ABO HAEMOLYTIC DISEASE OF THE NEWBORN

ABO Haemolytic Disease of the Newborn

By

E. R. Gold, M.D.

Research Fellow, Department of Child Health, University of Bristol

and

N. R. Butler, M.D., F.R.C.P., D.C.H.

Professor of Child Health, University of Bristol

BRISTOL
JOHN WRIGHT & SONS LTD
1972

ISBN 0 7236 0321 9

PRINTED IN GREAT BRITAIN BY JOHN WRIGHT AND SONS LTD.
AT THE STONEBRIDGE PRESS, BRISTOL BS4 5NU

Preface

We are concerned in this monograph only with ABO haemolytic disease of the newborn (HDN). We hope to have covered it more fully than if Rh and other kinds of HDN were included in their own right. Nevertheless, there are so many similarities that some overlap is inevitable. We are well aware that, even so, it has not been possible to give an exhaustive account of the whole literature but we hope nevertheless that it will be useful for blood-group serologists, obstetricians, and paediatricians.

The first chapter is devoted to hyperbilirubinaemia and jaundice, the second deals with the serology of ABO HDN in man, and the next with the clinical forms, haematological findings, and histopathology of the disease, leading on to the final chapter on interactions between mother and foetus and ABO incompatibility.

An appendix contains a chapter on diagnostic techniques and chapters on historical aspects of HDN and HDN in animals.

In the chapter on diagnostic techniques, much help has been afforded by the book entitled *Techniques in Blood Grouping* by Dr. C. C. Bowley and the late Dr. I. Dunsford, and in the chapters 'Historical and General Aspects of HDN' and 'HDN in Animals' by the publication *Comparative Aspects of Haemolytic Disease of the Newborn* by Dr. G. F. Roberts.

We wish to express our appreciation to these authors.

ABO HDN can only be well understood in the wider immunological context of interactions between mother and foetus and in relation to the physiopathology of neonatal jaundice, so no apology is necessary for the discussion of these basic subjects in the first and last chapters of the book.

Many problems of ABO HDN are still unsolved. Among these are the frequent lack of a positive direct antiglobulin test with the red cells of a newborn suffering from ABO HDN, the specificity of cross-reacting antibody, the structure and configuration of the combining site of pathogenic anti-A and anti-B and of the A and B antigens, and the topochemistry of the latter in the cell membrane. There are also still a considerable number of cases of neonatal jaundice of unknown aetiology. At the present

rate of progress in basic immunology and immunochemistry, it is reasonable to hope that soon much more will be known about the serology of ABO HDN and about the aetiology of neonatal jaundice.

We thank Dr. D. Voak and Mr. Gerald Keen for help and advice in preparing the manuscript.

E. R. G.
N. R. B.

Bristol, 1972

Contents

ABO HAEMOLYTIC DISEASE OF THE NEWBORN

CHAPTER 1

HYPERBILIRUBINAEMIA AND JAUNDICE

THE earliest mention of jaundice was probably by Hippocrates in 400 B.C.; Galen attributed jaundice to stones in the biliary tract and Avicenna about A.D. 1000 in his Canon distinguished jaundice due to obstruction from jaundice due to other causes.

A connexion between haemoglobin and bile-pigments was first established by Virchow (1847). He described the formation of haematoidin (equivalent to bilirubin) from haemoglobin in blood extravasation. Thirty years later confirmation was forthcoming of increased formation of bilirubin after haemolysis (Tarchanoff, 1874).

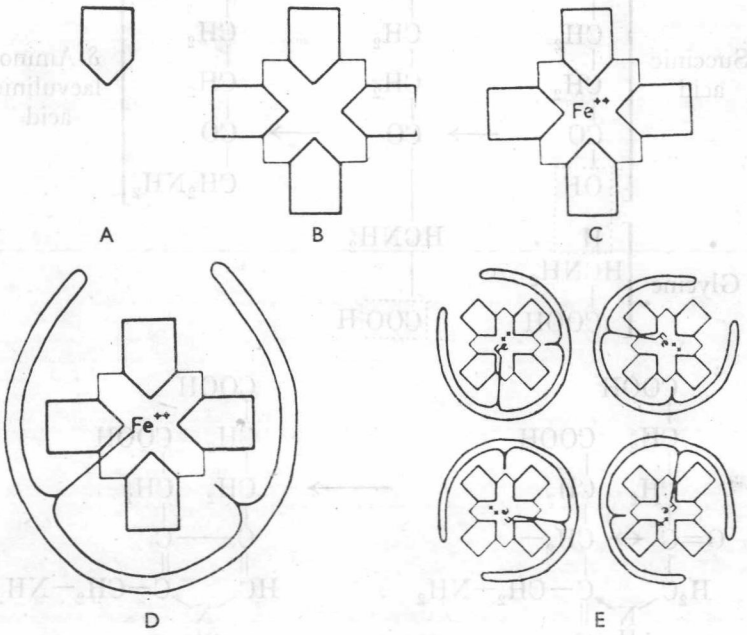


Fig. 1.—Steps in the development of haemoglobin (*by kind permission of Lehmann and Huntsman*, 1966).

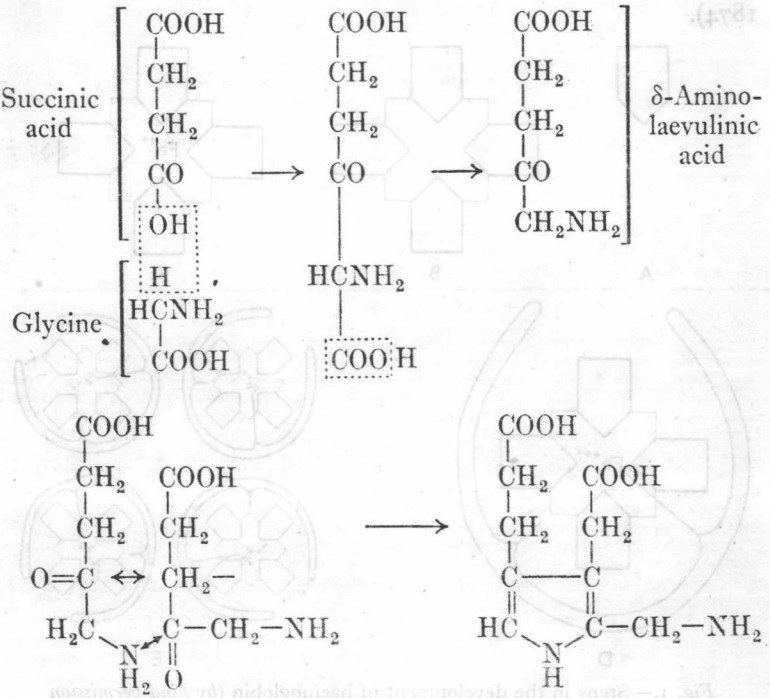
Before discussing jaundice due to ABO haemolytic disease of the newborn (ABO HDN) it is necessary to mention some elementary aspects of synthesis and breakdown of haemoglobin, to give a brief outline of bilirubin metabolism and of the pathogenesis of the various forms of jaundice, and to try to define the meaning of 'physiological jaundice'.

Synthesis and Breakdown of Haemoglobin

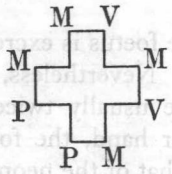
Haemoglobin consists of four haem-globin units forming one complex molecule (*Fig.* 1).

The haem disk measures 14×17 Å and consists of a porphyrin ring with an iron atom at its centre. Its synthesis is assumed to proceed along the following lines. The basic units are succinic acid and glycine; they combine, lose first water and then carbon dioxide, and form delta-aminolaevulinic acid.

Two molecules of delta-aminolaevulinic acid condense into porphobilinogen which is a pyrrole ring structure:



Then four porphobilinogen molecules combine, thus forming the porphyrin ring:

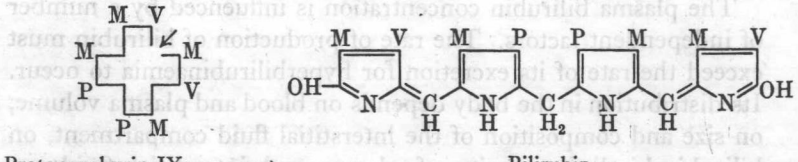


M = Methyl H_3C-
V = Vinyl $H_2C=CH-$
P = Propionic $HOOC-CH_2-CH_2-$

The porphyrin ring of haem has two vinyl, four methyl, and two propionic acid groups. There exist fifteen different arrangements of these groups. The porphyrin ring of haem is called 'protoporphyrin IX'. We shall see later that the two propionic acid groups are important for the excretion of bilirubin.

The fate is different for the three basic parts of human haemoglobin—globin, porphyrin, and iron. When, after a life span of approximately 120 days, the senescent red cell is finally taken up by the reticulo-endothelial system, iron and globin are re-utilized but the haem is catabolized. The waste product, bilirubin, is excreted as pigment dissolved in the bile.

When the iron atom is removed from the porphyrin ring, the latter is opened by a series of chemical reactions and transformed into bilirubin. Bilirubin is a straight-chain complex consisting of four pyrrole rings (tetrapyrrole) with two hydroxyl groups.



The passage of bilirubin from the cells of the reticulo-endothelial system into the blood-stream is probably an active process; once in plasma it is conjugated with albumin, its intravascular carrier, and is called 'prehepatic bilirubin'. We shall see later in CHAPTER 4 that the knowledge of this binding leads to an important therapeutic application.

Distribution of Bilirubin

Placental Clearance

A large part of the bilirubin produced by the foetus is excreted through the placenta even in severe Rh HDN. Nevertheless, the prenatal concentrations of foetal bilirubin are usually twice as high as those of the mother's. On the other hand, the foetal mechanism of excretion is more efficient than that of the neonatal liver, which is shown by the fact that the postnatal peak of physiological hyperbilirubinaemia rises still higher. Probably no significant conjugation occurs in the placenta. As is the case with most biological membranes, transplacental passage of bilirubin is not just a simple physicochemical process of diffusion. This is exemplified by the lack of jaundice in infants born to jaundiced mothers and is also supported by simultaneous measurements of bilirubin in maternal venous blood, in retroplacental blood, and in cord blood.

Body Distribution of Bilirubin after Birth

Bilirubin is normally found in plasma, in interstitial tissue fluids, and inside the cells. In deep jaundice it is also present in ocular fluids, milk, urine, sweat, and semen. The concentration of bilirubin in brain and the problem of the blood–brain barrier will be discussed under KERNICTERUS, p. 27.

The plasma bilirubin concentration is influenced by a number of independent factors. The rate of production of bilirubin must exceed the rate of its excretion for hyperbilirubinaemia to occur. Its distribution in the body depends on blood and plasma volume, on size and composition of the interstitial fluid compartment, on bilirubin-binding capacity of plasma and interstitial fluid, on diffusibility and solubility of bilirubin, on local differences in vascular permeability, and, finally, on affinity of tissues for bilirubin.

The intravascular and extravascular compartments both contain bilirubin which is mostly bound to albumin. Their volume is approximately identical and they are in a dynamic equilibrium. There is a great variability of blood and therefore also of plasma volume in the newborn infant. Neonates also have variable weight-loss and fluctuating states of hydration. All these factors contribute to the lability of the distribution of bilirubin in the newborn body fluids.

The importance of albumin binding of bilirubin is illustrated by the fact that certain drugs successfully compete with bilirubin for binding sites and so displace bilirubin from its complex with albumin. The disturbed equilibrium between plasma and tissues is rapidly restored by an increase in bilirubin levels in the interstitial fluid. Thus in spite of excess production or decreased excretion of bilirubin, the serum bilirubin level may be deceptively low. Rapid re-equilibration in the opposite direction takes place during exchange transfusion. It is well known that after an exchange transfusion the plasma bilirubin may rise again considerably, sometimes necessitating another exchange transfusion. In some cases of HDN this is explained by continuing destruction of the red cells of the newborn. However, the same so-called 'rebound phenomenon' has also been observed in non-haemolytic jaundice and is then caused by influx of bilirubin due to vascular permeability.

The affinity of tissues for bilirubin varies; unconjugated bilirubin is lipophilic (*see also* KERNICTERUS, p. 27) so that fat and elastic tissue show a great affinity for bilirubin. Although jaundice and hyperbilirubinaemia mostly occur simultaneously, there may not be complete parallelism between plasma bilirubin levels and the degree of jaundice. This was shown convincingly by Davidson, Merrit, and Weech (1941). When the plasma bilirubin level was high or, alternatively, fell below a certain level, jaundice was respectively pronounced or absent, but in the intermediate range discrepancies were observed. At relatively low levels of bilirubin some of the infants tested were jaundiced, whereas conversely at much higher levels not all the infants were icteric. Lack of parallelism is also observed at the onset and during the disappearance of jaundice. In HDN jaundice is often present when the plasma concentration of bilirubin is rising, but during recovery the depth of skin jaundice often does not correspond to serum bilirubin levels as bilirubin can still be firmly bound by albumin. A patient may remain icteric for a few days after his serum bilirubin concentration has returned to normal. During exchange transfusion, however, visible jaundice decreases even when the plasma concentration of bilirubin rises due to influx of bilirubin from the tissues.

Odell, Natzschke, and Storey (1966) gave rats a load of bilirubin which exceeded the excretory capacity of the liver. They found that approximately 68 per cent of the injected bilirubin was

bound by non-adipose tissue. It is nevertheless possible that fat may play a role as a reservoir for holding bilirubin, indeed the poorly developed adipose tissue of premature infants may be a factor which contributes to the raised bilirubin levels observed in such infants.

Very little is known about intracellular bilirubin. It must be in dynamic equilibrium with bilirubin in adjoining tissue spaces and its concentration depends on the properties and amount of substances in which it is dissolved or to which it is bound. In severely jaundiced infants it is present in high concentration and has been found precipitated in crystalline form.

Metabolism of Bilirubin

Excretion of Bilirubin

Unconjugated bilirubin is excreted by the kidney at most only in traces, because it is not water-soluble. It has also been speculated that some bilirubin may be transformed into 'bilirubinoids' and then further degraded to substances at present unidentified; these products are excreted in bile and in urine or through the gut. So a new equilibrium between production and excretion (though at a higher level) could conceivably be established.

Preliminary observations in cases of hereditary hyperbilirubinaemia in horses and cattle seem to show that such mechanisms exist. Observations on bilirubin metabolism in a few infants suffering from the Crigler-Najjar syndrome can hardly be explained otherwise.

Bilirubin Metabolism in the Liver

Excretion of bilirubin is a complex and active process which is still incompletely understood. It consists basically of dissociation from albumin, uptake by liver cells, intracellular transport to the microsomes where conjugation takes place, and finally secretion into bile canaliculi.

Conjugated bilirubin is perhaps stored temporarily before being secreted. It is assumed that dissociation of bilirubin from albumin occurs outside the hepatocyte, though the actual mechanism is as yet unknown.

Arias, Gartner, Cohen, Ezzer, and Levi (1969) assume that in type I, chronic, non-haemolytic, unconjugated hyperbilirubinaemia with glucuronyl-transferase deficiency, bilirubin may perhaps be excreted across the intestinal mucosa because in such

cases the bile was colourless but the stools were coloured. Uptake of bilirubin by the liver is faster than that of albumin (Odell, 1967). Uptake of bilirubin may be due to the acceptors described by Levi, Gatmaitan, and Arias (1969). They report the detection of two hepatic cytoplasmic organic anion acceptor proteins Y and Z; relative deficiency of Y, the major binding protein, was found in foetal and newborn guinea-pigs.

In rats Grodsky (1967) found that unconjugated bilirubin was concentrated inside the liver cell and was associated with a macromolecule of 40,000–50,000 molecular weight, immunologically distinct from albumin.

The next step is conjugation with glucuronic acid with the help of glucuronyl transferase (transglucuronylase). This reaction takes place in the microsomes, and transfers glucuronic acid from uridine diphosphate glucuronic acid (UDPGA) to bilirubin and to other ester and phenolic acceptors (*Fig.* 2).

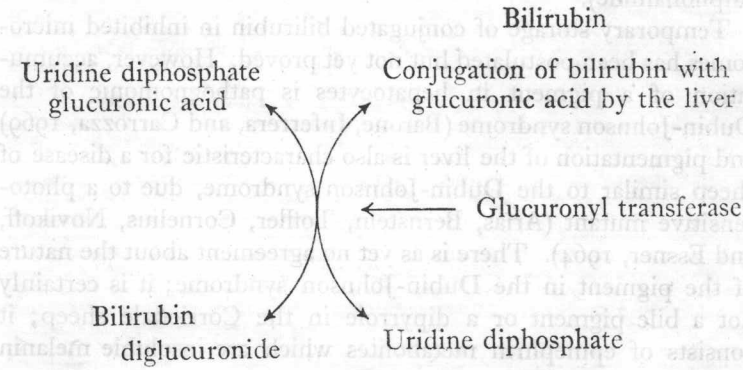


Fig. 2.—The conjugation of bilirubin with glucuronic acid by the liver (*after Sherlock*, 1962).

By this process the two propionic groups on the side-chains of bilirubin are esterified. Monoglucuronide can already be formed in serum. Earlier attempts at conjugation with glucuronic acid were unsuccessful because they were performed with free glucuronic acid which does not react.

Although the terminal transferase step is anaerobic oxidation, a source of high energy is necessary for the overall process. A small amount of bilirubin may also be bound to sulphate but this is no substitute for conjugation with glucuronic acid.

So far four types of conjugation have been described: (1) esters (e.g., bilirubin), (2) ethers (e.g., *o*-aminophenol, 4-methylumbelliferone), (3) N-linkage (e.g., aniline), (4) S-linkage (e.g., sulphanilamide). Species differences exist and in addition to the four groups there exist also substrate differences; Gunn rats whose acyl conjugation of bilirubin is deficient can nevertheless conjugate diphenylacetic acid.

On the other hand, ester and ether conjugation may be performed by the same enzyme. The existence of one transferase with structural differences and also of different transferases has been postulated.

Conjugation with glucuronic acid is a prerequisite for secretion of bilirubin into the bile. Bromsulphthalein, however, can be excreted unchanged as a conjugate of glutathione and indocyanine green. 'Detoxication' can also be achieved by the liver through oxidation (e.g., phenylbutazone), reduction (e.g., chloramphenicol), hydrolysis (e.g., pethidine), and acetylation (e.g., sulphonamide).

Temporary storage of conjugated bilirubin in inhibited microsomes has been postulated but not yet proved. However, accumulation of a pigment in hepatocytes is pathognomonic of the Dubin-Johnson syndrome (Barone, Inferrera, and Carrozza, 1969) and pigmentation of the liver is also characteristic for a disease of sheep similar to the Dubin-Johnson syndrome, due to a photosensitive mutant (Arias, Bernstein, Toffler, Cornelius, Novikoff, and Essner, 1964). There is as yet no agreement about the nature of the pigment in the Dubin-Johnson syndrome; it is certainly not a bile pigment or a dipyrrole in the Corriedale sheep; it consists of epinephrin metabolites which are insoluble melanin polymers.

The final step of bilirubin metabolism in the liver is secretion into the bile canaliculi. Bile secretion has been studied by Hargreaves and Lathe (1963).

Formation of bile must be distinguished from secretion into it. With regard to concentration in plasma and bile three categories of substances can be recognized according to whether these are: (1) present in bile in lesser amounts than in plasma, (2) present in approximately identical concentration in bile and plasma, and (3) present in bile at higher concentrations than in plasma. All endogenous and exogenous substances which are concentrated in bile have been termed 'cholephilic substances' by Hargreaves

et al. (1963). They include bromsulphthalein, used in liver-function tests, and also substances used in radiographic examination of the biliary tract. Bilirubin belongs to category (3); it is secreted in high concentration by an active energy-consuming process.

Bile formation is due to two simultaneous processes: (*a*) the formation of a fluid similar to deproteinized plasma, and (*b*) secretion of cholephilic substances into this fluid. Formation of sufficient amounts of bile is necessary for a normal bile flow in the canaliculi, thus preventing intrahepatic biliary stasis. The primary events of bile production are secretion of bile-salt and of the major inorganic anions, chloride and bicarbonate; in a perfused liver, this is independent of hydrostatic pressure of blood. Under certain experimental conditions bile flow and secretion of cholephilic substances can be dissociated.

The various stages of the journey of bilirubin from plasma to bile according to present knowledge are shown in *Fig.* 3.

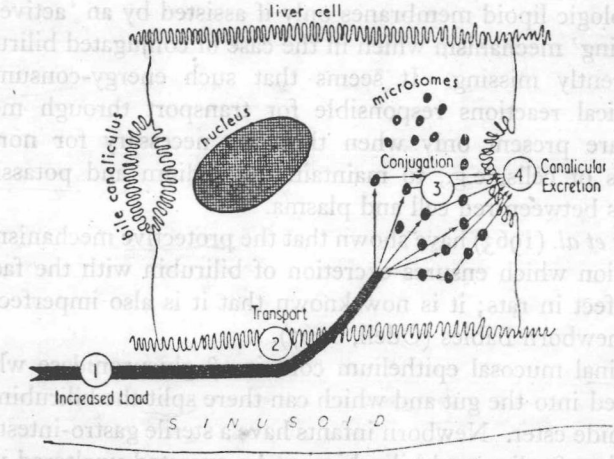


Fig. 3.—Jaundice could theoretically result from (1) an increased load of bilirubin, (2) defective uptake and transport within the liver cell, (3) defective conjugation in the hepatic microsomes, (4) defective canalicular excretion or a mechanical block in the bile-duct system. (*By kind permission of Sherlock and the 'British Medical Journal'*, 1962.)

It is conceivable that small amounts of unconjugated bilirubin reach the biliary tree and it has been speculated that unconjugated bilirubin is reabsorbed from the biliary tree (Lester and Schmid, 1963). From a teleological point of view, such absorption would

ensure that only conjugated bilirubin reaches the gut and is excreted by the faeces. In the colon, conjugated bilirubin is deconjugated and degraded by the normal bowel flora to urobilinogen (previously called 'stercobilinogen') and most of it is excreted by the faeces, though small amounts are absorbed and re-excreted by a normal liver. A small fraction, less than 4 mg. daily, escapes excretion by the liver and is excreted by the kidneys. If large amounts of bilirubin are excreted, as for instance in haemolytic anaemias, much urobilinogen is absorbed; the liver is often unable to handle such a heavy bilirubin load and large amounts of urobilinogen are excreted by the kidneys. Urinary urobilinogen is oxidized to urobilin.

It is now known that only unconjugated bilirubin is absorbed from the gut (Lester *et al.*, 1963), being non-polar and lipid-soluble; conjugated bilirubin is highly polar and water-soluble. Polar organic substances with a molecular weight higher than 200 can cross biologic lipoid membranes only if assisted by an 'active' or 'facilitating' mechanism which in the case of conjugated bilirubin is apparently missing. It seems that such energy-consuming biochemical reactions responsible for transport through membranes are present only when they are necessary for normal functions of cells, e.g., to maintain the sodium and potassium gradients between red cell and plasma.

Lester *et al.* (1963) have shown that the protective mechanism of conjugation which ensures excretion of bilirubin with the faeces is imperfect in rats; it is now known that it is also imperfect in certain newborn babies (Odell, 1967).

Intestinal mucosal epithelium contains β-glucuronidase which is secreted into the gut and which can there split the bilirubin diglucuronide ester. Newborn infants have a sterile gastro-intestinal tract before feeding and bilirubin can be excreted unaltered with the faeces for many weeks (Brodersen and Hermann, 1963). Its reabsorption is enhanced by intestinal obstruction. It is also obvious that early feeding initiates the bacterial degradation of bilirubin in the meconium and facilitates excretion by increasing the motility of the bowels (Wennberg, Schwartz, and Sweet, 1966). Ross, Waugh, and Malley reported in 1937 that a series of 14 jaundiced babies had less bilirubin and urobilin in stools and urine than a series of 21 non-jaundiced infants; this was interpreted by Ulström and Eisenklam (1964) as supporting evidence for absorption of bilirubin from the gut. More

convincing are the results of their own experiments with bilirubin isotopically labelled with ^{14}C. They could reduce previously normal serum bilirubin levels with activated charcoal if it was given in the first 4 hours after birth. Such individual variations in the early absorption of bilirubin could be a contributing factor to neonatal hyperbilirubinaemia. This recirculation of bilirubin has been called by the same authors 'enterohepatic shunting of bilirubin'.

Eighty per cent of the bilirubin excreted by the liver is derived from the breakdown of haemoglobin originating from mature red cells in the blood, and the remainder from tissue haem which includes liver haem. The chromoproteins cytochrome C, catalase, tryptophane pyrrolase, and myoglobin have rapid turnover rates in adults; Vest, Strebel, and Hauerstein (1965) have shown that after infusion of normal infants with ^{15}N-labelled glycine, 21–25 per cent of the bile-pigment excreted in the faeces was not derived from the haemoglobin of mature circulating red cells. This is more than double the amount of early (shunt) bilirubin excreted in the faeces by adults.

The bone-marrow is another source of extravascular bilirubin derived from haem precursors, haem, or intramedullary haemolysis at least in a condition described variously as 'shunt hyperbilirubinaemia' or 'idiopathic dyserythropoietic jaundice' (Israels, Sunderman, and Ritzmann, 1959; Berendsohn, Lowman, Sundberg, and Watson, 1964).

A crude diagram of bilirubin metabolism is presented in *Fig.* 4. A comprehensive monograph is available to provide further information about bilirubin metabolism (With, 1968). Selected aspects are reviewed in *Bilirubin Metabolism* (Bouchier and Billing, 1967).

Pathogenesis of Hyperbilirubinaemia and Neonatal Jaundice

It is often difficult and sometimes impossible to correlate exactly disease entities or syndromes with well-defined pathological mechanisms. Nevertheless knowledge of pathogenesis is necessary for successful treatment and the ultimate aim is to find the primary biochemical lesion.

A broad pathogenetic classification of hyperbilirubinaemias is given in *Table I* (*see page* 13).

We shall now discuss briefly some diseases and syndromes in the order of the consecutive steps of bilirubin metabolism,