LIVER AND PREGNANCY

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Introduction

This volume deals with the influence of normal and pathological pregnancy on the liver.
In the literature one often comes across the term "liver of pregnancy".
The following questions are considered: What are the changes in the biochemistry of liver function which occur during pregnancy? What is the influence of pregnancy on the structure of the liver?
There are many case reports to be found in the literature which record disturbances from the clinico-biochemical and from the histological and electron-microscopic points of view.
We have drawn on an extensive patient material with as many clinical and clinico-chemical data as possible to obtain a picture of liver function during pregnancy. The histological and electron-microscopic aspects have been included to amplify our clinico-chemical data.
No progress can be made in the understanding of liver function during pregnancy without a thorough knowledge of bilirubin, bile acid, cholesterol and serum lipid metabolism in pregnancy.
It is hoped that this study of the liver in pregnancy and especially of the disease which we have called "hepatogestosis" (pruritus and/or jaundice during pregnancy) will be a useful contribution to the field.

Contents

Chapter 1

Symptoms of jaundice and pruritus during pregnancy

Bilirubin metabolism in pregnancy

A study of the significance of serum bilirubin as a test of hepatic function in pregnancy requires investigation of the question whether a pregnant woman metabolizes bilirubin differently from a nonpregnant woman, and if so, in what way. An attempt will be made in the following pages to deduce information about the influence of pregnancy on bilirubin metabolism from a survey of bilirubin metabolism in the nonpregnant woman.

The serum bilirubin level in pregnancy is determined by the supply to the serum, the clearance from the blood, and the compartment in which bilirubin is found.

Supply of bilirubin to the serum depends on: the maternal and foetal production of bilirubin (the so-called 'early' bilirubin); the reflux of bilirubin from the liver cell into the bloodstream; the return of bilirubin from the tissues and intestine into the bloodstream; the hepatic liver flow; the permeability of the placenta to bilirubin; and the passage of bilirubin from the amniotic fluid into the maternal bloodstream. The first of these factors (maternal and foetal production of bilirubin) involves bilirubin arising from circulating erythrocytes, as well as the production of haemoglobin by mother and foetus and the destruction of haemoglobin in the reticuloendothelial system (RES), in the bloodstream and extravascularly.

Clearance of bilirubin from the bloodstream depends on: the transmission of bilirubin to the liver cell; the conjugation (and deconjugation) of bilirubin in the liver cell (UDPGA, glucuronyl transferase, glucuronidase); the passage of bilirubin from the liver cell into the biliary ductules; the transport of bilirubin through the biliary passages to the intestine; the hepatic blood flow; the excretion of bilirubin by the kidneys; the disappearance of bilirubin in the tissues; and the disappearance of bilirubin in the amniotic fluid and the foetus.

The various factors determining the compartments in which bilirubin is found, will be discussed with regard to the effect which pregnancy exerts or may exert upon them.

The supply of bilirubin to the serum

Bilirubin present in the body originates only partly from red blood cells, either in the blood circulation or extravascularly. That is, it does not arise entirely from the final break-up of circulating erythrocytes in the RES; 10-30% may result from the breakdown of red blood cell precursors in the bone marrow and from heme pigments such as myoglobin or cytochromes. This percentage may be increased, e.g. in pernicious anaemia (London and West, 1950) and in congenital porphyrinuria (Gray *et al*, 1950). Megaloblastic anaemia of pregnancy is rarely of the Biermer-Addisonian type (Knipscheer, 1965), but a similar development may be expected; Knipscheer makes no mention of

increased serum bilirubin. In cases of megaloblastic anaemia of pregnancy, he found a significantly higher incidence of liver dysfunction than in the control groups.

Bilirubin does not arise solely from circulating erythrocytes, however, as shown by experiments in which ^{15}N-glycine was administered orally to a group of subjects (London *et al.*, 1950; Gray *et al.*, 1950). Within 10 days ^{15}N-stercobilin appeared in the faeces. In healthy subjects this so-called early fraction amounted to 10-20% of the total stercobilin. In cases of pernicious anaemia and porphyruria the fraction far exceeded 10-20%. Israels *et al.* (1967) differentiated four types of bilirubin, according to origin. Types I and II, which appear during the first 24 hours, arise from the major tissue heme proteins and their precursors. Type III, which appears between the third and the fifth days, arises from erythropoiesis. Type IV, which appears after 100 days, originates from the erythrocytes. The liver itself is an important source of early bilirubin (Robinson, 1967). Vest (1967) found, by means of investigating faecal excretion of ^{15}N in bile pigments in the faeces of newborn infants that had been given ^{15}N-glycine, that at least 21-25% of the bile pigment recovered from the faeces does not arise from the circulating erythrocytes. In premature infants this percentage even exceeded 30. Production of this bilirubin depends on the production of haemoglobin within and outside the bone marrow in the mother and the foetus, and on the destruction of erythrocytes in the RES, in the bloodstream and in extravascular blood collections.

Maternal production of haemoglobin

Crosby and Akeroyd (1952), in calculating the amount of bilirubin that is presented to the liver each day for excretion, used the following values. The volume of red blood cells in an adult weighing 70 kg amounts to 2,250 ml, which corresponds to a total haemoglobin of 750 mg if the serum haemoglobin exceeds 16 mg%. If 1/120 of the erythrocytes are destroyed each day, 625 mg of haemoglobin, i.e. the amount present in 18.18 ml of red blood cells, are involved. The normal daily production of haemoglobin is therefore 6.25 g.

Klatskin (1963) calculated that, for a blood volume of 5 l, a haemoglobin value of 15 mg and an erythrocyte life span of 100 days, 7.5 g of haemoglobin are destroyed daily and must therefore be manufactured. Sherlock (1962) estimated a daily production of 6.25 g, and Verschure (1963) estimated 7.5 mg. It is likely, although not certain, that the erythrocyte life span in the pregnant woman is not different from that in the nonpregnant woman. The blood volume and, although to a lesser extent, the erythrocyte volume are increased by one litre in pregnancy (Evers, 1961). With adequate iron saturation this means an increase in total haemoglobin by about 150 g%. The daily production then amounts to 7.3 to 9 mg. Normally the bone marrow of the pregnant woman is therefore more active (hyperplastic?) than that of the nonpregnant woman.

There are limits to the maximum productive capacity of the bone marrow which become manifest in diseases which tax the erythropoietic tissue due to a continuously accelerated rate of breakdown of blood. These diseases may also occur or become overt during pregnancy.

Crosby and Akeroyd (1952) calculated that the bone marrow in two male patients with chronic haemolytic anaemia could produce haemoglobin at the rate of 0.60-0.65 mg/kg body weight/day. One patient had hereditary spherocytosis and the other hereditary haemolytic anaemia that differed somewhat in its characteristics from the known types. (The normal daily production of haemoglobin in an adult is about 0.09 mg/kg body weight.) The findings of these workers indicate that a reduction from 120 to 15-20 days of erythrocyte life-span can be compensated by the bone marrow in order to maintain a normal serum haemoglobin level. Thus there is a 'reserve' capacity in

the body for about 6-7 times the regular daily production. On the basis of values reported in the literature, they consider the reserve capacity to be slightly less in sickle cell anaemia and markedly less in pernicious anaemia, Cooley's anaemia and paroxysmal nocturnal haemoglobinuria (3-4 times less than normal). It is not known whether the bone marrow in pregnant women possesses the same potential maximum performance demonstrated by Crosby and Akeroyd in their patients, but there is no evidence to indicate the contrary.

Foetal production of haemoglobin

Haemoglobin production by the foetus is a process which increases during the nine months of pregnancy, although the lack of precise details rules out an accurate description of this process. Since the duration of pregnancy exceeds the erythrocyte life span in humans, a critical period may be presumed to occur – probably at the end of the first trimester or early in the second. Judged by the rate at which a healthy newborn infant produces bilirubin – about three times as fast as an adult, according to Mollison (1948) – it may be concluded that the site of production of haemoglobin is three times as extensive during intrauterine life. The need for this increase may, at least in part, be accounted for by the shorter life span of erythrocytes (Vest, 1967) and higher haemoglobin concentration of the blood in the foetus.

If the haemoglobin content in a newborn baby weighing 3 kg and possessing a blood volume of 300 ml is taken to be 18 g% and the erythrocyte life span to be 90 days (as against 16.6 g% and 120 days respectively in an adult weighing 70 kg and possessing a blood volume of 4.5 l), the newborn infant requires 9/5x4/3, i.e. more than twice as much erythropoietic tissue per kg body weight as the adult to meet the daily requirement of substitutional haemoglobin. If the 60 ml of blood contained in the placenta is added, the daily production of haemoglobin from the foetus at the end of pregnancy is approximately 1/90x360x18.8 g, i.e. more than 1 g. Depending on the blood volume, this amount will be somewhat smaller earlier in the pregnancy.

A hyperplastic bone marrow is present in the foetus suffering from excessive blood destruction, and extramedullary erythropoiesis is marked in the liver, spleen and kidney. However, it is not known if the excessive breakdown of blood can be completely compensated by the erythropoietic tissue in order to prevent anaemia. If it is assumed that the maximum potential production capacity is the same as in the adult, i.e. 6 times the normal capacity, the maximum daily production of haemoglobin in the foetus would be about 6 g.

Destruction of haemoglobin in the RES

Production and destruction of haemoglobin are balanced in order to maintain the haemoglobin concentration of the blood at a normal level. Under normal conditions the circulating erythrocytes are haemolyzed 'at the end of their lives' (about 120 days) in the RES (in a pregnant woman 7.3 to 9 mg/day, in the full term foetus about 1 mg/day). Excessive destruction may take place in the bloodstream as well as in the RES. Erythrocytes that accumulate extravascularly are also a source of bilirubin.

Crosby (1956) infused healthy subjects with increasing amounts of haemoglobin, and was able to calculate that the maximum capacity of the RES to transform haemoglobin into bilirubin was 45-50 mg/day. Maximum production and maximum destruction in the RES therefore seem to run parallel (6-7 times normal).

It is not known if the same process occurs in the pregnant woman. If the increase in erythrocytes in the maternal blood is taken into account, then the daily supply of bilirubin to the liver in pregnancy must be increased by about 50 mg. Observations in

newborn infants provide an indication of the amount of foetal haemoglobin available each day for bilirubin, which has to be cleared by the maternal liver. If the blood volume in a newborn infant is taken to be 300 ml and the total haemoglobin to be 54 g (Hb 18 g%), then 0.5 g is transformed into 17 mg of bilirubin, provided that 1% is destroyed each day. What happens if erythroblastosis foetalis is present (Zuelzer and Brown, 1961; Van Leusden, 1963). If the haemolysis increases by 1%, the haemoglobin level falls by 1 g instead of by 0.5 g/day, and becomes 17.7 g% instead of 18 g%. A small or imperceptible reduction in haemoglobin leads to a doubling of bilirubin production; instead of 17 mg, 34 mg are supplied to the maternal liver for daily elimination. If at birth a child has a bilirubin level of 8 mg% which rises to 20 mg% within 2 hr, this rate of increase implies a bilirubin production of about 20 mg/hr or 500 mg/day. All this only holds true for the newborn infant. If this calculation is transposed to the unborn child with an excessive breakdown of blood, a doubling of the daily blood destruction produces a breakdown of haemoglobin of 1 g, and a sixfold increase results in a breakdown of about 3 g, i.e. about 100 mg of bilirubin a day. The total haemoglobin would be reduced from 54 g to 51 g and the haemoglobin content from 18 g to about 17 g% if no compensation has occurred in its production. The maximum capacity for haemoglobin production in the newborn child is not known. If it is assumed to equal that of the adult, i.e. 6 times the normal, the maximum amount which may be produced daily by the destruction of foetal haemoglobin for transmission and excretion each day to the maternal liver with rhesus antagonism, is about 170 mg in excess of the amount handled by the liver of the nonpregnant woman each day. In erythroblastosis foetalis there is a marked increase in extramedullary haematopoietic tissue; it is not known whether this increase is accompanied by a similar increase in the RES. Since foetal bilirubin has to be cleared by the maternal liver, hyperbilirubinaemia would develop as soon as the supply exceeds the body's ability to handle it. Does this occur in the case of the healthy liver? Information in the literature (Hoffbauer, 1963; Kloosterman, 1947) suggests a difference between serum bilirubin of pregnant women with moderate and severe forms of the disease. The question arises whether this phenomenon should not be attributed to cases in which intravascular breakdown of haemoglobin occurred prior to the death of the foetus. In other cases, it may rather be assumed that the increased breakdown of haemoglobin in erythroblastosis foetalis results only in an additional supply of bilirubin to the liver, causing hyperbilirubinaemia, when the liver function is not optimal.

Neveaulima and Ekland (1963) found that no increase in the indirect bilirubin reaction in the maternal blood occurs in rhesus immunization. Of 30 pregnant women with serum bilirubin levels in excess of 1 mg% described by Ikonen (1964), there were two with rhesus antagonism. Both exhibited generalized itching of the skin. These two patients between them experienced five pregnancies; in all there were rhesus antibodies, and in all the foetus died *in utero*. In the one patient generalized itching accompanied all three pregnancies, while in the other it accompanied only the pregnancy associated with jaundice.

In his thesis Kloosterman (1947) determined serum bilirubin in 45 pregnant women, out of a group of 58 suffering from erythroblastosis, and in 28 suffering from habitual foetal death. In five out of a group of 45 women who had experienced at least two stillbirths each, and in one out of a group of 19 women who had experienced only one stillbirth each, the Van den Bergh reaction was in excess of 1 U; the maximum value was 1.6 U. No increase was shown in the group of 28 cases with 'habitual foetal death'.

Destruction of haemoglobin due to intravascular haemolysis

For conditions that are accompanied by intravascular haemolysis which may also be

associated with pregnancy, see Chapter 3.

The serum haemoglobin level rises. If more than 30 ml of blood is broken down, the haemoglobin content rises above 150 mg%, which results in haemoglobinuria.

The blood contains methalbumin and traces of methaemoglobin. If the haemoglobin breakdown exceeds the capacity of the liver to dispose of bilirubin, jaundice results; the spleen need not be enlarged. In cases of incompatible blood transfusion the haemolysis is of this type. If haemoglobin present in 30 ml of blood were converted into bilirubin, the liver would be loaded with about 0.3x16x35 = about 17 mg of bilirubin. This additional load could be disposed of by a healthy liver without any increase in the serum bilirubin level, depending on the reserve capacity of the liver. However, if the amount of blood destroyed is tripled, this may not be the case.

The normal daily production of bilirubin is considered to be 200-300 mg, the maximum depends on the maximum amount of haemoglobin that can be produced. This is about 6 times the normal daily amount. Thus a maximum of about 1,500 mg of bilirubin could be presented to the liver for disposal each day. In severe cases of rhesus antagonism, traces of methalbumin (the albumin complex of haematin) may be found in the serum. This indicates that intravascular haemolysis may also occur with this clinical picture. An acute breakdown of 25% of foetal blood will then result in a haemoglobin level of about 13.1 g% and the production of about 450 mg of bilirubin. The rare instances of erythroblastosis foetalis in which the mother becomes clinically jaundiced include cases in which such an acute breakdown of foetal blood takes place. It is an ominous sign; Scott (1940) pointed out the parallel between the occurrence of generalized oedema and jaundice in pregnant women suffering from rhesus antagonism and the imminent intrauterine death of the foetus.

From these findings it appears that severe haemolysis in the foetus is practically certain to represent a cause of maternal jaundice. In the cases reported by Ikonen (1964) itching was present as well. This concurrence of symptoms is readily explicable on the basis of preexistence of hepatic dysfunction. Another explanation is that this dysfunction is a result of the intrauterine event; this seems less likely if one bears in mind that generalized oedema may be present in the mother at the same time.

Destruction of haemoglobin in extravascular blood accumulations

Red blood cells are also destroyed in extravascular accumulations of blood, and they may be a source of bilirubin appearing in the blood, probably through the formulation of haematin. A well-known example is neonatal cephalhaematoma. It must be assumed that retroplacental haematomas constitute a similar source of bilirubin production.

Reflux of bilirubin from the liver cell into the bloodstream

In the presence of a normal supply of bilirubin and a normal handling capacity of the liver, the serum bilirubin does not exceed a certain level (this level varies according to the method of determination used).

In the dog this handling capacity is so great that scarcely any bilirubin is found in the serum. When the excretion of bilirubin from the liver into the bile is obstructed, serum bilirubin rises and conjugated bilirubin appears in the blood. Part of the bilirubin that reaches the liver cell, either before or after prior conjugation and deconjugation, is then returned to the blood. Under normal conditions, i.e. normal breakdown of erythrocytes, normal liver function and normal bile flow, according to Weber and Schalm (1962), bilirubin is present in the bloodstream only in the free unconjugated form. Thus it seems improbable that there is also a significant reflux of conjugated bilirubin into the blood under normal circumstances. Broderson and Jacobsen

(1968) found that with a certain technique the concentration of conjugated bilirubin in the blood of female donors was 1/30 and that of male donors 1/32 of free bilirubin. While this difference is significant, these amounts are so small that the presence of conjugated bilirubin in the blood, estimated by the usual methods, should be regarded as evidence of hepatic disorder.

Return of bilirubin from the tissues to the bloodstream

Little attention has been paid, at least in the adult, to the quantitative effect of this 'store' on serum bilirubin. In those types of jaundice associated with pregnancy, the impaired clearance of bilirubin by the liver will cease at the end of the pregnancy. Following pregnancy the tissues supply bilirubin to the blood. In the newborn the amount of bilirubin in the tissues, calculated by Van Kessel *et al.* (1967), Seelen *et al.* (1967) and Seelen and Van Kessel (1967) on the basis of results obtained with exchange transfusions, is certainly not negligible in respect to the amount already present in the bloodstream. Drugs such as salicylates and sulphonamide preparations may adversely affect the binding of bilirubin to albumin and produce a reduction of serum bilirubin by transmitting bilirubin to the tissues.

Lester and Schmid (1963) concluded from experiments in which unconjugated and conjugated bilirubin labelled with ^{14}C was introduced into the gastrointestinal tracts of patients that only free bilirubin is reabsorbed into the blood.

Brandt (1957, 1958) studied the breakdown of bilirubin in the intestine by means of serial stool examination for urobilinogen compounds according to Terwen-Watson's method, and found that with permanent complete biliary obstruction the excretion rate of urobilinogen was less than 5 mg/24 hr. Excretion was normal in constitutional hyperbilirubinaemia (100-200 mg/24 hr) and much increased in haemolytic jaundice.

Hepatic blood flow

'Because the liver has two different sources of blood, studies on the relationship of blood flow to liver function have proved to be surprisingly difficult, and much remains to be solved,' Horner-Andrews (1957) wrote in an issue of the *British Medical Bulletin* devoted especially to physiological and clinical aspects of liver function. Progress made since that time is reviewed by Sherlock (1968) in her book. However, no progress is described regarding hepatic blood flow in pregnancy. The only specific study was that of Munnell and Taylor (1947) who concluded that pregnancy produces no changes. They found that the blood flow in healthy pregnant women averaged 1,554 ml/1.73 m^2, as against 1,578 ml in nonpregnant women. The blood volume as well as the stroke volume was increased. In the absence of pregnancy the hepatic blood flow amounts to 35% of the stroke volume, as compared to only 28% during pregnancy; the difference is shortcircuited through the placenta.

If this is true, it may be expected that disorders of pregnancy accompanied by impaired flow through the placenta indicate a greater strain on the maternal liver. On the other hand, a cardiac liver due to congestive heart failure in pregnancy will not improve and may frequently be associated with necrotic foci which cause conjugated bilirubin to leak back from the liver into the bloodstream (Schalm, 1957). In the author's series of pregnant women there were three with cardiac defects, in all of whom the course of the serum bilirubin level, which was not markedly elevated, was erratic.

Van Gendt and Luyendijk-Elshout (1957), in a series of 50 children under 8½ years of age with congenital cardiac anomalies, found disturbances in the vasculature of the liver. Hepatic blood flow plays an important role in the changes that occur in the circulation in the changeover from intrauterine to extrauterine existence. The left half of the liver is

relatively much larger in the foetus than it is after birth because the umbilical vein empties into the left branch of the portal vein; thus the left half of the liver is better irrigated with blood than the right half. In 97% of infants, the ductus venosus closes within two months of birth and the greater part of the blood is carried along the portal vein so that the right half of the liver receives the most and probably the best blood. The right lobe of the liver then increases in size and the left lobe diminishes (Schalm, 1957).

Heart operations are often followed by jaundice; liver disease may have been preexistent. The influence of anaesthesia, drugs and blood transfusions should not be overlooked (Sherlock, 1968).

Permeability of the placenta to bilirubin

During pregnancy the foetus is an additional source of bilirubin. This bilirubin is normally cleared through the placenta and the maternal liver. This clearance is related to the degree of permeability of the placenta to bilirubin and/or its conjugate. Schenker *et al.* (1964) demonstrated in guinea pigs that unconjugated bilirubin passes freely between the mother and foetus through the placenta, but that conjugated bilirubin does not. Grodsky *et al.* (1963) found that some free bilirubin was transmitted from the foetus to the mother in the rat (although not enough to account for the transport regularly expected). Wynn (1953), in his studies in guinea pigs and rabbits, failed to establish any permeability of the placenta to free bilirubin. Lester *et al.* (1963) found that it passed from the foetus to the mother in the rhesus monkey, the placenta of which is very similar to that in humans. These investigators had great difficulty in maintaining intact the connection between placenta and uterus during their experiments, and it seems likely that an artificial leak existed. In perfusion experiments on human placentas following delivery, Page (1960) established a permeability to bilirubin. At a meeting of the Dutch Gynaecological Association in 1963, Van Leusden, also on behalf of Stolte, Seelen and Van Kessel, discussed experiments concerning placental permeability to bilirubin in the rhesus macaque; he had mentioned this subject before in his thesis (Van Leusden, 1963). Stolte *et al.* transferred free bilirubin to a foetal interplacental vessel, and the bilirubin level in the maternal blood was then examined in another vein. An increase was observed, mainly in the indirect bilirubin, by use of Malley-Evelyn's technique. The integrity of the connection between the uterus and placenta was confirmed by simultaneous injection of ^{51}Cr-labelled erythrocytes which did not pass.

During passage through the placenta, bilirubin becomes dislodged from the albumin to which it is bound. No indications for conjugation by the placenta were found, which corresponds with Van Leusden's finding in his *in vitro* experiments in 1963. Schenker *et al.* (1967) introduced cannulae into the gall bladder and a peripheral vein of pregnant rhesus monkeys as well as into the amniotic sac and the interplacental vessels. These workers were able to show that ^{14}C-labelled bilirubin was transmitted from the foetus to the mother, probably even in foetal blood values below 1 mg%. Elimination from the foetal blood was at least 5 times as slow as that from adult monkey blood. In another experiment it was shown that only 3.3.% of the dose of conjugated ^{14}C-bilirubin injected into the foetal circulation crossed the placenta.

The serum bilirubin level in the foetus is usually 7 mg% higher than that in the mother (Hsia *et al.*, 1963). The bilirubin in the umbilical blood is unconjugated (Smith *et al.*, 1959).

Passage of bilirubin from the amniotic fluid into the maternal bloodstream

In 1933 Bevis described the results of examination of 205 specimens of amniotic fluid obtained prior to birth from 98 pregnant women, 41 of whom had children suffering from erythroblastosis foetalis. Direct bilirubin was present in none, not even in the

presence of meconium. The amount of bilirubin varied from 0 to 7.5 mg%. The examination of amniotic fluid obtained by abdominal paracentesis was introduced to the Netherlands by the Nijmegen University Department of Gynaecology in 1957, and Seelen presented a preliminary account of the findings in rhesus antagonism at the 1961 meeting of the Dutch Gynaecological Association. Seelen and Van Kessel (1967) reported on the difficulties of prognosis. In 1961 Hoogeveen described his experience.

No study appears to have been made of the question whether bilirubin passes from the amniotic fluid through the membrane to the maternal blood. Since meconium contains bilirubin (Childs *et al.*, 1958), unconjugated bilirubin will be transported from the amniotic fluid to the mother in view of the presence, established by electron microscopy, of meconium in the amnion. Bilirubin present in the serum of the foetus is chiefly in the unconjugated form, presumably due to inability of the foetal liver (and the placenta) to conjugate it. If the amniotic fluid contains bilirubin, this also occurs mainly in the unconjugated form. Jansen *et al.* (1969) found unconjugated bilirubin in the amniotic fluid and foetal urine in seven out of eight cases of rhesus immunization.

Clearance of bilirubin from the bloodstream

The disappearance of bilirubin from the liver cell depends on: the transmission of bilirubin to the liver cell, the conjugation (and deconjugation) of bilirubin in the liver cell, the passage of bilirubin glucuronide into the biliary ductules, and the transport of bilirubin through the biliary passages to the intestine.

Bilirubin clearance tests may indicate the effect of the various factors. They were introduced by Bergman and Eilbott in 1927. Recently Billing *et al.* (1964) have studied them in detail. Schalm *et al.* (1962) carried out a series of experiments in normal, eviscerated and nephrectomized rabbits following loading with unconjugated bilirubin. They found that, even in the absence of liver and kidneys, conjugated bilirubin continued to be produced. A small amount was present in the blood, and this amount was exceeded many times in intact rabbits following the injection of bilirubin. The fact that bilirubin glucuronide may be found in the blood in man with an intact or almost intact liver was demonstrated by Schalm and Weber in three patients through gall bladder drain, following the intravenous administration of 600, 1,000 and 600 mg of bilirubin respectively. By means of chromatography they proved that overloading of the liver with bilirubin entails a reflux of bilirubin glucuronide into the bloodstream. This fact had already appeared likely from the diazo reaction in cases of haemolytic anaemia and following the injection of bilirubin in normal subjects (Tisdale *et al.*, 1959). The presence of bilirubin in the urine in these circumstances must be interpreted in the light of the glucuronide-forming capacity of the kidneys. It appears from these results that the excretion of bilirubin glucuronide is the limiting factor in the excretion of bilirubin into the bile, not the glucuronide-forming capacity of the liver. One of the three patients loaded with bilirubin by Schalm and Weber (1962) had serum bilirubin of 0.5 mg% before the experiment; there was no conjugated bilirubin in the blood nor was there marked liver dysfunction. The dose of bilirubin administered amounted to 1,000 mg within half an hour. Serum bilirubin was shown to remain elevated 18 hours after loading (1.25 mg% conjugated, 2.5 mg% total, determined chromatographically). The other two patients had bilirubin levels of 0.6 mg% total (0.35 mg% combined) and 1 mg% total (0.65 mg% combined) respectively. The first patient with the least amount of liver damage showed a bilirubin level 18 hours later of over 1 mg%, of which about 75% remained combined, and after 42 hours of just over 0.5 mg% total of which 75% was combined.

For the second patient these values were 1.8-1 mg% at the 18th hour and 1-0.5 mg% at

the 42nd hour. These results therefore indicate that minor damage to the excreting capacity of the liver may cause bilirubin retention with the normal daily amount increased 2-3 times.

The latency time between bilirubin loading and the excretion of conjugated bilirubin in the bile is about 1½ hours. Soffer (1933) carried out 31 bilirubin tolerance tests in 21 pregnant women. He found that, of 11 women examined in the first four months, one showed abnormal retention four hours after the injection of one mg per kg body weight. In the last five months of pregnancy only one of the 10 pregnant women showed normal retention. Ten pregnant women examined both in the first and in the second half of pregnancy. One showed abnormal retention during both periods and two showed no abnormality, while the seven others appeared to have a greater retention in the second half. The amount of bilirubin excreted by the liver is, within certain limits, proportional to the square of the concentration in the blood (Weech, 1941).

Lathe and Walker (1958) found that the conjugated capacity of liver suspensions (0.4 mg/g wet weight/hr) corresponded quantitatively with the excreting capacity of the liver, and they assumed this fact to be an indication that the conjugating capacity is the limiting factor in the excretion of bilirubin into the biliary ductules.

The conjugation of bilirubin with glucuronic acid in liver microsomes (endoplasmic reticulum) results in reduction with the aid of glucuronyl transferase to uridine diphosphate glucuronic acid (UDPGA).

Shibata *et al.* (1966) found that the glucuronyl transferase activity of rats in the last trimester of pregnancy amounted to 170% of the level in the liver of nonpregnant rats.

The normal value was reached gradually within two weeks of delivery. Despite the increased capacity to conjugate bilirubin, the maximum rate of excretion of bilirubin in the bile (Tm) did not alter.

Many substances may influence the activity of glucuronyl transferase, either in a competitive or a noncompetitive way. The influence of steroids (which are also conjugated with glucuronic acid) is not competitive as Lauritsen (1968) recently assumed. It is not known whether in the pregnant woman any of the stages by which bilirubin passes from the serum into the bile is different. However it is clear that all drug-induced, mechanical or infectious factors that interfere with this transport are also implicated in pregnancy.

On account of the potential consequences of pregnancy, importance is attached to infectious hepatitis, particularly the type that does not heal optimally. The most innocent late complication of hepatitis is posthepatitic hyperbilirubinaemia (Schalm, 1964); the only abnormality is then a moderate elevation of the serum bilirubin level, which consists exclusively of the conjugated type. 'It must be assumed,' wrote Schalm, 'that, as a residual phenomenon of the previous hepatitis, the liver cell takes up unconjugated bilirubin from the blood only at higher concentrations'. While this disturbance may exist for years, it usually disappears. It is due to the same mechanism as that underlying the condition of constitutional familial hyperbilirubinaemia (Meulengracht's syndrome), i.e. a disturbance in the wall of the liver cell. The bilirubin appears capable of invading the cell only under a higher 'pressure' than normal. It is not known whether pregnancy may influence the permeability of the cell wall to bilirubin.

In considering the factors which determine the capacity of the liver to handle bilirubin, little or no attention has been paid to the permeability of the cell wall to bilirubin. The conjugating capacity of the cell contents is considered to be the limiting factor in the excretion of bilirubin into the biliary ductules, and the role of the cell wall tends to be neglected. Is it justifiable to speculate over whether posthepatic cell wall

damage might exist, of such a nature that it permits a normal supply of bilirubin to be handled without producing manifest bilirubinaemia, but which interferes with the handling of an increased supply (such as exists in pregnancy), resulting in a moderate elevation of serum bilirubin of the unconjugated type?

A posthepatitic hyperbilirubinaemia occurring as a late sequel of hepatitis points to the presence of 'still existing damage of the liver parenchyma requiring continued treatment' (Schalm, 1964), if conjugated bilirubin remains present in the serum. If treatment is not instituted, chronically progressive hepatitis or cirrhosis may result. Of 31 patients mobilized within four months of the onset of the disease, Rot (1963) found one patient with an increased thymol turbidity level (10 U), the only residual phenomenon attributable exclusively to the infection; and of the 22 patients mobilized more than four months after the onset, he found one patient with a thymol turbidity level of 8 U and three with increased serum gamma globulin levels. All of these 53 patients, who suffered from severe progressive hepatitis, had been carefully treated. However, treatment need not be considered inadequate if it is simply less carefully administered. The point is, how often does suboptimally healed hepatitis, which is subclinical in everyday life, become overt due to pregnancy? The particular liver functions that would then be subjected to strain are the handling of bilirubin and the production of certain fractions. If during pregnancy conjugated bilirubin appears in the bloodstream, the possibility must be considered of conjugated bilirubin flowing from the liver cell into the biliary ductules, i.e. cholestasis, the same picture as is encountered in so-called cholestasis of pregnancy. The effect of pregnancy on the passage of bilirubin glucuronide from the liver cell to the bile capillaries and on the inability of the slightly damaged transmission mechanism to cope with an augmented bilirubin load can be evaluated only by bilirubin clearance tests both during and after pregnancy.

The liver bile possesses a bilirubin content of about 250 mg/l (Baumgärtel, 1969). Deenstra and Vink (1951; see also Vink, 1954) used the technique of adding ascorbic acid, which very rapidly converts the diazo salts, to determine the 'one-minute percentage'. Ducee and Watson (1945) and Deenstra (1947, 1948) had demonstrated the value, for clinical purposes, of reading the reaction after one minute. Vink (1954) found that one molecule of bilirubin produces two molecules of azobilirubin, contrary to previously reported findings. According to Van den Berg, one unit of bilirubin signifies a concentration of serum bilirubin of 1:200,000 (For hepatic blood flow, see p.6.)

Excretion of bilirubin by the kidneys

Unconjugated bilirubin is not excreted in the urine. According to Hartogh (1935) the threshold value of the renal excretion of conjugated bilirubin is approximately four units (thus about 2 mg%). The excretion of bilirubin glucuronide is influenced by the bile acid salts.

At the beginning of a virus infection when the serum concentration of direct reacting bilirubin is low it is possible to demonstrate bilirubin in the urine, whereas later on when the serum level is many times higher, it is absent from the urine (Neefe and Reinhold, 1946; Watson and Hofbauer, 1947). Hartogh (1935), who reported a case of recovering catarrhal jaundice with a total serum bilirubin of 11 U (about 5.5 mg%) in which Huppert-Salkowski's test revealed no bilirubin in the urine, and another case of diminishing jaundice associated with syphilis of the liver with a serum level of 9 U, reviewed the effect of absorption of indirect bilirubin on the serum protein. It is not known what effect is exerted by the combination of bilirubin with a mucoprotein in the urine (Heikel *et al.*, 1947). It is curious that in the Gunn rat, a species that lacks the ability to conjugate bilirubin with glucuronic acid, the capacity for excreting injected bilirubin

glucuronide in the urine is reduced.

Bilirubin crystals may be demonstrated at the tips of the renal papillae in this animal (Schmid *et al.*, 1958). The amount of bilirubin eliminated from the body in the urine is slight. According to Schachter (1959) the clearance of the bilirubin-bilirubin diglucuronide complex (which he called monoglucuronide) is 0.05-0.16 ml/min and that of the diglucuronide 0.41-0.96 ml/min.

Owen (1967), from experiments in chicks, concluded that conjugated bilirubin reaches the urine mainly through the capillary wall of the glomerulus and not by tubular excretion. Experiments performed by means of stop-flow techniques and following partial occlusion of the ureter in both intact dogs and in dogs without gall bladders and with patent biliary passages yielded confirmatory results. In icteric patients a clear correlation appears to exist between creatinine clearance and the excretion of bilirubin. Creatinine clearance varied from 24 to 89 ml/min (average 56 ml) and bilirubin clearance averages 0.24 ml/min. Kloosterman (1947) reported a series of cases in whom all children with severe jaundice who died were shown to have spleens weighing about 260% normal weight and livers about 170% normal weight. Microscopic examinations revealed a large amount of haematopoietic tissue, and it appeared that marked hepatosplenomegaly was always associated with pronounced erythroblastosis. In the cases of foetal hydrops the spleen was about 500%, the liver about 200% and the heart 160% normal weight. Part of this weight, of course, must be attributed to the hydrops. In 12 autopsy cases of erythroblastosis foetalis, some information is provided on renal involvement. In eight there were no signs indicative of the presence of bilirubin (about 3, 30, 36, 72, 81, 96, 96 and 216 hours respectively after birth). In the remaining four there were indications of the deposition of bilirubin in the kidneys:

Case 1 (about 3 hours after birth): bile pigment in tubular epithelium;

Case 2 (about 48 hours after birth): renal cortex markedly yellow, especially between the pyramids;

Case 3 (about 48 hours after birth): convoluted tubules stained vivid brown (jaundice);

Case 4 (about third day of life): cross-section corresponding to the medullary rays, yellow opaque stripes directed towards the cortex like a tuft of hair.

Potter (1947) confirmed that 'the cells of the kidney tubules may contain large amounts of pigment when the skin is deeply jaundiced.'

Study of the renal excretion of bilirubin is hindered by the presence of substances bound by the diazo reagent in the same way as bilirubin. As Heirwegh *et al.* (1966) discovered, these substances may constitute as much as half of the diazotizable pigments if the bilirubin values are low. Simultaneous determination of these agents results in an overestimation of bilirubin clearance. Allowing for this, De Groote *et al.* (1966) found that clearances of bilirubin and creatinine correlated well, which favours the theory of glomerular filtration. This mechanism implies that clearance is independent of the blood level, and this was found to be the case indeed in patients with cirrhosis and hepatitis, but not in patients with intrahepatic or extrahepatic biliary obstruction. This difference can be explained by the potentiating effect of bile acid salts (which aggravate the clearance of bilirubin in cirrhosis) or the 'filtered load' of bilirubin probably by influencing its solubility. The lowest clearance, about 0.1 ml/min, was found in patients with hepatitis, while slightly less than 0.15 ml/min was found in those with cirrhosis and about 0.2-0.55 ml/min in those with extrahepatic obstruction. Only one case of intrahepatic obstruction could be investigated; clearance was nearly 0.3 ml/min. The difference between hepatitis and cirrhosis was attributed to a change in the ratio between free and protein-bound bilirubin.

Hartogh (1935) referred to the difficulty of determining the renal threshold value for

bilirubin since it seems likely that a small amount of the substance is always excreted in the urine. Indeed, Gotterman and Kupfer (1966) obtained a positive (indirect) result with the Van den Berg reaction following concentration of the urine in 70% of healthy subjects, but this need not be due to the presence of bilirubin. Electrophoretically conjugated bilirubin in plasma migrates almost entirely with the albumin, but a small portion is also present in the β-globulin range (Fulop and Sandson, 1967).

The addition of gluco-deoxycholate and, to a lesser extent, of glycocholate resulted in displacement of part of the bilirubin to the α-globulin range, where part of the bile acid salts are to be found, both in the serum and in the urine. The addition of taurocholate caused no such displacement, notwithstanding its disappearance from the β-globulin range. Salicylates and p-aminohippurates increased the dialysis of conjugated bilirubin in the same way as bile acid salts, but they did not interfere with the electrophoretic pattern. The presence of urinary bilirubin at the onset of viral hepatitis and its absence later on, with unaltered serum bilirubin levels, might be explained, according to Fulop and Sandson (1967), by the following: (1) early in the disease there is a relatively larger amount of bilirubin glucuronide in the serum; and (2) the transport by means of albumin is not optimal.

Abei and Iber (1967) and Billing *et al.* (1967) found in experimental animals that, after ligation of the bile ducts, bilirubin leaves the body chiefly in the urine. Abei and Iber, who injected ^{14}C-bilirubin into dogs, did not attempt to establish the relation between free and bound bilirubin in the plasma. Billing and his colleagues, who used the method of Weber and Schalm (1962) to establish this in experiments in rats, found that conjugated bilirubin was present in the plasma within two minutes where only 12-16% of the dose administered was present. During the first 20 minutes the specific activity of the injected free bilirubin partially decreased, probably as a result of uptake in the liver. Thereafter ^{14}C-bilirubin became gradually diffused through the extravascular pool. Balance was not attained for 24 hours, when only three per cent of ^{14}C-bilirubin appeared in the plasma. Eighty per cent of total ^{14}C-bilirubin appeared in the plasma in the conjugated form within 30 minutes. In this way bilirubin is returned to the blood. After balance was attained between the various pools at 24 hours, ^{14}C-bilirubin (T½ 60-50-50 hr) fell and ^{14}C-bilirubin diglucuronide (T½ 38-30-37 hr) exponentiated. The shorter half life of the conjugate can be attributed to urinary excretion.

Disappearance of bilirubin in the tissues

Part of the excess bilirubin is taken up by the tissues; in erythroblastosis foetalis the foetal tissues contain not inconsiderable amounts of bilirubin (see p.5.)

Disappearance of bilirubin in the amniotic fluid and the foetus

Bilirubin from the maternal bloodstream does not reach the foetal bloodstream or tissues through the placenta in any quantity, if at all. It is considered possible that excess bilirubin produced by the foetus may reach the maternal blood via the placenta, and the amniotic fluid (in an unconjugated form) via the foetal membranes; this is certainly so if the capacity for conjugation and excretion of the maternal liver is under pressure.

The compartments in which bilirubin is found

It is not known whether the amount of bilirubin in the bloodstream and liver is altered during pregnancy. Bilirubin is transported in the blood mainly in an albumin-bound form. While the albumin falls during pregnancy, the effect of this reduction on serum bilirubin will be of little, if any significance. For details of liver bilirubin, reference is made to the