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DRUGS AND LIPID METABOLISM[1]

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Reduction of the concentration of serum lipids as a means of preventing or arresting the progress of atherosclerotic disease is the underlying stimulus for much of the current research effort on mammalian lipid transport and on the structure and function of serum lipoproteins. In this country, the importance attached to this effort is underscored by the recent establishment of Lipid Research Clinics and by the programs of many of the Specialized Centers of Research in Arteriosclerosis supported by the National Heart and Lung Institute. Emphasis in the past has attached both to dietary programs [i.e. the Diet-Heart Study (1) and several "secondary prevention" studies (2–4)] and to drug trials [i.e. the U.S. Veterans Administration Drug-Lipid Study (5), the Cooperative Coronary Heart Disease Drug Project, and controlled trials of clofibrate (6–8)]. The former have confirmed that serum cholesterol levels can be reduced on a sustained basis by controlling the intake of dietary cholesterol and saturated fats, while the latter have shown that reduction of the levels of cholesterol, triglycerides, or both can be achieved by oral agents that are tolerated for years. The effort involved in these studies implies provisional acceptance by many investigators of a causal relationship between the level of certain serum lipids and the development of the atherosclerotic lesion and its ischemic complications. Although such a relationship is supported by studies of the pathogenesis of the atherosclerotic plaque in experimental animals, by epidemiological studies among and within population groups, and by the association of ischemic arterial disease with hereditary hyperlipidemic disorders, proof that either prevention or amelioration of coronary heart or peripheral atherosclerotic vascular disease follows successful lipid-lowering remains to be obtained.

Since the subject of drugs and plasma lipids was treated in this series in 1966 (9), it has been the main topic of two international meetings (10, 11) and a general text has appeared (12). In addition, review articles have dealt briefly with the many agents that have received clinical trial (13, 14). This review will summarize our current understanding of the function of plasma lipoproteins in lipid transport and related aspects of lipid metabolism as a basis for understanding the mechanism of action and clinical use of hypolipidemic drugs. We will deal

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specifically with only two: clofibrate, because of widespread current interest, and cholestyramine, because of its promise in the treatment of the most refractory of the primary hyperlipidemic syndromes, familial hyperbetalipoproteinemia. A detailed discussion of the actions and use of a third agent, nicotinic acid (15), and a general survey of hypolipidemic drugs, including several α-aryloxyisobutyric acid derivatives and other compounds resembling clofibrate (16), have recently appeared.

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LIPID METABOLISM

Lipid Transport in Lipoproteins

Lipids largely exist in blood plasma in combination with specific proteins to form large molecular complexes. These lipoproteins, of which several distinct classes can be defined, serve to transport fat (mainly triglycerides) and cholesterol (mainly as esters of long-chain fatty acids) in the blood. Fat is also transported in the form of "free" (unesterified) fatty acids (FFA) bound tightly to albumin. In man and other mammals, concentrations of two of these classes, the triglyceride-rich very low density (pre-beta) lipoproteins (VLDL) and the cholesterol-rich low density (beta) lipoproteins (LDL) correlate positively with the incidence of atherosclerotic disease.

The function of plasma lipoproteins in triglyceride transport is well documented and its qualitative and quantitative features are partially understood (17). Dietary fat is absorbed after partial hydrolysis in the small intestine. After reesterification to form triglycerides in the endoplasmic reticulum of the mucosal cells, it is packaged into spherical droplets (chylomicrons) in which the fat is coated with a monolayer of phospholipids, specific proteins, and cholesterol. These droplets are concentrated in the Golgi apparatus of the cell and secreted into interstitial fluid. They then enter the lacteals of the intestinal villus and are transported through the lymphatic system to the blood. Upon reaching the capillary bed of several extrahepatic tissues, the chylomicrons are adsorbed to the endothelial surface, evidently forming an enzyme-substrate complex with the enzyme, lipoprotein lipase. As a result of the action of this enzyme, the triglycerides are hydrolyzed and the liberated fatty acids are taken up locally into the tissues to be esterified and thus stored or to be oxidized for energy needs. Alternatively, these fatty acids can recycle as FFA in blood and enter tissues elsewhere. Smaller triglyceride-rich lipoproteins (VLDL) are secreted from the liver and transported to extrahepatic tissues in an analogous manner. The fatty acids of these triglycerides are derived from three sources: (*a*) from fatty acids transported to the liver as FFA from extrahepatic tissues—these fatty acids may be derived from chylomicron triglycerides as above or from the hydrolysis of triglyceride stores in adipose tissue; (*b*) to a limited extent from the direct uptake of chylomicron triglycerides (see below); (*c*) from de novo synthesis in the liver from carbohydrate (in man this process is limited except during the sustained intake of extremely carbohydrate-rich diets).

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Transport of exogenous triglycerides in chylomicrons is highly efficient so that chylomicronemia persists only for a few hours after each meal containing fat and this process can account for transport of as much as several hundred grams of triglyceride daily. In contrast, transport of triglycerides in hepatogenous VLDL, though continuous, is more limited, amounting to about 20 grams daily in normal individuals. In states of genetically determined or acquired deficiency of lipoprotein lipase, the capacity to transport triglycerides is quite limited and chylomicronemia may persist from meal to meal (exogenous hyperlipemia). In other more common forms of hyperlipemia, not accompanied by overt deficiency of lipoprotein lipase, the capacity to transport triglycerides in VLDL appears to be limited specifically (endogenous hyperlipemia) so that concentrations may be substantially increased when rates of production are in the normal range. In these states relatively small increases in production, such as accompany increased caloric intake, may lead to inordinately high triglyceride levels.

The level of VLDL and its constituent triglycerides can be affected by hormones that increase (norepinephrine, growth hormone) or decrease (insulin) the supply of fatty acids that are used for hepatic triglyceride synthesis (18). Several chemicals such as carbon tetrachloride and ethionine impair the hepatic synthesis or secretion of VLDL and lead to fatty infiltration of the liver and hypolipemia (19), while hormones such as insulin affect the level of triglyceride-rich lipoproteins by influencing the activity of lipoprotein lipase in extrahepatic tissues (20). Thus, our knowledge of the pathways of transport of triglycerides in blood plasma and its regulation provides a reasonbly firm basis for evaluating the action of drugs that reduce triglyceride levels.

The function of plasma lipoproteins in cholesterol transport is much less well understood. Consequently the precise mechanisms by which cholesterol levels in blood are regulated are poorly defined. For example, although a positive cholesterol balance produced by feeding cholesterol is accompanied by increased cholesterol levels in blood plasma and a negative balance by reduced levels (21), it is not clear how these changes in cholesterol concentration are brought about. The major cholesterol-bearing lipoproteins are the low density lipoproteins (LDL). The cholesterol-rich LDL normally carry about two-thirds of the cholesterol in human blood plasma. Present evidence suggests that these macromolecules are not secreted into the blood plasma as such, but rather that they are produced during the process of removal of triglycerides from VLDL. After triglycerides have been removed from VLDL in extrahepatic tissues, certain protein constituents ("C" apoproteins) are transferred to high density lipoproteins (HDL). After further processing, probably in the liver, the remaining protein ("B" apoprotein), appears in LDL. Triglycerides in LDL also appear to be derived from VLDL and some cholesteryl esters may be as well. However, the remainder is probably transferred to LDL from HDL. The HDL appear to constitute a separate lipid transport system for cholesterol. Recent studies indicate that HDL are secreted from the liver and, presumably, from the small intestine in the form of micellar aggregates containing "free" (unesterified)

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cholesterol, phospholipids and specific "A" apoproteins.[2] The liver also secretes an enzyme, lecithin-cholesterol acyltransferase, that acts upon this complex to esterify the cholesterol at the expense of the fatty acyl moiety of the beta-carbon of the phospholipid, lecithin. The highly apolar cholesteryl esters formed move into the interior of the HDL particle or are transferred to lipoproteins of lower density, mainly LDL, while the more highly polar lysolecithin produced is transferred to albumin and thus removed from the complex. It has been postulated that this process results in net transport of cholesterol from various tissues to the liver in the following manner (22). As cholesterol is removed from the surface of the HDL particle, it is replaced by cholesterol derived from various surfaces with which the lipoprotein comes in contact in the blood or interstitial fluid (other lipoproteins and the membranes of red blood cells and various other cells). The cholesteryl esters formed are presumably removed from the blood when HDL and LDL are catabolized in the liver. The rate at which lecithin-cholesterol acyltransferase esterifies cholesterol in vitro suggests that this transport could amount to about 2.5 g daily (22). Isotopic studies of the rate of synthesis of cholesteryl esters suggest that such a process could account for most of the cholesteryl esters transported in blood. On ordinary diets, transport of intestinal cholesterol in chylomicrons probably accounts for less than half this amount. Studies in experimental animals indicate that chylomicron cholesteryl esters are transported by quite a different pathway (23). After removal of triglycerides in extrahepatic tissues through the action of lipoprotein lipase, a smaller particle is formed that contains no more than 10–20% of the original complement of triglyceride but essentially all of the cholesteryl esters in its smaller core. These constituents are rapidly removed by the liver (as in the case of VLDL, the formation of partially degraded chylomicrons is accompanied by transfer of "C" apoproteins to HDL but it has not been established that the small amount of "B" apoprotein in chylomicrons contributes to the formation of LDL). Cholesterol is also secreted from the liver in VLDL. The amount entering the blood by this route is uncertain, because the content of cholesterol in newly secreted human VLDL is not known, and at least some of that present in plasma VLDL may be transferred from HDL as described above, but it could be appreciable. Whether cholesteryl esters of VLDL are removed by the liver in a manner analogous to that for chylomicrons is also uncertain. Furthermore, we remain largely ignorant of the sites from which cholesterol used in the lecithin-cholesterol acyltransferase reaction is derived. Thus, the extent to which cholesterol transported in the blood is derived from its presumed site of removal, the liver, as opposed to various extrahepatic sites, is uncertain.

Cholesterol Metabolism

Although our understanding of cholesterol transport in the blood and its regulation is limited, certain aspects of the regulation of body cholesterol stores have been uncovered by studies of cholesterol turnover and balance (21). Cho-

[2] Hamilton, R. L., Williams, M. C., Havel, R. J. Unpublished observations.

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lesterol derived from the diet, the bile, and desquamated epithelial cells is absorbed in the small intestine. It leaves the body in the bile as such and after conversion in the liver to the "primary" bile acids (mainly cholic and chenodeoxycholic acids in man) and in desquamated skin. About half of the cholesterol in the intestinal lumen is ordinarily absorbed, mainly in the jejunum, with the fraction descreasing as the amount of dietary cholesterol increases. Approximately 98% of the bile acid is absorbed, mainly in the terminal ileum, to take part in an enterohepatic circulation. In humans ingesting 1 g of cholesterol daily, of which 0.5 g may be absorbed, cholesterol balance is maintained by excretion of about 0.5 g cholesterol and 0.5 g bile acids in the stool and 0.1 g in desquamated skin, suggesting that about 0.6 g has been synthesized in the body. Most of this synthesis is thought to occur in liver, intestinal mucosa, and skin but the amount occurring in other tissues has not been determined. The rate-limiting step of cholesterol synthesis is the reduction of β-hydroxy-β-methyl glutaryl CoA to mevalonic acid. In liver, this step is inhibited by dietary cholesterol. In the terminal ileum, it is inhibited by bile acids. Bile acids also inhibit the rate-limiting step (7-α-hydroxylation) in the conversion of cholesterol to bile acids in liver (24).

CLOFIBRATE

Background

Clofibrate (ethyl-α-p-chlorophenoxyisobutyrate) is probably the most widely used hypolipidemic drug, both in this country and in Europe. Its acceptance is based upon its ability to reduce levels of both cholesterol and triglycerides (reflecting effects upon LDL and VLDL, respectively) and upon its low order of toxicity. It was originally developed in England and was first considered to act by displacing androsterone, a steroid hormone with hypolipidemic effects, from albumin so as to increase its biological activity (25). Preliminary studies in man suggested that a combination of clofibrate with androsterone was more effective than clofibrate alone (26) and the combination (atromid) was originally marketed. Subsequent studies demonstrated that clofibrate alone was equally effective (27, 28).

Pharmacodynamics and Toxicity

Clofibrate is rapidly and completely absorbed (25). It is hydrolyzed, apparently by plasma and tissue esterases, and appears in the blood plasma as the anion, which is bound to proteins, mainly albumin. Limited amounts are found in tissues such as the liver and it is largely confined to the extracellular spaces. Most of the drug is excreted in urine, largely as the glucuronide. Its half-life in man is about 12 hours. In rodents, it uniformly produces enlargement of the liver associated with increased size and number of mitochondria and smooth endoplasmic reticulum, and especially a proliferation of microbodies (peroxisomes) (29–31). The latter are altered in that content of catalase is normal but that of uric

acid oxidase is reduced (29). These changes are reversible. In dogs and monkeys hepatomegaly produced by the drug is relatively slight (32). In man, some increase in transaminase activity and decrease in alkaline phosphatase activity of plasma may occur (27, 33) and, rarely, increases in serum creatine phosphokinase have been associated with a reversible muscular syndrome (34).

Mechanism of Action: Rats

Clofibrate uniformly reduces the concentration of plasma cholesterol and triglycerides in the rat, and most studies of its actions have been performed in this species. Thorp, who first demonstrated the hypolipidemic action of clofibrate, has attempted to relate it to the association of the drug with plasma proteins (35). When fed at a level of 0.25% in the diet, the plasma concentration of clofibrate is about 1 mM (36). In fed rats, this is accompanied by reduction in the concentration of FFA (36, 37). Since clofibrate can reduce the release of FFA from adipose tissue in vitro (38), it is presumed to impede the movement of FFA from adipose tissue to the blood. Such an action would be expected to inhibit hepatic triglyceride synthesis by decreasing hepatic uptake of FFA. In turn, this should decrease synthesis and secretion of VLDL; LDL levels would fall subsequently. However, the rate of transport of FFA has not been measured in such rats and it is possible that the reduced levels reflect an increase in turnover rate rather than reduced fat mobilization. Concentration of glycerol in plasma is moderately reduced in rats given clofibrate for 2 weeks (39), consistent with decreased fat mobilization, but it is not affected when plasma triglyceride level falls during the first day of treatment. In fasted rats treated with clofibrate and given a large dose 1 hour before sampling, plasma FFA level is moderately reduced and the increase in FFA concentration produced by adrenaline is inhibited (36).

Binding of clofibrate to plasma proteins also increases the proportion of unbound thyroxine (35, 40). This is accompanied by increased uptake of thyroxine in the liver. Some metabolites of thyroxine may be affected similarly (35). The hypolipidemic action of clofibrate and its effects on hepatic size and architecture could be related to the induction of localized "hepatic hyperthyroidism." In support of this hypothesis, the hypolipidemic effects of clofibrate are absent in thiouracil-fed rats and usually in thyroidectomized animals (41). Furthermore, the activity of mitochondrial α-glycerophosphate dehydrogenase increases several-fold in treated rats (29, 42), an effect resembling that of hyperthyroidism, and oxygen consumption of liver slices is increased (42). The effect on α-glycerophosphate dehydrogenase is abolished by thyroidectomy and restored by small doses of thyroid hormone (42). However, several drugs that also displace thyroxine from plasma proteins have no hypocholesterolemic effect in the rat (43), and certain effects of clofibrate, such as proliferation of microbodies (43) and prevention of fatty liver produced by feeding orotic acid (44), persist in thyroidectomized animals. Neither thyroid hormone nor clofibrate increase hepatic α-glycerophosphate dehydrogenase in guinea pigs, chickens, or pigeons (42); effects of clofibrate on plasma lipids in these species have not been reported.

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The effects of administering various amounts of thyroxine with clofibrate suggest that it intensifies certain stimuli provided by a given amount of thyroid hormone (42). Intraperitoneal injection of 250 mg/kg of clofibrate doubles hepatic mitochondrial α-glycerophosphate dehydrogenase in 4–6 hours, together with stimulation of mitochondrial protein synthesis; this is associated with reduction by half of hepatic concentration of α-glycerophosphate (45). When livers of animals given such doses of clofibrate are perfused with either labeled palmitate or glucose, the secretion of labeled triglycerides is also reduced by about one-half (45). These studies suggest that the effect of clofibrate on mitochondrial α-glycerophosphate dehydrogenase is causally related to its hypotriglyceridemic action through inhibition of hepatic triglyceride synthesis. Other studies confirm the decrease in α-glycerophosphate level (42) and hepatic triglyceride synthesis is reduced in treated rats (39). However, feeding 0.25% clofibrate in the diet inhibits the incorporation of labeled glycerol into hepatic triglycerides in as little as 6 hours with no change in the level of hepatic α-glycerophosphate and addition of 5 mM clofibrate to liver homogenates inhibits the incorporation of α-glycerophosphate into triglycerides (39). Additional studies have shown that this is associated with substantial inhibition of acyl-CoA-α-glycerophosphate acyltransferase with no effect on fatty acyl CoA synthetase, phosphatidic acid phosphatase or diglyceride acyltransferase (46). A minor part of this effect could be attributed to displacement of palmityl CoA from albumin present in the incubation mixture with increased binding to microsomes, but the remainder appeared to reflect direct inhibition of the initial reaction of the synthesis of glycerolipids. These studies suggest that the hypotriglyceridemic effect of clofibrate in rats could be the result of decreased synthesis and secretion of VLDL. Although measurements of accumulation of triglycerides secreted from perfused livers support this interpretation (47, 48), the results are difficult to interpret because heparin, which can release lipolytic activity from rat liver, was present in the perfusion fluid. Evidence for decreased synthesis of protein of VLDL plus LDL and of HDL has been obtained in one study of clofibrate-treated rats (49). However, in another study in which clofibrate was fed to rats made hyperlipemic by feeding diets high in sucrose, the synthesis of VLDL-protein appeared to increase during the first two days. Thereafter, as the concentration of triglycerides fell, the synthetic rate returned to control levels (50).

Other enzymatic changes observed in livers of rats treated with clofibrate suggest that the capacity for fatty acid synthesis may be decreased. These include reduced activity of acetyl CoA carboxylase, fructose-1,6-diphosphate aldolase, and glucose-6-phosphate dehydrogenase (51, 52). Purified acetyl CoA carboxylase is inhibited by one-half by addition of 1 mM clofibrate (53). The inhibition of the enzyme is competitive for acetyl CoA and isocitrate. In apparent conflict with these observations, conversion of acetate and tritiated water to hepatic triglycerides is increased in treated rats (54). Content of glycogen in liver has generally been found to be decreased (32, 51). Glycogen content of liver is unchanged in pair-fed animals but conversion of glucose to glycogen is reduced (51). Changes in enzymes that could influence ketogenesis have also been described. The

activity of β-hydroxy-β-methyl glutaryl CoA reductase is reduced (55) while that of mitochondrial acetoacetyl CoA deacylase is increased (56). These changes could be related to the observed increase in production of ketone bodies from acetate (55, 57) and pyruvate (55) in liver slices. However, the importance of enhanced activity of acetoacetyl CoA deacylase has been questioned (55) and no change has been observed in the concentration of acetoacetate in the blood of treated rats (56).

Some of the changes in mitochondrial activity in livers of treated rats could be related to increased content of mitochondria (58). These mitochondria have normal oxidative activity and respiratory control. The greater content of mitochondria could also account for the reported increase in hepatic content of NAD and of NAD:NADH (59). Mitochondria from rats fed clofibrate also have increased capacity to degrade the side chain of cholesterol under some, but not all, conditions of incubation (60), but its effect on the microsomal 7-α-hydroxylation of cholesterol, generally considered to be the rate-limiting step in the conversion of cholesterol to bile acids, has not been reported. An increased oxidation of cholesterol could also reflect a "hyperthyroid" state of the liver. However, in striking contrast to the situation in hyperthyroidism, hepatic cholesterol synthesis is substantially decreased, mainly by inhibition at the rate-limiting step of reduction of β-hydroxy-β-methyl glutaryl CoA to mevalonic acid (55, 57). The relationship between the decrease in cholesterol synthesis and increase in ketogenesis is not clear since the precursor pools for these two processes may be distinct (61). Surprisingly, the concentration of ubiquinone, the isoprenoid side chain of which is derived from mevalonate, progressively increases in the liver of rats fed clofibrate as the concentration of cholesterol in plasma falls (62, 63). This may be attributed in part to an increase in synthesis from mevalonate and in part to decreased catabolism (63, 64). Since feeding ubiquinone to rats reduces the concentration of cholesterol in plasma and inhibits hepatic cholesterol synthesis in a manner similar to clofibrate, it has been suggested that clofibrate's hypocholesterolemic action is the result of the accumulation of ubiquinone in the liver (62, 63).

Although numerous studies show decreased conversion of precursors to cholesterol in rats fed clofibrate in their diet for several days to weeks, studies have not been reported during the first day of feeding when the concentration of cholesterol falls rapidly. In animals fed a diet high in sucrose, this fall is attributable to a reduction in concentration of HDL. Reduced synthesis of HDL protein accompanies this change, which occurs as early as 12 hours after clofibrate is fed, but accelerated removal of HDL may also occur (50).

These studies in the rat suggest that both the hypocholesterolemic and hypotriglyceridemic effects of clofibrate may result largely from direct actions on the liver. However, effects of clofibrate acid in vitro could result from a detergent effect unrelated to its actions in the intact animal. Furthermore, the general applicability of many of the studies of hepatic metabolism may be questioned because of the profound effects of sustained ingestion of the drug on the size and architecture of the rat liver. These changes show substantial species differences.

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For example, proliferation of microbodies occurs in male rats, mice, hamsters, dogs, and, possibly, in man, but not in female rats, guinea pigs, rabbits, squirrel monkeys, and chickens (30). Since the hypolipidemic effect is present in both female and male rats, comparative studies of hepatic enzymic activities and metabolism could be of considerable value, particularly in species such as primates in whom structural effects are slight. Further clarification would probably be achieved by systematic evaluation of the dose and time dependence of these effects in relation to reduction of plasma cholesterol and triglyceride levels.

Treatment of rats with clofibrate also affects lipid metabolism in adipose tissue. It substantially increases the activity of lipoprotein lipase in homogenates of epididymal fat pads (65) [although no increase was observed when the enzyme was assayed in extracts of acetone powders of the tissue (66)]. In addition, it promotes the uptake of labeled triglyceride fatty acids by the tissue in vitro (66) and increases the activity of fatty acid esterifying enzymes (67). The increased oral fat tolerance observed in rats fed clofibrate (44) may reflect these changes. Evidence for greater capacity for lipogenesis in adipose tissue of treated rats includes increased activity of hexose monophosphate shunt dehydrogenases, NADP-malic enzyme, and increased conversion of glucose and tritiated water to fatty acids in vitro (67). A possible explanation for these changes is a reduction in adenyl cyclase activity of the fat pad (68). An action on this system could also explain the reduced rate of fat mobilization in treated rat discussed earlier. Activity of lipoprotein lipase in homogenates of heart and aorta is reduced (69). Such reciprocal effects on lipoprotein lipase in rat adipose tissue and heart and on lipoprotein lipase and "hormone-sensitive" lipase in adipose tissue have also been observed in relation to nutritional state and the availability of insulin (70). The described effects of clofibrate in extrahepatic tissues are similar to those observed in the fed state or after administration of insulin, and it is reasonable to postulate that they contribute to the hypotriglyceridemic (but not the hypocholesterolemic) action of clofibrate in the rat. Recently evidence for an increased rate of removal of VLDL-protein from the plasma has been obtained in clofibrate-treated rats made hyperlipemic by feeding a diet high in sucrose (50).

Mechanism of Action: Man

Explanation of the effect of clofibrate on lipid metabolism in man must take into account a number of characteristic features. It uniformly reduces the concentration of VLDL in 2–5 days, which accounts for its hypotriglyceridemic effect (71). Content of cholesterol in VLDL is also reduced but in most individuals its hypocholesterolemic effect is related to a reduction of LDL (6, 72). When the fall in concentration of VLDL is large, the absolute level of LDL may rise so that reduction of plasma cholesterol may be slight or absent (72, 73). The effects of clofibrate on HDL (72, 74) (increased concentration with reduced content of triglycerides) differ from those observed in the rat and appear to be related to, and are probably dependent upon, the fall in concentration of VLDL. Clofibrate also increases the percentage of oleate and decreases the percentage of linoleate in plasma lipids (75); this change is most prominent in cholesteryl esters, but it also

occurs in phospholipids and triglycerides. Finally, clofibrate evidently mobilizes cholesterol from tissue stores; this has been shown best for xanthomatous deposits but there is also evidence that cholesterol content of other tissues decreases (76).

It has been proposed that, as in the rat, clofibrate displaces several substances from plasma proteins. At concentrations several-fold in excess of those existing ($\sim$0.5 mM) in serum of treated subjects, it decreases binding of thyroxine to prealbumin, but it alters binding to albumin only at very high concentrations, and has no effect on binding to thyroxine-binding globulin (77). No change in free thyroxine has been found in plasma of treated subjects (78) and the hepatic clearance of thyroxine and the flux of labeled thyroxine from plasma to liver are unchanged (79). In some studies, binding capacity of thyroxine-binding globulin has been found to increase (78, 80). Several studies suggest that the concentration of FFA in plasma is decreased in subjects treated with clofibrate (81–83). In these studies, the estimated contribution of clofibrate to the titratable acidity of an extract of plasma was usually subtracted without actual determination of its concentration and the fraction of clofibrate contained in the extract was assumed to be constant. Not all such studies have shown this effect (84), and in one study in which clofibrate was removed from the extract before titration, concentration of FFA in arterial plasma, the turnover rate of FFA estimated by isotope dilution, and the concentration of glycerol were unaltered (85). The fat-mobilizing effect of epinephrine (but not that of norepinephrine or isoproterenol) is reduced in treated subjects (83). Thus, there is presently no evidence that binding of clofibrate to plasma proteins alters the metabolism of thyroxine in man, but the rate of transport of FFA may be reduced under some conditions.

Hepatic metabolism in the postabsorptive state is systematically altered in both normal (85) and hypertriglyceridemic (86) individuals given 0.5 g clofibrate four times daily for 3 weeks. Oxidation of FFA taken up in the liver is increased, the fraction oxidized to ketone bodies rising by about 50%. No change, however, is observed in the fraction (about 18%) that is secreted as VLDL-triglycerides. Net production of triglycerides from the splanchnic region is not altered in treated hypertriglyceridemic individuals, indicating that the hypotriglyceridemic effect of the drug in man cannot be ascribed to inhibition of hepatic secretion of VLDL. Secretion of VLDL-triglycerides appears to be somewhat increased in treated normotriglyceridemic individuals in whom only part of these triglycerides are synthesized from FFA (85, 87). This may explain the observation (88) that clofibrate does not usually affect normal levels of plasma triglycerides. Net splanchnic glucose production is not affected by the drug, but the efficiency of uptake of the major glucogenic amino acids (alanine, glycine, and serine) is increased and their concentration in plasma is reduced.[3] These features of the utilization of the major substrates of splanchnic metabolism resemble those of untreated subjects fasted for 2–3 days.

Direct studies of the effects of clofibrate on hepatic enzymes in man have not

[3] Wolfe, B. M., Kane, J. P., Havel, R. J., Brewster, H. Unpublished observations.

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been reported. Changes in glycolytic enzymes of jejunal mucosa from subjects receiving the drug (decrease in glucokinase, glucose-6-phosphate dehydrogenase, fructokinase, and fructose phosphate aldolases) (89) resemble those observed in rat liver. However, incorporation of labeled acetate into plasma triglycerides and phospholipids is approximately doubled in treated individuals in the postabsorptive state (71), suggesting that hepatic lipogenesis is increased. In contrast, incorporation of labeled acetate into plasma cholesterol is decreased about 30% while incorporation of mevalonate is unchanged (90). These observations are consistent with reduced synthesis of cholesterol in the liver at the hydroxymethylglutaryl CoA reductase step or below. Changes in the slope of decay curves for plasma cholesterol after intravenous injection of labeled cholesterol (76, 91, 92) are also consistent with decreased synthesis. Evidence for decreased synthesis of cholesterol in jejunal mucosa has been obtained from examination of specific activities of plasma and tissue cholesterol after injection of labeled cholesterol (76). Synthesis of plasma cholesteryl esters is also reduced in proportion to their concentration, but the relative turnover rates of the cholesteryl esters of VLDL, LDL, and HDL and the fraction of esterified cholesterol in these lipoproteins are the same as those of untreated individuals (93).

Absorption of dietary cholesterol is not affected by clofibrate (76), but both biliary and fecal excretion of cholesterol are increased in most hypercholesterolemic subjects (76, 92). The increase in fecal excretion persists for many weeks and could account for removal of substantial quantities of cholesterol from the body. Compartmental analysis suggests that the content of cholesterol in pools that have a slow turnover rate is reduced when such subjects are treated for prolonged periods of time (76). The negative sterol balance produced by clofibrate may occur with little fall in concentration of plasma cholesterol (76). In contrast, cholesterol levels may be reduced in subjects with normal or slightly increased concentrations with no change in fecal excretion of sterols or bile acids (92, 94).

Rates of transport of triglycerides in blood plasma based upon measured net splanchnic production (85, 86) and estimates based upon isotope dilution (71) suggest that clofibrate improves the efficiency of removal mechanisms with little effect on influx into the blood. A nonisotopic method also indicated improved efficiency of removal in some hyperlipemic individuals maintained on a fat-free diet, but decreased influx was suggested to be the principal alteration accounting for the hypotriglyceridemic effect of the drug (84). However, post-heparin lipolytic activity of plasma (presumably reflecting tissue stores of lipoprotein lipase) was usually increased, consistent with greater efficiency of removal. This interpretation is supported further by the lack of effect of clofibrate on plasma triglycerides of individuals with genetically determined deficiency of lipoprotein lipase when they are maintained on either fat-containing or fat-free diets (76, 95, 96). Estimates based upon clearance of radioiodine-labeled LDL in four subjects with combined hypercholesterolemia and hypertriglyceridemia suggest that synthesis of LDL is reduced during treatment with clofibrate (97). These results are difficult to reconcile with the apparent lack of change in rate of transport of triglycerides.

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In summary, available information in man indicates substantial effects of clofibrate on hepatic intermediary metabolism, on excretion of cholesterol and bile acids, and upon removal of triglycerides in extrahepatic tissues. The effects upon the liver are not confined to lipid metabolism but rather suggest that the drug produces a different "set" of hepatic intermediary metabolism. Possibly, many of the enzymatic and metabolic changes observed in the rat liver and those suggested or observed in man are the result of such an alteration. The changes in fatty acid oxidation and uptake of amino acids are consistent with a more "fasted" state of the liver, as are the changes in glycolytic enzymes and in cholesterol synthesis. However, both the increased incorporation of labeled acetate into plasma triglycerides and phospholipids and the greater saturation of the fatty acids of plasma lipids suggest that lipogenesis is increased. Unlike the situation in rats, thyroid hormone does not appear to be diverted to the livers of treated subjects, but the possibility remains that the response of liver to thyroid hormones or to other substances is altered. In this connection, individuals with severe hypothyroidism, like hypothyroid rats, are reported to respond poorly to clofibrate (80).

The hypotriglyceridemic effect of clofibrate appears to result mainly from an extrahepatic action that is unrelated to its hypocholesterolemic effect. In subjects with genetically determined deficiency of lipoprotein lipase maintained on a fat-free diet, clofibrate has only a hypocholesterolemic effect (76) and in some subjects with endogenous hyperlipemia, substantial reduction of triglyceride level may occur with no change in cholesterol as levels of LDL rise while those of VLDL fall (72, 73). Our current knowledge of metabolic interrelationships of lipoproteins provides no clear mechanism by which a drug acting to increase the rate of conversion of VLDL to LDL could produce a hypocholesterolemic effect in normotriglyceridemic subjects. Thus, it appears that the hypocholesterolemic effect of clofibrate (reduction of LDL) is related to increased biliary excretion of cholesterol, decreased cholesterol synthesis, or both, but the mechanism is obscure. As noted in the following, increased excretion of bile acids, coupled with increased cholesterol synthesis, may also lead to reduced levels of LDL.

THERAPY

Although clofibrate reduces the level of VLDL more effectively than LDL, its principal use may be in individuals with mild to moderate hypercholesterolemia in whom the level of triglycerides is normal or only slightly elevated. There are several reasons for this. In a great majority of individuals, the expression of a genetic predisposition to hypertriglyceridemia appears to depend upon the presence of obesity or the ingestion of substantial amounts of sugars and ethanol, so that dietary therapy is both rational and effective (98, 99). In individuals with clearly defined familial elevation of VLDL (endogenous hyperlipemia), reduction of the level of VLDL by clofibrate is frequently accompanied by an increase in LDL such that there is little or no fall in the concentration of cholesterol (72, 73). In mixed hyperlipemia (combined elevation of VLDL and chylomicrons), clofibrate is generally less effective than diet alone or diet together with another

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drug, such as nicotinic acid (99). Clofibrate is particularly effective in primary dysbetalipoproteinemia where VLDL unusually rich in cholesterol accumulate (73, 99). In this rare disorder, reduction of VLDL is not accompanied by an increase in LDL and the response to the drug equals that of a low cholesterol reducing diet, so that clofibrate is quite useful in those individuals who fail to adhere to a dietary regimen. In clearly defined familial hyperbetalipoproteinemia where the level of plasma cholesterol in adults usually exceeds 350 mg/dl, clofibrate is of limited effectiveness, causing a fall of about 10% (72, 100, 101), considerably less than that obtained with an anion-binding resin as described later.

In individuals with mild to moderate primary hypercholesterolemia (250–350 mg/dl), which reflects mainly elevated concentration of LDL, often not clearly familial, either a low cholesterol, moderately low fat diet or clofibrate (6, 8) will reduce the concentration of cholesterol about 15%. Based upon the demonstrated relationship between the level of cholesterol and risk of coronary heart disease in middle-aged men, such a reduction should be associated with an approximately 20% lower incidence of this common disorder (102). During the past year, three reports of trials designed to test this hypothesis have appeared in which clofibrate was used. One of these trials (8), carried out in northern California, was mainly a "primary prevention" study, since 98% of subjects were free of coronary heart disease upon entry. It included 1068 men whose average age was 48, mean plasma cholesterol level 262, and triglyceride level 181. They were divided into two matched groups, one of which received 2 g clofibrate daily for 39 months on a single-blind basis. The incidence of nonfatal myocardial infarction was reduced about fourfold in the treated group and no treated individual developed angina pectoris. There was no difference in the death rate attributable to coronary heart disease. Treatment of the control subjects for a subsequent period of 28.7 months similarly reduced their incidence of nonfatal coronary heart disease. In those individuals whose initial serum cholesterol levels exceeded 260 mg/dl, the level fell 20% on treatment and in those with serum triglycerides exceeding 160, triglycerides fell 47%. Reduction of cholesterol was similar in subjects with hydercholesterolemia alone or with combined elevation of cholesterol and triglycerides. In two British trials from Newcastle (6) and Edinburgh (7), clofibrate was given to individuals with established coronary heart disease ("ary prevention"). The trials differed in certain details, but each was double blind and involved several hundred patients. In both studies mortality was significantly reduced in patients entering with the symptom of angina and in neither was the incidence of nonfatal infarction affected. Although certain aspects of the authors' interpretation of these trials have been criticized (103), the results, like the American primary prevention study, suggest that treatment with clofibrate is beneficial. In both trials, the level of cholesterol was reduced to a greater degree in women (15–20%) than in men (about 10%), an observation that has occasionally been made before (27). Indirect estimates of VLDL and LDL levels were made in the Newcastle study. These showed that LDL were reduced whenever they were elevated initially or when the level of VLDL was normal, while the level of VLDL fell when it had been elevated at entry. An important result of

both the Newcastle and California trials was the observation that the apparent beneficial effect of clofibrate on coronary heart disease did not correlate with its hypocholesterolemic or hypotriglyceridemic effects. This raises the possibility mentioned earlier that removal of cholesterol from slowly metabolized pools is unrelated to reduction of serum cholesterol; alternatively the benefit produced by the drug may be unrelated to its demonstrated effects on lipid metabolism. Effects of clofibrate on mechanisms related to blood coagulation and thrombosis and upon cell permeability have also been described (104).

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CHOLESTYRAMINE

Backround

Cholestyramine was first used in patients with cholestasis in whom distressing pruritis prompted efforts to lower the content of bile acids in plasma (105). Interest in the application of bile-acid binding resins to the treatment of hyperlipidemia quickly followed the demonstration by Tennent and associates (106, 107) of sustained reductions in the cholesterol concentration in plasma of dogs fed a polycationic resin. While the scope of this review is limited to cholestyramine it should be noted that similar effects have been demonstrated with other cationic polymers (108, 109).

Toxicity

Cholestyramine is a copolymer of styrene and divinylbenzene with trimethyl benzyl ammonium groups providing the exchange sites (107). The average molecular weight exceeds one million daltons, and studies with radiolabeled resin indicate that it is not absorbed from the gastrointestinal tract. While animal studies have demonstrated that extremely large doses may produce metabolic acidosis, no effects on acid-base balance have yet been observed with the amounts employed in humans (110, 111). Plasma sodium and potassium levels are unaffected and only minimal effects on nitrogen balance occur with daily doses up to 36 g in man (112). It was originally reported that cholestyramine produced a positive calcium balance in man due to increased absorption from the gut (113). However this conclusion was based on the measurement of fecal calcium content during very brief periods of collection, so that the results could have been influenced by an effect of the resin on transit time in the gut. Nevertheless mean daily urine calcium excretion increased 21% during treatment. In recent studies in the rat there was no demonstrable effect on absorption of ^{47}Ca during short term administration of the resin (114). During prolonged treatment, absorption of calcium might be affected by changes in absorption of vitamin D. Whether some abnormality of calcium metabolism is causally related to the unusual calcifications of the biliary tree reported following treatment with cholestyramine in a few subjects with cholestasis (115, 116) is uncertain, since serum calcium levels were found to be unchanged during treatment in patients with intrahepatic cholestasis (117) and hyperbetalipoproteinemia (118).

Mild, reversible steatorrhea occurs with large doses. While fecal triglyceride

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loss is negligible with daily doses below 15 g (119, 120), twenty or more grams of neutral fat may appear in the stool per day at twice that dose (112, 120). The absorption of radioiodine-labeled triolein is impaired (120, 121) at the larger dose, but that of labeled oleic acid is not (120). Weight loss related to steatorrhea is prevented by a compensatory increase in caloric intake (112). The absorption of medium chain triglyceride at a daily intake of up to 150 g remains normal during treatment with 30 g per day (122). Preexisting steatorrhea appears to be aggravated by small doses (123). Studies of the effect of cholestyramine on fat absorption in the rat showed a significant correlation between saturation of the fatty acids and fecal loss of triglyceride (124). Thus, selective malabsorption of saturated triglycerides may contribute to the effect of high doses of the resin on serum cholesterol level.

The effect of the resin on absorption of fat-soluble vitamins parallels closely its effect on triglyceride absorption (125). Absorption of vitamin K_1 in the dog was unimpaired at a daily dose of 200 mg per kg, but was significantly diminished above twice that dose (126, 127). Absorption of vitamin K would appear to be adequate in practice since there is no change in prothrombin time with daily doses up to 24 g for one year in hyperlipidemic subjects free of preexisting malabsorption or liver disease (121). Hypoprothrombinemia with hemorrhage has been observed, however, in the presence of biliary cirrhosis (128) and with intestinal disease (129). Absorption of vitamin A is affected similarly to that of vitamin K. Plasma vitamin A levels were normal following the administration of a test meal containing 4 g of cholestyramine. When the content of resin in the meal was increased to 8 g, the concentration of the vitamin in plasma was reduced (130). However, plasma vitamin A and carotene levels were unchanged in hyperlipidemic patients after 5–9 months of therapy with daily doses ranging from 12 to 24 g (121). In rats on a diet containing 4% cholestyramine, absorption of vitamin D_3 was reduced by half (114). Recent studies of absorption of α-tocopherol in the rat indicate a direct relationship to absorption of triglyceride. On a diet containing 5% cholestyramine, no steatorrhea was observed and tocopherol absorption was normal when dietary fat was composed of medium chain triglyceride; when long chain triglyceride was given, fat absorption fell by half and tocopherol uptake was but a third of the pretreatment value. Plasma and liver tocopherol values were significantly diminished on both dietary regimens, however (131). It may be concluded that, with the exception of subjects who have malabsorption of lipids prior to treatment, significant deficiency of fat-soluble vitamins is unlikely at daily doses up to 24 g. Dietary supplementation is advisable at higher dosage where appreciable diminution of absorptive efficiency may be expected.

Binding of drugs has been observed in vitro, including some cationic compounds and molecules without charge. In contrast, the resin has little affinity for some anionic compounds (132, 133). In some cases the interaction of resin with anionic drugs appears to retard the rate of absorption without decreasing the total amount absorbed. Warfarin (132), digitalis glycosides (134, 135), and thyroxin (136) are known to bind to cholestyramine. Reduction of the half-life of

digitoxin has been demonstrated in man (135) during treatment. Absorption of thyroxine in man is markedly impaired, while that of inorganic iodide is unaffected (136). Clinical hypothyroidism with a late rise in serum cholesterol level was observed in a patient requiring thyroid hormone replacement (136). A 4-hour interval between the administration of thyroid hormone and the resin appears to permit normal absorption. Because of a moderate binding affinity of the resin for tetracyclines (132), impaired absorption may be expected with these compounds as well. Recently impairment of absorption of iron both in the inorganic form and as heme has been demonstrated in the rat (137).

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Mechanism of Action

All known metabolic effects of the polymer are thought to be related directly or indirectly to sequestration of bile acids. Binding affinity for these anions is relatively great compared to that for many other small anionic molecules (132). While ionic interaction appears to be the most important bonding force, secondary bonding mechanisms are also present which increase in magnitude with the hydrophobicity of the molecule (138). Thus the affinity of the resin for bile acids is reduced by introduction of hydroxyl functions into the ring structure.

The affinity of the resin is such that the bulk of bile acids presented to the resin in the gut are removed from the enterohepatic circulation and appear in the stool. Since bile acids inhibit the microsomal 7-α-hydroxylase enzyme system of liver, considered to be the rate-limiting step in the conversion of cholesterol to bile acids (24), sequestration of bile acids would be expected to increase this process. This effect is now well established (24, 139, 140). The most striking metabolic effect of cholestyramine in experimental animals and in man is a manyfold increase in fecal excretion of bile acids. In the rat, increases up to thirty times the normal rate have been observed (124, 141) and, in the mouse, reduction in size of the circulating bile acid pool has been observed with a fourfold increase in turnover of bile acids (142). Increased fecal excretion of bile acids has been demonstrated in normal man (118), in patients with cholestasis (105, 143), and in subjects with primary hyperbetalipoproteinemia (144–146). The increases in these studies have varied from three- to fifteenfold. In one study, subjects with familial hyperbetalipoproteinemia were found to have smaller increases in bile acid excretion than did normal subjects, perhaps reflecting an abnormality in the metabolism of cholesterol (146). The total daily fecal loss of cholesterol (bile acids plus neutral sterols) in subjects receiving cholestyramine varies from 0.7–1.9 g. Since this clearly exceeds the normal daily elimination, body stores of cholesterol should decline in the absence of an increase in biosynthesis or absorption of cholesterol. Several observations in human subjects suggest that a compensatory increase in cholesterol biosynthesis limits or abolishes negative cholesterol balance. First, the increased rate of fecal excretion of bile acids over extended periods exceeded the known content of cholesterol in the body (145). Second, the size of the rapidly exchangeable pool of cholesterol was not significantly altered during therapy with 12 g of cholestyramine per day despite large increments in fecal bile acid excretion (147). In the latter study the rate of entry

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into the rapidly exchangeable pool of cholesterol increased from 0.98 to 1.98 g per day. This approximates the turnover rate for all body cholesterol, as direct egress from slowly exchangeable pools is small. Evidently, the increased loss of cholesterol as bile acid stimulates an increase in cholesterol biosynthesis in liver as a result of decreased absorption of cholesterol and, possibly, of bile acids as well (148). Cholesterol biosynthesis is increased similarly during biliary drainage (149). Increased cholesterol synthesis has also been demonstrated in the intestine during treatment with cholestyramine (150). While the cholesterol of serum lipoproteins is a part of the rapidly exchangeable pool, determinants other than pool size may regulate the level or the composition of lipoproteins and hence cholesterol content of plasma. Plasma cholesterol levels may fall without significant change in the size of the rapidly exchangeable pool during treatment (147). The observation that xanthomata may regress during therapy without detectable change in plasma cholesterol level (151) suggests that a small negative sterol balance gradually depletes the slowly exchangeable pool even though serum lipoprotein levels are maintained at a high level by some specific impedance to their metabolism. In most studies in normal man and in the dog, plasma cholesterol levels fall during cholestyramine therapy. In several rodent species, however, cholesterol levels are unchanged despite profound alteration in bile acid excretion (152). The turnover rate of esterified cholesterol in each major ultracentrifugal class of lipoprotein is unique, LDL having the lowest rate. Within a given lipoprotein class, however, the rate for different esters is closely similar in normal and hyperlipidemic humans, a finding that is unchanged during cholestyramine treatment (93). Although bile acids are required for intestinal absorption of sterols (123, 153), fecal excretion of neutral sterols is either unchanged or only slightly increased with cholestyramine therapy (118, 144–146). Possibly the diversion of cholesterol to bile acids during therapy diminishes the importance of such a mechanism.

Among the plasma lipoproteins of man it is the LDL fraction that shows the greatest response (112, 154). Plasma cholesterol levels and LDL-cholesterol fall rapidly during the first 2 weeks of therapy (99) but the values may continue to decrease for at least 1 year (121). Pretreatment levels are regained within 3 weeks after termination of therapy. The effect of cholestyramine upon triglyceride levels in plasma of fasting humans is variable. In general little effect is seen (112, 154), but increased triglyceride levels have been reported in normal subjects (155) and in patients with hyperbetalipoproteinemia (156). Decreased levels were reported in one study (121). HDL-lipids are unchanged (99, 112, 156). The disappearance of radioiodine attached to the protein moiety of homologous LDL has been studied in normal subjects and in patients with primary hyperbetalipoproteinemia (157). Ingestion of 24 g of cholestyramine per day produced a 32% reduction in mean plasma cholesterol level with no change in the rate of synthesis of apoprotein in normal or hyperlipidemic subjects (approximately 15 mg per kg per day in both groups). However, the mean daily fractional catabolic rate of the apolipoprotein increased from 22 to 32%. How removal of cholesterol from the enterohepatic circulation reduces the concentration of LDL is unknown.

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THERAPY

Cholestyramine has been administered to adult humans in daily doses of 12–36 g. The dosage is often limited by gastrointestinal symptoms, especially constipation. High intestinal obstruction requiring surgical treatment occurred in a subject with cholestasis (158). Many subjects complain of epigastric distress, abdominal fullness, and increased bulk of stools, and diarrhea occurs occasionally. Most patients tolerate a daily dose of 15 g for prolonged periods and many tolerate daily doses of 25 g or more. Because the effect on serum cholesterol level increases in most subjects as doses are increased to between 25–35 g, doses in this range may be used in patients with severe or relatively resistant hyperlipidemia.

Reduction of serum cholesterol levels has been observed in most forms of primary hyperlipidemia as well as in normal man (111). Because other regimens are more effective in the various forms of endogenous hyperlipemia, cholestyramine is most useful in severe forms of primary hyperbetalipoproteinemia (usually familial) for which it is one of the most effective agents available. Considerable clinical heterogeneity exists among subjects with primary hyperbetalipoproteinemia. Plasma cholesterol levels range from the upper limits of normal to values approaching 1000 mg/dl, and responses to diets low in saturated fat and cholesterol vary from no significant change to an approximation of normal values. Levels of serum cholesterol exceeding 350 mg/dl or presence of xanthomata, especially of the tendons, usually indicates familial disease. A rare form of the familial disorder with serum cholesterol levels in excess of 500 mg/dl, extensive xanthomatosis, and predisposition to coronary vascular disease very early in life, often with atheromatous involvement of the aortic valve, is thought to represent the homozygous state. The interpretation of clinical results is most meaningful in groups of patients that are clearly defined with respect to familial occurrence, severity, and response to diet. It is not yet known whether the response of patients with associated increases in VLDL differs from that of subjects with isolated hyperbetalipoproteinemia.

While response of serum cholesterol levels has generally been employed to evaluate effectiveness, the observation that xanthomata can regress in the absence of detectable change in cholesterol content of plasma during therapy with cholestyramine (151) suggests that proper evaluation may require some index of sterol balance. Evidently, small net changes in balance extending over a protracted period may be of clinical benefit.

The effect of cholestyramine on serum lipids in patients with familial hyperbetalipoproteinemia has been evaluated in several short-term studies (99, 112, 156). The average reduction of total plasma cholesterol in the 60 subjects of these studies, who received 12–24 g of the resin per day, is 25%. Reductions of LDL-cholesterol of 27% (99) and 40% (112) were observed on daily doses of 16 and 32 g respectively. Several reports of smaller studies of the effect of cholestyramine over extended periods in patients with apparent heterozygous familial hyperbetalipoproteinemia have appeared. All of 7 such subjects in one study maintained reductions of plasma cholesterol levels of 20–50% for periods up to 4 years on a

daily dose of 13.3 g of resin (118). Decreases of mean serum cholesterol levels of 43, 24, and 20% have been reported in three studies of presumed heterozygotes (121, 154, 159) in whom duration of treatment ranged from 6 weeks to over 1 year with daily doses of 8–36 g. In another study of 20 subjects with tendon xanthomata, probably representing heterozygous familial hyperbetalipoproteinemia, who received 24 g daily, mean plasma cholesterol level fell from 393 to 292 mg/dl over a 12-month period (160).

Even among subjects with presumed heterozygous familial hyperbetalipoproteinemia, response to cholestyramine is variable. Serum cholesterol levels fall to the normal range in a few subjects while others show no response even while receiving 36 g of resin per day. The resin lowers serum cholesterol levels more effectively than stringent restriction of dietary cholesterol and saturated fats (99). Dietary indiscretion may vitiate the effect of the resin but this question has not been studied systematically. Response among subjects with mild hyperbetalipoproteinemia is similar to that observed with the classical heterozygous disease (160).

The putative homozygous form of hyperbetalipoproteinemia is considered to be most resistant to therapy. Of eleven cases treated with cholestyramine alone (118, 145, 151), plasma cholesterol level fell in only one. However, the mass of xanthomata decreased in several cases (151). Early reports indicate that combination of nicotinic acid with a bile acid-binding resin may be a more effective approach to these subjects. In one study (99) five of six such patients had a sustained reduction in serum cholesterol of 33% below the level achieved with resin alone, but of four subjects in another group only one had a sustained reduction (161).

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LITERATURE CITED

1. Final Report of the National Diet-Heart Study Group. 1968. *Circulation* 37: Suppl. 1:1–428
2. Leren, P. 1970. *Circulation* 42:935–42
3. Turpeinen, O. et al. 1968. *Am. J. Clin. Nutr.* 21:255–76
4. Dayton, S., Pearce, M. L., Hashimoto, S. D., Dixon, W. J., Tomiyasu, W. 1969. *Circulation* 40: Suppl. 11: 1–63
5. Schoch, H. K. 1969. *Advan. Exp. Med. Biol.* 4:405–20
6. Five-year Study by a Group of Physicians of the Newcastle upon Tyne Region. 1971. *Brit. Med. J.* 4:767–75
7. Report by a Research Committee of the Scottish Society of Physicians. 1971. *Brit. Med. J.* 4:775–84
8. Krasno, L. R., Kidera, G. J. 1972. *J. Am. Med. Assoc.* 219:845–51
9. Pinter, K. G., van Itallie, T. B. 1966. *Pharmacol. Rev.* 6:251–60
10. Proc. Third Int. Symp. on Drugs Affecting Lipid Metabolism. 1969. *Advan. Exp. Med. Biol.* v. 4, eds. W. L. Holmes, L. A. Carlson, R. Paoletti. New York: Plenum Press. 681 pp.
11. Proc. Fourth Int. Symp. on Drugs Affecting Lipid Metabolism. 1972. *Advan. Exp. Med. Biol.* v. 26, eds. W. L. Holmes, R. Paoletti, D. Kritchevsky. New York: Plenum Press. 359 pp.
12. Casdorph, H. R. 1971. *Treatment of the Hyperlipidemic States*, Springfield: C. C. Thomas. 434 pp.
13. Kritchevsky, D. 1968. *Ann. N. Y. Acad. Sci.* 149:1059–67
14. Kritchevsky, D. 1971. *Fed. Proc.* 30: 835–40

15. Gey, K. F., Carlson, L. A. 1971. *Metabolic Effects of Nicotinic Acid and its Derivatives*, Bern: H. Huber. 1251 pp.
16. Bencze, W. L., Hess, R., de Stevens, G. 1969. *Forschr. Arzeneimittelforsch.* v. 13. ed. E. Jucker, 217–92. Basel: Birkhaeuser
17. Havel, R. J. 1972. *Advan. Exp. Med. Biol.* 26:57–70
18. Havel, R. J. 1963. *Physiol. for Physicians* 1(6):1–6
19. Farber, E. 1967. *Advan. Lipid. Res.* 5:119–83
20. Havel, R. J. 1965. *Handbook of Physiology, Section 5*, eds. A. E. Renold, G. F. Cahill, Jr., 499–508. Baltimore: Williams & Wilkins
21. Nestel, P. J. 1970. *Advan. Lipid Res.* 8:1–39
22. Glomset, J. A. 1968. *J. Lipid Res.* 9: 155–67
23. Bergman, E. N., Havel, R. J., Wolfe, B. M., Bøhmer, T. 1971. *J. Clin. Invest.* 50:1831–39
24. Mosbach, E. H. 1969. *Advan. Exp. Med. Biol.* 4:421–41
25. Thorp, J. M. 1962. *Lancet* 1:1323–26
26. Oliver, M. F. 1962. *Lancet* 1:1321–23
27. Hellman, L. et al. 1963. *Ann. of Intern. Med.* 59:477–94
28. Butterfield, W. J. H. 1963. *J. Atheroscler. Res.* 3:427–590
29. Hess, R., Staubli, W., Riess, W. 1965. *Nature* 208:856–58
30. Svoboda, D., Grady, H., Azarnoff, D. 1967. *J. Cell Biol.* 35:127–52
31. Kaneko, A., Sakamoto, S., Morita, M., Onoe, T. 1969. *Tohoku J. Exp. Med.* 99:81–101
32. Platt, D. S., Thorp, J. M. 1966. *Biochem. Pharmacol.* 15:915–25
33. Green, K. G., Inman, W. H. W., Thorp, J. M. 1963. *J. Atheroscler. Res.* 3:593–616
34. Smith, A. F., MacFie, W. G., Oliver, M. F. 1970. *Brit. Med. J.* 2:86–88
35. Thorp, J. M., Cotton, R. C., Oliver, M. F. 1968. *Progr. Biochem. Pharmacol.* 4:611–17
36. Barrett, A. M., Thorp, J. M. 1968. *Brit. J. Pharmacol.* 32:381–91
37. Cenedella, R. J., Jarrell, J. J., Saxe, L. H. 1968. *J. Atheroscler. Res.* 8: 903–11
38. Barrett, A. M., 1966. *Brit. J. Pharmacol.* 26:363–71
39. Adams, L. L., Webb, W. W., Fallon, H. J. 1971. *J. Clin. Invest.* 50:2339–46
40. Ruegamer, W. R , Ryan, N. T., Richert, D. A., Westerfeld, W. W. 1969. *Biochem. Pharmacol.* 18:613–24
41. Best, M. M., Duncan, C. H. 1964. *J. Lab. Clin. Med.* 64:634–42
42. Westerfeld, W. W., Richert, D. A., Ruegamer, W. R., 1968. *Biochem. Pharmacol.* 17:1003–16
43. Azarnoff, D. L., Svoboda, D. J. 1969. *Arch. Int. Pharmacodyn.* 181:386–93
44. Westerfeld, W. W., Elwood, J. C., Richert, D. A. 1972. *Biochem. Pharmacol.* 21:1117–25
45. Pereira, J. H., Holland, G. F. 1970. *Atherosclerosis. Proc. Second Int. Symp.*, ed. R. J. Jones, 549–54. New York: Springer-Verlag. 706 pp.
46. Lamb, R. G., Fallon, H. J., 1972. *J. Biol. Chem.* 247:1281–87
47. Azarnoff, D. L., Tucker, D. R., Barr, G. A. 1965. *Metabolism* 14:959–65
48. Mishkel, M. A., Webb, W. F., 1967. *Biochem. Pharmacol.* 16:897–905
49. Gould, R. G., Swyryd, E. A., Avoy, D., Coan, B. 1967. *Progr. Biochem. Pharmacol.* 2:345–57
50. Segal, P., Roheim, P. S., Eder, H. A. 1972. *J. Clin. Invest.* 51:1632–38
51. Zakim, D., Paradini, R. S., Herman, R. H. 1970. *Biochem. Pharmacol.* 19: 305–10
52. Maragoudakis, M. E., Hankin, H., Wasvary, J. M. 1972. *J. Biol. Chem.* 247:342–47
53. Maragoudakis, M. E. 1969. *J. Biol. Chem.* 244:5005–13
54. Gould, R. G., Swyryd, E. A., Coan, B. J., Avoy, D. R. 1966. *J. Atheroscler. Res.* 6:555–64
55. White, L. W. 1971. *J. Pharmacol. Exp. Ther.* 178:361–70
56. Burch, R. E., Curran, G. L. 1969. *J. Lipid Res.* 10:668–73
57. Avoy, D. R., Swyryd, E. A., Gould, R. G. 1965. *J. Lipid Res.* 6:369–76
58. Kurup, C. K. R., Aithal, H. N., Ramasarma, T. 1970. *Biochem. J.* 116:773–79
59. Platt, D. S., Cockrill, B. L. 1966. *Biochem. Pharmacol.* 15:927–35
60. Kritchevsky, D., Tepper, S. A., Sallata, P., Kabakjian, J. R., Cristofalo, V. J. 1969. *Proc. Soc. Exp. Biol. Med.* 132:76–82
61. McGarry, J. D., Foster, D. W. 1969. *J. Biol. Chem.* 244: 4251–56
62. Krishnaiah, K. V., Inamdar, A. R., Ramasarma, T. 1967. *Biochem. Biophys. Res. Comm.* 27:474–78
63. Krishnaiah, K. V., Ramasarma, T. 1970. *Biochem. J.* 116: 321–27
64. Lakshmanan, M. R., Phillips, W. E. J., Brien, R. L. 1968. *J. Lipid Res.* 9:353–56

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65. Tolman, E. L., Tepperman, H. M., Tepperman, J. 1970. *Am. J. Physiol.* 218:1313–18
66. Nestel, P. J., Austin, W., 1968. *J. Atheroscler. Res.* 8:827–33
67. Giocoli, G., Tepperman, H. M., Tepperman, J. 1971. *Proc. Soc. Exp. Biol. Med.* 138:512–16
68. Greene, H. L., Herman, R. H., Zakim, D. 1970. *Proc. Soc. Exp. Biol. Med.* 134:1035–38
69. Grafnetter, D., Mrhová, O., Janada, J. 1971. *Arch. Int. Pharmacodyn.* 190:159–73
70. Robinson, D. S. 1970. *Comprehensive Biochemistry*, eds. M. Florkin, E. H. Stotz, 51–116, Amsterdam: Elsevier. 398 pp.
71. Sodhi, H. S., Kudchodkar, B. J., Horlick, L. 1971. *Metabolism* 20: 309–18
72. Strisower, E. H., Adamson, G., Strisower, B. 1968. *Am. J. Med.* 45: 488–501
73. Wilson, D. E., Lees, R. S. 1972. *J. Clin. Invest.* 51:1051–57
74. Strisower, E. H., Nichols, A. V., Lindgren, F. T., Smith, L. 1965. *J. Lab. Clin. Med.* 65:748–55
75. Hagopian, M., Robinson, R. W. 1968. *J. Atheroscler. Res.* 8:21–27
76. Grundy, S. M., Ahrens, E. H., Jr., Salen, G., Schreibman, P. H., Nestel, P. J. 1972. *J. Lipid Res.* 13: 531–51
77. Chang, Y-H., Pinson, R., Malone, M. H. 1967. *Biochem. Pharmacol.* 16: 2053–55
78. McKerron, C. G., Scott, R. L., Asper, S. P., Levy, R. I. 1969. *J. Clin. Endocrinol.* 29:957–61
79. Musa, B. U., Ogilvie, J. T., Dowling, J. T. 1968. *Metabolism* 17:909–15
80. Harrison, M. T., Harden, R. McG. 1966. *Scot. Med. J.* 11:213–17
81. Macmillan, D. C., Oliver, M. F., Simpson, J. D., Tothill, P. 1965. *Lancet* 2:924–26
82. Rifkind, B. M. 1966. *Metabolism* 15: 673–75
83. Hunninghake, D. B., Azarnoff, D. L. 1968. *Metabolism.* 7:588–95
84. Bierman, E. L. et al. 1970. *Trans. Assoc. Am. Phys.* 83:211–24
85. Wolfe, B., Kane, J., Havel, R., Brewster, H. 1970. *Circulation* 42 (Suppl. 3):2
86. Wolfe, B. M., Kane, J. P., Havel, R. J., Brewster, H. 1971. *Clin. Res.* 19:795
87. Barter, P. J., Nestel, P. J., Carroll, K. F. 1972. *Metabolism* 21:117
88. Zakim, D., Herman, R. H. 1969. *J. Atheroscler. Res.* 10:91–95
89. Zakim, D., Herman, R. H., Rosensweig, N. S., Stifel, F. 1969. *Gastroenterology* 56:496–99
90. Sodhi, H. S., Kudchodkar, B. J., Horlick, L., Weder, C. H. 1971. *Metabolism* 20:348–59
91. Nestel, P. J., Hirsch, E. Z., Couzens, E. A. 1965. *J. Clin. Invest.* 44:891–96
92. Horlick, L., Kudchodkar, B. J., Sodhi, H. S. 1971. *Circulation* 43:299–309
93. Goodman, D. S., Noble, R. P. 1970. *J. Lipid Res.* 11:183-89
94. Mitchell, W. D., Murchison, L. E. 1972. *Clin. Chim. Acta.* 36:153–61
95. Hood, B., Bedding, P., Carlander, B. 1963. *J. Atheroscler. Res.* 3:509–17
96. Levy, R. I., Quarfordt, S. H., Brown, W. V., Sloan, H. R., Fredrickson, D. S. 1969. *Advan. Exp. Med. Biol.* 4:377–87
97. Scott, P. J., Hurley, P. J. 1969. *J. Atheroscler. Res.* 9:25–34
98. Havel, R. J. 1969. *Advan. Intern. Med.* 15:117–54
99. Levy, R. I. et al. 1972. *Ann. Intern. Med.* 77:267–94
100. Hood, B., Angervall, G., Cramér, K., Welin, G. 1968. *Acta Med. Scand.* 184:337–46
101. Nordøy, A., Gjone, E. 1970. *Acta Med. Scand.* 188:487–90
102. Kannel, W. B., Garcia, M. J., McNamara, P. M., Pearson, G. 1971. *Human. Pathol.* 2:129–51
103. Friedewald, W. T., Halperin, M. 1972. *Ann. Intern. Med.* 76:821–23
104. Furman, R. H. 1971. *Treatment of the Hyperlipidemic States*, ed. H. R. Casdorph, 268–96. Springfield: C. C. Thomas. 434 pp.
105. Carey, J. B., Jr. 1961. *J. Clin. Invest.* 40:1028
106. Tennent, D. M. et al. 1959. *Circulation* 20:969–70
107. Tennent, D. M., Siegel, H., Zanetti, M. E. 1960. *J. Lipid Res.* 1:469–73
108. Parkinson, T. M. 1967. *J. Lipid Res.* 8:24–29
109. Glueck, C. J., Steiner, P. M., Scheel, D., Ford, S. 1971. *Circulation* 44 (Suppl. 11):59
110. Gallo, D. G., Sheffner, A. L. 1965. *Proc. Soc. Exp. Biol. Med.* 120:91–93
111. Casdorph, H. R. 1971. *Treatment of the Hyperlipidemic States*, ed. H. R. Casdorph, 243–66 Springfield: C. C. Thomas
112. Howard, R. P., Brusco, O. J., Furman, R. H. 1966. *J. Lab. Clin. Med.* 68:12–20

113. Briscoe, A. M., Ragan, C. 1963. *Am. J. Clin. Nutr.* 13:277–84
114. Thompson, W. G., Thompson, G. R. 1969. *Gut* 10:717–22
115. Shaffner, F., Klion, F. M., Latuff, A. J. 1965. *Gastroenterol.* 48:293–98
116. Wells, R. F., Knopshield, J. H., Davis, C. 1968. *Am. J. Digest. Dis.* 13:86–94
117. Datta, D. V., Sherlock, S. 1963. *Brit. Med. J.* 1:216–19
118. Hashim, S. A., van Itallie, T. B. 1965. *J. Am. Med. Assoc.* 192:289–93
119. Danhof, I. E. 1966. *Am. J. Clin. Nutr.* 18:343–49
120. Hashim, S. A., Bergen, S. S., Jr., van Itallie, T. B. 1961. *Proc. Soc. Exp. Biol. Med.* 106:173–75
121. Bressler, R., Nowlin, J., Bogdonoff, M. D. 1966. *South. Med. J.* 59:1097–1103
122. Zurier, R. B., Hashim, S. A., van Itallie, T. B. 1965. *Gastroenterology* 49:490–95
123. Wessler, S., Avioli, L. A. 1968. *J. Am. Med. Assoc.* 206:1285–88
124. Harkins, R. W., Hagerman, L. M., Sarett, H. P. 1965. *J. Nutr.* 87:85–92
125. Whiteside, C. H., Harkins, R. W., Fluckiger, H. B., Sarett, H. P. 1965. *Am. J. Clin. Nutr.* 16:309–14
126. Kelley, K. L., Lehman, E. G., Robinson, H. J. 1963. *Fed. Proc.* 22: 434
127. Robinson, H. J., Kelley, K. L., Lehman, E. G. 1964. *Proc. Soc. Exp. Biol. Med.* 115:112–15
128. Visintine, R. E. et al. 1961. *Lancet* 2: 341–45
129. Gross, L. G., Brotman, M. 1970. *Ann. Int. Med.* 72:95–97
130. Longenecker, J. B., Basu, S. G. 1965. *Fed. Proc.* 24:375
131. Davies, T., Kelleher, J., Smith, C. L., Walker, B. E., Losowsky, M. S. 1972. *J. Lab. Clin. Med.* 79:824–31
132. Gallo, D. G., Bailey, K. R., Sheffner, A. L. 1965. *Proc. Soc. Exp. Biol. Med.* 120:60–65
133. Gallo, D. G., Sheffner, A. L. 1964. *Fed. Proc.* 23:323
134. Caldwell, J. M., Greenberger, N. I. 1971. *J. Clin. Invest.* 50:2626–37
135. Caldwell, J. H., Bush, C. A., Greenberger, N. J. 1971. *J. Clin. Invest.* 50:2638–44
136. Northcutt, R. C., Stiel, J. N., Hollifield, J. W., Stant, E. A., Jr. 1969. *J. Am. Med. Assoc.* 208:1857–61
137. Thomas, F. B., McCullough, F. S., Greenberger, N. J. 1971. *J. Lab. Clin. Med.* 78:70–80
138. Johns, W. H., Bates, T. R. 1970. *J. Pharm. Sci.* 59:329–33
139. Boyd, G. S., Scholan, N. A., Mitton, J. R. 1969. *Advan. Exp. Med. Biol.* 4:443–56
140. Boyd, G. S., Lawson, M. E. 1970. *Atherosclerosis. Proc. Second Int. Symp.*, ed. R. J. Jones. New York: Springer-Verlag. 286 pp.
141. Huff, J. W., Gilfillan, J. L., Hunt, V. M. 1963. *Proc. Soc. Exp. Biol. Med.* 114:352–55
142. Beher, W. T., Beher, M. E., Rao, B. 1966. *Proc. Soc. Exp. Biol. Med.* 122: 881–84
143. Carey, J. B., Jr., Williams, G. 1961. *J. Am. Med. Assoc.* 176:432–35
144. Moore, R. B., Crane, C. A., Frantz, I. D., Jr. 1968. *J. Clin. Invest.* 47:1664–71
145. Moutafis, C. D., Myant, N. B. 1969. *Clin. Sci.* 37:443–54
146. Miettinen, T. A., 1970. *Atherosclerosis.* Proc. Second Int. Symp., ed. R. J. Jones. 558–61. New York: Springer-Verlag. 508 pp.
147. Goodman, D. S., Noble, R. P. 1968. *J. Clin. Invest.* 47:231–41
148. Wilson, J. D. 1972. *J. Clin. Invest.* 51: 1450–58
149. Myant, N. B., Eder, H. A. 1961. *J. Lipid Res.* 2:363–68
150. Dietschy, J. M. 1968. *J. Clin. Invest.* 47:286–300
151. Khachadurian, A. K. 1968. *J. Atheroscler. Res.* 8:177–88
152. Gallo, D. G., Bailey, K. R., Sheffner, A. L., Sarett, H. P., Cox, W. M., Jr. 1966. *Proc. Soc. Exp. Biol. Med.* 122: 328–34
153. Hyun, S. A., Vahouny, G. V., Treadwell, C. R. 1963. *Proc. Soc. Exp. Biol. Med.* 112:496–501
154. Fallon, H. J., Woods, J. W. 1968. *J. Am. Med. Assoc.* 204:1161–64
155. Weizel, A., Estrich, D. L., Splitter, S. B. 1969. *Proc. Soc. Exp. Biol. Med.* 130:149–50
156. Jones, R. J., Dobrilovic, L. 1970. *J. Lab. Clin. Med.* 75:953–66
157. Langer, T., Levy, R. I., Fredrickson, D. S. 1969. *Circulation* 40 (Suppl. 111):14
158. Cohen, M. I., Winslow, P. R., Boley, S. T. 1969. *N. Engl. J. Med.* 280:1285–86
159. Christensen, N. C., Mogensen, E. F. 1971. *Ugeskrift. Laeger* 133:2431–35
160. Connor, W. E. 1968. *Med. Clin. N. Am.* 52:1249–60
161. Moutafis, C. D., Myant, N. B., Mancini, M., Oriente, P. 1971. *Atherosclerosis* 14:247–58

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