Medical Grand Rounds

MEDICAL THERAPY OF CHOLESTEROL GALLSTONE DISEASE

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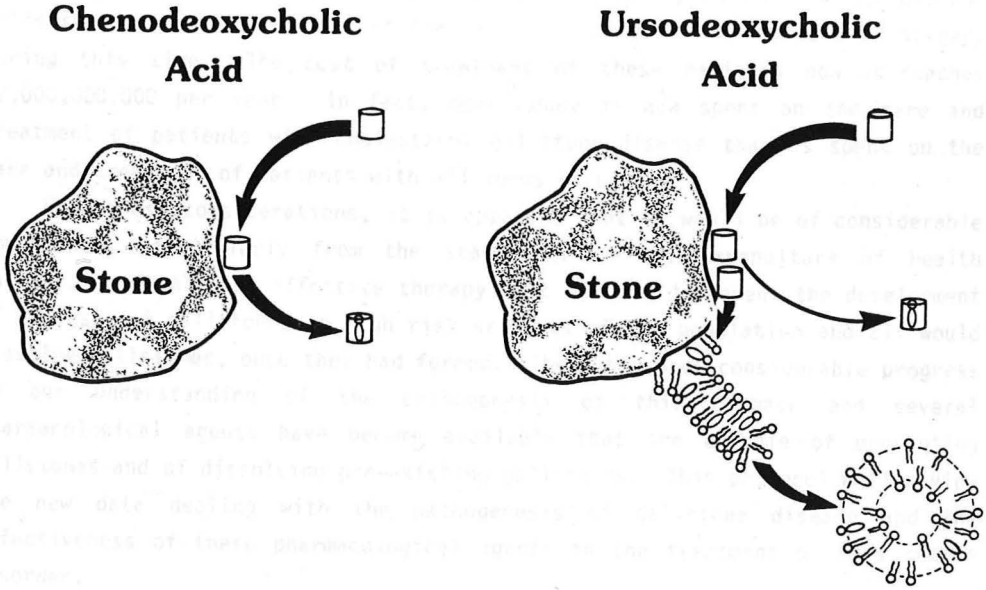


CHOLESTEROL GALLSTONE DISEASE

Gallstone disease represents one of the most common diseases of man, particularly in the Western world. It is also one of the leading causes for major surgery. Generally, gallstones fall into two categories: those that are composed principally of precipitated bilirubin and those that are composed principally of precipitated cholesterol. Of these two types of stones, cholesterol gallstones are by far the most important, and account for approximately 90% of the stones removed from gallbladders of patients in the Western world.

The true incidence of cholesterol gallstones is difficult to ascertain since at least half of such stones are said to be clinically asymptomatic. There are also marked differences in the incidence of stones in different ethnic groups. It is well established, for example, that in certain Indian groups in the Southwest nearly 70% of the women will harbor gallstones. Various other studies suggest that in the United States alone there are approximately 12,000,000 women with cholesterol gallstones and 4,000,000 men. These stones lead to clinically apparent gallbladder disease in approximately 8,000,000 patients each year. This, in turn, leads to approximately 400,000-500,000 cholecystectomies being performed each year, and approximately 5,000-8,000 patients die as a consequence of complications of gallstone disease or surgery during this time. The cost of treatment of these patients now approaches $2,000,000,000 per year: in fact, more money is now spent on the care and treatment of patients with cholesterol gallstone disease than is spent on the care and treatment of patients with all forms of cancer.

From these considerations, it is apparent that it would be of considerable importance, particularly from the standpoint of the expenditure of health dollars, to develop an effective therapy that 1) would prevent the development of cholesterol gallstones in high risk segments of the population and 2) would dissolve gallstones, once they had formed. There has been considerable progress in our understanding of the pathogenesis of this disease and several pharmacological agents have become available that are capable of preventing gallstones and of dissolving pre-existing gallstones. This protocol will review the new data dealing with the pathogenesis of gallstone disease and the effectiveness of these pharmacological agents in the treatment of this common disorder.

A) GENERAL FEATURES OF CHOLESTEROL METABOLISM

Since 90% of all gallstones are composed principally of precipitated cholesterol, gallstone disease must be considered as essentially a disorder of cholesterol metabolism in the body. Cholesterol is an essential constituent of all living tissues, and plays critical roles as a structural component of most biological membranes and as the immediate precursor for a number of essential vitamins, sterol hormones and bile acids. It is of critical importance, therefore, that the cells of all major tissues in the body be assured a continuous supply of this substance. To meet this need, a complex series of transport, biosynthetic and regulatory mechanisms has evolved. Generally, cholesterol can be acquired from the environment through the absorption of dietary cholesterol or synthesis de novo from acyl-CoA within the body. More cholesterol usually enters the body through these two mechanisms than is used during normal metabolic turnover so that the excess must be metabolized and/or excreted to prevent a potentially hazardous accumulation of sterol. Unfortunately, mammalian tissues do not possess enzymes capable of extensive degradation of the sterol nucleus. The best that can be accomplished is to modify certain of the substituent groups on the hydrocarbon tail or on the ring structure of the sterol molecule. Hence, cholesterol must be excreted from the body either as the unaltered molecule or after biochemical modification to other steroid products such as bile acids or hormones.

The general features of cholesterol balance that must be taken into consideration in man and in various experimental animals are shown diagrammatically in Fig. 1. The body pool of cholesterol in the adult remains

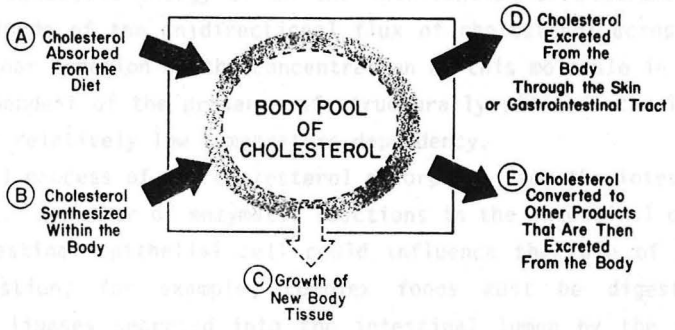


Fig. 1

essentially constant. The content of sterols in various tissues varies markedly, however, from about 0.5 g/kg muscle to 15 g/kg brain but averages about 1.4 g/kg tissue for the body as a whole. Thus, a 70 kg man contains about 100 g cholesterol while a .2 kg rat has only .3 g of total sterol.

New cholesterol can be added to the body pool from only two sources. Either preformed sterol is absorbed from dietary sources across the gastrointestinal mucosa or, alternatively, the cholesterol molecule is synthesized de novo from acyl-CoA in a variety of different tissues within the body. The sum of these processes constitutes the total input of cholesterol into the body pool each day. Similarly, there are only two major pathways for the removal of cholesterol from the body. The unmodified cholesterol molecule may be lost directly from the body pool. This takes place through the sloughing of oily secretions and cells from the skin, through the desquamation of cells from the stomach, small intestine and colon and through the movement of cholesterol into pancreatic, gastric, intestinal, and cannalicular secretions. Of these various routes, secretion of cholesterol through the cannalicular membrane of the hepatocyte is of greatest quantitative importance. Alternatively, the cholesterol molecule may first be metabolized to another product, such as a bile acid, which in turn is secreted from the body through the urine or gastrointestinal tract.

The first of the two major sources for sterol in the body pool is dietary cholesterol absorbed through the gastrointestinal tract. Every animal is capable of absorbing dietary cholesterol to at least some degree although there are remarkable differences among the various species in the rate of such intestinal transport. Most data indicate that cholesterol movement into the intestinal epithelial cell is a passive process that does not depend upon the expenditure of metabolic energy or on the intervention of membrane receptors. Thus, the magnitude of the unidirectional flux of cholesterol across the brush border is a linear function of the concentration of this molecule in the luminal fluid, is independent of the presence of structurally related sterol molecules, and manifests a relatively low temperature dependency.

The overall process of net cholesterol absorption from the intestinal lumen is very complex. A number of enzymatic reactions in the intestinal contents and within the intestinal epithelial cell could influence the rate of net uptake. Following ingestion, for example, complex foods must be digested by the peptidases and lipases secreted into the intestinal lumen by the pancreas in

order to release the largely unesterified dietary cholesterol. The small amount of dietary cholesteryl esters is hydrolyzed by another pancreatic enzyme, cholesteryl esterase. This unesterified cholesterol from the diet, along with the unesterified cholesterol reaching the intestinal lumen from the bile, is then solubilized in the complex structure of a mixed micelle or liposomal vesicle. Following the movement of these carriers up to the brush border, dietary sterol diffuses into the cystosolic compartment of the intestinal absorptive cell where it presumably mixes with the pool of newly synthesized cholesterol. A large portion of this intracellular cholesterol pool is esterified to long-chain fatty acids and incorporated into the structure of the nascent chylomicron. This lipoprotein particle is then secreted from the epithelial cell by an exocytotic process, enters the intestinal lymphatic system and eventually reaches the circulating blood.

While few data are available delineating the velocity of each of these steps in different animals, measurements have been made of the overall rates at which dietary cholesterol is absorbed in several species. As summarized in Table 1 these data suggest that there are remarkable differences in the amount

TABLE 1. *Comparison of rates of cholesterol absorption in four different animal species*[a]

Species	Representative body weight (kg)	Dietary cholesterol intake (mg/day)	Cholesterol absorption rate	
			Per animal[b] (mg/day)	Per kg body weight[c] (mg/day/kg body weight)
Rat	0.2	50–300	44–102	220–510
Rabbit	1.5	465–500	450	300
Dog	10.0	1,100–1,600	70–1,000	70–100
Man	70.0	300–2,950	140–280	2–4

[a] Representative values are shown for the amount of cholesterol absorbed from the diet in four different species. Data are presented in terms of the absolute milligram cholesterol absorbed per day in an animal of average weight[b] and expressed as the milligram sterol absorbed per kilogram body weight[c].
These data were adapted from a variety of studies

of cholesterol that can be absorbed by man and by different experimental animals. A 70 kg man, for example, can absorb several hundred mg of cholesterol per man. A much smaller animal such as 0.2 kg rat or 1.5 kg rabbit may absorb nearly as such sterol. These differences are made more apparent when the rate of net cholesterol absorption is expressed per kg of body weight. On a

relatively high cholesterol intake man absorbs only about 2 to 4 mg of cholesterol/day/kg body. In contrast, other species such as the rat, rabbit and dog can absorb from 35 to 50 times this amount. On the basis of such findings it has been postulated that this limited capacity to absorb cholesterol may be one of the major mechanisms that protects man against the detrimental effects of excessive dietary cholesterol intake.

The second major source for cholesterol in the body pool is de novo synthesis of sterol by the major organ systems. The rate at which cholesterol is synthesized within the body of man or the experimental animal has been measured by two different types of procedures. One method involves measuring sterol balance across the body. With this technique, the amount of cholesterol secreted from the body in the feces as neutral (cholesterol and its bacterial degradation products) and acidic (bile acids) sterols is quantitated in the steady state. After taking into account the amounts of cholesterol that are eaten in the diet and lost from the skin or converted to sterol hormones, and after correcting for any sterol that may be completely degraded by intestinal bacteria, it is possible to calculate the rate of total cholesterol synthesis per day in the experimental subject.

The rate of whole body sterol synthesis can also be measured in vivo by assaying the rate at which [^{3}H]water is incorporated into sterols. Furthermore, by assuming that 1.45 µg atoms of carbon are incorporated into cholesterol for every µg atom of ^{3}H, it is possible to convert such incorporation data into absolute rates of cholesterol synthesis.

Using these methods, rates of whole-body cholesterol synthesis have been measured in man and in a variety of animals under conditions where dietary cholesterol intake was low. These values are summarized in Table 2. As is apparent, the absolute amount of cholesterol synthesized varies markedly from animal to animal and even differs between animals of similar weight, e.g., rat and hamster or guinea pig and squirrel monkey. As was the case with species differences in cholesterol absorption, these variations in rates of sterol synthesis are emphasized by expressing the data as the amount of cholesterol synthesized per kg body weight. Thus, man can synthesize about 9 mg of cholesterol/day/kg body weight, while the rat is capable of making over 13 times more sterol, or about 118 mg/day/kg body weight. In general, there is an inverse, although imperfect, relationship between the rate of whole animal

TABLE 2. *Comparison of rates of whole animal cholesterol synthesis in eight different animal species*[a]

Species	Representative body weight (kg)	Rates of cholesterol synthesis	
		Per animal[b] (mg/day)	Per kg body weight[c] (mg/day/kg body weight)
Rat	0.2	23.6	118
Hamster	0.15	5.8	39
Squirrel monkey	0.6	20.6	34
Rabbit	1.5	46.1	31
Guinea pig	0.5	11.2	22
Baboon	25.0	525.0	21
Dog	10.0	120.0	12
Man	70.0	630.0	9

[a] Representative values are shown for the amount of cholesterol synthesized in the whole body of eight different species. Data are presented in terms of the absolute amount of cholesterol synthesized per day in an animal of average weight[b] and expressed as the milligram cholesterol synthesized per day per kilogram body weight[c].

This table was compiled from data determined either by external balance techniques and reported from a number of different laboratories or by determining the rate of incorporation of [^{3}H]water into cholesterol *in vivo* and then calculating the absolute rate of cholesterol synthesis

sterol synthesis and body weight. The larger animals and man generally synthesize much less sterol per unit weight than the smaller animals, particularly the rat.

From these data on the rates of cholesterol absorption (Table 1) and rates of cholesterol synthesis (Table 2), it is possible to begin to appreciate the quantitative importance of each of these input processes as sources for the body pool of cholesterol. Obviously, when man or an experimental animal is maintained on a cholesterol-free diet, 100% of the sterol in the body pool, circulating in the plasma and secreted into bile must ultimately be derived from cholesterol synthesized endogenously. As the amount of cholesterol in the diet is increased, the extent to which the body pool is derived from these exogenously sources will vary and, in a given species, will be largely determined by the amount of cholesterol that can be absorbed from the gastrointestinal tract relative to the amount that can be synthesized under a particular experimental circumstance. In man, for example, the low rate of cholesterol absorption (2 to 4 mg/day/kg body weight) relative to the capacity for endogenous sterol synthesis (9 mg/day/kg body weight) would suggest that even on a fairly high cholesterol intake, most of the body pool would still be derived from endogenous synthesis. This conclusion is supported by experiments in which human subjects were fed a diet high in radiolabeled cholesterol until

the isotopic steady state was achieved. It could then be calculated directly that approximately 60% of the plasma cholesterol pool was still derived from endogenous synthesis, despite the intake of relatively large amounts of dietary cholesterol. In contrast, when the rat is placed on a high cholesterol diet, it absorbed much more dietary cholesterol relative to the rate of endogenous synthesis so that less than 10% of plasma cholesterol is derived from newly synthesized sterols.

B) CHOLESTEROL SYNTHESIS IN THE LIVER

Rates of cholesterol synthesis have been measured in liver specimens obtained from a variety of animals species that have been maintained on a low intake dietary cholesterol. As summarized in Fig. 2, large variations were seen among the different species when these rates of synthesis are expressed per gram of liver tissue. The rat again manifests an extremely high rate of cholesterol synthesis reflecting the high rate of whole-body sterol synthesis found in this same species (Table 2). Rates of sterol synthesis are much lower in other species, including man. Another point to be emphasized is that there is no general correlation between the rate of hepatic cholesterol synthesis and the relative amounts of cholesterol secreted into the bile of each of these species or of their respective propensities to develop cholesterol gallstones.

Fig. 2

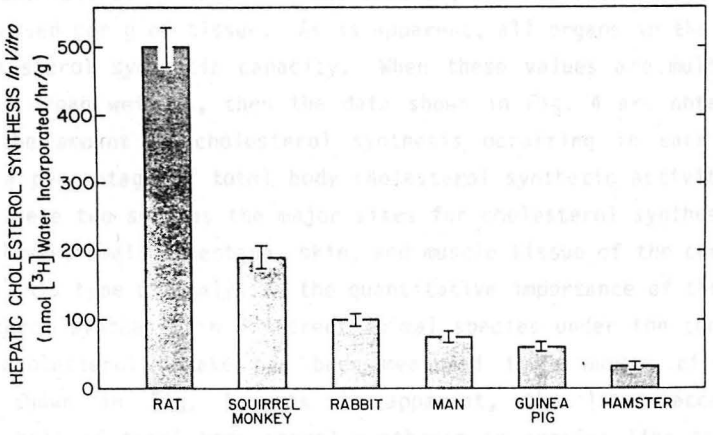
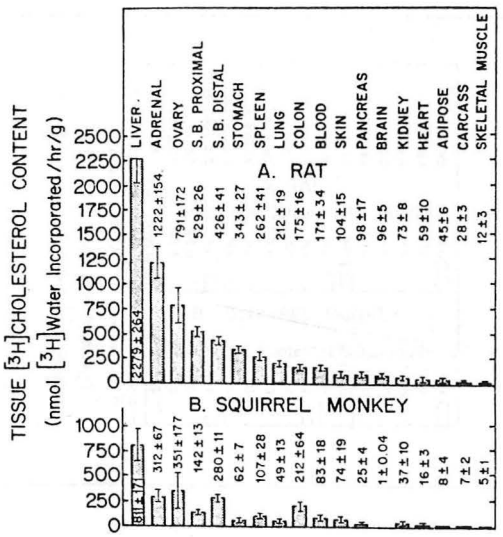


Fig. 3



These comparisons, although interesting, do not answer the fundamentally important question about the quantitative importance of the liver to whole body sterol synthesis in each of these species, particularly under in vivo conditions. Such data are now, however, available. The data in Fig. 3 shows the rates of cholesterol synthesis in two species, the rat and monkey, when such data are expressed per g of tissue. As is apparent, all organs in the body have at least some sterol synthetic capacity. When these values are multiplied by the respective organ weights, then the data shown in Fig. 4 are obtained. In this figure the amount of cholesterol synthesis occurring in each organ is expressed as a percentage of total body cholesterol synthetic activity. As is apparent, in these two species the major sites for cholesterol synthesis in the body are the liver, small intestine, skin, and muscle tissue of the carcass.

Based on this type of analysis, the quantitative importance of the liver to total body sterol synthesis in different animal species under the condition of low dietary cholesterol intake has been measured in a number of different species, as shown in Fig. 5. As is apparent, the liver accounts for approximately half of total body sterol synthesis in species like the rat and squirrel monkey but much lower percentages in other species, including probably man.

Fig. 4

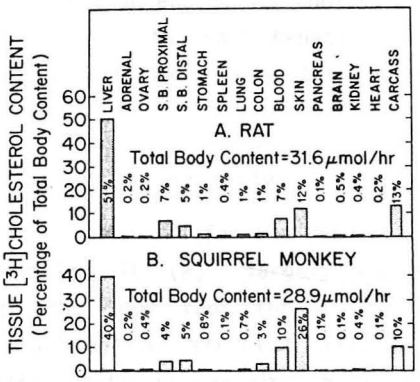
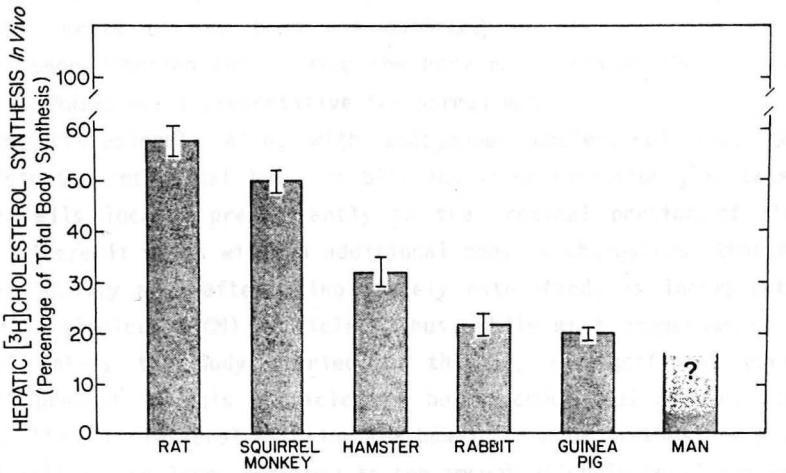

TISSUE [3H]CHOLESTEROL CONTENT
(Percentage of Total Body Content)
A. RAT
Total Body Content=31.6μmol/hr
LIVER 51%
ADRENAL 0.2%
OVARY 0.2%
S.B. PROXIMAL 7%
S.B. DISTAL 5%
STOMACH 1%
SPLEEN 0.4%
LUNG 1%
COLON 1%
BLOOD 7%
SKIN 12%
PANCREAS 0.1%
BRAIN 0.5%
KIDNEY 0.4%
HEART 0.2%
CARCASS 13%
B. SQUIRREL MONKEY
Total Body Content=28.9μmol/hr
40%
0.2%
0.4%
4%
5%
0.8%
0.1%
0.7%
3%
10%
26%
0.1%
0.1%
0.4%
0.1%
10%

Fig. 5


HEPATIC [3H]CHOLESTEROL SYNTHESIS In Vivo
(Percentage of Total Body Synthesis)
RAT
SQUIRREL MONKEY
HAMSTER
RABBIT
GUINEA PIG
MAN
?

In external balance studies performed in normal man, cholesterol feeding has been reported to have an inconstant effect on total body synthesis. Several investigators have reported studies in which cholesterol feeding had either no effect or only a modest effect on endogenous cholesterol synthesis rates. Such a result also implies that in man hepatic cholesterol synthesis is quantitatively less important to total body sterol synthesis than in some other animal species.

C) CHOLESTEROL TRANSPORT THROUGH THE PLASMA

The liver remains the key organ for the regulation of cholesterol balance within the intact animal: it (a) largely compensates for changes in cholesterol input into the body from the diet, (b) synthesizes various lipoprotein particles which deliver sterol to certain peripheral tissues, (c) takes up other lipoprotein particles carrying cholesterol from the extrahepatic tissues back to the liver, and (d) secretes cholesterol and bile acids from the body. The movement of cholesterol through the plasma and its targeted uptake by specific tissues is articulated by special classes of lipoproteins interacting with specific cell surface receptors present on the parenchymal cells of many organs. The major pathways for the transport of cholesterol among the various tissue compartments of the body are outlined in Fig. 6. The amounts of cholesterol seen entering and leaving the body pool through the various input and output pathways are representative for normal man.

Dietary cholesterol, along with endogenous cholesterol that has been secreted into the intestinal lumen in bile and other secretions, is taken up by absorptive cells located predominantly in the proximal portion of the small intestine. There it mixes with an additional pool of cholesterol that has been synthesized locally and, after being largely esterified, is incorporated into the nascent chylomicron (CM) particle. Thus, while most exogenous or dietary cholesterol enters the body carried in the CM, a significant portion of cholesterol present in this particle may be of endogenous origin, since the amount of biliary cholesterol entering the bowel lumen or synthesized within the intestinal wall may be large compared to the amount of cholesterol available for absorption from the diet.

The nascent particle contains predominantly apoproteins A-I (apo A-I) and B (apo B). Once it enters the lymph, however, the CM acquires apoproteins E

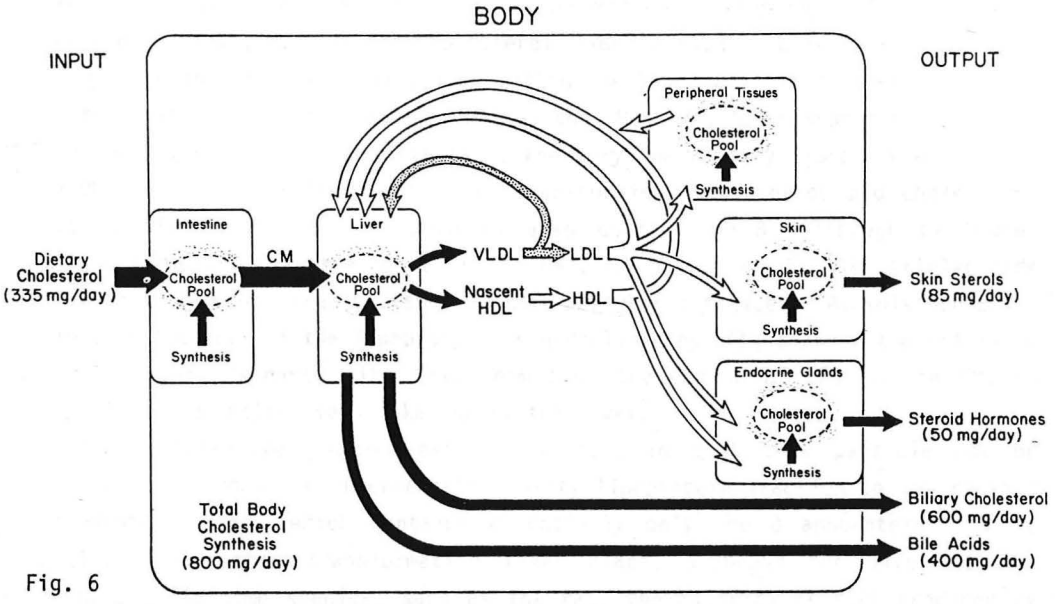


Fig. 6

(apo E) and C (apo C) through interaction with other lipoproteins, such as high density lipoproteins. This family of C apoproteins serves two important functions. First, the presence of large amounts of apo C, relative to apo E, appears to prevent the uptake of the particle by the liver. Second, one component of this family, apo C-II, activates the enzyme lipoprotein lipase. This enzyme is situated on the luminal surface of capillaries found predominantly in muscle and adipose tissue and rapidly hydrolyzes much of the triglyceride present in the core of the CM. This liberates large amounts of free-fatty acid that are then taken up and stored or metabolized in adjacent tissues. As the triglyceride in the core of the chylomicron is removed, the particle becomes smaller in size and loses some of its surface components, including unesterified cholesterol, phospholipid and apoproteins A-I and C. Presumably, because of the decrease in the ratio of apo C to apo E, the partially metabolized CM or CM remnant, is recognized by the hepatocyte and is

rapidly and essentially quantitatively cleared by the liver. This uptake occurs by way of a high-velocity, saturable transport system that depends upon the presence of receptors on the liver parenchymal cells (the chylomicron remnants receptor). Thus, much of the cholesterol from the diet or bile that is absorbed across the intestine or synthesized within the bowel wall is delivered directly to the liver by this mechanism. Just as the CM serves to transport triglyceride and cholesterol out of the intestine, the very low density lipoprotein particle (VLDL) serves a similar function in transporting triglyceride and cholesterol out of the liver. These particles also contain apo B (although of higher molecular weight than the apo B in the CM), apo C and apo E. The triglyceride carried in VLDL is largely disposed of in peripheral tissues. As this non-polar lipid in the core of the lipoprotein is hydrolyzed by LPL, a remnant particle is formed. This remnant, like that formed by the action of LPL on the CM, is rapidly and quantitatively taken up by the liver.

An alternative pathway exists for VLDL in that this particle may be metabolized through an intermediate density lipoprotein fraction to low density lipoprotein (LDL), which contains essentially only the B apoprotein. It is unclear where this transformation takes place, although the liver may be involved. In some species, such as the rat, the majority of VLDL produced by the liver is metabolized through the remnant pathway whereas in man, a much larger proportion of the VLDL is metabolized to LDL.

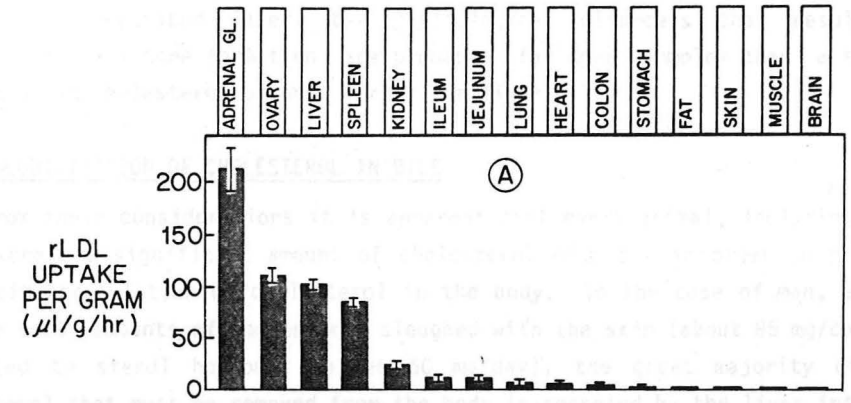
Many tissues of the body, including the liver, possess specific cell surface receptors that recognize and bind lipoproteins containing the B and/or E apoproteins. These binding sites are referred to as LDL receptors.

As is apparent in Fig. 7, most tissues possess LDL receptors although the highest rates of LDL uptake are seen in the liver, endocrine glands and intestine. Such data are now available in a number of different species and similar findings have been observed. When such rates of uptake are again multiplied by organ weight, it has been found in all species in which data are available that the liver accounts for the uptake of 55-75% of the circulating LDL. Thus, while a small portion of the cholesterol acquired by peripheral organs (Fig. 6) comes from the uptake of LDL, most tissues synthesize the majority of sterol that they require for maintenance of cell membrane structures.

Since these peripheral tissues do acquire more cholesterol through synthesis and LDL uptake than they require, this excess cholesterol must, ultimately, be returned to the liver for excretion. Presumably, high density lipoproteins play a key role in this process although the actual transport processes responsible for the movement of cholesterol from these peripheral tissues back to the liver remain very poorly understood.

Thus, from these considerations, it is apparent that the liver receives net contributions of cholesterol from the diet, from local synthesis and from cholesterol synthesized in the peripheral tissues and carried back to the liver (? in HDL). It is this net contribution of cholesterol to the liver (equaling about 1,000 mg/day in man) that ultimately must be excreted in the bile.

Fig. 7



D. MAINTENANCE OF CHOLESTEROL HOMEOSTASIS IN THE BODY

Obviously, since the input of dietary cholesterol into the body may vary over a wide range, the amount of cholesterol reaching the liver in chylomicrons may vary dramatically on a day-to-day basis. Mechanisms exist, however, that adapt for such changing loads of dietary cholesterol and so maintain relative homeostasis within the intact animal. When large amounts of cholesterol are ingested in the diet, there is suppression of the rate of cholesterol

synthesis in the intestinal epithelial cells and, in particular, in the liver. If these decreases in rates of cholesterol synthesis just balance the increased load of cholesterol absorbed from the diet then the net input of sterol to the liver is maintained at a constant value. Under these circumstances, the rates of VLDL production and circulating LDL-cholesterol levels remain constant and the amount of sterol that must be secreted in the bile remains unchanged. Conversely, if there is an increased demand in the body for cholesterol, the rate of cholesterol synthesis in the intestine and liver can also increase to meet this demand. Again, if this adaptive response in synthesis is adequate to meet the new demand then the circulating levels of cholesterol in the plasma again remain unchanged, as does the rate of biliary cholesterol secretion. The important point to emphasize is that the rate of biliary cholesterol secretion cannot, in some simplistic sense, be directly related to the rate of dietary cholesterol absorption or to the rate of cholesterol synthesis within the body. As will be described later, the physiological disorders that result in cholesterol gallstone formation are probably far more complex than a simple disruption in cholesterol balance across the liver.

E. SOLUBILIZATION OF CHOLESTEROL IN BILE

From these considerations it is apparent that every animal, including man, must excrete a significant amount of cholesterol each day in order to prevent the rapid accumulation of cholesterol in the body. In the case of man, except for the small amounts of cholesterol sloughed with the skin (about 85 mg/day) or converted to sterol hormones (about 50 mg/day), the great majority of the cholesterol that must be removed from the body is secreted by the liver into the bile. In man, this amounts to approximately 1,000 mg/day. However, cholesterol is a very hydrophobic molecule that possesses only a single hydroxyl group that is capable of hydrogen bonding with water. Hence, it is very insoluble in an aqueous environment. If 1,000 mg of cholesterol were secreted directly into the aqueous environment of normal hepatic bile the cholesterol molecules would associate through hydrophobic bonding and immediately form crystals that would precipitate within the small biliary radicals.

For this reason, special mechanisms have evolved for solubilizing cholesterol, at least temporarily, in bile. To accomplish this solubilization, a portion of the cholesterol destined for export out of the liver cell is first

converted to bile acids. This biochemical conversion involves four specific biochemical transformations, the function of which is to convert the very hydrophobic cholesterol molecule into a far more hydrophilic one that can interact readily with water molecules through hydrogen bonding. These biochemical transformations are illustrated in Fig. 8. First, the size of the sterol molecule is reduced. Cholesterol contains 27 carbon atoms. During the formation of bile acids the terminal three carbon atoms are cleaved from the molecule and the C-24 carbon is oxidized to an acid group. Second, the double-bond that exists in cholesterol between the 5th and 6th carbon atom is saturated and the hydrogen atom at this location is turned upward into the beta position. Third, a varying number of hydroxyl groups are then added to the sterol ring structure at various locations. These are commonly in the alpha configuration although in some animals hydroxyl groups also are present in the beta configuration. Finally, before the newly synthesized bile acid molecule is excreted from the liver cell, it is conjugated at the C-24 position with either taurine or glycine. This reduces the pKa for the acid from approximately 6.5 in the unconjugated bile acid to 3.5 and 1.5, respectively, in glycine and taurine conjugated bile acids. The net effect of these biochemical transformations is to convert cholesterol, a molecule that is essentially totally insoluble in water, into a strong amphipathic bile acid molecule. As illustrated in Fig. 9, such amphipathic molecules have polar and nonpolar regions asymmetrically distributed over the surface of the molecule. When placed in water, the hydrophobic surface is forced out of solution and will associate through hydrophobic

Fig. 8

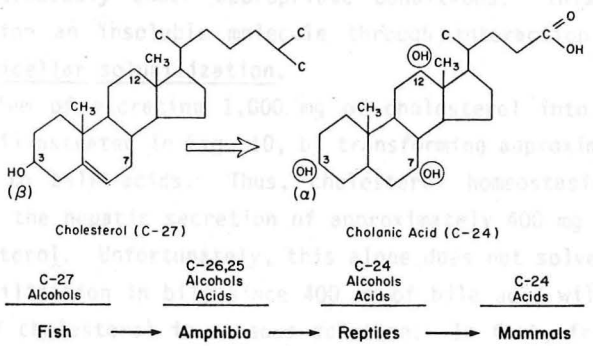
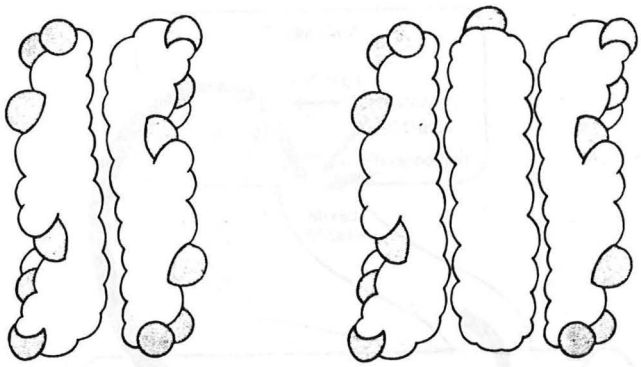


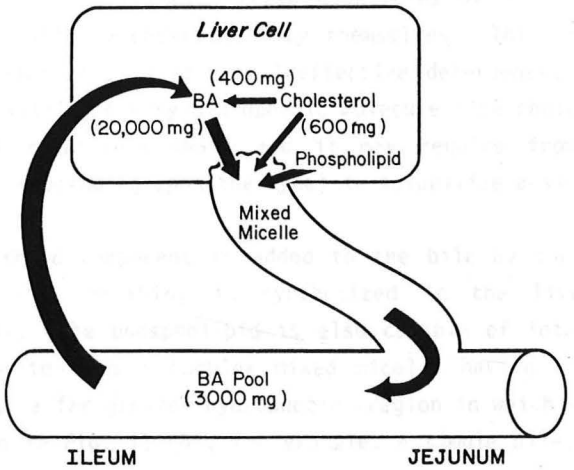
Fig. 9



bonding with the hydrophobic surface of adjacent molecules. The hydrophilic groups (the acid group and hydroxyl groups) stick outward into the aqueous environment where they tightly bond to water. Hence, this "association colloid" or simple micelle gains solubility in water. Since the energy state of the molecules in the simple micelle is lower than when these molecules are disbursed in monomolecular form, such micelles form spontaneously in aqueous solution (i.e., this is a negative free-energy process). Another property of these micelles is also illustrated in the right side of Fig. 9. Under certain circumstances, very insoluble, hydrophobic molecules may be inserted into the interior of the simple micelle. Since the hydrophobic surfaces of such an insoluble molecule can readily interact with the hydrophobic, interior surfaces of the bile acid molecules, such a structure is again thermodynamically stable and will form spontaneously under appropriate conditions. This process of bringing into solution an insoluble molecule through interaction with simple micelles is called micellar solubilization.

Thus, the problem of excreting 1,000 mg of cholesterol into the bile is partially solved as illustrated in Fig. 10, by transforming approximately 400 mg of the cholesterol to bile acids. Thus, cholesterol homeostasis in man is actually achieved by the hepatic secretion of approximately 400 mg of bile acid and 600 mg of cholesterol. Unfortunately, this alone does not solve the problem of cholesterol solubilization in bile since 400 mg of bile acid will not nearly solubilize 600 mg of cholesterol in aqueous solution. In fact, from 50 to 100 times more bile acid would be required than is actually synthesized each day to

Fig. 10



fully solubilize 600 mg of cholesterol through the process of micellar solubilization. Two additional processes are required. First, mechanisms were evolved to reutilize the relatively small amount of bile acid that is synthesized each day. This was accomplished by the development of reabsorptive processes located principally in the ileum, that reclaim essentially all bile acid that reaches the gastrointestinal tract. Thus, as shown in Fig. 10, the total bile acid pool present in normal man equals approximately 3,000 mg. Since this pool is reabsorbed and reutilized 6-7 times each day, the actual amount of bile acid secreted across the cannalicular membrane of the liver cell equals approximately 20,000 mg/day. This intestinal absorption and resecretion process is referred to as the enterohepatic circulation of bile acid. Thus, even though only 400 mg of bile acid is actually synthesized in the liver cell each day, fully 20,000 mg may actually be secreted. This process, it should be emphasized, is critically dependent upon the active transport sites in the gastrointestinal tract that are capable of reabsorbing nearly all bile acid reaching the terminal ileum. Any disease process or surgical procedure that alters ileum function leads to rapid loss of bile acids from the body and to rapid depletion of the circulating bile acid pool. Even though synthesis in the liver may increase 3- to 4-fold this compensatory increase in synthesis can still only supply approximately one tenth the amount of bile acid normally fluxing through the enterohepatic circulation. While the enterohepatic

circulation effectively amplifies the effectiveness of bile acids in solubilizing cholesterol in bile, even these quantities of bile acid are not sufficient to fully solubilize cholesterol by themselves. This is due to the fact that bile acids alone are relatively ineffective detergents, particularly when it comes to solubilizing a very hydrophobic molecule like cholesterol. The bile acid micelle is relatively small and it may require from 50 to 400 molecules of bile acid (depending upon the type) to solubilize a single molecule of cholesterol.

Consequently, a third component is added to the bile by the liver cell. Phospholipid, principally lecithin, is synthesized in the liver and also secreted into the bile. The phospholipid is also capable of interacting with the bile acid micelle to form a complex mixed micelle having a far greater volume and, therefore, a far greater hydrophobic region in which to solubilize cholesterol. As shown in Fig. 11 (B), for example, a simple bile acid micelle

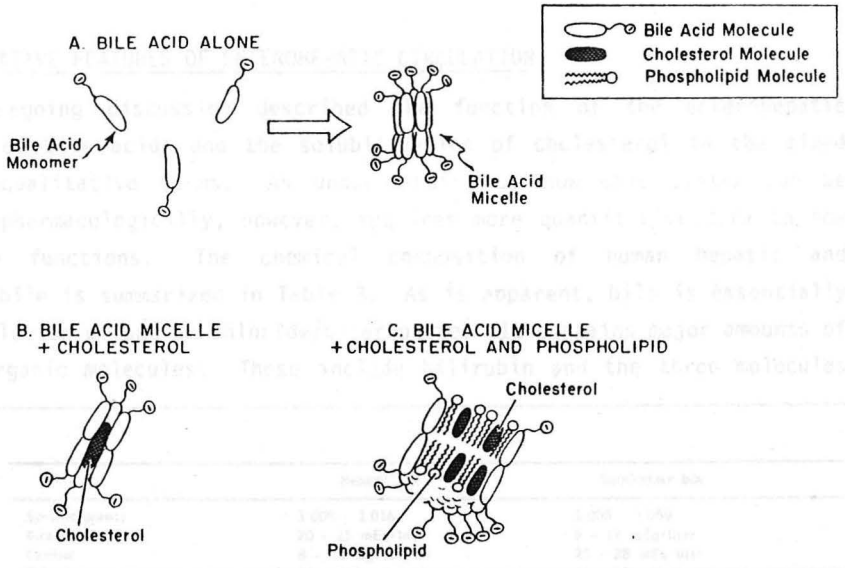


Fig. 11

may consist of only 20-30 bile acid molecules: such a structure is barely able to solubilize one molecule of cholesterol. With the addition of phospholipid, however, (C) a far larger structure is formed. The fatty acid chains of the phospholipid molecules adhere together through hydrophobic bonding and form a double layer with their hydrophilic groups projecting outward into the aqueous

environment. The exposed hydrophobic surfaces of this cylinder are coated by interaction with the hydrophobic surfaces of the bile acids. Since the hydrophilic groups of the bile acids project outward into the water this very large mixed micelle is stable in an aqueous environment. Such a mixed micelle is capable of solubilizing much more cholesterol than the bile acid micelle alone.

Thus, the problem of excreting 1,000 mg of cholesterol each day is accomplished through the interaction of three separate processes. These include the conversion of approximately 400 mg of cholesterol to bile acid, the amplification of the physiological effect of this small amount of bile acid through the enterohepatic circulation and the enhancement of the solubilizing effect of bile acids by the addition of phospholipids to the bile. In this manner, the cholesterol can be transported in a thermodynamically stable, soluble form through the hepatobiliary tree until it reaches the intestinal lumen.

F. QUANTITATIVE FEATURES OF ENTEROHEPATIC CIRCULATION

The foregoing discussion described the function of the enterohepatic circulation of bile acids and the solubilization of cholesterol in the mixed micelle in qualitative terms. An understanding of how this system can be manipulated pharmacologically, however, requires more quantitative data on how this system functions. The chemical composition of human hepatic and gallbladder bile is summarized in Table 3. As is apparent, bile is essentially a watery solution of sodium chloride/bicarbonate. It contains major amounts of only four organic molecules. These include bilirubin and the three molecules

Table 3

	Hepatic bile	Gallbladder bile
Specific gravity	1.008 - 1.016	1.008 - 1.059
Bicarbonate	20 - 25 mEq/liter	8 - 12 mEq/liter
Calcium	8 - 11 mg/100 ml	25 - 28 mEq/liter
Chloride	90 - 100 mEq/liter	16 - 19 mEq/liter
Bilirubin	17 - 71 mg/100 ml	50 - 1000 mg/100 ml
Water	97 - 98%	84%
Mucins		1 - 4%
Bile acids	1.24 - 1.72 gm/100 ml	2.3 - 7.7gm/100 ml
Cholesterol	86 - 176 mg/100 ml	100 - 900 mg/100 ml
Fatty acids	101 - 438 mg/100 ml	80 - 1600 mg/100 ml
Lecithins	250 mg/100 ml	350 mg/100 ml

involved in cholesterol secretion (bile acids, phospholipid and cholesterol). The bile is concentrated in the gallbladder, but the relative concentrations of the major organic constituents do not change appreciably.

The bile acids present in bile are a complicated mixture of different specific bile acids that have very different physical chemical characteristics. These characteristics profoundly influence the ability of bile to solubilize cholesterol. As illustrated in Fig. 8, during the conversion of cholesterol to bile acids, one of the principle biochemical modifications is the addition of hydroxyl groups at various points on the steroid nucleus. These hydroxyl groups form, on average, two hydrogen bonds each with water and so greatly increase the hydrophilicity of the molecule. Since the hydroxyl groups may be added at nearly any position on the sterol molecule and in either the beta (up, out of the plane of the molecule) or alpha (downward) positions, many isomeric forms are possible. Fortunately, in man, there are only a relatively small number of bile acids of biological or pharmacological importance. The structure of these bile acids is summarized in Table 4.

TABLE 4

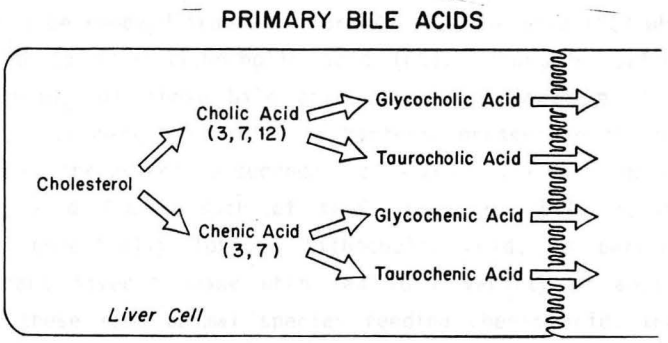
NUMBER OH GROUPS	SYSTEMATIC NAME	COMMON NAME	ABBREVIATION AFTER CONJUGATION TO GLYCINE OR TAURINE
1	3α OH cholanic acid	Lithocholic acid	TLC, GLC
2	3α, 7α diOH cholanic acid	Chenic acid	TCD, GCD
2	3α, 12α diOH cholanic acid	Deoxycholic acid	TDC, GDC
2	3α, 7β diOH cholanic acid	Ursodeoxycholic acid	TUDC, GUDC
3	3α, 7α 12α triOH cholanic acid	Cholic acid	TC, GC

There are four bile acids of particular importance to understanding the physiology of human bile. In man, for practical purposes, bile acids are modified by changing the number of hydroxy groups at the 3, 7 and 12 positions. There is one monohydroxy bile acid where the hydroxyl group is added in the 3α position. This bile acid is called lithocholic acid. There are two dihydroxy bile acids found in man in which the hydroxyl groups are added in either

the 3α, 7α or 3α, 12α positions: these bile acids are called chenic acid and deoxycholic acid, respectively. There is only one trihydroxy bile acid in man and this compound has hydroxyl groups in the 3α, 7α and 12α positions and is called cholic acid. While not present in any significant amounts in normal man, ursodeoxycholic acid has become important as an agent for dissolving gallstones and is an isomer of chenic acid having hydroxyl functions in the 3α and 7β positions. Some of these bile acids are actually synthesized in the human liver while others represent the products of bacterial degradation of bile acid within the lower smaller intestine and colon of man.

By definition, primary bile acids are those bile acids that are made in the liver cell directly from cholesterol. In man there are, for practical purposes, only two primary bile acids. These include chenic acid (CD) and cholic acid (C). Since each of these primary bile acids is conjugated to both glycine and taurine, the four primary bile acids appearing in human bile include TCD, GCD, TC, and GC. Once the primary bile acids are excreted into the

Fig. 12



gastrointestinal tract, they come into contact with a variety of bacteria living in the lower small intestine and colon. These organisms, particularly the anaerobic organisms, possess two groups of enzymes that are capable of modifying the chemical structure of the bile acids. In particular, these organisms have enzymes that will deconjugate the bile acids and enzymes which will remove the hydroxyl group present in the 7α configuration. It should be noted that there are very few species of bacteria in the human intestine which can remove the hydroxyl group in the 7β position (as is present on ursodeoxycholic acid). As a result of contact with these bacteria the various conjugated primary bile acids will be metabolized to unconjugated C and CD. A portion of these unconjugated

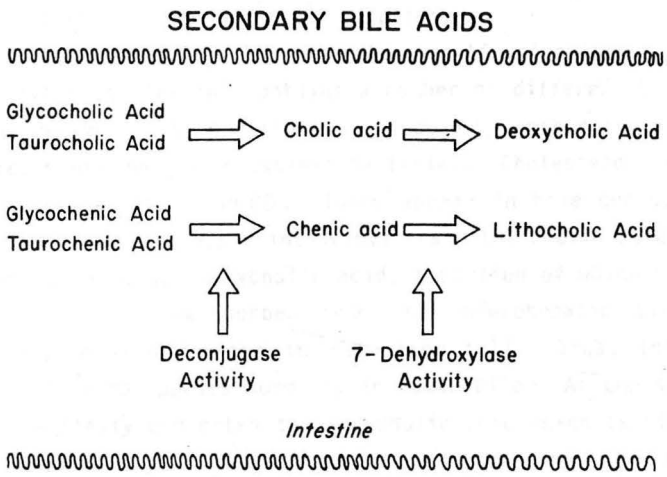


Fig. 13

bile acids will be subjected to further degradation and, for example, the 7α hydroxy group will be removed from C to form deoxycholic acid (DC) while CD will be dehydroxylated to form lithocholic acid (LC). Thus, by definition, the secondary bile acids are those bile acids that are formed by the metabolic degradation of the primary bile acids by bacteria present in the normal human intestine. In man, the principle secondary bile acids are deoxycholic acid (DC) and lithocholic acid (LC). Both of these secondary bile acids are very hydrophobic and potentially toxic. Lithocholic acid, in particular, will produce significant liver disease when fed to a variety of animal species. Furthermore, in these same animal species feeding chenic acid, the immediate precursor for lithocholic, leads to excessive lithocholic acid production and liver disease. It becomes of considerable importance, therefore, that lithocholic acid not be captured in the enterohepatic circulation, but essentially be quantitatively excreted once formed. This is accomplished by a third modification of the bile acid molecule. Once lithocholic acid is formed in the gastrointestinal tract, it is reabsorbed and carried back to the liver in the portal blood. There it is not only conjugated to glycine or taurine, but in addition, is sulfated. This makes the molecule very hydrophilic and prevents its subsequent reabsorption from the gastrointestinal tract once it has been reexcreted in the bile. Man, in particular, is relatively resistant to the

toxic effects of lithocholic acid feeding (or chenic acid feeding) because of a high capacity to carry out the sulfation reaction.

Thus, as summarized in Fig. 14, the enterohepatic circulation of bile acids in man is relatively complex and contains a number of different bile acids that result from the metabolism of the steroid molecule by both enzymes in the liver cell and enzymes contained in intestinal bacterial. Cholesterol is metabolized to the primary bile acids C and CD. These appear in bile conjugated to both taurine and glycine. In the gastrointestinal tract the cholic acid is converted to the secondary bile acid, deoxycholic acid, a portion of which is excreted in the feces while some is reabsorbed into the enterohepatic circulation and conjugated to glycine and taurine in the liver cell. Thus, this conjugated secondary bile acid also appears normally in human bile. At the same time, the chenic acid is partially converted to lithocholic acid which is either excreted in the feces or partially reabsorbed into the enterohepatic circulation. The lithocholic acid that reaches the liver cell is conjugated to glycine or taurine and, in addition, is sulfated. This very polar, sulfated bile acid is then excreted essentially quantitatively, in the feces. As a result of these complex interactions, in the steady state, the bile acid pool in man is made up of approximately 45% cholic acid, 37% chenic acid, 15% deoxycholic acid, and only 1-3% lithocholic acid. This composition can, of course, be radically changed by

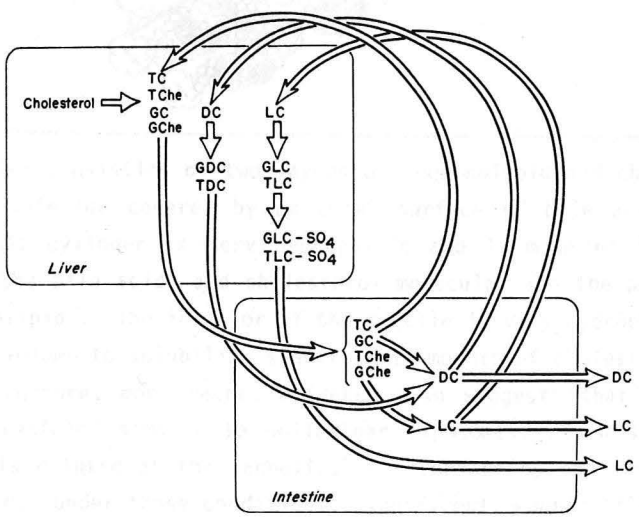


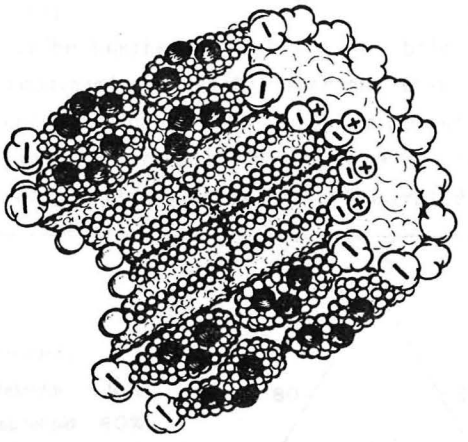
Fig. 14

feeding exogenous bile acids. In general, such treatments result in partial suppression of bile acid synthesis in the liver and replacement of these various normal bile acids by the particular bile acid that was fed orally.

G. QUANTITATION OF THE DEGREE OF BILIARY CHOLESTEROL SATURATION

From these considerations it is apparent that bile is a very complex mixture of detergent molecules (the various bile acids), swelling amphipaths (phospholipids) and an insoluble amphipath (cholesterol). In some fashion, not wholly understood at this time, these three groups of molecules must be secreted across the hepatocyte and come together in a mixed micelle of very specific composition. As illustrated diagrammatically in Fig. 15, this mixed micelle is

Fig. 15



essentially a cylinder consisting of two layers of phospholipid and cholesterol in the hydrophobic interior covered by an outer surface of bile acids. The outer surface of this cylinder is very hydrophilic and is made of the polar hydroxyl groups on the bile acids and cholesterol molecules and the polar head group of the phospholipids. The interior of the micelle is very hydrophobic and contains sufficient volume to solubilize significant amounts of cholesterol. In addition to this structure, more recent evidence also suggests that bile may contain unilaminar vesicles similar to unilaminar liposomes. Such structures may form when bile is diluted at the cannalicular interface by the inflow of a large volume of fluid. Under these conditions, significant amounts of bile acid

must leave the mixed micelle to maintain the critical micelle concentration in the aqueous phase. The mixed micelle becomes "bile acid poor" and spontaneously forms unilaminar vesicles consisting principally of phospholipids and cholesterol. These vesicles are very large, of the order of 400 angstroms, while mixed micelles are much smaller, of the order of 50 angstroms.

In order to make such structures as the mixed micelle it is obviously necessary to have the right proportions of the three types of lipid molecules present in bile. That is to say, it is critically important that the relative concentrations of bile acid, phospholipid and cholesterol be appropriate in order to bring about stable solubilization of the cholesterol molecules. One of the major advances in this field came with the recognition of this important quantitative relationship.

In order to describe whether or not a given bile sample will solubilize cholesterol in a thermodynamically stable fashion, it is necessary to take into consideration the concentration of the three components of the system, bile acids, phospholipids and cholesterol. This is usually done by plotting the data in a triangular reference system such as that illustrated in Fig. 16. In such a

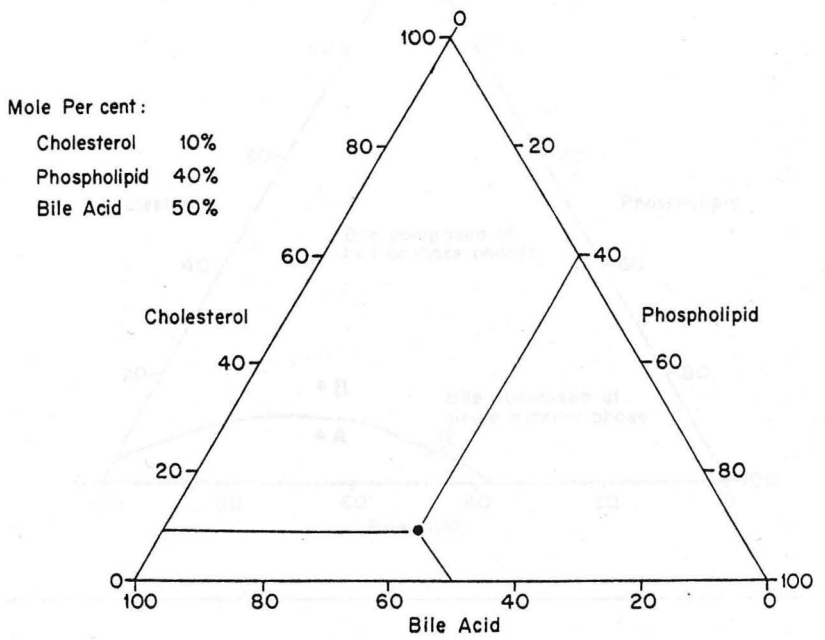
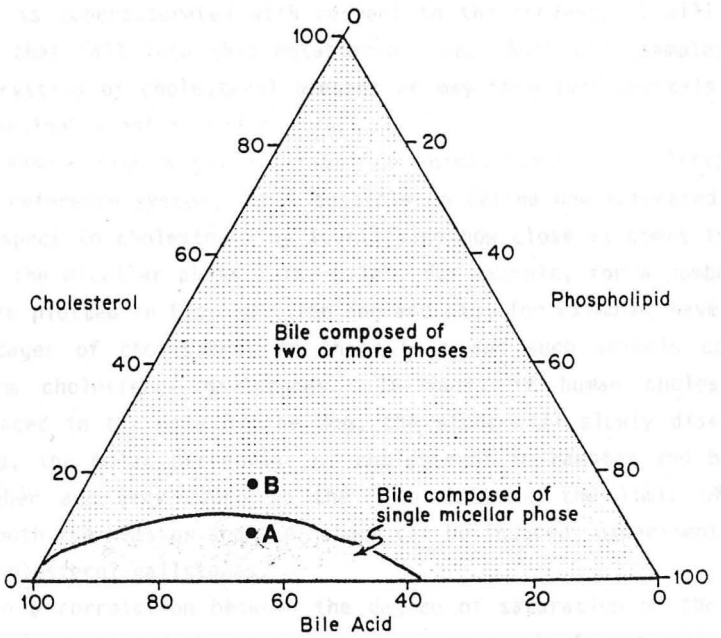


Fig. 16

system the absolute concentration of the three components is determined and the relative concentration of each component is then expressed as a percentage of the total concentration. In the example shown in Fig. 16, for example, 10% of the molecules in the bile sample are cholesterol, 40% are phospholipid and 50% are bile acids. These values represent the molar percentages of each of the three components. Since the sum of these molar percentages equals 100%, the composition of this bile sample can be plotted as a single point in the triangular reference system. Conventionally, phospholipids are plotted on the right axis, bile acids on the lower axis and cholesterol on the left axis.

Thus, the composition of any possible mixture of these three components can be represented as a single point within the triangle. However, it was recognized a number of years ago that stable, mixed micelles of the type illustrated in Fig. 15 were formed only when the composition of the bile sample fell within very narrow limits. These limits are shown as the shaded area in the left lower corner of the triangle illustrated in Fig. 17. Thus, for

Fig. 17



example, bile sample A would be found to contain a relatively uniform population of mixed micelles that were very stable and that would hold the cholesterol in solution without any crystallization taking place. In contrast, any bile sample whose composition falls outside this shaded zone invariably had two or more phases present. One of these phases was usually a microcrystaline phase of cholesterol monohydrate since these solutions contained relatively more cholesterol than could be effectively solubilized by the bile acid and phospholipid.

More recent work has indicated that the situation is more complex than suggested by this simple model. There is clearly another region of bile compositions that lie just above and along the limiting line defining the micellar phase. This region defines an area of metastability that is not truly in thermodynamic equilibrium. There is an excess amount of cholesterol present in these samples that would crystalize out under appropriate conditions. However, these samples can remain stable for long periods of time and probably contain a mixture of mixed micelles and unilaminar vesicles. Many patients who secrete bile that is supersaturated with respect to the cholesterol will have bile compositions that fall into this metastable area. Such bile samples may have gross microcrystals of cholesterol present or may form such crystals when an appropriate "seeding" agent is added.

When a bile sample from a given patient or animal species is plotted on such a triangular reference system, it is possible to define how saturated that sample is with respect to cholesterol by identifying how close it comes to the limiting line for the micellar phase. The biles, for example, for a number of animals species are plotted in Fig. 18. The dog and pig, for example, have very low molar percentages of cholesterol in their bile and such animals do not spontaneously form cholesterol gallstones. In fact, if human cholesterol gallstones are placed in the bile of the dog, the stone will slowly dissolve. On the other hand, the molar percentage of cholesterol in hamster and baboon bile is much higher and very close to the line defining the limit of the micellar phase. both the hamster and the baboon can be induced, experimentally, into developing cholesterol gallstones.

There is also a correlation between the degree of saturation of the bile with respect to cholesterol and the incidence of spontaneously formed gallstones in human populations. As illustrated in Fig. 19, for example, Scandinavian

Fig. 18

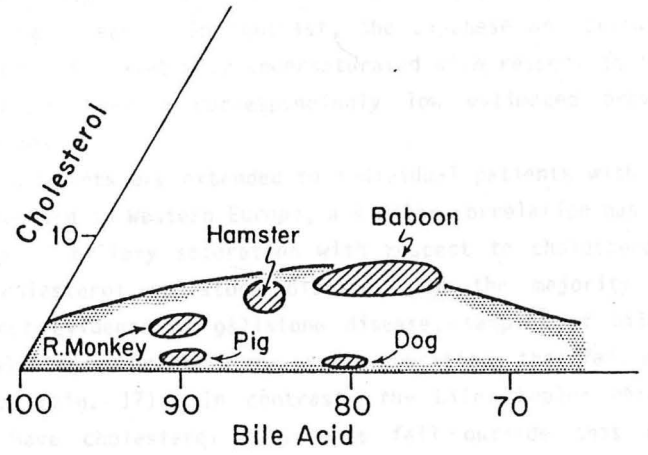
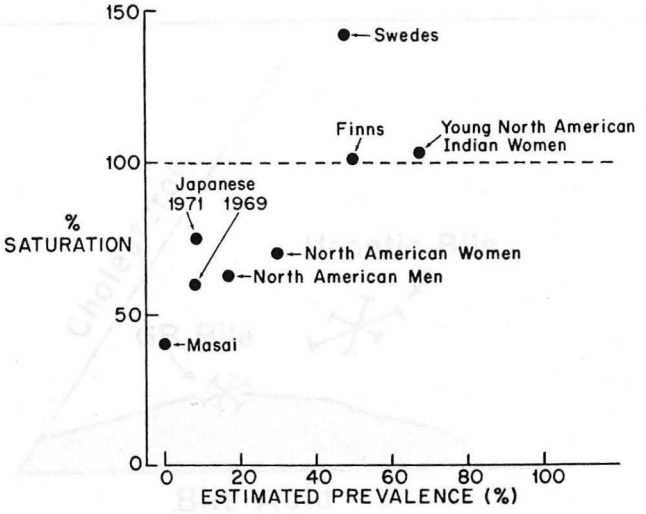

Cholesterol
10
Hamster
Baboon
R.Monkey
Pig
Dog
100
90
80
70
Bile Acid

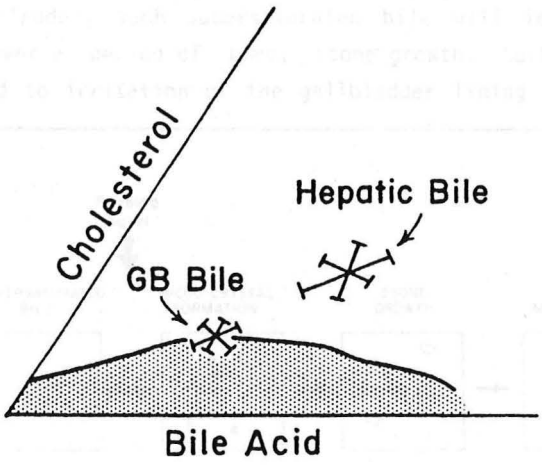
Fig. 19


150
100
50
0
% SATURATION
Swedes
Finns
Young North American Indian Women
Japanese 1971
1969
North American Women
North American Men
Masai
0
20
40
60
80
100
ESTIMATED PREVALENCE (%)

groups such as Swedes and Finns and some groups of Southwestern American Indians (the Pima) have a bile which is saturated or even supersaturated with respect to cholesterol. These populations have an exceedingly high prevalence of cholesterol gallstone disease. In contrast, the Japanese and certain African tribes have bile which is relatively undersaturated with respect to cholesterol and these populations have a correspondingly low estimated prevalence of cholesterol gallstones.

When such measurements are extended to individual patients with gallstones in the United States and in Western Europe, a similar correlation has been found between the degree of biliary saturation with respect to cholesterol and the development of cholesterol gallstone disease. In the majority of normal individuals, without evidence of gallstone disease, samples of bile obtained from either the gallbladder or the liver have compositions that fall well within the micellar phase (Fig. 17). In contrast, the bile samples obtained from patients who do have cholesterol gallstones fall outside this range. As illustrated in Fig. 20, for example, in a large group of patients with cholesterol gallstones, the composition of the bile obtained from the gallbladder fell at or above the line illustrating maximal cholesterol solubility

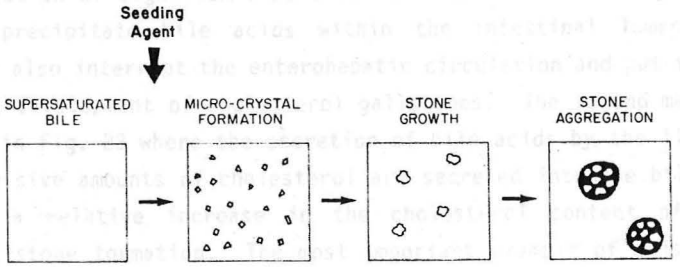
Fig. 20



The results obtained with the hepatic bile obtained from the same patients was even more striking and revealed that nearly twice as much cholesterol was present as could be effectively solubilized in a mixed micelle by the amounts of bile acid and phospholipid present in the samples, i.e., these hepatic biles were very supersaturated with respect to cholesterol.

The presence of such supersaturated bile now appears to be a prerequisite for the formation of cholesterol gallstones. However, not all individuals with such supersaturated bile will go on to develop stones. It is now clear that some bile samples that are supersaturated with respect to cholesterol are, nevertheless, relatively stable. Presumably, the cholesterol in these samples is carried in a metastable particle such as a unilaminar liposome. Furthermore, the gallbladder may contribute significantly to gallstone formation either by exuding substances (like mucin) that act as the seeding agent for promoting crystallization from the supersaturated bile or, alternatively, may even secrete substances that inhibit such crystallization. Thus, the current concepts concerning the formation of cholesterol gallstones are illustrated in Fig. 21. The primary defect appears to be the production of bile by the liver which contains more cholesterol than can be solubilized in a thermodynamically stable form. Some patients may produce such supersaturated bile most of their life and never develop stones; however, in the presence of an appropriate environment within the gallbladder, such supersaturated bile will lead to microcrystal formation and, over a period of time, stone growth. Such small stones or "gravel" may lead to irritation of the gallbladder lining and to exudation of

Fig. 21

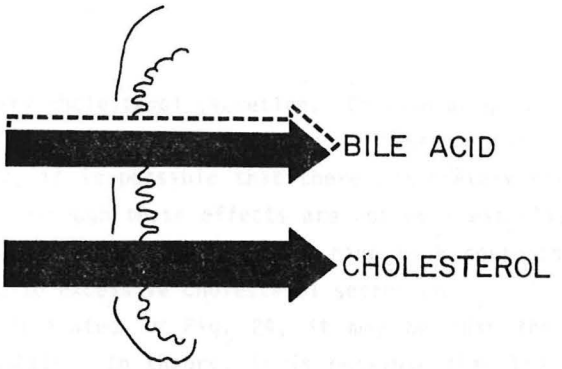


mucinous materials. These proteinaeous materials, along with free-fatty acids and calcium lead to aggregation of the small cholesterol stones into the larger, faceted aggregate stones typical of cholesterol gallstone disease.

H. PHYSIOLOGICAL DEFECTS RESULTING IN SUPERSATURATED BILE AND CHOLESTEROL GALLSTONE FORMATION

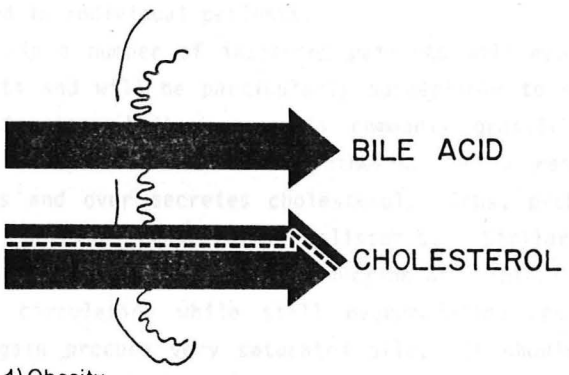
Since the fundamental defect in cholesterol gallstone disease is the production of bile by the liver which is supersaturated with respect to cholesterol, the next question of importance is what physiological defects lead to the production of such supersaturated bile. The three general possibilities are illustrated in Figs. 22, 23 and 24. The first possibility (Fig. 22) is that the daily secretion of bile acid is less than normal. If the rate of biliary cholesterol secretion remains normal then the bile will necessarily become supersaturated with respect to cholesterol because of the relative decrease in the output of bile acid. As already discussed (Fig. 10) the rate of secretion of bile acid by the liver cell is dependent on both the rate of bile acid synthesis and the integrity of the enterohepatic circulation. Thus, disruption of these processes invariably leads to the production of supersaturated bile. This is seen, for example, in certain groups of American Indians who have a defect in bile acid synthesis that leads to a smaller-than-normal bile acid pool and supersaturated bile. Any patient who has ileal dysfunction (and, hence, interruption of the enterohepatic circulation) is at risk for gallstone formation. This includes individuals who have a chronic disease of the terminal ileum (such as Crohn's disease or ileal tuberculosis) or patients who have had surgical a resection of significant portions of the ileum. Finally, any drugs that bind or precipitate bile acids within the intestinal lumen (such as cholestyramine) also interrupt the enterohepatic circulation and put the patient at risk for the development of cholesterol gallstones. The second major defect is illustrated in Fig. 23 where the secretion of bile acids by the liver may be normal but excessive amounts of cholesterol are secreted into the bile. Again, this leads to a relative increase in the cholesterol content of bile and cholesterol gallstone formation. The most important example of this defect is seen in obese patients. In general, the rate of cholesterol synthesis by the body increases with body weight and this appears to be reflected by an increase

Fig. 22



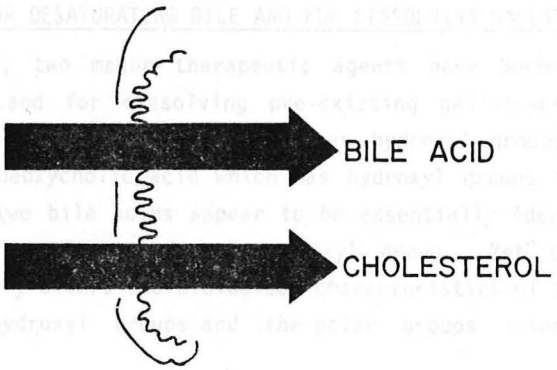
1) American Indian
2) Ileal Dysfunction
3) Drugs That Bind or Precipitate BA

Fig. 23



1) Obesity
2) Drugs
3) Diets

Fig. 24



in the rate of biliary cholesterol secretion. Certain drugs also lead to marked increases in biliary cholesterol secretion. These included compounds like clofibrate. Finally, it is possible that there are dietary effects on biliary cholesterol output, although these effects are not well established. Possibly, diets that are rich in cholesterol or that have high contents of unsaturated fatty acids may lead to excessive cholesterol secretion.

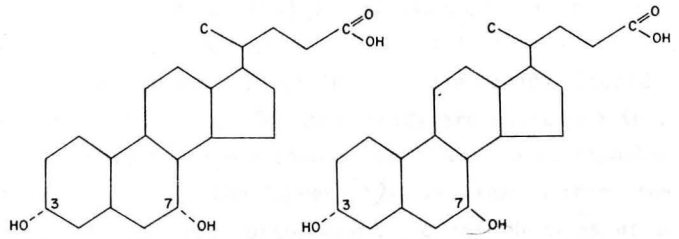
Finally, as illustrated in Fig. 24, it may be that the defect in bile formation is more subtle. In theory, it is possible that the rate of biliary secretion of bile acids and cholesterol is normal but the types of bile acids in the bile acid pool have changed in such a way that they solubilize cholesterol less well. Such a defect has not yet been clearly established in man although current work is underway to better define the hydrophobic/hydrophilic balance of the bile acids found in individual patients.

Unfortunately, in a number of instances patients will manifest more than one of these defects and will be particularly susceptible to stone formation. For example, the American Indian woman is commonly grossly obese and, in addition, has the defect in bile acid synthesis. As a result, she under secretes bile acids and over secretes cholesterol. Thus, probably 60-80% of these women will develop cholesterol gallstones. Similarly, the obese individual who is fasted to promote weight reduction will interrupt his/her bile acid enterohepatic circulation while still overproducing cholesterol. Such individuals will again produce very saturated bile. It should be emphasized that these various physiological abnormalities are extremely common in Western populations so that a very large segment of the people is at risk with respect to gallstone production.

I. AGENTS USED FOR DESATURATING BILE AND FOR DISSOLVING GALLSTONES IN SITU

Very recently, two major therapeutic agents have become available for desaturating bile and for dissolving pre-existing gallstones. As shown in Fig. 25, these include chenic acid which has hydroxyl groups in the 3α, 7α positions and ursodeoxycholic acid which has hydroxyl groups in the 3α and 7β positions. These two bile acids appear to be essentially identical and differ only in the configuration of the 7 hydroxyl group. Yet this very subtle difference profoundly alters the biological characteristics of this steroid. In chenic acid the hydroxyl groups and the polar groups associated with the

Fig. 25

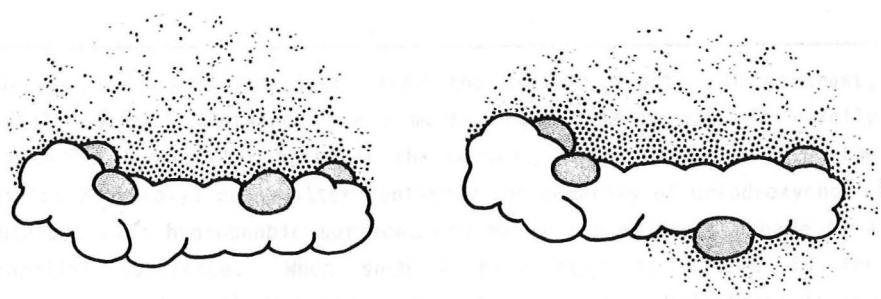


3αOH, 7αOH Cholanic Acid
Chenodeoxycholic (Chenic) Acid
Chenix®(Rowell)

3αOH, 7βOH Cholanic Acid
Ursodeoxycholic Acid
Deursil-RR®(Giphармex, Falk)

conjugated amino acid at the C-24 position all project on to one side of the planar molecule so that, as illustrated in Fig. 26, this compound has one surface which is very hydrophilic and readily interacts with water and another surface which is very hydrophobic and readily interacts with membranes and cholesterol. Thus, chenic acid is a very potent amphipathic detergent. In contrast, when the hydroxyl function in the 7 position rotates into a beta configuration, it totally disrupts the hydrophobic surface by allowing hydrogen bonding to take place on both sides of the molecule. As a result of this subtle

Fig. 26



Chenodeoxycholic Acid

Ursodeoxycholic Acid

change there is a profound alteration in the hydrophobic/hydrophilic balance in this molecule and it behaves biologically very differently from chenic acid. This change is most dramatically illustrated using reversed-phase, high-performance liquid chromatography. As illustrated in Fig. 27, this procedure involves separation of different bile acids according to their hydrophilic/hydrophobic balance. The bile acids are dissolved in a hydrophilic phase and allowed to move through a column coated with a hydrophobic phase. The more hydrophobic a bile acid, the longer it is retained within the column. As illustrated by the chromatogram, ursodeoxycholic acid behaves as a very hydro-

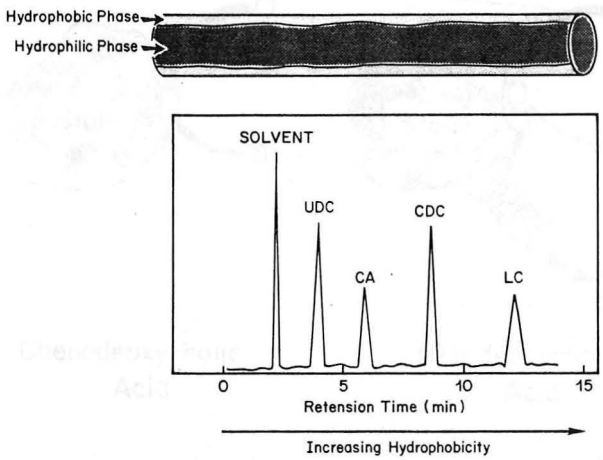


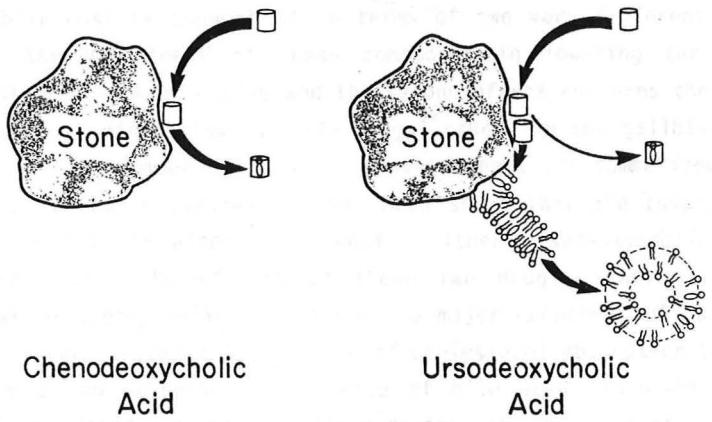
Fig. 27

philic molecule and comes out just after the solvent front. In contrast, chenodeoxycholic acid is retained for a much longer time because it readily interacts with the hydrophobic phase in the column. Thus, merely shifting the position of the 7 hydroxyl group alters entirely the capacity of ursodeoxycholic acid to interact with hydrophobic surfaces and makes the molecule behave as a very hydrophilic substance. When such a bile acid is placed in the enterohepatic circulation, it does not readily interact with cholesterol in the liver to mobilize it into the bile, it cannot interact with lipids and cholesterol in the intestine to promote their absorption and it cannot interact with membrane receptor sites to turn on intestinal secretion.

Finally, data also suggest that ursodeoxycholic acid cannot interact with the regulatory sites in the hepatocyte that control the rate of bile acid synthesis.

In addition to these very marked differences in behavior, chenic and ursodeoxycholic acid dissolve gallstones by different mechanisms and at different rates. These two mechanisms are illustrated diagrammatically in Fig. 28. When a gallstone is perfused with a solution containing mixed micelles of chenodeoxycholic acid, the mixed micelles diffuse up to the surface of the stone, interact with cholesterol molecules on the surface and then diffuse away.

Fig. 28



Essentially, the rate at which this occurs is determined by the rate of diffusion of the mixed micelles through the aqueous bile surrounding the stone. In contrast, when the bile is made up predominantly of ursodeoxycholic acid micelles a second process seems to be quantitatively very important in stone dissolution. As the mixed micelle comes in contact with the stone the hydrophobic attraction between the phospholipids in the micelle and the cholesterol in the stone is greater than the attraction of the phospholipids for the ursodeoxycholic acid: again, this is a manifestation of the hydrophilic character of UDC. As a consequence of this, the residency time of the micelle at the interface is prolonged and phospholipid molecules are transferred from the micelle structure to the surface of the stone. These phospholipid molecules interact with the cholesterol to form liquid crystals that rapidly penetrate and

disintegrate the stone and "bud off" as unilaminar vesicles. Thus, because of the very hydrophilic nature of the ursodeoxycholic acid micelles there is much less effective micellar solubilization from the stones, but there is rapid dissolution of the stones through the formation of liquid crystals. Hence, under in vitro conditions cholesterol is much more rapidly solubilized by ursodeoxycholic acid than by chenodeoxycholic acid, and the same situation appears to also take place in vivo.

J. CLINICAL EFFECTS OF CHENODEOXYCHOLIC AND URSODEOXYCHOLIC ACID

The clinical effectiveness of these two bile acids in altering the physiology of bile must be thought of in terms of two very different effects: one effect is the usefulness of these compounds in lowering the relative cholesterol content in hepatic bile and the second effect concerns the efficacy of these compounds in dissolving cholesterol gallstones in the gallbladder and, to some extent, in the common bile duct. These effects are summarized in Fig. 29 and Table 5. In human studies, it has been shown that the composition of bile can be significantly altered by feeding either ursodeoxycholic acid or chenodeoxycholic acid. The effects of these two drugs, however, are very different. When ursodeoxycholic acid becomes a major component of human bile, there is a significant decrease in the rate of cholesterol absorption (Table 5). There is little or no decrease in the rate of bile acid synthesis and less cholesterol is recruited out of the liver during the movement of these bile acids from the sinusoidal to the cannalicular surface. As a consequence of all

Fig. 29

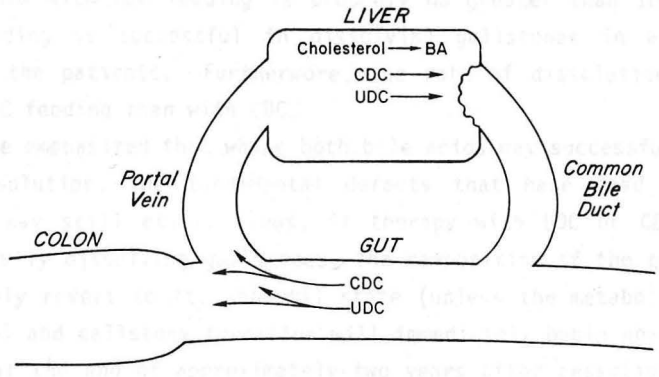


Table 5

	UDC	CDC
Cholesterol Absorption	↓	↓
Bile Acid Synthesis	↓	↓
Recruitment of Hepatic Cholesterol	↓	↓
Biliary Cholesterol Saturation	↓	↓
Gallstone Dissolution	40-60%	10-20%
Recurrence Rate	50%	50%

of these effects, feeding UDC results in a significant decrease in the degree of cholesterol saturation in the bile. With the CDC feeding there is little or no change in the rate of cholesterol absorption. There is marked suppression of endogenous bile acid synthesis and there is little change in the amount of cholesterol recruited from the liver during passage of the bile acids through the enterohepatic circulation. Again, as a consequence of all of these effects, biliary cholesterol saturation is reduced although the effect is probably quantitatively less than the effect seen with UDC feeding. Thus, both UDC and CDC feeding lead to a decrease in the degree of saturation of bile with cholesterol. There is a striking difference, however, between these two bile acids in terms of their effectiveness in bringing about gallstone dissolution. The success rate with CDC feeding is probably no greater than 10-20% overall, while UDC feeding is successful in dissolving gallstones in a far greater percentage of the patients. Furthermore, the rate of dissolution is probably faster with UDC feeding than with CDC.

It must be emphasized that while both bile acids may successfully result in gallstone dissolution, the fundamental defects that have lead to gallstone precipitation may still exist. Thus, if therapy with UDC or CDC is stopped after successfully dissolving gallstones, the composition of the patient's bile will immediately revert to its abnormal state (unless the metabolic defect has been corrected) and gallstone formation will immediately begin again. Thus, in most series, at the end of approximately two years after cessation of therapy,

at least 50% of the patients will manifest recurrent gallstones. This recurrence rate is probably the same in patients treated with UDC or CDC.

The potential toxicity of these two agents is summarized in Table 6. As discussed earlier, CDC is a substrate for bacteria that have the 7α dehydroxylase enzyme. Hence, when CDC is fed, large amounts of lithocholic acid

Table 6

	UDC	CDC
Liver Damage	None	⊕ (?)
Elevation of LDL	None	⊕
Diarrhea	None	⊕
Calcification of Stones	7-10%	7-10%

are formed. In experimental animals this leads to very serious liver damage. While the liver of man has a great capacity to detoxify lithocholic acid by sulfation, there is, nevertheless, significant liver function abnormalities seen in fully one third of the patients who are placed on CDC. In contrast, the human intestine contains few bacteria that are capable of removing the 7β hydroxyl group from ursodeoxycholic acid; hence, feeding this bile acid does not generate large amounts of lithocholic acid and there is no evidence that it produces liver toxicity in either animals or man. Since UDC feeding does not inhibit bile acid synthesis and actually decreases the rate of cholesterol absorption, there is no elevation of LDL-cholesterol during therapy with this agent. In contrast, CDC feeding suppresses bile acid synthesis and has little effect on cholesterol absorption so that it is likely that after long-term feeding there will be a progressive rise in the circulating levels of plasma cholesterol, particularly in the LDL fraction. Since UDC is so hydrophilic, it does not interact with the colonic membrane and produce diarrhea, whereas CDC feeding commonly produces a bothersome diarrhea by increasing the rate of colonic electrolyte and water secretion.

Thus, in summary, both UDC and CDC will desaturate bile but UDC is far more effective in bringing about dissolution of pre-existing gallstones. Furthermore, UDC apparently is not toxic to the liver, does not elevate circulating LDL levels and does not produce diarrhea. There seems to be little

question, therefore, that UDC is the superior agent for altering bile physiology. Unfortunately, at this time, only CDC is available in the United States even though UDC has been used extensively in Europe for a number of years.

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APPENDIX

THE FOLLOWING INFORMATION IS FOR THE CLINICAL USE OF CHENIX, A PREPARATION OF CHENIC ACID, WHICH IS THE ONLY BILE ACID CURRENTLY APPROVED IN THE UNITED STATES FOR THE DISSOLUTION OF GALLSTONES.

How and Why CHENIX® Works: Bile Acid Metabolism/Mechanism of Action

Bile is, among other things, the major pathway for the excretion of cholesterol, a water-insoluble substance normally held in solution within micelles of bile salts and lecithin. An excess of cholesterol or a deficiency of bile salts or lecithin causes bile to become super-saturated with cholesterol. When this occurs, microscopic crystals of undissolved cholesterol can often be detected in fasting gallbladder bile samples. These crystals may stay suspended and cause no problem; however, when certain as-yet-undefined conditions exist, the crystals precipitate to initiate cholesterol gallstone formation.

The supersaturation of bile with cholesterol may occur under several conditions. It appears that abnormalities in hepatic enzyme systems may play an important role in cholesterol gallstone formation. If there is an increase in HMG CoA-reductase activity, cholesterol synthesis is increased. A decrease in 7-alpha hydroxylase activity can also result in higher concentrations of cholesterol. Other disorders that may contribute to the supersaturation of cholesterol in bile include obesity, hyperlipidemia and diabetes mellitus. Drugs such as estrogen, oral contraceptives and clofibrate may induce increased cholesterol saturation. In advanced liver disease or disease of the terminal ileum, bile salt deficiencies may result, leading to lithogenic bile.

Supersaturation of bile, alone, is not sufficient to produce cholesterol stones, although it is probably the most important factor. Changes within the gallbladder, such as development of a nidus for cholesterol precipitation and pH alterations can contribute to the organic matrix necessary for gallstone formation.

CHENIX® at Work

CHENIX® (chenodiol) is a therapeutic agent composed of chenodeoxycholic acid, a naturally occurring human bile acid. Administered in therapeutic doses, to carefully selected patients, CHENIX® is capable of decreasing biliary cholesterol saturation. Though cholic acid, a primary bile acid, can expand the bile acid pool, it is not capable of cholesterol desaturation.

CHENIX® acts primarily through suppression of hepatic cholesterol synthesis and subsequent secretion.[1] Chenodiol reverses the process of cholesterol gallstone formation by reducing the molar ratio of cholesterol to bile acid and lecithin in bile. Cholesterol gallstones gradually dissolve in the daily flow of fresh unsaturated bile. Therefore, a well-functioning gallbladder is necessary for gallstone dissolution therapy with CHENIX.

CHENIX® is well absorbed from the small intestine, and taken up by the liver. There, chenodiol is converted to its taurine and glycine conjugates, becomes part of the bile acid pool and enters the enterohepatic circulation. At steady state, an amount of chenodiol (approximately equal to the daily dose) escapes to the colon and is converted to lithocholic acid. About 80% of the lithocholate is excreted in the feces, and the remainder is conjugated in the liver. During CHENIX therapy, lithocholic acid is increased from 1% to 4% of the bile acid pool.

It is important to note that both desaturation of bile and clinical dissolution of cholesterol stones with chenodiol have been shown to be dose related. This factor, plus proper patient selection and careful monitoring are essential to successful CHENIX® therapy.

Diagnosis: Radiography and Ultrasonography

Many cases of gallstones are discovered coincidentally when x-rays or other tests are conducted as part of a routine medical examination. In other cases, though, the patient presents with obvious clinical problems. Intermittent mild pain or sudden terrifying pain in the stomach, accompanied by vomiting may be the initial symptoms. Often the patient has suffered for years with nagging symptoms or distention or discomfort following the ingestion of meals, high in fat content. In more severe cases, if gallstones have obstructed the flow of bile, jaundice may be evident.

When gallstones are suspected, the diagnosis can be confirmed either by x-ray or ultrasound technique. In many health centers, sonograms have replaced cholecystograms in the diagnosis of gallstones. However, a cholecystogram is necessary prior to CHENIX therapy to determine the presence of a functioning gallbladder and radiolucent stones. If the gallbladder fails to opacify on oral cholecystogram, the patient is not a candidate for CHENIX therapy. A sonogram may be suitable for detecting the presence of gallstones, but an oral cholecystogram (OCG), and perhaps a plain film of the abdomen to detect subtle calcification, is needed to determine the prospect of successful gallstone dissolution.[2-4] An oral cholecystogram should include several films, including one view with the patient upright, using a horizontal beam, to detect floatable stones.

It is important to note that floatability of stones cannot be assessed by ultrasound in the absence of dye. Chemical analysis has shown floating stones to be essentially pure cholesterol. Prediction of gallstone type is essential to proper patient selection for CHENIX therapy. The following examinations will help determine the CHENIX candidate:

1 **Oral cholecystogram** – This is highly accurate in determining the presence of gallstones and estimating stone size, when the gallbladder is satisfactorily outlined.[4] Most important, it reveals whether or not the gallbladder is functioning. It has been suggested, in order to avoid the need for a repeated cholecystogram (due to poor opacification of the gallbladder), that patients be given single doses of the contrast agent on two consecutive days, followed by the radiogram on the third day.[5]

2 **Ultrasonography** – Sonograms, too, are highly accurate in detecting gallstones and with very small stones may offer a better screening method than the cholecystogram. However, sonograms cannot determine gallbladder function or stone lucency. Patients should fast for at least 6 hours prior to the sonographic examination. This insures distention of the gallbladder. Sonography is not effective in the patient whose gallbladder is in an unusual site or hidden by overlying structures.[6]

A major advantage of sonography is that it is noninvasive. Also, ultrasound waves have no known harmful effects.

Patient Selection

Radiolucency and functioning gallbladder are the keys to proper patient selection. CHENIX® can dissolve gallstones that are primarily comprised of cholesterol. It is indicated for the dissolution of radiolucent stones in well-opacifying gallbladders in patients expected to need surgical intervention, ie cholecystectomy. These patients must be at risk of surgical morbidity or mortality because of systemic disease or age. Patients should have normal liver function.

Proper patient selection is an essential factor in the successful outcome of CHENIX therapy. Below, the key criteria for the CHENIX candidate are discussed.

- Radiographic features – Radiolucent stones may have rims or centers of opacity representing calcification. Pigment stones and partially calcified radiolucent stones do not respond to chenodiol. Floatable stones are exclusively cholesterol stones. Among nonfloatable radiolucent stones, cholesterol stones are likely to be smooth surfaced, less than 0.6 cm in diameter and to occur in numbers less than 10. CHENIX is recommended for patients whose stones measure less than 15 mm in diameter. The likelihood of successful dissolution is far greater if the stones are floatable.
- Surgical risk – CHENIX is suitable therapy for the patient considered a high surgical risk. Overall surgical morbidity and mortality increase with age. Other complicatons that may make the patient a surgical risk include cardiopulmonary disease, diabetes, chronic obstructive lung disease and obesity.[7] Relatively young patients might be better treated by surgery rather than CHENIX, because treatment with chenodiol, if successful, is associated with a high rate of recurrence. The long-term consequences of repeated courses of chenodiol in terms of liver toxicity and elevated cholesterol levels are not known.
- Patients must have a functioning gallbladder.
- Female patients must not be pregnant during treatment.
- Patients must have normal liver function, with no evidence of prior liver disease. Refer to WARNINGS and ADVERSE REACTIONS in official directions for use.

Dosage and Duration of Therapy

As mentioned previously, successful dissolution of gallstones is dose related. The following guidelines are recommended for proper dosage and duration of therapy:

- Dose range – CHENIX® should be administered in doses of 13 to 16 mg/kg in two divided doses, morning and night.
- Initial therapy – Begin with 500 mg/day for the first two weeks.
- Step-up therapy – Increase by 250 mg/day until recommended or maximum tolerated dose is reached.
- Temporary dosage adjustment – If diarrhea occurs during initial therapy or through the course of treatment, it can be controlled by temporary dosage adjustment until symptoms abate. After this, the previous dose is usually tolerated.

Duration of CHENIX® therapy is based on progress determined through clinical monitoring. Oral cholecystograms or sonograms will indicate whether dissolution is taking place. Complete dissolution should be confirmed by a repeat test after one to three months of continued CHENIX® therapy. Sonograms have not been evaluated for detecting partial dissolution but are perhaps more sensitive for confirming total dissolution.

Most patients who eventually achieve complete dissolution will show partial (or complete) dissolution at the first on-treatment cholecystogram. CHENIX® should be discontinued if there is no response by 18 months. Safety of use beyond 24 months has not been established.

Monitoring Therapeutic Progress

- Dissolution:
  - OCG or sonogram at 6-to 9-month intervals.
  - Complete dissolution should be confirmed by a repeated cholecystogram 1 to 3 months later.

while patient continues on CHENIX® therapy.
  - If dissolution is not seen by 9 to 12 months, the likelihood of success is greatly reduced.

- Enzyme levels:
  - Monitor SGOT and SGPT monthly for the first 3 months.
  - Monitor SGOT and SGPT at 3 month intervals thereafter.
  - Minor elevations (1½ to 3 times upper limits of normal) will usually return to normal, but those persisting longer than 3 to 6 months require discontinuation of CHENIX® until levels return to normal. At that time, CHENIX therapy can resume usually without incident.
  - Major elevations (over 3 times the normal) require discontinuation of therapy and usually reoccur on challenge.

- Serum cholesterol:
  - Monitor serum cholesterol at 6-month intervals.
  - It may be advisable to discontinue CHENIX® if cholesterol rises above the acceptable limit for a given patient.

- Bile acid diarrhea:
  - Patients should be instructed to anticipate the possible occurrence of diarrhea.
  - It occurs in 30% or more of the patients.
  - Temporarily lowering daily dosage of CHENIX® by 250 mg will often resolve the problem.[7]
  - Higher doses are usually tolerated in a week or two.
  - Diarrhea has required discontinuation of therapy in about 3% of the patients.

- Biliary cholic/acute cholecystitis:
  - Patients should be instructed to report symptoms immediately.
  - In such cases, evaluate the need for surgery.
  - In all studies with chenodiol there has been no indication that *partial dissolution* has led to these complications.

- Recurrence:
  - May be expected within five years in 50% of the cases.
  - After confirmed dissolution, treatment is generally stopped.
  - Annual OCG or sonograms can be used to monitor the patient. Radiolucency and gallbladder function should be reestablished before starting another course of treatment with CHENIX®.
  - Prophylactic dose has not been established, and reduced doses are not recommended.
  - Low cholesterol or carbohydrate diets have been reported to reduce biliary cholesterol in some cases.
  - Weight control is recommended.

Patient Compliance and Aids to Compliance

Compliance is a critical factor in successful CHENIX® therapy. As dissolution of radiolucent stones generally requires 6 to 24 months of treatment, patients must have a knowledgeable commitment to therapy.

Multiple factors can influence patient adherence to the prescribed course of CHENIX® treatment[8]:

- Simple dosing regimen—Compliance may be enhanced by simple dosage schedules.
- Duration of treatment—Compliance failure may increase with long-term treatment.
- Side effects—Compliance failure increases in the presence of side effects.

Who is The Non-Compliant Patient?

Socio-demographic characteristics of the non-compliant patient do not conclusively indicate a "patient type". Some studies suggest that females (78% of gallstone patients) may be less compliant than males. However, several other personal factors may contribute to compliance[8]:

- Patient satisfaction with medical care.
- Support from family and friends.
- Stable living situation.

Compliance Starts in The Office

The physician-patient relationship can play an important role in achieving compliance. Patients must be informed and willing partners in treatment. Physicians who question compliance in a nonjudgmental, nonthreatening manner are likely to receive a more reliable response from patients.

It should be remembered that long waiting times in the office are a deterrent to treatment. Also, the quantity and the quality of time that is spent with the patient during an office visit can influence compliance. In discussions with patients, the issue of compliance deserves significant time. Other office personnel can reinforce this discussion. Futhermore, it has been suggested that simply paying more attention to patients increases compliance.

Rewarding and reinforcing compliance has been shown to be effective. Recognition and praise from the physician are positive "reinforcers" that may help to sustain or enhance compliance.

If the patient misses an appointment, contact should be made to reschedule. Efforts toward increasing compliance begin with initial treatment, but should be continued throughout treatment. With each patient visit, compliance should be evaluated and discussed.

Explain, Inform and Discuss

A sample checklist describes methods the physician can use to enhance compliance.

Compliance Checklist

	Explain gallstones
	Inform how CHENIX® works
	Suggest dosing dose administration to events or times of the day
	Establish most simple dosage schedule possible (b.i.d. or once daily - please see full prescribing information)
	Encourage patient to keep a written record of progress, appointments and dates for periodic laboratory tests or OCG

Obesity and Diet

Obese patients tend to have a high prevalence of gallstones. In addition they are likely to have bile secretory characteristics favoring cholesterol saturation. The obese patient is generally considered a surgical risk.

As a result of these factors it would seem that the obese patient is a suitable candidate for CHENIX® therapy. However, it should be noted that these patients tend to require higher doses of chenodiol. Weight reduction is a suggested plan for these patients. If weight loss occurs, dosage requirements are lower and chances for successful dissolution increase.[1] For patients who are considering weight reduction it may be helpful to substitute high fiber foods for high caloric intake, wherever possible.

In the case of the non-obese patient a change in diet may play a role in successful dissolution, although the effects are probably minor. Low cholesterol and high fiber diets may enhance progress.[1]

The National Cooperative Gallstone Study

The United States National Cooperative Gallstone Study (NCGS) was a double-blind study conducted to determine the efficacy and safety of randomly allocated chenodiol or placebo.[2] The study, administered over 2 years, involved 916 patients with radiolucent gallstones. Since the publication of the study in 1981, there have been some questions about its results. The discussion that follows seeks to clarify these issues.

- Dosage – The NCGS dosage regimen was inadequate in view of what now is known about chenodiol.[3] The NCGS "high" fixed dose of 750 mg/day provided on average only 9.0 mg/kg/day for the men in the study, and only 10.6 mg/kg/day for the women, accounting for the low dissolution rates reported (13.5% total, 40.8% total plus partial). In a subgroup of 29 patients less than 100% ideal body weight, hence receiving higher body weight doses, there were 36.1% total dissolutions and 76.3% total plus partials. While these NCGS patients were mainly "thin women," Rowell Laboratories' analysis of NDA data has shown insignificant differences in responses of thin vs obese patients and men vs women, within groups receiving the same body weight doses of chenodiol. Doses in the range from 13 to 16 mg/kg/day have produced total dissolution rates of 28% to 38%, disregarding stone size or bouyancy, and 42% to 60% in patients with stones <15 mm in diameter. Floatable stones have double the dissolution rate of small nonfloatable stones (70% or more vs 35%).
- Many Plusses of the NCGS – Despite the shortcomings of the NCGS, this well-controlled trial has given a wealth of information on cholelithiasis and on chenodiol. The biopsy study which preceded the major clinical trial was the most extensive yet attempted, and showed that cholelithiasis patients have substantial subclinical liver pathology, ie, 63% of untreated patients with electron-microscopic evidence of intrahepatic cholestasis. Baseline demographic and clinical characteristics collected on the 916 patients entered into the NCGS give a most complete picture of radiolucent gallstones on which to design future studies.

  Despite the relatively low doses, much was learned of the potential hepatotoxic effect of chenodiol, put into proper perspective by inclusion of the placebo group, ie, while chenodiol produced dose-related serum aminotransferase elevations, a significant number of elevations occurred in the placebo group, and the number of major elevations were not significantly different in treated and placebo groups. Of particular importance, placebo patients with floatable stones were found to have significantly more problems (biliary pain and cholecystectomy) than patients with nonfloatable stones over the two-year course of NCGS; treatment with chenodiol provided a much higher dissolution rate for floatable stones than nonfloatable stones, and reduced both biliary pain and cholecystectomies in the group with floatable stones.[4]

Benefit to Risk Assessment

Treatment with CHENIX should be determined with a full understanding of the potential benefits and risks of the drug compared to alternative courses of management.

1. **Surgery** – Cholecystectomy, the surgical removal of the gallbladder (containing gallstones), is the customary and established treatment for gall-

stones when treatment is indicated. It is generally agreed that symptomatic gallstones or complications of gallstones, such as acute cholecystitis or choledocholithiasis are best treated by surgery.

Benefits—Surgery is an effective treatment for gallstones, relieving symptoms in the majority of patients. The mortality for these surgical procedures in patients free of other diseases and under 70 years of age is under 1.0%.

Risks—When diseases of other organ systems are present, the mortality with surgery is increased, and should common duct exploration be required in conjuction with cholecystectomy, the surgical mortality has a fourfold increase.[1] Nonfatal complications of this operation and of anesthesia occur in at least 7% of patients. These include wound infection, pneumonia, phlebitis, urinary tract infection, bile duct injury, retained gallstones and pancreatitis.[1]

2. **Observation**—When symptoms are minor and nonspecific, some physicians recommend observation rather than surgery as the better and safer course.

Benefits—Although precise information is not available, about 50% of patients with gallstones and minor symptoms observed for 10 to 20 years do not develop cholecystitis or require cholecystectomy.

Risks—About 50% of patients with gallstones and minor symptoms observed for 10 to 20 years develop cholecystitis and require cholecystectomy. Complications of gallstones increase with time. The complications include obstruction or infection of the bile ducts, pancreatic inflammation, fistula formation or perforation and, remotely, malignancy of the gallbladder. Once complications of gallstones have occurred, the mortality and nonfatal complications of operation are increased.

3. **Gallstone Dissolution with CHENIX**—Cholesterol gallstones may be successfully dissolved with chenodiol in patients who have a functioning gallbladder.

Benefits—Successful dissolution of gallstones will eliminate the gallstones while avoiding the risk of surgery and its attendant complications and costs. The probability of success is enhanced by careful patient selection, correct dosage and active programs designed to encourage patient compliance.

Risks—Gallstone dissolution with CHENIX may not prevent complications of gallstones (ie, acute inflammation of the gallbladder, jaundice, or severe pain), and surgery may still be necessary. These complications, once they occur, have an increased mortality and nonfatal complication of surgery. Persistent liver enzyme elevations during CHENIX therapy has been reported in 3% of patients. In these cases, CHENIX therapy should be discontinued. Elevated CHENIX therapy is accompanied by dose-related diarrhea in approximately 35% of the patients, and discontinuation of CHENIX because of failure to control diarrhea is to be expected in about 3% of the patients treated.

Successful gallstone dissolution with CHENIX is highly dependent on stone size and number, composition (cholesterol, noncalcified cholesterol) and floating versus nonfloating stones. For patients with floating stones, dissolution rates of 70% or more can be expected, while nonfloating stones in some studies had a success rate of only 27%. The course of therapy may take up to two years for successful dissolution and there is a 50% recurrence of gallstones within five years of dissolution.

(1) Package Insert.
(2) Stoll, Protocol NCGS Document #113, [illegible]

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