Bile salts and liver disease in childhood

NORMAN B. JAVITT
M.D., Ph.D.

Gastroenterology, New York Hospital—Cornell University Medical College

Summary
Recognition of neonatal liver disease has been heavily dependent on the occurrence of jaundice. In most instances the jaundice is related to specific disturbances in bilirubin transport and other tests of liver function are normal. In contrast, hepatitis and other liver diseases not specifically affecting bilirubin transport often go undetected unless jaundice occurs.

The development of practical methods for the estimation of bile acids in serum has permitted an evaluation of hepatic excretory function in neonates and children independent of bilirubin excretion. Since bile acid excretion by the liver each day greatly exceeds bilirubin excretion it was not surprising to find that elevations in serum bile acids occur regularly in anicteric hepatitis. Because the excretion of bile acids generates canalicular bile flow a reduction in the capacity to excrete bile acids intimates the presence of cholestasis. Early cholestasis is not associated with hyperbilirubinaemia but as cholestasis becomes more severe, jaundice occurs and the possibility of biliary atresia arises in neonates. Serum bile acid patterns in neonates being evaluated for biliary atresia indicate two distinctive patterns. Those infants with severe cholestasis and patent bile ducts usually have predominantly cholic acid in serum. This observation is consistent with the bile acid patterns found in intrahepatic and extrahepatic cholestasis occurring in adult life. Infants found to have extrahepatic biliary atresia have marked elevations in the proportion of chenodeoxycholate in serum. Since elevations of chenodeoxycholate in serum are associated with hepatitis, the findings are consistent with the view that extrahepatic atresia is a rare sequela of hepatitis.

Studies of bile acid metabolism and excretion give promise of providing further insight on the pathogenesis of cholestatic liver disease.

My interest in the metabolism and excretion of bile salts is an outgrowth of an interest in the excretory function of the liver (Anderson and Javitt, 1974). Both the liver and the kidney have metabolic functions and excretory functions. Usually the kidney is viewed almost exclusively as an excretory organ and the liver as a metabolic organ. However, the kidney metabolically is a small liver, even to the extent of glucose synthesis, or BSP conjugation or hydroxylation of vitamin D. Similarly, the liver has an important excretory function and actually bile contains small amounts of all the compounds that are ultrafilterable at the glomerulus.

In addition, the liver excretes a number of compounds into bile in high concentration, that normally appear in only trace amounts in urine.

Figure 1 shows the organic anions present in bile and the amount excreted over a 24 hr period. Note that bilirubin is a true pigment in that the relatively small amount in bile is responsible for its striking colour. Actually it is the colourless bile salts that represent the major organic anions in bile. The difference between bilirubin and bile salt as an excretory load on the liver becomes even more striking when one considers the enterohepatic circulation.

Fig. 1. Pool size and enterohepatic circulation of bile salt and bilirubin. Less bilirubin than bile salt is reabsorbed from the intestine and therefore bilirubin becomes an even smaller proportion of the total anion load.

Most of the bile salt entering the intestine is reabsorbed whereas most of the bilirubin is excreted. No data are available on what fraction of the bilirubin might be reabsorbed from the intestine in a physiological setting, but assuming that a fourth or 25% of the bilirubin is reabsorbed and reexcreted in bile this is considerably less than the 90% or more reabsorption of bile acids from the intestine. Therefore, in terms of the daily excretory load on the liver, the bile salts greatly exceed bilirubin.
From a quantitative point of view, therefore, one would guess that disturbances in hepatic excretory function are more likely to be detected by changes in bile acid excretion (Kaplowitz, Kok and Javitt, 1973) rather than changes in bilirubin excretion. However, it is only recently that we have had the technical capability of estimating changes in bile acid excretion and therefore practically all our concepts of hepatic excretion have been based on bilirubin metabolism. The situation is changing rapidly and it is already apparent that estimation of serum bile acid levels is a far more sensitive way of evaluating hepatic excretory function than the estimation of serum bilirubin levels. In particular a lot of liver disease in infancy and childhood is anicteric, and better methods must be found for early recognition of the problem.

Figure 2 illustrates this point. Dr Saul Krugman was kind enough to give us thirty-six sera of patients exposed to hepatitis B virus. All of the samples were 0.2 ml which is the smallest volume ever analysed for bile acids. We used our published method (Ali and Javitt, 1970) by just proportionally reducing all the materials. Sample size is therefore really no longer any reason for not obtaining more information in neonates. When we sent Dr Krugman our results he compared them to the SGOT levels in serum and the antigaemia. Although the antigaemia always occurred earlier, an elevation in serum bile acid occurred in all the patients close to the time of transaminase increase. Needless to say, none of the sera had elevations in bilirubin. This is probably the first evidence that disturbances in hepatic excretory function occur regularly in anicteric hepatitis.

The neonatologist has a special interest in bilirubin, perhaps more in its conjugation than its excretion. The problem of Kernicterus in the neonate is most serious, and fortunately good preventive techniques have been developed. The neonatologist may not know what the bile acid concentrations are in the sera of these infants, and, parenthetically, whether they contribute in any way to the diffusibility of bilirubin into the central nervous system. However, we assume, perhaps incorrectly, that serum bile acid level is always normal and that the only abnormality is in bilirubin metabolism. This indeed, is a definition of hyperbilirubinaemia, an abnormality exclusively in bilirubin metabolism. Except in the newborn period, hyperbilirubinaemia per se, is a benign disorder quite compatible with a long and colourful life. (Except perhaps for the very rare problem of the Crigler-Najjar syndrome.)

It would be nice if we knew as much about bile acid metabolism in the newborn as we know about bilirubin metabolism, but there is very little information available. There follows an outline of the present concepts.

Figure 3 illustrates the studies of Back and Ross (1973). It shows the proportions of mono-, di- and trihydroxy bile acids in normal human meconium obtained from thirteen premature neonates and eleven full term neonates. The origin of all the bile acids and liver disease in childhood

![Figure 2](http://pmj.bmj.com/first_published_as/10.1136/pgmj.50.584.354) on 1 June 1974. Downloaded from http://pmj.bmj.com/ on April 26, 2022 by guest. Protected by copyright.

**Figure 2.** Serum abnormalities following exposure to hepatitis B virus. Serum bile acid levels were analysed in four individuals exposed to hepatitis B virus. In each instance an abnormal elevation occurred usually following the elevation in SGOT. ---, antigaemia; XXX, G0uT; OOOO, bile acid. Symbols extend to first day test became abnormal.

**Figure 3.** Proportion of mono-, di- and trihydroxy bile acids in human meconium. (See Back and Ross, 1973.) Solid, premature; hatched, full term.
acids is not known. Some of them could have been transported across the placenta and others made in the foetal liver. In either event most of the bile acids found were considered to be synthesized in the liver and not the result of intestinal bacterial activity.

If this premise is correct it becomes very difficult to explain the presence of monohydroxy bile acids according to the currently proposed theory of bile acid synthesis (Danielsson and Tchen, 1968).

The metabolic pathway for the synthesis of bile acids from cholesterol is as follows. The cholesterol molecule can be divided into two parts, a steroid ring and a hydrocarbon side chain. The current belief is that in the adult, the metabolism of cholesterol to bile acid begins with the addition of a hydroxy group at the seven position to form 7α-hydroxycholesterol. Once this rate limiting step occurs, there is a very rapid transformation of the steroid moiety into either di- or trihydroxycoprostone and then oxidation of the side chain to a C-24 bile acid. The important point to bear in mind in this pathway is that bile acids with only one hydroxyl group are not formed as intermediates. The end products are the dihydroxy bile acids, chenodeoxycholic acid and a trihydroxy bile acid, cholic acid.

Although this theory of bile acid synthesis may be entirely correct for the adult, it raises some questions in regard to foetal and neonatal life. In order to explain the in vivo production of monohydroxy bile acids one has to postulate an alternate pathway. One possibility is that bile acid synthesis from cholesterol begins with side chain oxidation of cholesterol to 26-hydroxycholesterol. This alternate pathway has been investigated in our laboratory by a series of studies in both man and animal. Emerman (Emerman, Wachtel and Javitt, 1968) was able to isolate

![Diagram](http://pmj.bmj.com/)

**FIG. 4.** Effect of bile salts on bile flow. Infusion of monohydroxy bile salts into rats causes a prompt reduction in bile flow. Di- and trihydroxy bile salts cause an increase in bile flow and when given together with monohydroxy bile salts prevent the cholestatic effect. The mechanism is believed to be related to precipitation of monohydroxy bile salts in the biliary tree and is prevented by micellar solubilization.
monohydroxy bile acids from animals given 26-
hydroxycholesterol, chiefly, the one found in
the urine and meconium of infants. Dr Karl Anderson
has shown that in man both 7α- and 26-hydroxy-
cholesterol are metabolized to chenodeoxycholic and
cholic acids (Anderson, Kok and Javitt, 1972). More
recently, Dr Uri Lavy (Lavy, Burstein and Javitt,
1973) has found that normal human meconium con-
tains relatively large amounts of 26-hydroxy-
cholesterol but no 7α-hydroxycholesterol. Thus we
believe that both pathways may exist in man.

Our interest in the metabolic pathways for bile
acid synthesis came about when we found that mono-
hydroxy bile acids had a special effect on bile flow
(Javitt and Emerman, 1968). They completely
suppress flow when given in large amounts. This
phenomenon is illustrated in Fig. 4.

If one infuses a monohydroxy bile acid into a rat
or hamster, bile flow immediately falls and a chole-
static syndrome ensues and lasts from 24 to 48 hr
followed by a complete recovery. Di- and trihydroxy
bile acids always increase bile flow and will prevent
the cholestasis caused by monohydroxy bile acids
by forming soluble complexes with them.

With all this work behind us, we began to investi-
gate the cholestatic syndromes occurring in the new-
born period, thinking that some of this very exciting
experimental work must prove to be the patho-
physiological mechanism underlying neonatal choles-
tatic liver disease. We have made some interesting
observations, perhaps more important than we were
planning. However, we have not as yet established a
metabolic error in bile acid synthesis as a cause of a
neonatal cholestatic syndrome.

Let us review our studies in infants which have
been confined almost exclusively to an analysis of
serum bile acid patterns.

Since the normal liver has a great capacity to
remove bile acids from the blood, the normal level
in serum is about 1–2 μg/ml. At this very low
level we can not be as certain of the identification of
each bile acid in serum. However, in various liver
diseases the concentration rises from 10 to 200 fold
and at these levels we can be quite certain about the
proportion of each bile acid in serum although we
are less certain how accurately the proportions in
serum reflect the proportions in the gallbladder or
the intestines.

In considering serum bile acid patterns in diseases
of the liver and biliary tract one can evaluate both
the proportion of each bile acid in serum and the
total concentration in serum.

Figure 5 summarizes our experience in evaluating
adult human liver disease. If the ratio of chenodeoxy-
cholate to cholate is less than 0·4 we can be certain
we are dealing with hepatitis or cirrhosis. If the ratio
is greater than 4 we can be quite certain we are deal-
ing with a cholestatic syndrome. By a cholestatic
syndrome we mean that there is a disturbance in bile
flow with little or no evidence of cell necrosis. The
disturbance may be related to mechanical obstruc-
tion or some metabolic failure in the flow generating
system of the liver cell. In hepatitis and cirrhosis the
total levels are less than obstructive disease. Clearly
there is a large group in which the ratio and the totals
do not give us a great deal of confidence in the diag-
nosis.

Now when we began to evaluate serum bile acid
patterns in the newborn period (Javitt et al., 1973),
we accidentally came across some very striking
patterns that puzzled us greatly.

Figure 6 shows a serum bile acid pattern of an
infant that is being followed at New York University
Medical Center and was our first experience. We use
an internal standard that has a retention time longer
than any of the normal bile acids and therefore it is
unlikely that we would miss one in our analysis.
Note that the serum contained more than 90% chenodeoxycholate with a rather high total of 133
μg/ml. The child was about 3 months old and had
been explored and found to have extrahepatic biliary
atresia. A pattern like this does not exist in an adult.
The only pattern that comes close to it, is that seen
in advanced, pre-terminal cirrhosis, where one sees
almost all chenodeoxycholate but the total is much
lower. The lower total in cirrhosis is presumably
related to the very low synthesis of bile acids.

Naturally, we began to analyse further specimens
from around the country and rather quickly and most
surprisingly it became apparent to us that infants

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Hepatitis cirrhosis</th>
<th>Indeterminate</th>
<th>Cholestasis intra or extra hepatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholate/chenodeoxycholate</td>
<td>&lt; 0·4</td>
<td>0·4–4</td>
<td>&gt; 4</td>
</tr>
<tr>
<td>Total (μg/ml)</td>
<td>&lt; 40</td>
<td>40–100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

FIG. 5. Serum bile acid patterns in adult liver disease. Bile acid patterns can occasionally be diagnostically helpful, but often there are values that are indeterminate.
finally as cirrhosis develops, become predominantly chenodeoxycholate again.

Since our initial observation we have had the opportunity to analyse serum bile acid patterns in twenty-five infants and children sent to us from all over the U.S. and the results are summarized in Fig. 7. The surprise is that extrahepatic atresia is segregating with hepatitis and not with other cholestatic syndromes. We think that our data strongly suggest the view that some paediatricians have had for a long time. Extrahepatic atresia may be, in most instances, an acquired sequelae of neonatal hepatitis and not a primary foetal abnormality in the anatomical formation of the biliary tree.

If we accept as a working hypothesis that at least some types of extrahepatic biliary atresia are a consequence of hepatitis, how does this affect our approach to the problem? It is certain that one approach must be a co-operative one. It is a rare disease with a variable course and unless physicians develop together some uniform therapeutic approach we will never know which treatment may be most successful. This does not refer to a uniform surgical approach by rather a uniform medical approach. Many of these neonates really have anicteric hepatitis and the first sign of jaundice is evidence of partial atresia. At first only a portion of the duct system may become occluded and if the inflammation subsides the remaining patent system compensates and the jaundice disappears. In others, the closing of the ducts follows a relentlessly progressive course. If this hypothesis is correct, then we need some way of detecting anicteric hepatitis early, perhaps routine immunoassay for serum bile acids. There are other aspects of our studies.

There are a group of neonates who develop jaundice as part of a cholestatic syndrome early in life and often the hyperbilirubinaemia will fall spontaneously to levels of 3–4 mg/%. Although they have patent bile ducts, they are often explored and it is common practice to do some type of venting procedure usually a choledochoenterostomy. Many of these infants will respond to either phenobarbitone or cholestyramine and surgery should not be done until a diagnostic or therapeutic trial of these medications is carried out (Morrissey and Javitt, 1973).

<table>
<thead>
<tr>
<th></th>
<th>Hepatitis extrahepatic atresia</th>
<th>Indeterminate</th>
<th>Cholestasis patent bile ducts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio</td>
<td>&lt; 1</td>
<td>?</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Cholate/chenodeoxycholate Total (µg/ml)</td>
<td>30–130</td>
<td>?</td>
<td>100–400</td>
</tr>
</tbody>
</table>

Fig. 7. Serum bile acid patterns in neonatal liver disease. Neonates with hepatitis and extrahepatic biliary atresia segregate together.
Figure 8. Effect of cholestyramine feeding in infants (solid line) and children (interrupted line) with patent bile ducts and with extrahepatic biliary atresia. Cholestatic syndromes with patent bile ducts tend to have cholate/chenodeoxycholate ratios greater than one and rise progressively on cholestyramine feeding (from L. Schiff, Diseases of the Liver, Fourth Ed.) (in press.) --- patent bile ducts; - • - , extrahepatic biliary atresia.

Figure 9. Neonatal cholestatic syndromes; cholestatic syndromes are considered to be disturbances in the canicular flow generating mechanism of the liver.

any effect it may have on total bile acid excretion. Other agents, such as ethacrynic acid (Shaw et al., 1972) and ouabain (Graf, Korn and Peterlik, 1972), also increase canicular bile flow without apparently affecting bile salt excretion. These observations imply the existence of ionic pumps that may be governed by mechanisms independent of bile salt concentration. Instances of neonatal cholestasis have been reported in which the patients respond to phenobarbital and not cholestyramine (Ballow et al., 1973) and perhaps these infants represent examples of defects in bile salt nondependent flow.

Other instances occur in which cholestyramine appears to have a much more beneficial effect than phenobarbital (Sharp and Mirkin, 1972). Since the only established effect of cholestyramine is its capacity to bind bile acids it implies that it may be correcting some aspect of bile salt transport and is therefore related to bile salt dependent flow.

Although monohydroxy bile acids have been found as the conjugated and sulphated derivative in the urine of infants with cholestatic syndromes, we have no precise idea of whether they initiate or perpetuate the cholestasis. Preliminary studies in animals (Javitt, 1973) indicates that the conjugated and sulphated compound do not affect bile flow but the situation could be very different in man.

References


Professor Aagenaes of Oslo made a plea that the term 'Neonatal Hepatitis' or 'Neonatal Hepatitis Syndrome' be dropped from the nomenclature of neonatal liver disease since the word 'hepatitis' implies infection which appears to be a rare cause of neonatal obstructive jaundice. He suggested that a more neutral term such as 'Intrahepatic cholestasis' would be a more correct term for this clinical entity.

Dr Danks of Melbourne felt it was very difficult to get an all-embracing terminology. He used the term 'Neonatal Hepatitis' for children in whom liver biopsy showed a degree of cholestasis with liver cell necrosis and some reaction to these two abnormalities in the form of inflammatory cell infiltrate, fibroblastic proliferation and the development of multinucleated cells. This definition would exclude patients with mild disease who did not have a liver biopsy and would include such well defined genetic entities as galactosaemia or hereditary fructose intolerance; conditions which could not be distinguished on histological grounds. He thus found it simplest to use the term 'Neonatal Hepatitis' to cover everything, including those with predominant liver cell necrosis or predominant cholestasis, and any other variations.