J. clin. Path. (1955), **8**, 130.

THE THREE SERUM BILE PIGMENTS IN OBSTRUCTIVE JAUNDICE AND HEPATITIS

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(RECEIVED FOR PUBLICATION SEPTEMBER 17, 1954)

Many workers have observed that in obstructive jaundice and hepatitis hyperbilirubinaemia is due mainly to a rise in those pigments which give a " direct " reaction in the van den Bergh test. Cole, Lathe, and Billing (1954) have shown that there are two water-soluble pigments which give this reaction. They appear to be chemically related to bilirubin, but their exact structure is still unknown and they have been temporarily called pigment I and pigment II. Pigment II is the more polar of the two and is the principal pigment found in bile.

The metabolic relationship between the three pigments is not fully understood and it was hoped that a knowledge of the amounts of pigment I and pigment II in serum might give further information on this subject. Using the method described in the previous paper (Billing, 1955), the sera from 17 jaundiced patients have therefore been analysed for bilirubin, pigment I, and pigment II.

The clinical diagnosis of obstructive jaundice does not necessarily mean that the obstruction of the bile ducts is complete and there is also the possibility of some additional liver pathology being present. The effect of bile duct obstruction on bile pigment metabolism can be more easily studied if the obstruction is produced experimentally under standardized conditions. An analysis has therefore been made of the sera from a small series of rats, whose bile ducts were tied, and the results obtained will be compared with those from the pathological sera.

Methods and Materials

The bile pigment determinations were made as previously described (Billing, 1955). The human sera were obtained from 14 patients with obstructive jaundice of varied aetiology and three patients who had infective hepatitis. Obstructive jaundice was produced experimentally in seven rats by ligating the bile ducts under light anaesthesia (Weinbren, 1953). After periods of three, six, and 11 days the animals were sacrificed and bile pigment determinations were made on their sera.

Results

Obstructive Jaundice and Hepatitis.—The findings for the sera from 14 cases of obstructive jaundice and three cases of hepatitis are given in Table I. There was some variation in the proportions of the pigments, but in all instances the dominant pigment was pigment I, which accounted for 38 to 62% (mean 49%) total bile pigments. Bilirubin formed 18–39% (mean 25%) and pigment II formed 15–35% (mean 26%) total bile pigments. No association was found between the level of total bile pigments and the proportion of any one pigment in the serum although when the obstruction was of long standing the proportion of indirect bilirubin appeared to be greater.

TABLE I
BILE PIGMENTS IN OBSTRUCTIVE JAUNDICE AND HEPATITIS

No.	Clinical Diagnosis	Total Bile Pigments (mg./100 ml. Serum)	Bilirubin (mg./100 ml. Serum)	Pigment I (mg./100 ml. Serum)	Pigment II (mg./100 ml. Serum)
1	Obstructive jaundice	9	2·6	4·4	2·0
2	,, ,,	11	2·6	6·1	2·3
3	,, ,,	11	2·9	5·1	3·0
4	,, ,,	12	3·0	4·6	4·4
5	,, ,,	12	2·2	6·0	3·8
6	,, ,,	15	3·2	8·5	3·3
7	,, ,,	18	4·1	11·2	2·7
8	,, ,,	21	4·0	9·8	7·2
9	,, ,,	24	4·6	10·3	9·1
10	,, ,,	26	7·5	10·4	8·1
11	,, ,,	27	7·8	10·5	8·7
12	,, ,,	30	11·7	13·8	4·5
13	,, ,,	31	10·2	12·1	8·7
14	,, ,,	39	13·7	19·1	6·2
15	Hepatitis ..	8	2·0	4·0	2·0
16	,, ..	30	7·2	13·5	9·3
17	,, 12/11/53	29	7·0	16·2	5·8
	,, 19/11/53	39	13·7	18·3	7·0
	,, 4/12/53	12	2·8	6·6	2·6

It was only possible to follow one case of hepatitis throughout the illness, and in this instance it was observed that when the total bile pigment concentration fell the proportion of bilirubin also fell. In

this small series no differences in the pattern of pigments given by sera from cases of hepatitis and obstructive jaundice could be distinguished.

Biliverdin was not detected in any specimens of fresh sera, although it was occasionally observed on the chromatograms of sera from cases of obstructive jaundice which had been left at room temperature for more than 24 hours.

Bile Duct Obstruction in Rats.—Unlike the sera from patients with obstructive jaundice, the chief pigment in the sera from rats with experimental obstruction of the bile ducts was pigment II (Table II) which accounted for 47 to 68% (mean 54%)

TABLE II
BILE PIGMENTS IN EXPERIMENTAL OBSTRUCTIVE JAUNDICE IN RATS

Days after Ligation of Bile Duct	Total Bile Pigments (mg./100 ml. Serum)	Bilirubin (mg./100 ml. Serum)	Pigment I (mg./100 ml. Serum)	Pigment II (mg./100 ml. Serum)
3	12	1·7	4·7	5·6
3	10	1·9	2·8	5·3
3	8	1·3	2·5	4·2
6	5	0·5	1·1	3·4
6	5	0·7	1·7	2·6
11	19	3·2	5·5	10·3
11	11	2·2	3·3	5·5

total bile pigments. Bilirubin was responsible for 10 to 20% (mean 16%) total bile pigments and pigment I for 22 to 39% (mean 30%) total bile pigments.

Discussion

There is some evidence to suggest that pigment I is an intermediate compound in the formation of pigment II from bilirubin since pigment I *in vitro* can be converted by gentle heating and evaporation to bilirubin and pigment II. Also, when diazotized in the van den Bergh test, bilirubin forms one azo compound (pigment A) and pigment II a different azo compound (compound B) while pigment I forms both pigments A and B (Billing, 1954).

If a rat, whose bile duct has been cannulated, is given an infusion of bilirubin, at a rate which exceeds the excretory capacity of the liver for bilirubin, then the direct reacting pigment which gradually appears in the serum is almost entirely pigment I while the pigment in the bile is pigment II (Weinbren and Billing, 1954). In newborn babies with haemolytic disease, directly reacting pigments are sometimes found in the blood in addition to bilirubin, and in several instances it has been noted that pigment I, without any pigment II, is present in amounts up to 60% of the total bile pigments. The conversion of bilirubin to pigment II for excretion in the bile, therefore, appears to take place in two separate stages.

In human bile more than 80% of the pigments present are found to be pigment II, and it was therefore anticipated that this would be the chief pigment responsible for the rise in serum bile pigments in obstructive jaundice. The results have shown that although this is so in the sera from rats with experimental obstructive jaundice, in sera from patients with obstructive jaundice and hepatitis the rise in the bile pigments is largely due to an increase in pigment I.

This finding might be explained on the basis of a species difference governing the renal threshold of the two pigments, but this is unlikely, since in human urine pigment I is usually present in greater amounts than pigment II. A more probable explanation is that in the diseased human liver, in addition to the obstruction preventing the excretion of the bile pigments, there is also some impairment in the conversion of pigment I to pigment II. A defect in the conversion of bilirubin to pigment I may also occur since bilirubin was present in larger amounts in human than in rat serum, and in long-standing cases of obstructive jaundice relatively greater proportions were present. The possibility that pigment II may be reconverted to pigment I or bilirubin by some organ in the body must be considered, but there is at present no evidence that such a conversion can occur.

Summary

The sera from 14 cases of obstructive jaundice and three cases of hepatitis have been analysed for bilirubin and the two pigments which give a direct reaction in the van den Bergh test (pigment I and pigment II). In all the sera the proportion of pigment I (38–62% total bile pigments) was greater than that of pigment II (15–35% total bile pigments) or bilirubin (18–39% total bile pigments).

A comparison of the results obtained from human sera and from rats with experimental obstructive jaundice has been made.

In obstructive jaundice, it is suggested that, in addition to the obstruction preventing the excretion of the pigments into the bile, there is also an impairment in the conversion of bilirubin to pigment I and of pigment I to pigment II.

The author acknowledges the receipt of a personal grant from the Medical Research Council. She wishes to thank Dr. K. Weinbren for ligating the bile ducts of the rats, and Dr. S. Sherlock and other medical colleagues for providing blood from their patients.

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