

BILE PIGMENTS OF JAUNDICE *

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(Submitted for publication March 3, 1959; accepted August 21, 1959)

Current concepts concerning the formation and metabolism of bile pigments hold that bilirubin is formed from the catabolism of hemoglobin by the reticuloendothelial system, mainly in bone marrow and spleen, and that the bilirubin is then transported to the liver where it is modified and excreted via the biliary system. These concepts are based largely on the studies by Mann, Magath and Bollman (1, 2) of the effect of total hepatectomy on mammals which show that after complete extirpation of the liver, indirect-reacting bile pigment was formed and accumulated in the serum progressively during the survival period of the animal. Bollman and Mann (3) noted that as the amount of bilirubin in the blood increased after hepatectomy, the van den Bergh reaction changed from an indirect to a direct reaction and bilirubin began to appear in the urine.

Since the original work of van den Bergh, Snapper and Muller (4, 5) on the application of the Ehrlich diazo reaction to the quantitative measurement of the serum bilirubin and their observation of the differing character of the reaction in various types of jaundice, many investigators have sought to determine the fundamental basis for these differences. Despite a voluminous literature on the subject which has accumulated for more than 40 years, disagreement and lack of exact knowledge are still evident. In this paper we can cite only recent surveys encompassing the many concepts which have been proposed (6-9).

Recently the studies of Cole and Lathe (10) have provided a new and important approach to

these problems. By the technic of reverse phase chromatography using silicone-treated kieselguhr, these workers were able to separate from protein-free extracts of icteric serum a nonpolar, indirect-reacting pigment which in all its characteristics resembled pure crystalline bilirubin. Also isolated was a polar, direct-reacting pigment which differed from the indirect-reacting bilirubin in being water soluble and in spectroscopic absorption.

With the use of a different solvent system, these same workers with Billing demonstrated that the direct-reacting fraction could be separated into two pigments which they termed "I and II" (11). Evidence was presented, based on the differing chromatographic behavior of the diazotized pigments, that Pigment I appeared to represent an intermediate structure between the indirect-reacting pigment and Pigment II (12).

More recently, Billing and Lathe (13) showed that Pigment II is the diglucuronide of bilirubin and that Pigment I is probably the monoglucuronide form. Their work has been confirmed and amplified (14-16). Recently, sulfate conjugates of bilirubin have been demonstrated in animal bile. It has been proposed that a small percentage of bilirubin is normally excreted in this form (17). The chromatographic methods used in this study (see below) do not distinguish the sulfate fraction. The entire subject has been thoroughly reviewed recently (18).

In light of this new and fruitful approach to the chemistry of bilirubin, we decided that some of the concepts of bilirubin metabolism as well as certain clinical aspects of the problem of jaundice should be re-examined by the technic of reverse phase partition chromatography. The work presented in this paper includes studies of 1) the pigment composition of bile of the normal human, dog and rat; 2) serum and urinary pigments in the dehepatized dog and rat; 3) fistula bile in the dog and rat following injections of hemoglobin and bilirubin; 4) serum pigments in animals with ex-

* This paper is based, in part, on the theses submitted by Drs. Hoffman and Whitcomb to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine.

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perimentally produced extrahepatic biliary obstruction; 5) serum pigments in animals with hepatocellular necrosis induced by carbon tetrachloride and by ethionine; 6) serum pigments in 147 patients with hepatocellular and extrahepatic obstructive jaundice, including chromatography of the bile in 12 patients with hepatocellular jaundice; 7) serum pigments in patients with various other types of jaundice; 8) serum and biliary pigments in eight patients with constitutional hepatic dysfunction (Gilbert's disease); and 9) the serum and bile pigments in the homozygous, congenitally jaundiced rat (Gunn strain).

METHODS USED IN STUDY OF BILE PIGMENTS

Kieselguhr was prepared according to the method of Howard and Martin (19). For the solvent systems and preparation of samples of blood, bile and urine, the methods of Cole, Lathe and Billing (11) were employed, with one exception. This was concerned with the preparation of serum from dehepatized dogs in which it was characteristically difficult to redissolve the dried protein-free serum pigment extracts prior to chromatographic separation. The pigments seemed bound to the ammonium sulfate crystals and, despite much agitation, incomplete re-entry into solution occurred. After it was found that such extracts were much more promptly and completely redissolved if the ammonium sulfate was eliminated from the preliminary protein precipitation, this modification was employed for most of the subsequent studies on hepatectomized dogs. In some instances, the ammonium sulfate was reduced in quantity but not deleted. The chromatographic column devised by Billing (20) was employed in the present studies.

The pigment fractions were measured by placing the pigment-containing segments of the column in separate, stoppered glass tubes. Five ml. of an alcoholic diazo solution was then added to each tube and the tubes were shaken thoroughly. The diazo solution was prepared as follows: Solution A: 1 Gm. of sulfanilic acid dissolved in 15 ml. of concentrated HCl, diluted to 1 L. with distilled water. Solution B: 0.5 Gm. of sodium nitrite (NaNO_2) in 100 ml. of distilled water. This solution was made daily before use. Mixed diazo reagent: 10 ml. of Solution A plus 0.3 ml. of Solution B. This reagent was used within 10 to 20 minutes after mixing. After standing with occasional further shaking for 25 minutes, the diazotized solution of pigment was removed from each tube by filtering through sintered glass filters, and the tubes and filters were washed with 5 to 10 ml. of absolute methanol to remove all traces of pigment. The diazotized solutions of pigment then were measured in cuvettes at 540 $m\mu$ with the Coleman Universal Spectrophotometer, Model 14. Calculation of the amount of pigment was made from the same cali-

bration curve as that employed for the measurement of serum bilirubin, the values being corrected for a final volume of 9 ml., which was the volume used for measurement of total bilirubin in the serum bilirubin method employed. In presenting our results, Pigments I and II are expressed as percentages of the total direct-reacting bilirubin which additively they represent.

The method is at best difficult and time consuming. The chief technical difficulties are concerned with packing of the column and the achievement of clean-cut separation of the three pigments on the column. Experience and extreme care were needed to overcome these problems. All of the determinations in this study were performed by two of us (H. N. H. and F. F. W.).

The reproducibility of our results is comparable to that cited by Billing (20) and by Baikie (21). The concentration of bilirubin did not influence the reproducibility provided that the concentration of total bilirubin in the sample was at least 3 mg. per 100 ml. Recovery studies indicated that 90 per cent of the pigments in the protein-free extract were recovered from fractionation on the column whereas approximately 60 to 70 per cent of the original serum pigment was ultimately recovered after column separation. The losses of Pigments I and II during this procedure are apparently equal (20). Since our estimations have been concerned with the relative proportions of Pigments I and II and not their absolute values, the losses during protein precipitation and column separation do not influence our results.

Determinations of serum bilirubin were carried out on all serum tested. A modification of the Malloy-Evelyn method was used, with 1 minute and 15 minute direct readings and measurement of total bilirubin after the addition of alcohol (22).

Solutions of bilirubin for injection were prepared as follows: for dogs, 150 mg. of crystalline bilirubin was dissolved in 25 ml. of distilled water and 2 ml. of 0.1 N NaOH; for rats, 10 mg. of bilirubin was dissolved in 1.0 ml. of normal saline solution and 1.0 ml. of 0.1 N NaOH. The total volume was injected intravenously in bile fistula studies, and half the volume was administered to dehepatized rats. Suspensions of hemoglobin were prepared as follows: 15 Gm. of hemoglobin (from fresh dog erythrocytes hemolyzed in distilled water) was suspended in 120 ml. of normal saline solution for intravenous use in dogs. A similar preparation of rat hemoglobin was given to rats for which the usual dose was 300 mg. in 2 ml. of normal saline solution.

For the dehepatized dogs, solutions of bilirubin were injected intravenously within two hours of operation. Injections of hemoglobin (one to three 10 ml. aliquots) were given either 30 minutes prior to hepatectomy or within two hours after removal of the liver. The chromatographic determinations of serum pigment were all carried out at the termination of the experiments, which varied from 12 to 40 hours after injection.

The number of animals and patients studied and any special procedures taken to prepare them and to obtain test material will be described with the results.

RESULTS

Composition of bile

Bile obtained at operation from four human gall bladders and fistula bile from seven dogs and four rats were studied chromatographically. In all instances, most of the pigment in the bile was Pigment II. In human bile this fraction represented a mean of 73.1 per cent of the total, whereas in the bile of the dog and the rat, Pigment II was 75.1 and 84.8 per cent of the total, respectively (Table I).

Dehepatized dogs

Fourteen dogs were dehepatized after the method of Grindlay and Mann (23). They were maintained postoperatively by a saline-glucose solution administered by a constant injection apparatus. Ten dogs were given injections of hemoglobin or bilirubin. Two of the 14 dogs also underwent bilateral nephrectomy. Only dogs surviving 14 or more hours after operation were included in the present series.

Chromatography using the butanol pH 6 system invariably disclosed the presence of indirect-reacting bilirubin and one direct-reacting pigment. The latter was the dominant pigment in most instances. Further studies of the direct-reacting pigment fraction revealed that its mobility was

somewhat less than that of known fractions of Pigment II, and that diazotization produced Pigment A and B bands, other characteristics of Pigment I (12). Additional chemical studies, that is, alkaline or enzymatic hydrolysis of Pigments A and B, were not carried out. The two hepatectomized, nephrectomized dogs yielded pigment ratios similar to those of the other animals, indicating that renal tissue was not responsible for the conjugation of bilirubin in the absence of the liver. Pigment patterns were not appreciably altered by injections of hemoglobin or bilirubin (Figure 1). The pigment in urine also was shown to be Pigment I.

Dehepatized rats

By a similar method 10 white Sprague-Dawley rats were hepatectomized. Of these, three were also eviscerated, and five, in addition to hepatectomy, underwent evisceration and nephrectomy. Injections of bilirubin were given to four of the animals after operation. All were maintained by continuous infusions of glucose in saline solution in the tail veins and were sacrificed approximately 24 hours after operation.

Indirect-reacting bilirubin, Pigment I and small amounts of Pigment II were present in the serum of all rats with intact kidneys. In contrast, Pigment II was absent in all of the nephrectomized

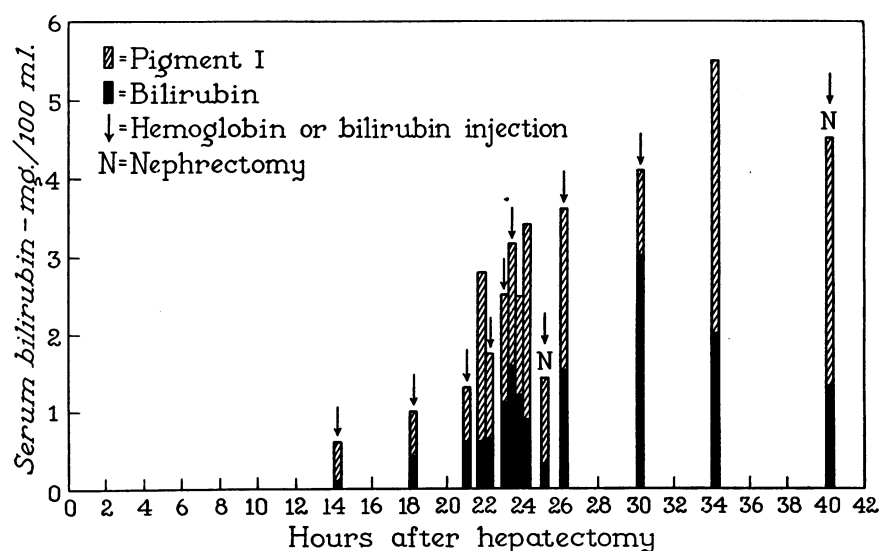


FIG. 1. BILE PIGMENTS IN SERUM OF 14 DOGS AFTER TOTAL HEPATECTOMY
 The word bilirubin is used to indicate the indirect-reacting bilirubin.

TABLE I
*Composition of pigment in bile**

Type	Pigment	
	II	I
	%	%
Human		
1	70.5	29.5
2	72.0	28.0
3	86.0	14.0
4	64.0	36.0
Mean	73.1	26.9
Dog		
1	70.0	30.0
2	75.0	25.0
3	76.0	24.0
4	79.0	21.0
5	70.5	29.5
6	76.4	23.6
7	79.0	21.0
Mean	75.1	24.9
Rat		
1	90.3	9.7
2	80.0	20.0
3	80.0	20.0
4	89.0	11.0
Mean	84.8	15.2

* Pigments I and II are expressed as per cent of total pigment content.

animals. Indirect-reacting bilirubin was the predominant pigment in all except one of the preparations. As in the dehepatized dogs, pigment patterns were not appreciably altered by postoperative injections of bilirubin (Figure 2).

Bile following injections of hemoglobin and bilirubin

Biliary fistulas were surgically created by cannulation of the common bile duct following cholecystectomy in three dogs. The bile was collected over ice and protected from the light. It was studied chromatographically before and after injections of bilirubin and hemoglobin. Six white Sprague-Dawley rats were subjected to the same procedure.

All animals showed a two- to sixfold rise in total excretion of bile pigment following injections of bilirubin or hemoglobin. A twofold increase was present in all dogs and a sixfold increase in three of the six rats. There was a three- to tenfold rise in concentration of Pigment I, and a one- to sevenfold rise in concentration of Pigment II.

Bilirubin was absent in all the dogs used as controls and present in traces in all except one of the rats used as controls. After the injections, there was a four- to twelvefold rise in excretion of bilirubin in five of the rats, and some bilirubin appeared in the bile of all dogs. Control levels appeared in approximately 48 to 72 hours in all of the animals. The overall results following injection of bilirubin or hemoglobin were similar in all respects. The bile-pigment pattern with respect to Pigments I and II remained normal in all of the rats following the injections but was variable in the three dogs.

Biliary obstruction in dogs

Twenty-two dogs were utilized in a study of the composition of bile pigment of peripheral blood following ligation of the common bile duct and cholecystectomy. In some animals hepatic lymph obtained by cannulation of the hepatic lymph vessels was studied.

In the first 72 hours after obstruction, there was a prompt elevation of direct-reacting pigment in the blood and hepatic lymph with a predominance of Pigment II (56 to 84 per cent of the total). This predominance, though in lesser degree, persisted for the first three to five weeks.

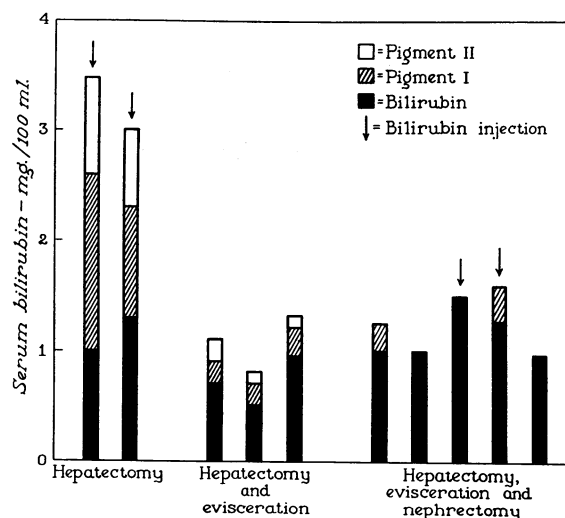


FIG. 2. BILE PIGMENTS IN THE SERUM OF 10 RATS AFTER TOTAL HEPATECTOMY ALONE OR WITH EVISCERATION OR WITH EVISCERATION AND BILATERAL NEPHRECTOMY

Bilirubin as used in this picture is the indirect-reacting type.

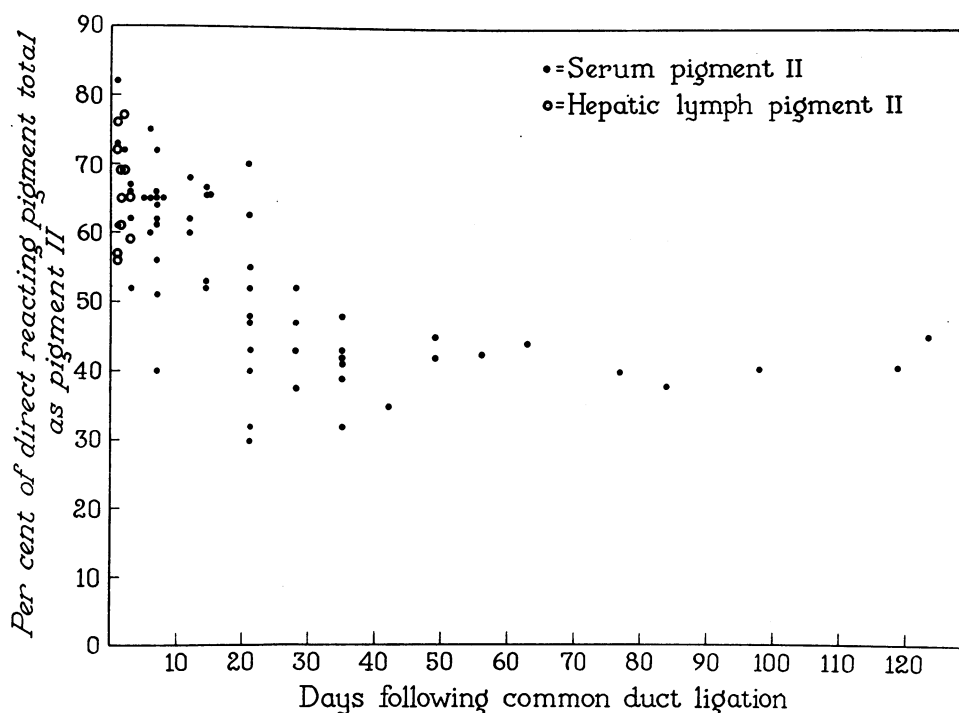


FIG. 3. DIRECT-REACTING BILE PIGMENTS IN SERUM AND HEPATIC LYMPH OF DOGS AFTER LIGATION OF THE COMMON BILE DUCT

Thereafter, measurements revealed that Pigment I was the chief pigment in serum, ranging from 56.5 to 68 per cent of the total direct-reacting pigment (Figure 3).

Experimental hepatocellular jaundice

Two dogs were fed ethionine (15 mg. per Kg. of body weight per day) which was mixed with the normal kennel ration. The dogs became jaundiced after 12 to 15 days. Two other dogs were given carbon tetrachloride (1 cc. per Kg. per day) by gastric tube until jaundice developed, which occurred in three to four days. The serum and bile were studied after jaundice appeared.

In all four dogs the serum showed a predominance of Pigment I in quantities ranging from 57 to 69 per cent of the total direct-reacting pigment. The average concentration of total serum bilirubin was 3.34 mg. per 100 ml. In all animals, the chromatographic pattern of the bile was normal.

Hepatocellular and obstructive jaundice

Serum from 147 jaundiced patients was studied. These patients were chosen almost solely by

the criterion of a value for direct-reacting bilirubin of at least 3 to 4 mg. per 100 ml. of serum. In most of the cases, the diagnosis was unknown to us at the time the tests were done. Bile from 12 patients with hepatocellular jaundice who had elevated values for Pigment I in the serum was studied chromatographically. Eight patients had hepatitis and four had cirrhosis. Samples of bile were obtained by duodenal drainage in all except two cases in which it was obtained at necropsy by aspiration of the gall bladder.

Included in the 147 cases were 91 cases of extrahepatic obstructive jaundice. The etiologic factors in these 91 cases included stone or stricture of the common duct, carcinoma of the bile ducts, ampullary carcinoma and carcinoma of the pancreas. All diagnoses were substantiated at either operation or necropsy. In 80 of the 91 cases (87.9 per cent), Pigment II was the predominant pigment (Figures 4 and 5).

Fifty-six cases of hepatocellular jaundice were included in the group studied. Of these, 29 were cases of acute or subacute hepatitis and the remainder represented chronic hepatic disease,

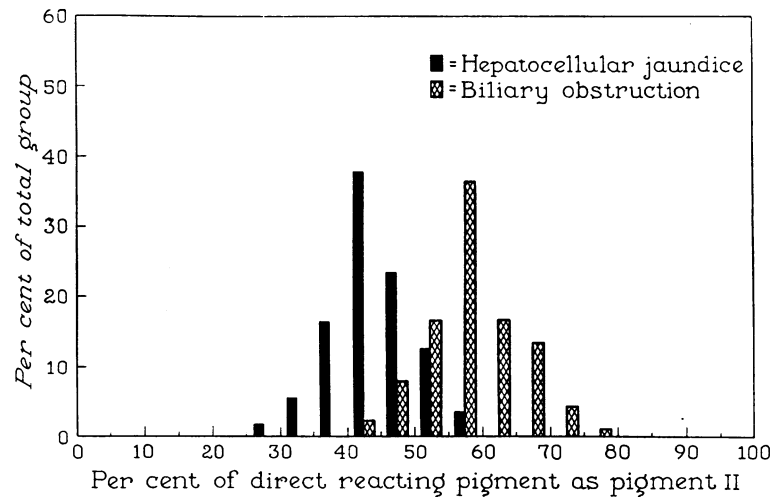


FIG. 4. PERCENTAGE OF PIGMENT II IN THE DIRECT-REACTING BILE PIGMENTS IN THE SERUM OF 147 PATIENTS WITH HEPATOCELLULAR AND EXTRA-HEPATIC OBSTRUCTIVE JAUNDICE

namely cirrhosis of the portal and postnecrotic types. In 47 of these 56 cases (83.9 per cent), Pigment I represented more than 50 per cent of the total direct-reacting pigment (Figure 4).

Normal bile patterns were observed in all of the 12 patients with hepatocellular jaundice whose

bile was studied chromatographically; this group included two patients who died in hepatic coma.

Other types of jaundice

A small number of patients with various other types of jaundice were studied.

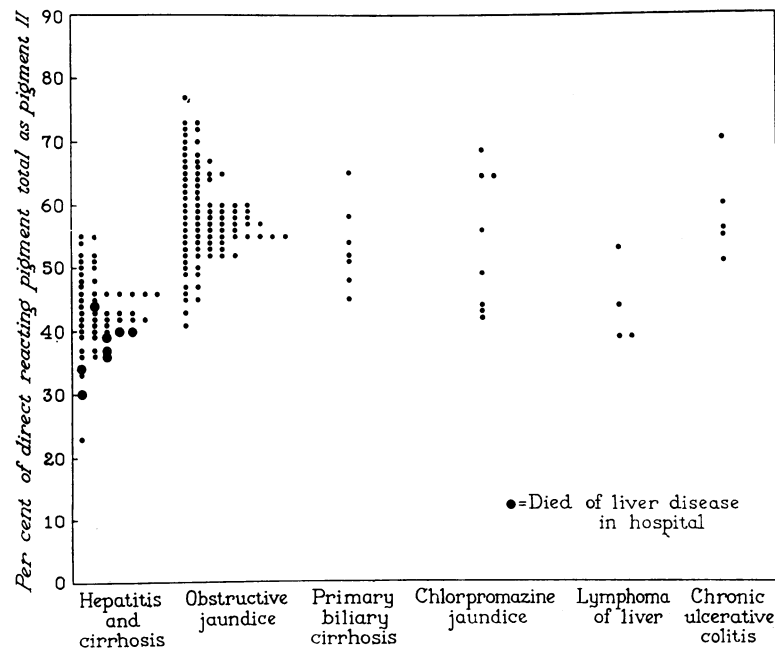


FIG. 5. DIRECT-REACTING BILE PIGMENTS IN SERUM OF PATIENTS WITH VARIOUS TYPES OF JAUNDICE

Eight patients with jaundice due to chlorpromazine were studied. In four of these, more than 50 per cent of the direct-reacting pigment in the serum was Pigment II, and Pigment I predominated in the remainder. Seven patients with primary biliary cirrhosis were studied chromatographically. In five, Pigment II constituted more than 50 per cent of the direct-reacting pigment in the serum. The chief direct-reacting pigment in the serum of the others was Pigment I. In three of the four patients with lymphoma in the liver, Pigment I predominated, whereas Pigment II was dominant in all five patients with chronic ulcerative colitis (Figure 5).

Constitutional hepatic dysfunction

Chromatographic studies were carried out on the serum from eight patients with constitutional hepatic dysfunction. The bile from five of these was studied also, and determinations of fecal urobilinogen were made on three of the patients whose bile was studied.

Bilirubin was the only pigment in the serum in each case. In those patients whose bile was studied, a normal pigment pattern was observed. Values for fecal urobilinogen were normal in the three cases.

The Gunn rat

Chromatography was performed on the serum and bile from two homozygous congenitally jaundiced rats. The serum from one of the rats was fractionated 24 hours after ligation of the common duct.

Bilirubin alone was found in the fractionated serum and bile. Traces of what appeared to be Pigment I were present in amounts too small to measure. The bile in both rats was pale yellow. Twenty-four hours after surgical obstruction in one of these rats, the serum showed a fourfold rise in bilirubin. The fractionation pattern, however, was unchanged from that which was present prior to obstruction.

DISCUSSION

It is generally agreed by workers active in this field that the studies of Cole, Lathe and Billing have provided long-sought answers to questions concerning the nature and behavior of bilirubin. At the same time, new questions as well as new

approaches to the problem of the physiologic interrelationships of the forms of bilirubin and the possible clinical applications have been created. We have sought to pursue some of these in the present study.

Cole, Lathe and Billing (11) observed that Pigment II was the dominant pigment in bile. Our studies indicate that this fraction constitutes more than 70 per cent of the total pigment in bile of human beings and dogs, and more than 80 per cent of it in rats. The remainder of the pigment in bile is Pigment I.

The failure to demonstrate Pigment II in the serum or urine in our dehepatized dogs indicates that its formation is a function of the liver. The presence and the progressive accumulation of direct-reacting Pigment I in addition to indirect (unconjugated) bilirubin in all our dehepatized dogs are not in accord with previous concepts of bilirubin metabolism which assign to the liver the role of transforming indirect bilirubin to direct bilirubin. Bilateral nephrectomy in two dogs at the time of hepatectomy did not appear to diminish the concentration of Pigment I. The continued presence of small amounts of Pigment II in dehepatized, eviscerated rats, however, with its disappearance after nephrectomy, suggests that the kidney of the rat possesses the capacity for conjugating bilirubin with glucuronic acid (Figure 2). Such conjugation has been demonstrated *in vitro* by Grodsky and Carbone (24), and Billing and Lathe (13), using tissue homogenates. In all other respects, the pigment patterns in the dogs and rats were similar. Inasmuch as Pigment I is thought to be the monoglucuronide of bilirubin, and inasmuch as it persists despite the elimination of the two known major organ sites of glucuronide conjugation, the precise extrahepatic origin of Pigment I in these experimental preparations remains unknown.

The evidence cited leading to the conclusion that Pigment I is formed in the dehepatized animal is qualified by the modification in protein precipitation cited earlier. When reduced amounts of ammonium sulfate were used, the pigment extracts appeared more soluble and a greater proportion of polar (direct-reacting) pigment was noted moving on the column. If fully prescribed quantities of ammonium sulfate were used, however, little or no polar pigment was recovered from the

column. These variations in yield of polar pigment that seemed to relate to alterations in the protein precipitation process could possibly be explained by the known greater affinity of conjugated bilirubin for denatured plasma protein.

It might be argued that, in accordance with the extraction procedure of Cole, Lathe and Billing (11), no direct-reacting (polar) pigment was detectable in the dehepatized dog. However, certain observations make us certain that such is not the case: 1) Bollman and Mendez (25) clearly demonstrated that, employing the chloroform, carbon tetrachloride, methanol, pH 6 solvent system which separates the direct and indirect-bilirubin fractions, a significant direct-reacting pigment fraction was present in the serum of dehepatized animals, and we have confirmed this many times. 2) By the van den Bergh test, direct-reacting pigment (1 minute fraction) was always demonstrable in the serum of such animals, the quantity varying from 10 to 50 per cent of the total measurement. 3) For many years direct-reacting bilirubin has been found in the urine after total hepatectomy (3). 4) In studies on the dehepatized rat, full amounts of ammonium sulfate were used, and Pigments I and II were present in all columns. These observations, we think, are strongly suggestive that polar (conjugated) pigment is formed in the absence of the liver and that it is Pigment I. The amount and nature of conjugates which may be present in this fraction, however, will require isolation and analysis, procedures that are not presently possible. For the present, the polar pigment of the hepatectomized dog appears chromatographically similar to Pigment I obtained from bile or from icteric sera of dogs or humans.

In the hepatectomized dogs and rats which were given injections of hemoglobin or bilirubin, the relative proportion of Pigment I to total bilirubin in the serum was not increased. However, injections of hemoglobin into dogs and rats with biliary fistulas resulted in increased excretion of Pigment I in the bile. This seems to indicate that at least some Pigment I can be formed within the liver. From these studies, it is hypothesized that Pigment II may be formed from bilirubin in at least two different sites. These are: 1) the extrahepatic formation of Pigment I from bilirubin and its subsequent conversion within the liver to Pigment II; and 2) the direct conjugation of

bilirubin by the liver to form Pigment I and its conversion thereafter to Pigment II.

The prompt appearance of pigments (predominantly Pigment II) in the blood in our animals following ligation of the common duct has been reported by Billing (26). This might be anticipated in accordance with the concept of regurgitation of bile from the biliary system into the circulation. Billing's observations, however, were confined to the first 12 days of obstruction, and no suggestion of the subsequent decline in the Pigment II fraction that was apparent in our series was noted. The predominance of Pigment I in our animals with chronic biliary obstruction may be explained by metabolic derangement of the parenchymal cells with impairment of the glucuronide-conjugating mechanism which resulted from the prolonged total obstruction.

In contrast to the greater accumulation of Pigment II following experimentally produced biliary obstruction, the jaundice in dogs which followed hepatic injury by carbon tetrachloride or ethionine was characterized by a predominance of Pigment I. This finding we have interpreted as suggesting strongly that jaundice due to damage of the parenchymal cells is due to impaired conjugation and excretion of most of the bilirubin as the diglucuronide, with resultant accumulation of the monoglucuronide.

The values for the pigment fractions in jaundiced patients were consistent with those obtained in experimentally produced jaundice in animals just discussed. In 87.9 per cent of the cases of extrahepatic obstruction, the major portion of the direct-reacting serum bilirubin was Pigment II. In contrast to the obstructive jaundice produced in dogs, prolonged human obstruction, which in some cases had lasted for more than six months, did not result in a loss of the predominance of Pigment II in the serum. The human liver appears to be more resistant to prolonged biliary obstruction than our earlier observations seemed to indicate (27). It was not possible to correlate the Pigment II values in the patients with obstructive jaundice with levels of serum bilirubin or alkaline phosphatase, or with the thymol turbidity, or with results of the cephalin-cholesterol flocculation tests.

Of the group with hepatocellular jaundice, 83.9 per cent showed a predominance of Pigment I in

the direct-reacting serum bilirubin. As in the group with obstructive jaundice, there was no positive correlation between the results of any of the conventional tests of liver function and the pigment values. However, the lowest values for Pigment II were obtained in the patients most severely ill with hepatitis and cirrhosis. Hospital deaths from liver disease in our series occurred largely within this group. Hence, it might be concluded that a low Pigment II value in a patient with parenchymal jaundice is an indication of the severity of the disease process. In a number of these patients the clinical and laboratory features did not strongly suggest hepatocellular jaundice. Chromatography in all of these cases, however, showed definite predominance of Pigment I, and a diagnosis of hepatocellular jaundice was eventually substantiated.

In contrast to the greater amounts of Pigment I in the serum of patients with infectious hepatitis, the pigment pattern of bile obtained by duodenal drainage in 12 of these patients did not differ from that of normal bile. It is possible that a minimal number of intact liver cells is able to excrete normally conjugated bile pigments and that the damaged cells are not only unable to conjugate but are also incapable of excreting conjugated pigments. Less likely is the possibility that Pigment II is formed by the epithelial cells of the extrahepatic biliary tract. A final explanation of this phenomenon awaits further study.

In most of our patients with primary biliary cirrhosis and in half of those with chlorpromazine jaundice (both of these conditions are examples of intrahepatic obstruction), the pigment pattern was similar to that seen in extrahepatic obstruction. However, in two patients with primary biliary cirrhosis and in four with chlorpromazine jaundice, Pigment I was dominant. Although our series is small, it indicates that in some cases pigment partition may be of diagnostic aid in demonstrating hepatocellular damage associated with intrahepatic obstruction and in differentiating it from extrahepatic obstructive jaundice.

The elevation of Pigment I in all but one of our patients with lymphomatous involvement of the liver is compatible with the presence of diffuse cellular dysfunction. In contrast, an obstructive pattern was present in all patients with jaundice secondary to chronic ulcerative colitis.

The results discussed above concerning obstructive and hepatocellular jaundice differ from those reported in the recent studies of Billing (26) and of Baikie (21). In 14 cases of obstructive jaundice and three of acute hepatitis, Billing observed that Pigment I in all instances was dominant. Baikie observed no consistent pattern of diagnostic value in 46 determinations in 12 patients with hepatocellular disease and two patients with extrahepatic biliary obstruction. Careful scrutiny of these data, however, shows that Pigment II was dominant in the majority of determinations in the group with extrahepatic biliary obstruction whereas Pigment I was more commonly elevated among the patients with parenchymal jaundice. It is difficult to explain the differences in our figures and those of Billing despite personal discussion of our respective results. It is our opinion that estimation of the serum bile pigment fractions offers a potential means of differentiating parenchymal from obstructive jaundice. However, the reproducibility and the technical difficulties inherent in the method limit its diagnostic value in its present form.

Recent studies have clarified the mechanisms involved in the conjugation of bilirubin by the liver. These have shown that glucuronic acid is transferred to bilirubin from uridine diphosphate glucuronic acid in a reaction catalyzed by the enzyme, glucuronyl transferase, which is present in the microsomes of the liver (24, 28, 29). Using liver homogenates, Carbone and Grodsky (30), Schmid, Hammaker and Axelrod (28), and Lathe and Walker (29) have demonstrated marked impairment in the conjugation of bilirubin in rats with congenital nonhemolytic jaundice due to a deficiency or absence of this enzyme system. The virtual absence of direct pigment in the colorless bile of similar rats in the present study is consistent with such a defect.

In our eight patients with constitutional hepatic dysfunction, no conjugated pigment was found in the serum. The normal composition of bile in these patients was intriguing, since Arias and London (31) have shown deficient glucuronyl transferase activity in the liver of two patients with this disease. It is of interest, however, that bile aspirated from the gall bladder of one of their patients gave a direct van den Bergh reaction. Bile from the other patient was not studied. In

addition, a similar defect in transferase activity was demonstrated in the three children with congenital nonhemolytic nonobstructive jaundice studied by Axelrod, Schmid and Hammaker (32). The bile, however, was colorless and contained only traces of unconjugated bilirubin. Serum bilirubin levels were much higher in these patients than in ours.

Since ratios of conjugated bilirubin in bile and values for fecal urobilinogen were normal in our cases of constitutional hepatic dysfunction, it is difficult to postulate a defect in the mechanism of excretion of bile pigment. Inasmuch as constitutional hepatic dysfunction is a nonspecific disease entity (33), our patients may not be identical to those described by the other authors. Quantitative differences in glucuronyl transferase activity or the presence of other conjugates of bilirubin may explain these differences.

SUMMARY AND CONCLUSIONS

A study of bile pigments employing the method of reverse phase partition chromatography has been presented. This has included a variety of studies on animals intended to define more clearly the metabolic pathways and interrelationships of the pigments, and an analysis of the pigment patterns observed in the serum and bile of humans and animals with various types of jaundice. The following conclusions have been drawn.

1. The pigment in bile of normal humans, dogs and rats is 75 to 80 per cent Pigment II, the remainder being Pigment I.
2. Pigment I and free bilirubin appear in the serum of the dog and the rat after total hepatectomy. Since essentially no Pigment II is formed in the absence of the liver, and as it is the dominant pigment in bile, it is concluded that the liver is the major site of formation of Pigment II.
3. In addition to the extrahepatic formation of Pigment I and its subsequent conversion by the liver to Pigment II, our studies suggest that some Pigment I is formed from bilirubin in the liver and converted thereafter to Pigment II.
4. Experimental biliary obstruction in animals is characterized initially by a predominance of Pigment II in the serum, a pattern reflecting that of normal bile. However, in animals with induced hepatocellular jaundice, Pigment I is the chief

serum pigment. This strongly suggests that damage to liver cells impairs the conjugation and excretion of the majority of the bilirubin as Pigment II, with the resultant accumulation of Pigment I.

The last hypothesis is strongly supported by a clinical study of jaundice in humans. Pigment II was the dominant direct-reacting pigment in the serum of 87.9 per cent of the patients with extrahepatic obstructive jaundice. Conversely, Pigment I was the chief pigment in the serum of 83.9 per cent of the patients with hepatocellular disease. Of interest is the observation that the pigment ratio in the bile of these patients was normal. The possible diagnostic and prognostic application of this approach to the clinical evaluation of jaundice has been discussed. In our opinion, the separation and measurement of serum bile pigment fractions is a potentially satisfactory method for the differentiation of parenchymal and obstructive jaundice. However, the technical difficulties inherent in the current method both limit its diagnostic accuracy and make its general use impractical at present.

5. Our studies confirm the presence of a defect in the conjugation of bilirubin in rats (Gunn strain) with congenital hyperbilirubinemia.

6. The results of our studies on patients with constitutional hepatic dysfunction do not support the concept of absent or defective glucuronyl transferase activity. Other possible interpretations of our results have been discussed.

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