Progress report

Bile acid metabolism in infants and children

Many clinical states are encountered in the infant and child which in the adult are known to be associated with disturbances of bile acid metabolism. In recent years there have been an increasing number of studies of bile acid metabolism in infants and children. This paper reviews the results of such studies so as to provide a comprehensive survey of the information available to date on bile acids in the neonate and older child, and to illustrate the clinical significance of altered bile acid metabolism during development.

Bile Acids in the Neonate

Bile acids occur only in vertebrate species and in all the vertebrates studied to date the biosynthesis of bile acids from cholesterol takes place in the liver. Cholic acid and chenodeoxycholic acid are the bile acids synthesized in the human liver and are referred to as the primary bile acids; deoxycholic acid and lithocholic acid are secondary bile acids derived from the primary bile acids by the action of bacteria normally present in the terminal ileum, caecum, and colon (fig 1). No more than traces of free bile acids have been found in bile from healthy mammals and in the human liver the bile acids are conjugated with glycine or taurine before their excretion into the bile.

The mean value of the ratio of the concentration of glycine-conjugated bile acids to that of taurine-conjugated bile acids found in studies of normal adult human bile is approximately 3:2; cholic acid accounts for approximately 40% of the total biliary bile acids, chenodeoxycholic acid 37%, deoxycholic acid 20%. In most studies only small amounts (<3%) of lithocholic acid conjugates have been detected in normal adult human bile.

The principal bile acids of human foetal gallbladder bile after 22 and 26 weeks' gestation are taurine-conjugated dihydroxy bile acids. After the 28th week taurine-conjugated cholic acid may be found together with small amounts of glycine conjugates. During the first week of life cholic acid is the predominant biliary acid the proportion between cholic acid and chenodeoxycholic acid being 2-5:1; within the first month the ratio falls to the normal value of 1-2:1.

FORMATION OF BILE ACIDS

It has not yet been firmly established that in the early stages of development bile acid formation occurs along the same pathways it does in adult life. A recent analysis of meconium samples from premature and full-term infants has confirmed the results of an earlier study and shown that in addition to the four common bile acids detailed in fig 1, ursodeoxycholic acid and
3 beta-hydroxy-5-cholenoic acid may often be present. The finding of 3 beta-hydroxy-5-cholenoic acid is of some significance as this bile acid has not been detected, to date, in the normal pregnant adult, nor in cord blood\(^9\), and it is therefore unlikely that its origin is the maternal liver or intestine. The amounts of both deoxycholic acid and 3 beta-hydroxy-5-cholenoic acid, each expressed as a percentage of the total amount of bile acids present, were significantly higher in the meconium samples from the premature infants than in those from the full-term infants\(^8\). These results are in accord with the observation that although deoxycholic acid has recently been detected in the intestinal contents of premature infants\(^{10,11}\) it has rarely been found in sterile samples of the bile\(^3,5,12\) or serum\(^3\) of full-term babies and infants less than 1 year old. Lithocholic acid, on the other hand, has often been detected in such samples\(^3,5,12,14\). All of this evidence supports the hypothesis that the synthesis of bile acids in the foetal liver may, in addition to the usually accepted pathway, occur via a pathway which has lithocholic acid and 3 beta-hydroxy-5-cholenoic acid as intermediates\(^{15}\) and which has been postulated as a minor biosynthetic sequence for bile acids in the adult human liver\(^{16}\) (fig 2). No pathway for the production of deoxycholic acid in the
human liver has yet been described. Therefore the detection of this bile acid in
the neonate may merely reflect maternal-placental transfer.

CONJUGATION OF BILE ACIDS
With regard to the conjugation of bile acids during development there is much
evidence to suggest that at birth taurine-conjugated bile acids predominate
and that the proportion of glycine conjugates increases with age\(^3,4,17\). Not all
of the factors determining bile acid conjugation have been defined. Conjugation
with glycine is the most advanced stage of conjugation in the evolutionary
sense\(^18\), a preference for glycine conjugation is usually found in herbivorous
animals whilst taurine conjugation is predominant in carnivores\(^1\) and the
transition in developing sheep\(^19\) from a preference for taurine conjugation to
that for glycine conjugation is usually explained on the basis of a change in
diet. It is not known if significant synthesis of taurine de novo does occur in
the human liver. One point of note, however, is that the taurine content of the
foetal liver is higher than that of the adult liver\(^20\). A new model, making use of
liver cell culture techniques for the study of bile acid conjugation by the foetal
liver has recently been described\(^21\) and future work with this model may
elucidate the factors determining conjugation.

THE FUNCTION OF BILE ACIDS
Conjugated bile acids are excreted from the liver, via the biliary canaliculi,
into the bile by what is generally believed to be an active transport
mechanism\(^22\). About 1 litre of bile is secreted by the adult liver each day and
passes into the gallbladder where it is concentrated to about one fifth of its
original volume.

The bile acids are present in bile micelles, ie, water-soluble polymolecular
aggregates with detergent-like properties\(^23\). Bile acids exhibit marked
choleretic effects and it has recently been suggested that it is the counter-ions associated with the micelles that are responsible for this property. The micelles in hepatic bile consist of bile acids, lecithin, and cholesterol and are associated with calcium and sodium ions, and it is generally accepted that bile acids play an important role in the hepatic excretion of lecithin and to a lesser extent cholesterol. When the gallbladder contracts the concentrated bile is discharged into the duodenum, where the biliary lecithin of the bile acid-lecithin-cholesterol micelle is readily cleared by pancreatic phospholipase to lysolecithin and fatty acid. The latter, together with the digestive products of triglyceride, ie, fatty acids and monoglycerides, contribute to the formation of new mixed micelles composed of bile acid, fatty acid, and monoglyceride. These mixed micelles are essential for the efficient transport and absorption of water-insoluble nutrients.

Another function of bile acids in the duodenum is illustrated by their effect on pancreatic function. Bile acids promote pancreatic lipolysis by solubilization of its products, activation of lipase, and activation of co-lipase. They also play a role in protein digestion since they activate enterokinase.

The Enterohepatic Circulation of Bile Acids

Bile acids may be absorbed to some extent from all regions of the gastrointestinal tract including the stomach, small intestine, and the large intestine. The principal site for bile acid absorption is the ileum; in the normal adult probably 80-90% of the conjugated bile acids are absorbed by an active transport mechanism in the ileum; the small amount of conjugated bile acids which escape ileal absorption are degraded by bacteria and absorbed by passive non-ionic diffusion in the colon.

After intestinal absorption the bile acids are transported to the liver via the portal vein. By means of this enterohepatic circulation the bile acids again get access to the intestinal lumen. The efficiency of the enterohepatic circulation and its clinical significance have received much attention in the adult, but little is known about this cycle in the infant. The size of the circulating bile acid pool in the human adult is about 3 g and the entire pool circulates at least twice during the digestion of a single meal. The small amount (approximately 3% each cycle; 300-700 mg/day) which escapes absorption during each cycle is replaced by biosynthesis from cholesterol. The rate of catabolism of cholesterol to bile acids is believed to be regulated by a feedback mechanism the exact nature of which has not been firmly established.

Although early studies of bile acid concentrations in the duodenal juice of infants, on samples obtained without gallbladder stimulation, found values much the same as those in older children and adults, more recent studies have suggested that these concentrations may often be relatively low. Concentrations of bile acids in the neonate gallbladder are much lower than those of the adult and these results are in accord with the relatively low levels of duodenal bile acids obtained after cholagogic stimulation with magnesium sulphate or during a milk feed. The results of all these studies support the view that the pool of circulating bile acids in the full-term newborn is much smaller than that in older children and adults. The results of a study of bile acid pool sizes in normal infants, using non-radioactive isotopic labelling techniques has confirmed this view. The mean cholic acid pool size in five full-term healthy neonates, at the time of commencement of the study
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days old, was found to be 41.4 mg. When expressed per square metre of body surface area the mean pool size was 290 ± 36 mg/m² compared to a cholic acid pool size in the adult of 605 ± 122 mg/m². The mean rate of cholic acid synthesis in the infants was found to be 22.7 mg/day, ie, 110 ± 20 mg/m²/day compared to that of 194 ± 28 mg/m²/day in the adult. A similar small pool size and synthetic rate of chenodeoxycholic acid was also found.7

As indicated above the rate of bile acid formation in the normal adult is equal to the amount of bile acids lost from the enterohepatic circulation and excreted in the faeces. Values for the faecal excretion of bile acids in neonates, 2-8 days old, have not been reported. First reports of faecal bile acids in children47,48, however, suggest that these values correlate well with weight; the mean value of total faecal bile acids in 18 normal infants and children was found to be 18.3 ± 8.2 mg/kg/72 hr48. These values would suggest that the synthesis rate of bile acids in the neonate exceeds the faecal loss and that the neonate represents a situation in which the enterohepatic circulation is not in a steady state. Therefore, although the foetal liver has been shown to be capable of bile acid synthesis and conjugation51 it is likely that the transition from intrauterine to extraterine existence is accompanied by the ‘step-up’ of bile acid synthesis and the beginning of growth of an effective bile acid pool. The finding of only trace amounts of bile acids in the urine of normal infants54 and serum bile acids comparable to those in normal adults50 indicates that the bile acid pool in the normal neonate is confined to the enterohepatic circulation. It must be noted, however, that there have been few studies of serum bile acids in normal infants, reported in the literature, and that this aspect of bile acid distribution in the neonate is not firmly established.

Bile Acids and Malabsorption in Children

MALABSORPTION AND THE NEONATE

As indicated above, during the normal absorption of fat the products of triglyceride hydrolysis are incorporated into a micellar solution. Bile acids from micelles when their concentration exceeds a certain critical value—the critical micellar concentration (CMC). The CMC of a mixture of bile acids under conditions closely stimulating those in the intestinal lumen is 1-2 mM53. Thus normal absorption of fat requires at least this concentration of bile acids in the lumen and it has been proposed58 that the concentration of bile acids in intestinal contents should be in excess of 4 mM, the lower limit of the range of values found during a meal53,54. Failure to achieve such concentrations may result in impaired fat absorption. The associated calorie loss may or may not present a problem since even in the total absence of bile acids about half of the dietary intake of fat may be absorbed. Some fat-soluble vitamins have, however, no alternative mode of absorption.

In particular the poor nutritional state of infants with intrahepatic cholestasis has been ascribed to malabsorption due to inadequate intestinal concentrations of bile acids55. Other studies have demonstrated that malabsorption of fat may be accompanied by malabsorption of vitamin A56 vitamin K57,58 and vitamin E59,60. It has further been suggested that inadequate micelle formation may affect the absorption of water-soluble nutrients due to an adverse change in intestinal transit time53,54. The assimilation of calcium and magnesium may also be affected in the presence of steatorrhoea due to the
formation of insoluble soaps and lack of enhancement of absorption by bile salts. In many newborn full-term infants the coefficient of fat malabsorption is low (80-90%) during the first year of life it gradually increases to >90%. In the premature infant the coefficient of fat absorption is even lower. Low concentrations, ie, <3 mM, of bile acids in the duodenal contents of fasting neonates have often been observed and a study of bile acid concentrations in the intestinal contents of eight healthy full-term newborns has indicated that the level of bile acids during the digestion phase is often less than that required for micelle formation, although the concentrations in the resting juice may be >3 mM. Similar observations have been made during a study of fat absorption in 18 premature infants and the bile acid pool size of premature infants has been shown to be much smaller than that of the full-term newborn. Such studies indicate, therefore, that the steatorrhoea often encountered in the premature and newborn is related to the inadequate solubilization of fat in the duodenum.

Other factors may also be involved; concentrations of pancreatic enzymes in the duodenal juice of infants vary greatly from one infant to another. Pancreatic function in these infants with low concentrations may be further reduced by lack of activation by bile acids and unhydrolysed fat may result in bile acid malabsorption. Increased losses of bile acids have been demonstrated in those premature infants fed with a cow's milk substitute relative to the losses observed in premature infants fed human milk. It is evident that the disadvantage of the premature infant with respect to the absorption of fat may be exacerbated by providing a diet with a high content of long-chain triglyceride.

MALABSORPTION AND THE CONTAMINATED SMALL BOWEL

Recent studies have demonstrated that bacterial contamination of the small intestine can occur in infants with malabsorption and failure to thrive, in the absence of a structural lesion of the bowel. Many of the studies of fat malabsorption in adults with small bowel contamination suggest that the malabsorption is also associated with increased deconjugation of bile acids in the small intestinal lumen. Free bile acids are not always detected in conditions of bacterial overgrowth and the principal event resulting in fat malabsorption appears to be the reduced jejunal concentration of conjugated bile acids. Although in adults bacterial overgrowth has not yet been shown to increase bile acid excretion, that increased formation of free and secondary bile acids may result in increased losses of bile acids from the enterohepatic circulation has been demonstrated in a 3½-month-old infant with bacterial overgrowth and severe steatorrhoea. Since the normal full-term infant is under stress to produce an adequate pool in the first few months of life, as might be expected increased losses due to a bacterial overgrowth result in a decreased pool size.

Unconjugated bile acids are also thought to play a role in the sugar malabsorption sometimes associated with an overgrowth of faecal organisms in the upper small intestine. The mechanism causing the latter association is not known but studies in rats in vitro and in vivo have suggested that unconjugated bile acids can adversely affect monosaccharide absorption. Sugar malabsorption has also been observed in bile duct-ligated rats and the role of conjugated bile acids in the presence or absence of bacterial over-
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growth in the aetiology of monosaccharide malabsorption merits further attention.

Bile Acid Metabolism in Infants with Liver Disease

FORMATION OF BILE ACIDS
The first report of the unsaturated bile acid 3 beta-hydroxy-5-cholenoic acid being present in patients with liver disease was made by Makino, Sjövall, Norman, and Strandvik, 1971. These authors reported the presence of this acid in the urine of infants with liver disease, including four infants with extrahepatic biliary atresia, in amounts < 32% of the total bile acids present. Small amounts of allolithocholic acid were also detected; lithocholic acid was found in the urine of only one patient. Later studies are in accord with this observation and 3 beta-hydroxy-5-cholenoic acid is believed to be present, as its taurine conjugate, in the urine of all infants with cholestasis whether due to extrahepatic biliary atresia or to intrahepatic cholestasis of infancy.

The clinical syndrome of cholestasis has long been associated with disturbances of bile acid metabolism. In contrast to the choleretic effect of the common primary bile acids, taurine conjugated 3 beta-hydroxy-5-cholenoic acid produces a marked reduction in biliary flow and bile acid secretion when infused into animals. It has been suggested that cholestasis is associated with the production of monohydroxy bile acids within the liver cell and the pathway suggested for their formation usually involves side chain oxidation of cholesterol as an early event (fig 2). In this regard it has been noted that the low incorporation of radioactivity into 3 beta-hydroxy-cholenoic acid following the administration of isotopic cholesterol indicates that cholesterol itself is not the direct precursor of this compound.

Another uncommon bile acid in man, also indicative of a disturbance of bile acid formation, is trihydroxycholestanic acid, and this acid has been detected in the duodenal juice of two infants with cholestatic jaundice; this acid, however, was not detected in the bile of three other patients with cholestasis and therefore does not appear to characterize bile acid metabolism in cholestasis.

CONJUGATION OF BILE ACIDS
Conjugation of bile acids in infants with cholestatic liver disease does not appear to be significantly impaired as labelled conjugated bile acids predominate in the urine after the administration of isotopic cholic acid, only trace amounts of free cholic acid being present. This finding is in accord with observations on conjugation of bile acids in adults with cholestatic liver disease. In addition to conjugation with glycine or taurine, bile acids in infants with cholestasis may often be conjugated at nuclear hydroxyl group sites with sulphate, and sulphation appears to be another common feature of intra- and extrahepatic cholestasis. The explanation for the presence of such sulphates is not established: compounds with two ionic groups are less easily absorbed from the intestinal tract than compounds with one ionic site; sulphated taurocholic acid is less readily absorbed from the intestinal tract than taurocholic acid as the former compound requires active
transport for its absorption and consequently faces competition for absorption at ileal active sites from the common primary bile acids which are present in larger amounts. Sulphation of bile acids in cholestasis appear to compensate for the fact that biliary excretion is much reduced and an increased amount of bile acid is displaced into the peripheral circulation. Excretion from the latter necessitates urinary elimination. The renal clearance of bile acid sulphates is some 100 times greater than the clearance of non-sulphated bile acids and only small quantities of sulphated bile acids may be found in the serum of patients with large amounts of sulphated bile acids in the urine.

**Intestinal Bile Acid Concentrations, Malabsorption, and Infants With Liver Disease**

As indicated above, cholestatic liver disease in children represents a situation in which the bile acid pool is dispersed largely in the systemic circulation. Serum bile acids and urinary bile acids are raised in infants with liver disease and an association between steatorrhoea and the impairment of bile acid excretion into the intestine has been frequently demonstrated. In view of these findings it is not surprising that infants with liver disease may often exhibit marked malabsorption of nutrient factors dependent for their absorption on bile acid solubilization in the intestinal lumen.

**The Diagnostic Value of Studies of Bile Acids in Infants With Liver Disease**

Neonatal hepatitis causing intrahepatic cholestasis and biliary atresia (extrahepatic cholestasis) account for more than 85% of all cases of prolonged obstructive jaundice in infants and may occur with equal frequency. Although many of the specific causes of intrahepatic cholestasis may be readily diagnosed some cases of neonatal hepatitis are extremely difficult to separate from those of extrahepatic biliary atresia. The establishment of bile flow by surgical intervention is indicated in all cases of congenital biliary atresia but such intervention in patients with intrahepatic cholestasis may be less than beneficial. Because of the unique role of the liver in bile acid metabolism it might be expected that the investigation of the latter would provide a valuable aid to differential diagnosis of intrahepatic cholestasis and biliary atresia.

As stated above, to date no bile acids have been detected in any specimens of plasma or urine from patients with biliary atresia which have not also been found in similar samples from patients with intrahepatic cholestasis. It would therefore seem that the formation of bile acids in these two syndromes occurs via the same pathways. Norman and Strandvik (1973) in a study of urinary bile acids in five infants with biliary atresia and 19 infants with intrahepatic cholestasis found no difference between the two groups with respect to half life and pool sizes of cholic acid, the mean daily excretion of cholic and chenodeoxycholic acids, and the ratio of urinary cholic to urinary chenodeoxycholic acid concentrations. In contrast to an earlier report it has recently been observed that the predominant bile acid in the plasma of infants with biliary atresia is chenodeoxycholic acid whereas that in the plasma of infants with intrahepatic cholestasis is cholic acid. The mechanism
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responsible for this qualitative difference in plasma bile acids is not known. It has, however, been suggested that the predominantly chenodeoxycholic acid pattern is related to the more rapid development of cirrhosis observed in patients with complete atresia.\(^{104}\)

Hepatic secretion of bile acids into bile varies considerably in infants with intrahepatic cholestasis and it may often be virtually non-existent.\(^8^4\) Hence although faecal bile acid concentrations in infants with intrahepatic cholestasis are often higher than those in infants with biliary atresia,\(^{106}\) in some cases they do not help to distinguish between the two groups.\(^8^4\) The same observation is true of duodenal bile acid determinations made in the fasting state.

Response to pancreozymin stimulation may help to separate some cases. Weber and Roy (1973)\(^{100}\) observed that four infants with biliary atresia showed no response to pancreozymin stimulation whereas a response was obtained from two infants with paucity of intrahepatic bile ducts and four infants with posthepatic cirrhosis. Any agent therefore which evokes gallbladder contraction may be some help in differentiating the two types of cholestasis.

Cholestyramine, an insoluble quaternary ammonium anion exchange resin, binds bile acids in the duodenum thus interrupting the enterohepatic circulation. One consequence produced by the resin in normal subjects is an increase in hepatic synthesis, primarily in glycocholic acid, accompanied by reduction in the secondary bile acid pool.\(^{106}\) Cholestyramine therapy has been shown to produce an improvement of liver function and general condition in infants with paucity of bile ducts and also in infants with post-hepatic cirrhosis.\(^{100}\) The efficiency of cholestyramine therapy would be expected to be related to the level of hepatic secretion before administration. A new method for distinguishing between intrahepatic and extrahepatic cholestasis in infancy has made use of this relationship.\(^104\) Patients with intrahepatic cholestasis who have predominantly cholic acid in their plasma show a marked response to cholestyramine indicated by an increase in the ratio of cholic acid to chenodeoxycholic acid concentration; patients with extrahepatic cholestasis who have predominantly chenodeoxycholic acid in their plasma show only a poor or no response.

Another form of treatment effective in lowering serum bile acid levels is phenobarbitone therapy.\(^{108,109,110}\) This effect may be explained by an increase in bile acid synthesis resulting in an increase in bile flow or by an increase in the bile acid-independent bile flow.\(^112\) It has recently been demonstrated that increased ileal absorption is not a major factor.\(^113\) Phenobarbitone therapy is effective in infants with intrahepatic cholestasis but not in infants with extrahepatic cholestasis. Since its effectiveness can be ascertained by following serum total bile acid levels, determination of the latter before and during therapy may provide a valuable aid to the differential diagnosis of intra- and extrahepatic cholestasis.

Assessment of Bile Acid Metabolism in Infants and Children

In the assessment of bile acid metabolism the knowledge of bile acid pool size and synthesis rate is highly desirable. Until recently such information has been obtained only by inference from the measurement of duodenal bile acid concentrations during a test meal and by determination of faecal bile acids. With the use of non-radioactive isotopes it is now possible to measure pool size and synthesis rate directly.\(^7\) In this regard it must be noted that the
assumptions implicit in any such study involving isotope dilution must be made, namely, that the bile acids are confined to one pool which is in a steady state during the period of study. It remains to be established whether these assumptions are justifiable in those situations where there appears to be a rapidly expanding pool, eg, in the neonate, or where the steady state is influenced by therapy, eg, phenobarbitone. In the latter circumstances quantitative and qualitative analysis of duodenal and serum bile acids before and after gallbladder stimulation provides a valuable diagnostic aid. The determination of faecal 3 alpha-hydroxy bile acids and of urinary 3 alpha-hydroxy bile acids before and after sulphate solvolysis enables the loss of bile acids from the enterohepatic circulation to be assessed. By means of such laboratory procedures it is thus possible to build up a comprehensive picture of bile acid metabolism in infants and children. For information on pathways of bile acids more sophisticated techniques involving the use of gas-liquid chromatography and mass spectrometry are almost certain to be required.

Summary

It is evident that adaptation to extrauterine life includes the rapid development of the production and secretion of bile acids by the neonatal liver. The factors determining the rate of this development are not known. In the course of normal development the increase in bile acid pool size occurs as a result of an increased synthesis of cholic acid and chenodeoxycholic acid accompanied by an increase in the proportion of glycine conjugation. The role of placental transfer of bile acids in the bile acid metabolism of the foetal liver is not clear. Although it has been demonstrated that the foetal liver is capable of synthesising and conjugating bile acids little is known of the development of the hepatic bile acid secretory mechanisms. If the formation and conjugation of bile acids occurs in the foetal liver at a stage when the secretion into the bile is undeveloped, it might be expected that placental transfer is important for the clearance of bile acids from the foetal circulation.

Malabsorption of fat in the premature and full-term neonate, in children with liver disease, and in children with cystic fibrosis has been shown to be associated with disturbance of bile acid metabolism. The influence of bacterial colonization of the large bowel on bile acid metabolism in the normal infant remains obscure and the nature of the relationship between malabsorption and deconjugation of bile acids with bacterial overgrowth of the small bowel has yet to be firmly established.

Much evidence has accumulated associating the presence of unsaturated mono-hydroxy bile acids with cholestatic liver disease in infants and the study of bile acid patterns in these patients has been recommended as a diagnostic aid, whilst cholestyramine has been suggested as an effective form of therapy. The study of bile acid metabolism in infants and children is obviously a rapidly expanding field and research during the next few years may well provide the answers to many of the problems associated with clinical states in infants and children.

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