dialkylamino ethoxy-steroids, 11 of which showed hypocholesterolemic activity. It was noted that these compounds not only reduced the level of serum cholesterol, but also decreased the level of total sterol. Phillips and Avigan (35) reported that a single large dose of U-18666A sig-

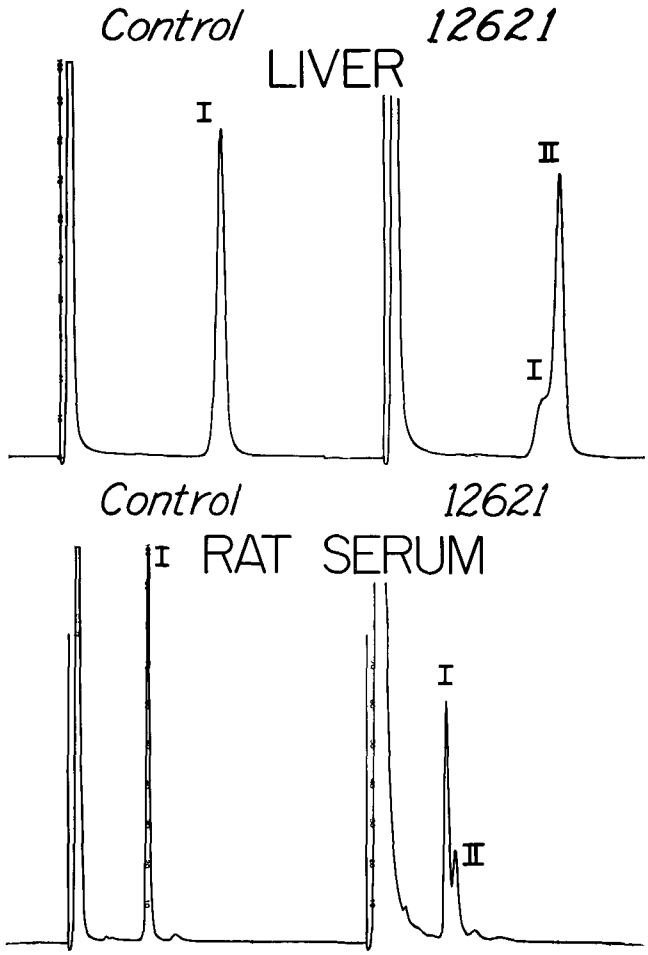


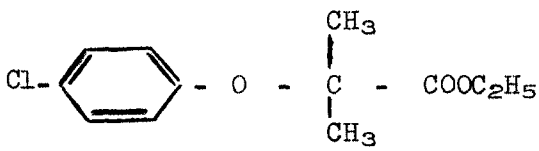
FIG. 3. Gas chromatograms of the nonsaponifiable fraction of serum and liver of a rat treated subcutaneously, once daily, with SK&F 12621 at a dose of 25 mg/kg. The peaks are: I Cholesterol, and II Desmosterol. Different columns were used for the analysis of serum and liver, hence, the difference in retention times.

nificantly reduced a triton-induced hypercholesterolemia in rats. Considartion of these findings suggest that, in addition to blocking the reduction of desmosterol, the 3-(β-dialkylamino ethoxy)-substituted steroids inhibit the endogenous synthesis of sterols.

Substituted Aliphatic Acids

A large number of these compounds have been reported to inhibit cholesterol biosynthesis. Since most of the work in this field prior to 1962 is adequately reviewed elsewhere (1–5) only studies with an interesting new compound will be considered here.

In 1962, Thorp and Waring (37) reported that a series of aryloxyisobutyric acids and related compounds reduce total lipid and cholesterol concentration in the liver and blood of rats. The most effective of these was ethyl-α-p-chlorophenoxyisobutyrate (C.P.I.B.), or its free acid. Subsequent studies suggested that it may enhance the metabolic activity of endogenous adrenal steroids; therefore, it was tested in combination with exogenous steroids. A hypocholesterolemic response was obtained in monkeys



C.P.I.B.

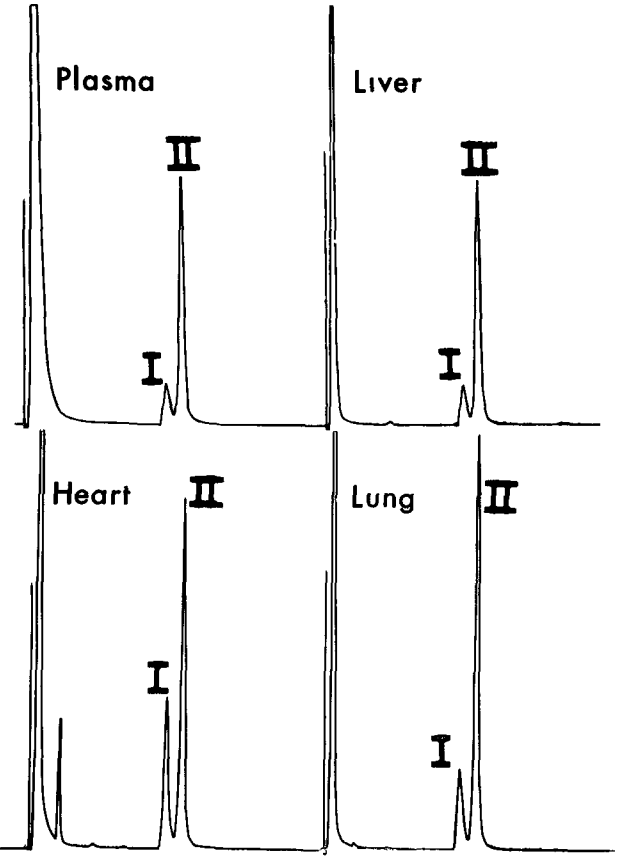


FIG. 4. Gas chromatograms of nonsaponifiable lipid from four tissues of a rat treated subcutaneously, once daily, with 3β(β-dimethylaminoethoxy)-androst-5-en-17-one at a dose of 25 mg/kg. The peaks are: I Cholesterol, and II Desmosterol.

when C.P.I.B. was administered orally in combination with androsterone ('Atromid') while administration of C.P.I.B. or androsterone alone had no effect (38). The mechanism whereby this compound renders androsterone active by mouth was postulated to involve displacement of part of the plasma protein-bound fraction of the steroid, and reduction of its rate of urinary excretion.

Oliver (39) reported that Atromid produced a significant depression of serum cholesterol and triglycerides in patients with ischemic heart disease. Moreover, he suggested from preliminary observations that C.P.I.B. alone had no effect on serum lipids of the human. At a later symposium (40) he presented clear evidence that the C.P.I.B. alone is as effective as Atromid in reducing serum cholesterol and triglycerides in man. More recent confirmation of the hypolipemic effect of C.P.I.B. without androsterone has come from the work of three other groups (41–43). The magnitude of this effect was found (42) to be greater on triglycerides than on cholesterol. Also, a striking sex difference was observed (43), with women showing a much larger hypolipemic response than men. These findings are of great interest since, as previously indicated, C.P.I.B. alone was effective in the rat. In our laboratory C.P.I.B. alone was found to induce a dose-related depression of cholesterol in the plasma and liver of mice.

The *in vitro* conversion of acetate-C^{14} into cholesterol by liver slices from rats treated with C.P.I.B. was reported (37) to be reduced by 30–50% compared with controls. It did not, however, appear to interfere with a triton-induced hypercholesterolemia in rats and presumably, therefore, with cholesterol

synthesis in the intact animal. Studies in our laboratory have shown that C.P.I.B. added to rat liver homogenates blocks the conversion of mevalonate to cholesterol at a presqualene stage. Although much remains to be learned concerning the mechanism of action of C.P.I.B., it is tempting to speculate on the basis of these *in vitro* data that at least part of its hypocholesterolemic activity may result from an interference with cholesterol synthesis. Certainly further studies along these lines are indicated.

Nicotinic Acid

The mechanism of action of nicotinic acid in lowering serum lipids has not been definitely determined, although many speculations have been advanced concerning this matter. Somewhat conflicting data have come from studies in rats, perhaps because nicotinic acid has no significant effect on serum cholesterol in this species. Merrill (44) using both *in vitro* and *in vivo* techniques reported that large doses of nicotinic acid increased hepatic cholesterol synthesis in the rat. Similar results were obtained by Hardy et al., (45) using liver slices from pretreated rats or chicks. Duncan and Best (46) found that administration of nicotinic acid to rats for 8–42 days had no effect on serum, liver or total carcass cholesterol, nor did it effect the incorporation of acetate-1-C^{14} into cholesterol. In contrast, Kritchevsky and Tepper (47) found that cholesterol synthesis from intraperitoneally administered acetate was significantly reduced in the liver of rats which had been fed 1% nicotinic acid for three weeks. Other evidence indicating that nicotinic acid interferes with cholesterol biogenesis comes from the work of Perry (48) and Gamble and Wright (49) who reported that high concn effectively inhibit cholesterol synthesis by rat liver *in vitro*. Also, Schade and Saltman (50) found cholesterol synthesis from acetate to be inhibited in liver slices from rabbits treated for long periods with nicotinic acid.

By far the most convincing evidence that nicotinic acid interferes with cholesterol synthesis comes from studies in humans. Parsons (51) studied the incorporation of acetate-1-C^{14}, administered orally, into serum free and esterified cholesterol and erythrocyte cholesterol in patients while they received nicotinic acid and again after a suitable control period on nicotinamide or a placebo. In seven patients, nicotinic acid caused an average inhibition of 65% in the incorporation of acetate into serum cholesterol when compared to the control periods. In a similar type of study Nunn et al., (52) noted an average reduction in cholesterol synthesis of 50% of pretreatment value. These studies together with others reported by Goldsmith (53) strongly support the concept that cholesterol synthesis is impaired by nicotinic acid.

Thyroid Hormones

The hypocholesterolemic effect of thyroxin and many of its analogs is well documented. The precise mechanism whereby these compounds lower blood cholesterol is by no means well understood. Many aspects of this problem are discussed throughly by Kritchevsky (54) in an excellent review of the influence of thyroid hormones and related compounds on the biosynthesis and degradation of cholesterol. The present discussion will be concerned only with the effect of these agents on the synthesis of bile acids from cholesterol.

Thompson and Vars (55) observed a lowering of biliary excretion of cholate in thyroid-fed, bile-fistula rats. Later, Erikson (56) showed that the thyroid-induced decrease in cholate in rat fistula bile was accompanied by a marked increase in chenodeoxycholate. Strand (57) demonstrated similar changes in bile-fistula rats treated with L- or D-triiodothyronine (L- or D-T_3). Essentially similar results were obtained by Lin et al., (58) when GLC was used to study fistula bile from L- or D-T_3 treated rats. In addition, the latter workers observed the presence of appreciable amounts of an acid more polar than cholic acid (component X) in the bile from treated animals. Although this acid has not been definitely identified some evidence was obtained indicating that it might be α-muricholic acid ($3\alpha,6\beta,7\alpha$-trihydroxycholanic acid). Quantitative studies by the various investigators using fistula rats have shown that total bile acid production is essentially the same in treated and control animals. Since the ratio between cholate and chenodeoxycholate is essentially reversed by L- or D-T_3 this means that the synthesis of cholic acid is markedly reduced. It would appear, therefore, that L- and D-T_3 favor the synthesis of chenodeoxycholate.

In an elegant extension of his investigations Strand (59) observed a cholate/chenodeoxycholate ratio of 3:2 in L- or D-T_3 treated intact rats as compared to 4:1 in untreated animals. In contrast to bile fistula rats the synthesis of cholate in the intact animals was unchanged by these thyroidal agents. The total bile acid pool in the treated animals, however, was approx doubled because of a 2- to 3-fold increase in the daily synthesis of chenodeoxycholate. Thus, as in the fistula animals, L- and D-T_3 have a marked effect on the synthesis of chenodeoxycholate in the intact rat. The relationship, if any, of this effect of L- and D-T_3 on bile acid synthesis to their effect on serum cholesterol remains to be elucidated.

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[Received April 15, 1964—Accepted July 8, 1964]

Gas Chromatography in Lipid Investigations

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Abstract

Biological problems involving long chain compounds and steroids can now be studied more effectively than ever before through use of GLC techniques. When combined with TLC methods for the separation of classes of compounds, these procedures are the most valuable analytical methods now known for lipid investigations. The fact that both qualitative and quantitative data may be obtained at the same time, and that the methods may be used to study complex mixtures at the microgram and sub-microgram level, suggests that many new applications will be found in the fields of chemistry, biology and medicine.

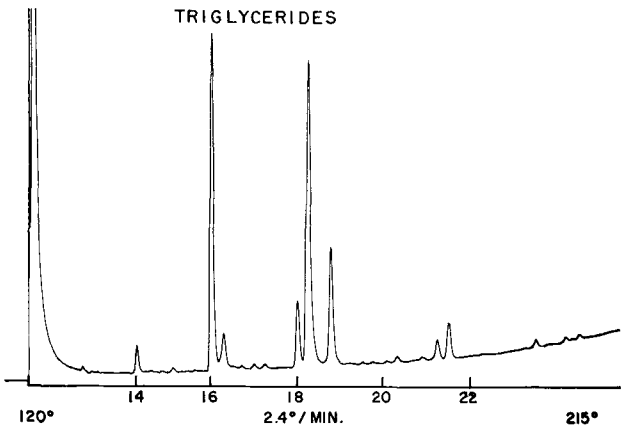

FIG. 1. GLC analysis of fatty acid methyl esters derived from triglycerides present in human arterial lesion tissue. The conditions were as follows: 12 ft x 4 mm glass coil; 1% EGSS-X (Applied Science Laboratories, Inc.) on 100–120 mesh acid-washed and silanized Gas-Chrom P; hydrogen flame ionization detector; temperature programmed separation starting at 120C. The position of saturated unbranched long chain esters is indicated on the chart. The compounds near C_{22} are C:20:3 and C:20:4 methyl esters.

The procedures described in this paper are chiefly those developed over the last few years in the laboratories of the authors. Thin-film columns prepared with diatomaceous earth supports have been used since 1960 in many studies. A new procedure, utilizing a thin-film column, was developed for the separation and estimation of long chain fatty acid methyl esters. The method has been used in conjuction with TLC separations using silica gel and silica gel-silver nitrate plates. The relationship between retention time behavior and the structure of steroids has been studied, and the "steroid number" concept has been used to described GLC properties of steroids. Procedures have been found for the inactivation of supports, for the modification of liquid phase properties, for the preparation of a variety of special derivatives useful in GLC work and for the study of several groups of steroids important in human metabolism. A number of quantitative analytical separations were also developed.

GAS-LIQUID CHROMATOGRAPHY (GLC) is the most valuable analytical technique now known for the study of long chain compounds and of steroids. When combined with TLC methods suitable for the separation of classes of compounds, these techniques provide a highly effective means of studying many biochemical and biological problems involving lipids and steroids in plants, animals and humans. In animals and in humans the effects of drugs, stress, dietary changes and many other experimentally devised circumstances, now can be studied with greater effectiveness than ever before.

At the present time, most laboratories using GLC procedures for the study of long chain compounds employ one or more variations of the polyester column separation methods developed in 1958–1959 (1–4). These methods are suitable for the study of tri-