Middle Articles

CLINICAL PROGRESS

Recent Advances in Jaundice

In the articles below three contributors discuss recent progress in the physiology and medical and surgical aspects of jaundice. Dr. R. P. H. Thompson emphasizes the need for electron-microscope and biochemical studies in the screening of new drugs for hepatotoxicity. Dr. Roger Williams considers that screening for Australia antigen may have an important role in long-term haemodialysis units. Mr. J. L. Dawson recommends that all patients undergoing surgery should have prophylactic treatment with mannitol.

Physiology

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Haem, a compound of iron and protoporphyrin, is the prosthetic group of haemoglobin and myoglobin. It is also found in cytochrome enzymes and in other haem compounds, chiefly in the liver. When haem is broken down the iron can be used again, but the protoporphyrin component cannot and is completely catabolized to bilirubin. Bilirubin is formed only from haem, but the mechanism of this degradation is still unknown. Studies using precursors of haem labelled with radioactive isotopes have shown that they are incorporated into protoporphyrin and later into its breakdown products—the bile pigments. The results of such experiments show that the bilirubin in bile may be divided into at least three fractions, derived mainly from the haem in the red cells, bone marrow, or the liver.

About 80-85% of the 250-300 mg. of bilirubin formed daily by an adult man comes from the breakdown of the haemoglobin in the red cells. This type of haem is degraded about 120 days after it has been synthesized, when the red blood cells die. This means that bile excreted four months after a single injection of labelled glycine has been given is found to contain the radioactive label. The remaining 15-20% of the radioactive glycine that is incorporated into bilirubin appears

another labelled precursor of haem—namely, aminolaevulinic acid—is used the pattern of labelling of bilirubin is quite different. With this precursor the early bilirubin is labelled predominantly; only small amounts enter red-cell haem. Further, this labelled bilirubin is excreted mainly in the first 24 hours, being derived from the hepatic haem compounds with a rapid turnover, rather than from haem in the bone marrow (Fig. 1).

In normal man the relative contributions of the hepatic and erythropoietic components to the early bilirubin are not known, but the latter probably predominates.¹ In patients with congenital, erythropoietic porphyria the erythropoietic early bilirubin is much increased, and in thalassaemia minor and pernicious anaemia this substance may be the main cause of jaundice. Nevertheless, in human liver disease the importance of the hepatic component of early bilirubin is not yet clear.

Bilirubin in the Blood

In blood bilirubin is firmly bound to albumin. Since in the adult the albumin in 100 ml. of blood can bind at least 40 mg., it is seldom saturated. Nevertheless, presumably some bilirubin can become detached from albumin and when it becomes bound to the proteins of the tissues gives rise to jaundice. Protein-bound drugs, such as salicylates and sulphonamides, compete with bilirubin for albumin, and hence displace bilirubin into the tissues. Because of this these drugs may precipitate brain damage or kernicterus when given to infants. The susceptibility of infants to kernicterus is probably due partly to their high unconjugated bilirubin levels in the presence of hypoxia and acidosis, which decrease the binding of bilirubin to albumin. The infant brain may also be damaged more easily by bilirubin than is that of adults, though kernicterus has been described in an adolescent.² It is not yet clear whether bilirubin itself is toxic to nerve cells in vivo.

Bilirubin does not normally pass into urine because it is firmly bound with albumin, and it is relatively insoluble in protein-free aqueous solutions. Being soluble in lipid it can in theory pass across the bowel-wall in both directions, and this potential route of excretion may be important in severe, congenital unconjugated jaundice (Crigler-Najjar syndrome).

A few minutes after the injection of labelled bilirubin over half of it is found free of albumin inside the liver cells. In vitro protein-bound bilirubin does not cross membranes unless albumin is added also to the dialysing solution.³ Similarly, therefore, a protein inside the cells that had a stronger affinity for bilirubin than albumin would facilitate the entry of

FIG. 1.—Pattern of incorporation of labelled glycine into the three major components of bilirubin in bile.

much earlier in the bile, within a few hours to ten days after the injection. This *early bilirubin* has two components. The first is derived from the breakdown of haem compounds which have been incorporated into red blood cells that have not been released from the marrow into the circulation. The second is derived from haem compounds in the liver which have never been incorporated into red cells. Nevertheless, if

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the pigment into the cells. A. J. Levi and his colleagues have recently described two such proteins in the cytoplasm of liver cells, which also bind other anions such as bromsulphalein.⁴ The existence of these binding proteins may explain why hepatic cells have such an outstanding affinity for bilirubin.

Bilirubin in the Liver

By itself bilirubin does not appreciably enter the bile to any extent. Nevertheless, after it has been taken up by the liver bilirubin is conjugated with glucuronic acid by the enzyme glucuronyl-transferase. This enzyme is found on the membranes of the endoplasmic reticulum, which when separated by centrifugation are known to the biochemist as microsomes. The conjugated bilirubin molecule is more water-soluble than previously, and can be excreted into bile. The endoplasmic reticulum probably bears a whole range of different glucuronyl-transferases that similarly conjugate compounds such as chloramphenicol, steroids, and thyroxine (Fig. 2).

FIG. 2.—Passage of bilirubin through the hepatic cell.

Unfortunately, up to the present determination of bilirubin glucuronyl-transferase has proved difficult, for only a small amount of the conjugated product is produced in the presence of an excess of unconjugated bilirubin.

Hepatic glucuronyl-transferase activity is low or absent in patients with congenital, moderate, or severe, unconjugated jaundice (Crigler-Najjar syndrome), who seem to be of two types.⁵ Type I patients have severe jaundice (bilirubin above 22 mg. per 100 ml.), some develop kernicterus, and they have no bilirubin in bile. Type II patients are less severely affected (bilirubin 8–22 mg. per 100 ml.), without kernicterus, and conjugated bilirubin is found in bile. In Type I patients, there is probably a recessive inheritance of a partial enzyme defect.

There is also recent evidence that the activity of this enzyme may be much lower than previously thought in patients with the mild unconjugated hyperbilirubinaemia of Gilbert's syndrome, in whom bilirubin levels are below 6 mg. per 100 ml.⁶ Moreover, the enzyme activity is probably also low in the livers of neonatal primates.⁷ The immaturity of a similar enzyme probably explains the toxicity of chloramphenicol to the newborn, for this drug is also conjugated with glucuronic acid.

Several drugs specifically inhibit glucuronyl-transferase activity when they are added to enzyme systems *in vitro*, but the relevance of these results to conditions *in vivo* is doubtful. Some children have been described who have developed jaundice when they were started on breast feeding. Examination of the breast milk of their mothers showed high concentrations of an unusual steroid, 3α , 20β -pregnanediol. This compound has been reported to inhibit glucuronyl-

transferase *in vitro*, and when administered to normal infants produces transient jaundice. Though the steroid may be the cause of this unusual syndrome, recent evidence is contradictory.⁸ A more severe and familial variety of neonatal jaundice (Lucey-Driscoll Syndrome) occurs in the babies of women whose serum during pregnancy strongly inhibits glucuronyl-transferase activity *in vitro*. The inhibitory factor has not been identified.

Some drugs damage microsomal enzymes as part of a more general injury to liver cells. Tannic acid and yellow phosphorus, for instance, probably first affect the endoplasmic reticulum—which, as seen by the electron-microscope, rapidly becomes abnormal—and then proceed to cause cell necrosis by an unknown mechanism. A few toxins, such as carbon tetrachloride or mushroom toxins, are even metabolized in the endoplasmic reticulum to give rise to products with increased hepatotoxicity. Since the patterns of membrane and enzyme changes are similar for most predictable hepatotoxins,⁹ electron microscopy and detailed biochemical studies should be included in the screening of all new drugs.

Secretion of Bilirubin

In general, organic compounds are excreted into bile if they are large and water-soluble, while smaller molecules (even after detoxication in the liver) are excreted into the urine. The molecular structures necessary for excretion via the bile are not yet known.

Once conjugated, bilirubin is rapidly excreted, presumably against a concentration gradient, across the microvilli of the canalicular wall into the bile. This secretion of bilirubin is probably the limiting step in its overall excretion, though the maximum excretory capacity of the liver for bilirubin is greatly above normal requirements. In the rare Dubin-Johnson syndrome there is a defect in the excretion of conjugated bilirubin and dyes such as bromsulphalein and indocyanine green (Fig. 3). It has been suggested that bile salts share an active transport mechanism with bilirubin, but in the Dubin-Johnson syndrome levels of conjugated bilirubin, but not bile salts, are raised in the blood and radiographic dyes increase bilirubin levels. All these dyes, other anions, and bilirubin may, therefore, have a common excretion mechanism which is separate from that for the bile salts.

FIG. 3.—Plasma levels of the dyes bromsulphalein (B.S.P.) and indocyanine green (I.C.G.) after their intravenous injection into a patient with the Dubin-Johnson Syndrome. The delayed rise of B.S.P. is due to the conjugated dye, but that of I.C.G. to the unchanged compound. Half-life-time ($7\frac{1}{2}$) of B.S.P., 6–4 minutes, and of conjugated bilirubin, 29 hours (derived from levels between 8 and 72 hours). $7\frac{1}{2}$ of I.C.G., 4 minutes. Doses: B.S.P. 5 mg. per kg. body weight, I.C.G. 0.5 mg. per kg. (Thompson and Williams, unpublished data).

Conjugated bilirubin is less firmly bound to albumin than is free bilirubin pigment, especially when the serum level of bile salts is raised—and some enters urine.³ When renal function is normal, this excretion prevents a massive rise in the blood bilirubin level. Since conjugated bilirubin is insoluble in fat it passes less well across the blood vessel wall into the brain,

which may be the reason that kernicterus does not occur in conjugated hyperbilirubinaemia.

Cholestasis

Cholestasis may be defined as stagnation of bile within the intrahepatic or extrahepatic biliary tree. Biochemically, there is retention of conjugated bilirubin, bile salts, and alkaline phosphatase; morphologically, there is at least dilatation of biliary canaliculi and loss of their microvilli, and later accumulation of bile pigment in the canaliculi (bile plugs).¹⁰ The mechanism underlying the canalicular lesion is unknown,¹¹ though a variety of hepatic diseases may cause it. Similar cholestasis has been produced by the administration of some bile salts, such as lithocholates, which are relatively insoluble and may precipitate in the canaliculi; hence qualitative changes in the metabolism of the bile acids could be important.¹²

Bile can be secreted against a hydrostatic pressure, and is composed of at least two fractions, to which excreted molecules are added. The first, produced by the liver cells, closely follows the secretion of the bile salts; the second (which may be secreted by the smaller biliary passages), is produced independently of the bile salts, but its production is increased by the hormone secretin. The rates of excretion of bilirubin or bromsulphalein and other dyes are not constant, but increase

as the volume of bile increases. Furthermore, drugs such as phenobarbitone which induce the synthesis of hepatic microsomal enzymes, also often increase the size of the liver, bile flow, and the biliary excretion of substances such as chlorothiazide and ICG which are not metabolized by hepatic enzymes.¹³ Conversely, drugs such as oestrogens and the fungal steroid icterogenin which produce cholestasis also decrease bile flow.

Many other steroid drugs, especially if their carbon atom 17 is substituted, produce cholestasis. A few women develop a similar jaundice while taking the contraceptive pill, and of the two components of the pill the oestrogen, rather than the progestogen, is probably the cause of this reaction¹⁴—which is observed more commonly in Scandinavia and Chile than elsewhere. Some of these women also develop itching and cholestatic jaundice in the last trimester of successive pregnancies, when the high levels of oestrogen are probably the cause. Though the mechanism of this steroid cholestasis is unknown, in the rat oestrone may increase the permeability of the biliary tree to fluid and reduce the flow of bile.¹⁵

Classification of Jaundice

Any classification of jaundice is difficult. The first consideration is whether unconjugated or conjugated bilirubin predominates in the blood, the latter being much the commoner cause of jaundice. Thus massive haemolysis will overload the normal capacity to excrete bilirubin and occasionally ineffective erythropoiesis may be a cause of jaundice. These are *prehepatic* causes of unconjugated jaundice (see Table).

The second group, *hepatic* causes of unconjugated jaundice, includes impairment of uptake of bilirubin, as may occur in Gilbert's syndrome or after the administration of a few drugs such as flavaspidic acid (male fern extract). The conjugation of bilirubin may be inadequate in the Crigler-Najjar syndrome, in normal neonates, and possibly in children born to the rare women who have high serum levels of 3 α , 20 β -pregnandiol or of the Lucey-Driscoll factor.

Hepatic causes of conjugated jaundice are the commonest group. In the rare Dubin-Johnson syndrome, there is an isolated abnormality of bilirubin secretion, while in intrahepatic cholestasis due to primary biliary cirrhosis in its early stages, intrahepatic biliary atresia, or steroid drugs secretion of all biliary constituents is held up. In drug or viral hepatitis and in cirrhosis there may be multiple defects, including haemolysis, impaired transport, and cholestasis due to widespread damage to the liver cells.

Posthepatic or extrahepatic obstructive jaundice may be due to factors such as gallstones, carcinoma of the pancreas, etc.

Classification of Jaundice

Predominant pigment in Blood	Anatomical	Physiology	Examples of Aetiology
Unconjugated	Prehepatic	Increased bilirubin production	{ Haemolysis Ineffective erythropoiesis ? Hepatic haem catabolism
	Hepatic	Hepatic uptake impaired Glucuronyl-transferase activity —low —absent —inhibited	{ ? Gilbert's syndrome Flavaspidic acid { Neonates Crigler-Najjar (Type I) " " (Type II) 3,20—pregnandiol Lucey-Driscoll syndrome
Conjugated	Hepatic	Secretion of bilirubin impaired Intrahepatic cholestasis Hepatocellular damage	Dubin-Johnson syndrome { Early primary biliary cirrhosis Intrahepatic atresia Drugs—for example: steroids chlorpromazine Drug or viral hepatitis Cirrhosis
	Posthepatic	Extrahepatic cholestasis	Gallstones or carcinoma

Medical Aspects of Investigation and Treatment

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In the past year exciting advances in the detection of the elusive hepatitis virus have been reported. Other interesting recent developments include classification of chronic hepatitis; the treatment of some varieties of this condition by immuno-suppressive agents; the use of enzyme inducing agents in treating unconjugated hyperbilirubinaemia. A better appreciation of the functional disturbance in chronic cholestasis, and of the value of immunological tests and of percutaneous trans-

hepatic cholangiography in its differential diagnosis has led to a more rational approach to management.

Acute and Chronic Hepatitis

Role of Australia Antigen

This antigen was first found by B. S. Blumberg and his colleagues¹⁶ in the serum of an Australian aborigine—hence its name, which gives little indication of its probable association or identity with the virus of infective hepatitis. Characteristically a precipitin reaction occurs when Australia antigen

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