DRUGS IN LIPID METABOLISM[1,2]

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In recent years, there has been an increased interest in drugs affecting lipid metabolism, which is mainly explained by the two following reasons: the first one is to alter lipid level in blood and tissues in relation to a number of human diseases, particularly atherosclerosis; the second, is to increase knowledge of the single steps concerning the synthesis, metabolism, and transport of the various lipid fractions, thanks to the development of new analytical methods.

In the present review particular emphasis will be given whenever possible, to drugs used at the clinical level. No attempts will be made to cover all the literature related to drugs in lipid metabolism since comprehensive reviews were recently published on the subject [Garattini & Paoletti (1); Steinberg (2)].

A suitable approach for a discussion on drugs affecting lipid metabolism may be made by dividing the subject into three main sections: (*a*) Drugs active on lipid synthesis; (*b*) Drugs interfering with lipid metabolism and excretion; (*c*) Drugs modifying lipid transport.

DRUGS ACTIVE ON LIPID SYNTHESIS

Most efforts have been directed towards interference with the endogenous formation of cholesterol.

A schema of the cholesterol synthesis is reported in Fig. 1. The elucidation of the single steps of this pathway is due to the efforts of several research groups [Bloch (3); Cornforth (4); Gurin & Crandall (5); Popjak (6); Porter (7)]. Cholesterol is the final product of a series of reactions, starting from a simple molecule, acetate. It is possible to distinguish: (*a*) an activation of acetate with coenzyme A, (*b*) the formation of a C-5 branched unit, (*c*) the addition of C-5 units up to the formation of squalene, (*d*) the cyclization of squalene (C_{30}) and the rearrangement of the steroid molecule up to the formation of cholesterol (C_{27}).

(*a*) *Inhibitors of acetate activation.*—Since the activation of acetate is a key reaction in cholesterol metabolism, fatty acids, proteins and carbohydrates, it seems probable that an inhibition of that step will result in a generalized interference with many metabolic routes. However, should an increased activation of acetate cause hypercholesteremia, or should a given

[1] The survey of the literature pertaining to this review was concluded in June 1962.

[2] Abbreviations used in this chapter include: ATP (adenosine triphosphate); CoA (coenzyne A); FFA (free fatty acids); HMG (hydroxymethylglutaric); ICS (inhibitor of cholesterol synthesis); TGL (triglycerides).

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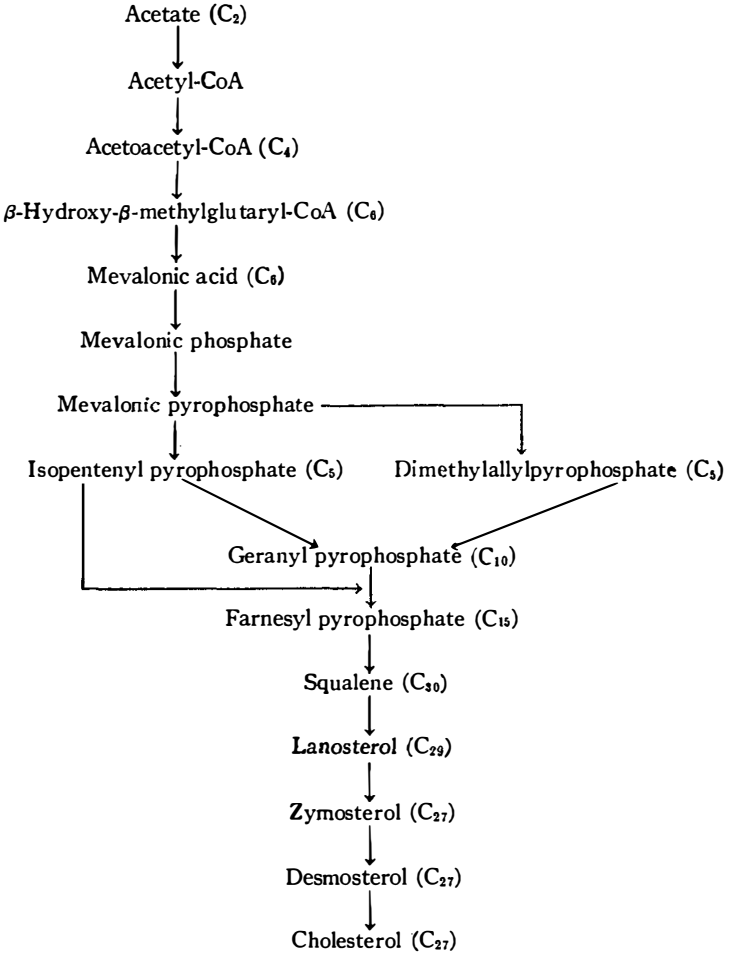

FIG. 1. Simplified schema of cholesterol synthesis.

drug also act at other sites in the sequence of cholesterol synthesis, then an inhibition at the level of acetate activation might contribute to the control of hypercholesteremia. Some aromatically substituted carboxylic acids are examples of this type of inhibitor. The first agent discovered was α-phenylbutyrate, a compound showing hypocholesteremic properties in rats and humans [Cottet *et al.* (8); Bargeton *et al.* (9); Rossi & Rulli (11); Cottet & Gros (10)]. The effect of this agent on humans was not fully confirmed, however, in subsequent clinical trials [Fredrickson & Steinberg (12); Oliver & Boyd (13); Grande *et al.* (14)]. According to a suggestion made in the original work of Cottet, it seems proved that α-phenylbutyrate interferes with the

acetylation processes. Milhaud & Aubert (15), and Garattini *et al.* (16, 17), showed that the acetylation of sulfanilamide *in vitro* (pigeon liver enzymes) or *in vivo* (rat) is inhibited by this agent. The previous data may account for the observed inhibition of labeled acetate into acetoacetate and total fatty acids [Steinberg & Fredrickson (18), 19)]. The inhibited enzyme is the acetokinase [Masters & Steinberg, (20); Rossi *et al.* (21)], and the type of inhibition is probably competitive. The transfer of acetyl-CoA to the substrate being acetylated (choline) is not inhibited by α-phenylbutyrate [Garattini *et al.* (22)].

After Cottet's discovery a large number of derivatives were synthesized and tested for their ability to reduce the acetylation process and serum cholesterol. It is impossible to draw conclusions here on the relationship between chemical structure and biological properties, and for this discussion the reader is referred to the following papers: (16, 17); Garattini *et al.* (23); Wagner-Jauregg (24); Wagner-Jauregg & Saner (25); Palazzo (26); Canonica *et al.* (27).

It is interesting that in the α-phenylbutyric acid molecule, increase of the number of carbon atoms between the phenyl and carboxylic groups or prolongation of the lateral chain in the α-position result in an increased inhibition of the acetylation (16).

Among the compounds having reached the clinical trials, α-biphenylbutyric acid [Garattini *et al.* (28)], difenesenic acid [Cavallini *et al.* (29); Garattini *et al.* (30); and β-benzalbutyrate (21), (27)] are stronger inhibitors of acetate activation than α-phenylbutyrate.

According to the investigations carried out *in vitro* (rat liver homogenates) by Wright *et al.* (31), the hypocholesteremic properties of vanadium salts [Curran (32); Mountain *et al.* (33)] may also be related to an inhibition of CoA for a reduced availability of adenosine triphosphate (ATP). This effect on ATP may result in other inhibitions in the sequence of cholesterol synthesis when ATP is required (i.e. the phosphorylations of mevalonic acid).

(*b*) *Inhibitors of mevalonic acid formation.*—In this series of reactions the various steps, when inhibited, seem to have different importance for the rate of cholesterol synthesis. For example, in the synthesis of acetoacetic acid [Lynen *et al.* (34)] an obligatory intermediate is hydroxymethylglutaric acid (HMG) and, therefore, inhibiting the formation of this metabolite will not lead specifically to the block of cholesterol synthesis. On the other hand, an inhibition of the step transforming HMG into mevalonic acid will probably affect only cholesterol synthesis since it has been shown that mevalonic acid is transformed almost quantitatively into cholesterol [Tavormina *et al.* (35)], the only alternative being the utilization for the synthesis of coenzyme Q [Rudney & Sugimura (36)]. The fact that the HMG→mevalonic acid reaction may be an ideal point for inhibition of cholesterol synthesis is stressed by a series of observations suggesting that the physiological control in liver is exerted at that level [Bucher *et al.* (37); Siperstein & Guest (38); Gould & Popjak (39)].

Δ^4-cholestenone depresses serum cholesterol in various animal species [(19), Tomkins *et al.* (40)] and inhibits the incorporation of labeled acetate into cholesterol [Tomkins *et al.* (41)]. The work of Steinberg & Fredrickson (19) and Bucher *et al.* (37) suggests that Δ^4-cholestenone influences mevalonic acid formation, probably in the conversion of hydroxymethylglutaryl-CoA to mevalonate. This pattern of inhibition is similar to that demonstrated by feeding cholesterol (38).

Another agent effective in lowering serum cholesterol in various experimental conditions is benzmalecene [Huff & Gilfillan (42); Garattini *et al.* (30)]; it is also effective on hyperlipemic or normolipemic patients [Page & Schneckloth (43); Bergen *et al.* (44); Sachs *et al.* (45); Furman & Howard (46); Furman *et al.* (47)]. The precise site of action of this agent is unknown. Besides the inhibition of mevalonic incorporation into cholesterol (42) to be discussed later on, benzmalecene may inhibit cholesterol synthesis, at a pre-mevalonic stage, after chronic treatment in mice [Holmes & Di Tullio (48)].

The investigation of Migicovsky's group, summarized by Migicovsky (49), led to the discovery of an endogenous inhibitor of cholesterol synthesis (ICS) present in mitochondria. This principle is active *in vitro* [Migicovsky & Wood (50); Scaife & Migicovsky (51)], and *in vivo* [Migicovsky (52)]. It does not affect the incorporation of mevalonic acid into cholesterol, or the synthesis of acetoacetate from acetic acid. Therefore, its point of inhibition lies between hydroxymethylglutaric acid and mevalonate.

(*c*) *Inhibition of steps between mevalonic acid and squalene.*—A number of agents reduce cholesterol synthesis (digitonine precipitable material) when 2-^{14}C-mevalonic acid is used as a precursor. α-Phenylbutyrate, and, to a larger extent α-biphenylbutyrate [Tavormina *et al.* (53); Garattini *et al.* (30); Paoletti (54)], and difenesenic acid (30), (54), decrease the incorporation of 2-^{14}C-mevalonic acid in both cholesterol and fatty acids, but the point of inhibition is not yet known. However, since
mevalonic acid are mostly prenoic acids [Christophe & Popjak (55)] it may be assumed that these compounds exert an effect between mevalonic acid and polyprenolpyrophosphates, the precursors of prenoic acids. Similar conclusions may be suggested also for benzmalecene [Holmes & Di Tullio (48)], nicotinic acid at high concentrations (30), and β-benzalbutyric acid (27).

Vanadium salts inhibit incorporation of mevalonic acids but not of squalene into cholesterol [Azarnoff & Curran (56)]. The inhibition probably takes place at the squalene synthetase level [Azarnoff *et al.* (57)].

1-*p*-Chlorophenylpentylsuccinate is a new hypocholesteremic drug [Palazzo *et al.* (58); Bizzi *et al.* (59)] which also prevents incorporation of mevalonic acid into both cholesterol and fatty acids (59).

The elegant investigation of the Holmes' group has elucidated the mechanism of action of two drugs having hypocholesteremic properties [Dick *et al.* (60)]. The two compounds β-diethylaminoethyl diphenylpropylacetate-HCl (SKF-525-A) and 2,2-diphenyl-1-(β-dimethylaminoethoxy)pentane HCl (SKF-3301-A) seem to have a similar mode of action. They inhibit C_5-

alcohol pyrophosphate utilization [Holmes & Bentz (61); Holmes & Di Tullio (48)] and, in addition, two other steps on the pathway from squalene to cholesterol. These compounds, however, do not accumulate anything but cholesterol in the tissues (48).

Recently, a large number of mevalonic acid analogues were synthesized for obtaining effective inhibitors of mevalonic acid utilization. Only partial results are available as yet. Derivatives of deoxymevalonic acid have been studied on microbiological tests [Stewart & Woolley (62)] but were not found active as inhibitors of cholesterol synthesis in mice [Stewart & Woolley (63)]. Weiss *et al.* (64) found that derivatives of 3-methylpentenoic acid reduce the conversion of mevalonic acid into cholesterol. Fluoro derivatives of mevalonic acid proved to be effective in inhibiting mevalonic acid utilization in liver [Tschesche & Machleidt (65); Singer *et al.* (66)], but no attempts have been made so far to elucidate their mode of action. The work of Kirschner *et al.* (67) suggests that 2-fluoromevalonic acid may be transformed into 5-pyrophosphoryl-2-fluoromevalonate. The latter inhibits the decarboxylation of 5-pyrophosphoryl mevalonate to isopentenyl pyrophosphate.

The analogues of farnesoic acid studied by Popjak *et al.* (68), particularly 3,7,11-trimethyldodecanoic acid, are strong inhibitors of mevalonic kinase, but they do not appear to be very effective *in vivo* [Popjak & Fletcher (69)].

Other terpenes interfere with cholesterol synthesis [Isler *et al.* (70); Pletscher *et al.* (71)]. Citronellal and linalol are more effective in inhibiting mevalonic than acetate incorporation into cholesterol [Gey *et al.* (72)].

Androstene derivatives and Δ^1-testololactone [Singer *et al.* (66)] block the incorporation of mevalonic acid into cholesterol, but the exact site of this inhibition is unknown.

Holmes & Di Tullio (48) found that the 4-chloro-17-α-methyl-19-nortestosterone (SKF 6612) inhibits the incorporation of mevalonic acid into polyprenols, and probably two other steps as well.

(*d*) *Inhibition of the steps between squalene and cholesterol.*—The possibility of exerting an inhibition in the last stages of cholesterol synthesis was stressed by the knowledge of the triparanol (MER-29) mechanism of action. Discovered by MacKenzie & Blohm (73) this compound has shown its ability to reduce the level of cholesterol in blood and tissues. Investigations carried out *in vitro* demonstrated that the incorporation of acetate into digitonin precipitable material was unaffected, but that cholesterol dibromide radioactivity was markedly reduced [Blohm *et al.* (74); Blohm *et al.* (75)]. From the start these data suggest that a decrease of cholesterol was correlated with an increase of another steroid, this latter being identified as 24-dehydrocholesterol (desmosterol) [Avigan *et al.* (76 to 78); Steinberg & Avigan (79), (80); Steinberg *et al.* (81, 82)].

Triparanol was shown to inhibit the transformation of desmosterol into cholesterol *in vitro* and *in vivo*, and desmosterol accumulates in blood and tissues during triparanol treatment (79, 80).

The measurement of cholesterol under triparanol administration becomes

faulty owing to the variation in desmosterol sensitivity according to the method used [Avigan *et al.* (78); Frantz *et al.* (83); Hollander *et al.* (84)].

Beside the primary effect on the desmosterol→cholesterol reaction, triparanol may act on other sites. For example, Holmes & Di Tullio (48) found that triparanol also blocks the synthesis before squalene cyclization. These data agree with other observations, i.e. after treatment with triparanol the synthesis of total steroids from acetate is slightly reduced (30).

The clinical meaning, and the therapeutic value of the blood cholesterol reduction by triparanol has been questioned (2). Furthermore, desmosterol can induce in rabbits atherosclerotic lesions as well as cholesterol [Avigan & Steinberg (85)]. Fluorotriparanol probably acts in a manner similar to that of triparanol [Delbarre *et al.* (86)].

It is interesting to note that, 3-β-(β-dimethylaminoethoxy)-androst-5-ene-17-one is also structurally related to triparanol, and inhibits the conversion of desmosterol to cholesterol (Gordon *et al.* (87)].

Other agents such as *tris*-(2-dimethylaminoethyl) phosphate-3HCl (SKF-7732-A_3), and its diethyl analogue (SKF 7997) show hypocholesteremic properties in dogs [Greenberg *et al.* (88)], and inhibit incorporation of mevalonic acid into digitonin precipitable sterols, but not into total non-saponifiable lipids [Holmes & Bentz (89)]. Further studies indicate that the site of inhibition lies somewhat between squalene and zymosterol (48). Therefore, with these agents, there is no accumulation of desmosterol.

Vitamin A deficiency induces a reduction of cholesterol synthesis from 2-^{14}C-mevalonic acid for a block between squalene and cholesterol which results in an enhancement of radioactivity in the squalene fraction [Gloor *et al.* (90)].

(*e*) *Miscellaneous.*—Since heparin and various heparinoids are able to prevent or counteract experimental hypercholesteremica [Bianchini *etal.* (91); Capraro *et al.* (92)] it has been wondered whether these agents, beside their well known effect on clearing reactions, may act also on the cholesterol synthesis. The results *in vitro* are quite difficult to interpret, but the data obtained after administration of a duodenal heparinoid *in vivo* show that a slight inhibition of acetate incorporation into liver cholesterol may occur (30).

Many natural, chemically altered, or synthetic heparinoids are now available. It would be interesting to submit these agents to a comparative analysis of their possible effects on cholesterol synthesis, particularly after prolonged administration *in vivo*.

Thyroxine and its derivatives although showing hypocholesteremic properties are not effective in blocking cholesterol synthesis (30), and even stimulate liver cholesterol synthesis.

3-Pyridine-acetic acid, similarly to nicotinic acid, lowers serum cholesterol [Fumarola *et al.* (93); Ginoulhiac *et al.* (94)], and reduces at high concentrations the rate of cholesterol synthesis *in vitro* either from acetate or mevalonate [Bizzi & Grossi (95)]. In humans, however, the decreased incor-

TABLE 1. Pattern of activity of some hypocholesteremic drugs.

Drug	*Biosynthesis from Acetate After Treatment In Vivo*	*Serum Cholesterol in Rats*	*Hypercholesteremia*		
			By Estrogens in Cockerels	*By Triton in Rats*	*In Suckling Rats*
Thyroxines	—	±	+	±	+
alpha-Biphenylbutyric acid	+	−	−	+	−
Difenesenic acid	+	−	−	+	±
Benzmalecene	+	−	+	±	±
Triparanol	+	+	+	±	−
Nicotinic acid	±	+	−	±	−
Heparinoid	±	−	+	+	−

− No effect, + Inhibition, ± Inhibition at toxic doses

poration of acetate into cholesterol seems to be an important factor in explaining the hypocholesteremic effect of nicotinic acid [Parsons (96)].

Phenylpiperidylate decreases serum cholesterol in mice [Kabara *et al.* (97)]. No attempts have been made to elucidate the site of inhibition.

Salicylates also inhibit cholesterol synthesis and, at the same time, reduce cholesterol levels [Reid (98); Tygstrup *et al.* (99)].

Chlorpromazine at high doses reduces cholesterol synthesis [Yakuboskaya & Kiseleva (100); Yakuboskaya & Rykovskaja (101)] but no definitive effects have been observed in patients [Denber & Teller (102)]. Reserpine and bretylium also reduce the serum cholesterol level [Somoza (103); Chrusciel (104)].

All these observations may represent interesting approaches to further investigations. Table I reports the effect of some hypocholesteremic drugs *in vivo*.

DRUGS INTERFERING WITH LIPID METABOLISM AND EXCRETION

While cholesterol is synthesized in most tissues, it is catabolized only in a few organs, i.e., liver, gonads, intestine. The main routes of cholesterol catabolism are: (*a*) the hepatic oxidation to cholalic acid and excretion as bile salts (taurine and glycine conjugates; (*b*) the direct excretion of cholesterol into the gastro-intestinal tract through the mucosa or with the bile; (*c*) transformation into steroid hormones. In addition, this section will consider the various effects of thyroxine and derivatives on cholesterol metabolism.

(*a*) *Cholesterol oxidation.*—The carbon atoms of the cholesterol side chain

terminal isopropyl group (C-25, C-27) are oxidized into carbon dioxide by rat and mouse liver mitochondria [Whitehouse *et al.* (105)] and $^{14}CO_2$ formation from cholesterol 26 ^{14}C is used in a system containing liver mitochondria as an indicator of cholesterol catabolism. Cholesterol oxidation *in vitro* is specifically depressed by the addition of bile salts. This is regarded as a physiological feedback control regulating the rate of cholesterol oxidation [Whitehouse & Staple (106)].

The elimination of the entero-hepatic circulation by cannulating the bile duct (106), or by feeding basic ion exchange resins antagonizes the intestinal reabsorption of bile salts [Whitehouse (107)], and increases cholesterol oxidation. The contrary may be expected by feeding bile acids: hypercholesteremia has been in fact observed in animals under this treatment [Portman & Stare (108)]. The feeding of bile acid binding resins, such as "cholestyramine" (MK 135), a quaternary ammonium styrene divinyl benzene copolymer, to animals and humans, decreases blood cholesterol, and enhances fecal excretion of both bile acids and neutral sterols [Bergen *et al.* (109); Tennent *et al.* (110)]. The blocked reabsorption of bile salts in the intestine with MK 135, and the subsequent displacement of the natural feedback control of cholesterol catabolism, represents an important approach in the pharmacological control of hypercholesteremia.

Other factors modify cholesterol oxidation; in particular a diet rich in unsaturated fats [Kritchevsky (111)] enhances cholesterol oxidation and reduces cholesteremia. However, there is seldom evidence of a correlation between factors modifying the oxidation of cholesterol and the actual levels of cholesteremia. A much greater cholesterol oxidation takes place in the liver of female rats than in that of the males [Kritchevsky *et al.* (112)], and this suggests that the mitochondrial enzyme systems active on cholesterol metabolism are indirectly controlled by the sex hormones.

The inhibition of steroid hydroxylation in the adrenal cortex, and of cholesterol catabolism in the liver are common to a number of drugs (metapirone, amphenone, SU-9055) [Whitehouse (107)]. Other drugs, like triparanol and SKF 525, inhibit both cholesterol biosynthesis and oxidation in liver. It is interesting that the same drugs are inhibitors of both mitochondrial sterol oxidation and microsomal steroid hydroxylation [Whitehouse (107)].

(*b*) *Biliary salt formation.*—Nearly 90 per cent of cholesterol is metabolized into bile salts [Bergström *et al.* (113)]. The degradation of cholesterol into bile salts is an oxidation process including inversion of the 3-beta-hydroxy-group, hydroxylation of the steroid nucleus at C-7, C-12 oxygenation, and C-24 reduction of the double bound, Δ^{5-6} activation and cleavage of the terminal isopropyl group of the side chain, and, finally, conjugation of the hydroxycholalic acids with glycine and taurine (a simplified schema of cholesterol metabolism is shown in Fig. 2). No drugs are known to be active on specific points of this series of reactions, except the thyroid hormones which control the rate of activity of the C12-hydroxylase.

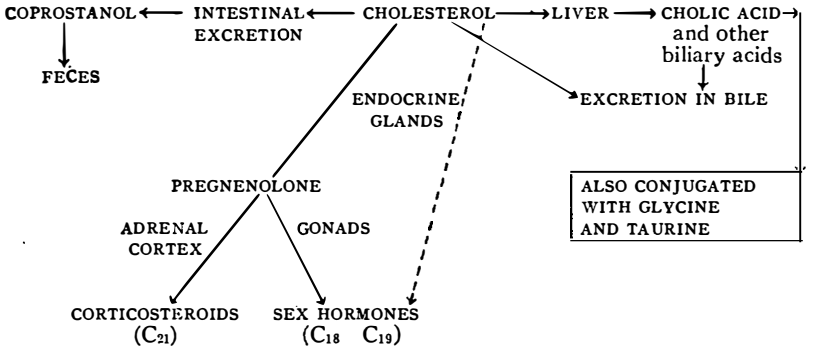

FIG. 2. Metabolic routes of cholesterol.

Dietary factors increase fecal excretion of bile salts [Bergström *et al.* (113)], but the technical conditions for experimenting upon bile excretion in animals fed on a restricted diet, make a detailed study difficult. Regulation of bile salts formation in the liver by enterohepatic circulation is shown by making a bile fistulas: under these conditions the quantity of bile salts excreted is increased up to 20 times [Thompson & Vars (114)]. A further demonstration of this homeostatic control is the finding that infusion of taurochenodeoxycholate in rats with a bile fistula eliminated entirely the increased bile salt formation [Bergström & Danielsson (115)]. Such an increase has no effect on cholesterol levels, because the increased bile salt excretion stimulates liver cholesterol biosynthesis.

(*c*) *Cholesterol excretion.*—An important route of excretion of cholesterol is through feces. Cholesterol is mainly transformed into coprostanol in the lower intestinal tract by microbiological reduction. This process is abolished by bacteriostatic agents given by oral route, and is absent in germ-free rats [Danielsson & Gustafsson (116)]. Bile salts and surface active agents reduce coprostanol formation in the rat [Wilson (117)], while its excretion is increased by feeding linoleic acid (117) or "phrenosine" a cephalin fraction of brain [Rosenheim & Webster (118)]. Many of the coprostanol precursors are formed in the intestinal mucosa and are not in equilibrium with serum cholesterol [Danielsson (119)]. Cholesterol is also excreted as such with the bile. Thyroxine increases this way of excretion in rats [Thompson & Vars (114)].

(*d*) *Cholesterol catabolism in other tissues than liver.*—The adrenal cortex, gonads, and placenta oxidize cholesterol to pregnenolone and isocaproil aldehyde. The main pathway of cholesterol catabolism is hydroxylation at C-20 and C-22, and cleavage of the side chain [Chaudhuri *et al.* (122)]. The formed pregnenolone is further metabolized into the C-19 steroid hormones. According to Dorfman (123) an alternative way is the direct synthesis of these hormones from cholesterol without an intermediate of 21 carbon atoms

such as pregnenolone. Heparin induces an increase of cholesterol and cholesterol products excretion [Engelberg & Lau (120)], and the same effect is shown by 1,4-dicaffeylquinic acid (Cynarine) [Preziosi *et al.* (121)]. Very little is known of factors, or drugs acting on this oxidative reaction other than hormonal treatments such as thyroxine and sex hormones [Berliner & Dougherty (124); Boyd (125)]. Vitamin A was also found to be directly active on cholesterol metabolism in the adrenals [Johnson & Wolf (126)].

Triparanol inhibits steroid genesis *in vivo*, and causes adrenal hypertrophy in rat after chronic treatment [Melby *et al.* (127)]. Δ^4 cholestenone feeding reduces adrenal secretion, induces accumulation of dihydrocholesterol in the adrenal cortex and may, therefore, inhibit cholesterol catabolism [Fredrickson *et al.* (128)].

(*e*) *Thyroid hormones and cholesterol metabolism.*—Thyroid deficiency induces an increase in blood cholesterol. Cholesterol plasma levels in humans and many mammalian species are constant from day to day, when the thyroid function is normal, but become very sensitive to dietary cholesterol in thyroid deficient humans [Hurthal (129)], and dogs [Entenman *et al.* (130)]. The increased plasma cholesterol levels in thyroid deficient cholesterol-fed dogs results in a deposition of cholesterol in the arterial walls [Steiner & Kendall (131)]. These observations led to the introduction of thyroid hormones as agents able to reduce increased cholesterol levels. However, L-thyroxine (L-T_4) and L-triiodothyronine (L-T_3), the physiological thyroid hormones, are not of practical use in this respect because the doses reducing blood cholesterol are too close to the doses raising basal metabolic rate (B.M.R.).

Lerman & Pitt-Rivers (132), utilized in a patient an analogue of thyroxine in which the alanine side chain was substituted with acetic acid, triiodothyroacetic acid (TRIAC). This compound was able to lower serum cholesterol levels without effect on the B.M.R. Various analogues have been tried both in pharmacological and clinical trials, and the results agree that the D-isomers of T_4 and T_3 [Boyd & Oliver (133); Greenberg *et al.* (134)], the formic analogues of T_4 and T_3, and the propionic acid analogue of T_3 are the most active in lowering plasma and liver cholesterol concentration without great effect on general metabolism [Best & Duncan (135); Cuthbertson *et al.* (136)].

Carefully selected doses of the thyroid analogues reduce human plasma (and not B.M.R.) cholesterol without raising the B.M.R. A large number of clinical investigations underline the selective effects on plasma cholesterol by TRIAC [Trotter (137); Hill *et al.* (138)], D-T_4 [Boyd & Oliver (139)]. It is important to know that there are two limitations to the clinical use of the thyroid analogues: an increase of B.M.R. occurs in some of the patients treated, and even when an increase of B.M.R. is not detectable, the oxygen consumption of the heart may be increased. This last point may explain the clinical observation of anginal pain in patients treated with doses of TRIAC insufficient to raise the B.M.R. [Ibbertson *et al.* (140)].

Thyroxine analogues able to influence cholesterol metabolism selectively are significant for a better understanding of thyroid regulation of lipid metabolism. An early hypothesis that some of these analogues are concentrated by the liver, and therefore more likely to act on cholesterol metabolism has been shown to be inexact after experimental testing [Duncan & Best (141)].

It is possible that the two effects of thyroxine on cholesterol and B.M.R. are completely independent and related to different parts of the molecule.

(*f*) *Thyroid hormone effect on cholesterol catabolism.*—It is well known that thyroid hormones may stimulate cholesterol synthesis [Byers *et al.* (142); Gould *et al.* (143)]. This stimulatory effect makes it difficult to explain the fall of plasma cholesterol in thyrotoxicosis. Thyroid hormones must, therefore, greatly increase excretion or conversion of cholesterol to other substances. This is demonstrated by the fact that plasma cholesterol disappears more quickly in thyrotoxic than in normal rats [Rosenman *et al.* (144)], and the same results have been obtained in humans injected with ^{14}C-labeled cholesterol and thyroid hormones [Duncan & Best (145)].

The increase in liver cholesterol synthesis could be related to a decrease of total or free cholesterol in liver [Gould *et al.* (143)], but total hepatic cholesterol is not changed or even slightly increased [Fletcher & Myant (146); Dayton *et al.* (147)]. Comparatively little information is available on the effect of thyroid hormones on removal of plasma cholesterol. The biliary excretion of cholesterol increases [Friedman *et al.* (148)], and the same is true for the excretion of total bile acids [Eriksson (149)]. This increase is due to a larger output of chenodeoxycholic acid which more than compensates for a fall of cholic acid excretion (149).

DRUGS MODIFYING LIPID TRANSPORT

Catecholamines, thyroxine and adrenocortical steroids regulate lipid mobilization from the depot-organs, lipid transport in blood, and peripheral utilization. Rapid progress has been achieved in this field after the discovery that albumin-bound free fatty acids (FFA) in blood plasma are a major factor in fat transport from adipose tissue [Dole (150)]. FFA in blood have a rapid turnover rate [Havel & Fredrickson (151)]; adipose tissue triglycerides (TGL) are the principal source of circulating FFA and these constitute the major lipid fraction transported in blood for utilization by tissues [Fredrickson & Gordon (152)]. There will be no discussion here of the effect of heparin on the clearing factor lipase [see the recent review by Robinson & French (153)].

Catecholamines.—These increase the circulating FFA. Intravenous doses of epinephrine as little as 0.05 μg/kg show an increase of FFA in man. This increase is evident as early as two minutes after the injection and persists for 10 to 15 min [Havel & Goldfien (154)]. The rise in the concentration of FFA produced by a single injection of epinephrine is also evident in dogs [Shafrir *et al.* (155)], monkeys [Goodman & Knobil (156)], and rats [Goodman &

Knobil (157)]. When epinephrine is infused intravenously, the increase of FFA is only temporary, because of the hyperglycemia also produced by this hormone [Shafrir & Steinberg (158)]. Hyperglycemia induces insulin hypersecretion and insulin is active in lowering FFA concentration.

Norepinephrine.—The hormone naturally present at the sympathetic nerve endings is norepinephrine. Single injections of norepinephrine produce rapid increase of plasma FFA concentration in the dog [Havel & Goldfien (154)] and man [Klein *et al.* (159)] similar to that of epinephrine. However, when given in continuous intravenous infusion, norepinephrine has a more lasting effect than epinephrine: the slight effect of norepinephrine on hepatic glycogenolysis may account for this difference.

Many other catecholamines have been tested in animal and man. Isoproterenol [Bruce *et al.* (160)] and alpha-methyl norepinephrine [Mueller & Horwitz (161)] have also been found very active in man. Bogdonoff *et al.* (162) have shown that many sympathomimetic compounds are ineffective, and that the most active compounds have an alpha-carbon amino and hydroxyl group in the positions 3rd and 4th of the benzene ring.

Both epinephrine [MacKay (163)], and norepinephrine [Aujard (164)] produce a fatty liver in rats. In the case of norepinephrine in dogs, a continuous intravenous infusion induces an increase of liver TGL without modification of cholesterol and phospholipid levels [Feigelson *et al.* (165)]. Several hours after injecting catecholamines an increase of all plasma lipids is observed, probably as a secondary effect of an alteration in hepatic lipid metabolism. The major increase is in plasma TGL concentration [Dury (166)]. In fed dogs, epinephrine shows a more rapid effect on plasma TGL concentration which is probably related to an interference with fat absorption [Shafrir *et al.* (155)].

Mechanism of action.—The mechanism by which catecholamines increase plasma FFA has been partially elucidated. Epinephrine [Gordon & Cherkes (167)], and norepinephrine [White & Engel (168)] increase both the rate of FFA release from adipose tissue when added *in vitro* and the concentration of FFA inside the tissue (168).

Both *in vitro* and *in vivo* experiments suggest that the increased release of FFA from adipose tissue is related to the increased FFA pool within the tissue; and therefore, the primary effect is on the triglyceride hydrolysis. The direct effect of norepinephrine on FFA release is also confirmed in the dog by the observation that the regional perfusion of omental fat with norepinephrine is able to increase the FFA concentration in the effluent blood [Paoletti *et al.* (169)].

The fat mobilizing action of epinephrine in adipose tissue requires neither glucose nor oxygen [Hagen & Ball (170)], while an addition of FFA increases the oxidation of C^{14} labeled glucose, and its conversion to glyceride glycerol [Cahill *et al.* (171)]. Not all of the FFA obtained by hydrolysis of depot TGL are released by the adipose tissue, because one part is re-esterified to TGL with alpha-glycerophosphate derived from glucose metabolism [Vaughan

(172)]. This explains the increase of glucose phosphorylation and, eventually, glucose uptake induced by catecholamines in the adipose tissue. The so-called calorigenic effect of epinephrine and norepinephrine is partially related to the increased oxygen consumption and oxidation of glucose required for esterifying fatty acids. Conditions and drugs affecting the action of catecholamines in tissues and the sympathetic nervous system may have a great influence on the pharmacological and physiological regulation of fat metabolism. Sympathetic denervation of fat tissue decreases fat utilization during starvation [Hausberger (173); and Sidman & Fawcett (174)]. Long lasting ganglionic blockade with hexamethonium [Havel & Goldfien (154)], or chlorisondamine [Paoletti *et al.* (169)] decreases the rate of mobilization of free fatty acids induced by anesthesia or cold exposure. The peripheral sympathetic nerve endings in white adipose tissue [Paoletti *et al.* (175)], and especially in the brown adipose tissue of rodents [Sidman *et al.* (176)], contain large amounts of norepinephrine. Surgical denervation [Weiner *et al.* (177)], or reserpine treatment [Paoletti *et al.* (175); Weiner *et al.* (177)], induces an almost complete disappearance of catecholamines in the tissue. The catecholamine depleted animals show great resistance to factors affecting lipid transport such as ACTH, morphine, cold exposure and chlorpromazine, and this is in agreement with the observation that the addition *in vitro* of an antiadrenergic agent like phenoxybenzamine reduces the release of free fatty acids by ACTH [Schotz & Page (178); Hamosh & Wertheimer (179)].

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* English translation will be announced in *Technical Translations*, issued by the Office of Technical Services, U. S. Department of Commerce, and will be made available by the Photoduplication Service, Library of Congress, and by the SLA Translation Center at the John Crerar Library, Chicago, Illinois.

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