The value of Jirgl's flocculation test in the diagnosis of jaundice

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SYNOPSIS Jirgl's serum flocculation reaction was examined in a series of 121 patients with varying types of liver disease. Positive results were found in 90% of patients with proven extrahepatic obstructive jaundice. Strongly positive reactions were also obtained in primary biliary cirrhosis and chlorpromazine jaundice.

One out of three cases of 'cholestatic' hepatitis gave a weakly positive reaction and the test may be of value in the diagnosis of this condition and in the rare recurrent conjugated hyperbilirubinaemia in which it is also negative.

Eighty-four per cent of cases of portal cirrhosis were negative and the finding of a positive result in this condition may indicate the presence of a hepatoma.

No correlation could be found either in intra- or extrahepatic obstructive jaundice between the degree of flocculation present and the severity of the obstruction as judged by serum bilirubin and alkaline phosphatase levels.

In 1957, Jirgl described a new serum flocculation test for distinguishing between obstructive and hepatocellular jaundice. Sera from 44 out of 46 patients with jaundice due to bile duct obstruction gave a positive result and no false positives were found in the remainder of the 691 sera examined. Since then confirmatory reports have appeared, mainly from continental workers, although the percentage of positive tests found has varied considerably. Daikos, Mattheou, and Athanasiadou (1959), for instance, reported 92% tests positive in obstructive jaundice in one series and 73% positive in a further series studied later (Daikos, Kourkoumeli-Kontomichalou, and Kekis, 1961). In both series false positive results were obtained in approximately 10% of patients with hepatocellular jaundice. Ragno and Baldi (1961) found essentially the same level but Guelke (1961), who examined sera from 226 patients with obstructive jaundice, found a positive result in only 51%. Recently Shimaoka and Firat (1962) in America have stressed the value of this test in the evaluation of difficult cases of jaundice in which the routine liver function tests gave equivocal or contradictory results.

Few of these reports give adequate clinical details and the reason for the variable but definite percentage of false negative results in obstructive jaundice is unknown. The use of this flocculation

test in the distinction of extrahepatic obstruction from the various types of intrahepatic cholestasis has never been properly evaluated and there are no previous reports of its use in Great Britain. In this paper we describe our findings in a series of 122 patients with widely differing types of liver disease. The place of this test in the differential diagnosis of jaundice has been assessed and an attempt made to determine the factors responsible for the degree of flocculation observed.

MATERIALS AND METHODS

All the patients were jaundiced at the time of study. They have been divided into six main groups as shown in Table I. The diagnosis was confirmed in the patients with cirrhosis either by liver biopsy or at necropsy. All the patients with extrahepatic obstruction had had a laparotomy and in the majority of cases, grouped together under the heading of intrahepatic cholestasis, obstruction of the extrahepatic biliary passages had been excluded by laparotomy or percutaneous cholangiography.

Most cases were studied on more than one occasion and the test was always repeated whenever a weakly positive reaction was obtained.

The method of analysis was as follows:

Serum, 0.8 ml., and 2 ml. 0.1N KOH are placed into a test tube, mixed, and stood at room temperature for 45 minutes, then 2 ml. of 20% sulpho-salicylic acid is added and the mixture shaken thoroughly. This is left for 10

TABLE I
PATIENTS STUDIED

Diagnosis	Number of Cases
<i>Acute hepatitis</i>	
Acute infective	13
'Cholestatic' hepatitis	3
Infectious mononucleosis with hepatitis	4
<i>Cirrhosis</i>	
Post-hepatic or cryptogenic	25
Alcoholic	7
Juvenile	10
Haemochromatosis	4
Cirrhosis with hepatoma	3
<i>Intrahepatic obstructive jaundice</i>	
Chlorpromazine jaundice	2
Primary biliary cirrhosis	21
Congenital biliary atresia	1
Aetiology unknown	1
<i>Extrahepatic obstructive jaundice</i>	
Carcinoma	13
Gallstones	2
Stricture	5
<i>Congenital hyperbilirubinaemia</i>	
Gilbert	1
Dubin-Johnson	1
Recurrent conjugated hyperbilirubinaemia	2
<i>Haemolytic jaundice</i>	4
<i>Total</i>	122

minutes at room temperature and then filtered. Of the filtrate, 2.5 ml. is mixed with 0.5 ml. tungsten reagent (5% phosphotungstic acid in 2N HCl), stood for 10 minutes at room temperature, then centrifuged at 500 *g* for 15 minutes. The supernatant is discarded, the inside of the tube wiped dry, and the sediment redissolved in 3.25 ml. 10% Na₂CO₃. 0.25 ml. Folin-Ciocalteu phenol reagent (Varley, 1958) diluted 1:3 with water is then added.

The flocculation may be read immediately as described by Jirgl (1957) or 12 hours later (Daikos *et al.*, 1959). At the beginning of the study the flocculation reaction was read at both times but subsequently only the immediate reaction was recorded. The result was read against a dark background using a strong incident light and reported as follows: β Negative = tube contents clear, + = slight turbidity, ++ = flocculation, +++ = precipitate.

The reaction was the same whether the serum was examined immediately after taking or after being kept at 0 to 4°C. for periods of up to one week.

Miscellaneous sera from 20 non-jaundiced patients with no evidence of liver disease gave consistently negative results.

RESULTS

The number of positive tests found in the various groups of patients is shown in Fig. 1 and the degree of flocculation recorded in Table II.

ACUTE HEPATITIS None of the 13 patients with acute infective hepatitis gave a positive reaction. The severity and duration of illness varied widely in these patients. The highest serum bilirubin recorded was 22.5 mg. per 100 ml. in a woman who was admitted during a relapse. Recovery was ultimately complete and the Jirgl test was consistently negative throughout the course of the illness.

Another three patients were regarded as having 'cholestatic' hepatitis. After a typical acute onset their symptoms had improved with the exception of jaundice and pruritus. Their jaundice showed all the features of intrahepatic obstruction and in each instance they had been deeply jaundiced for some weeks before admission. One of the two who were examined during the stage of deep jaundice (serum total bilirubin level 18 and 25 mg. per 100 ml. respectively) had a weakly positive Jirgl test, and the other case was negative. The third patient, who was studied at a time when the serum bilirubin was falling (total level 13.8 mg. per 100 ml.), also showed no flocculation.

The four patients with infective mononucleosis who had clinical and biochemical evidence of hepatitis had negative tests.

CIRRHOSIS Four of the 25 patients with post-hepatic and cryptogenic cirrhosis and one of four patients with idiopathic haemochromatosis gave a flocculation reaction (Fig. 1, Table II). In four of these five positive reactions the test was weakly positive (+) and in only one did a precipitate form (+++). These positive reactors were of some interest. In one the diagnosis may well have been wrong. She was a woman who had been regarded as having a post-necrotic type of cirrhosis and she died from variceal haemorrhage. At necropsy she was found to have a dilated thickened common bile duct full of stones. Although the histological changes in the liver at this stage were too advanced to make an accurate diagnosis, in retrospect it seems likely that this was a

TABLE II
ANALYSIS OF CASES WITH POSITIVE FLOCCULATION REACTION

Diagnosis	Number of Cases		Flocculation Reaction		
	Total	Positive	+	++	+++
'Cholestatic' hepatitis	3	1	1	—	—
Cirrhosis	46	5	4	—	1
Cirrhosis with hepatoma	3	3	2	—	1
Intrahepatic obstructive jaundice	25	24	1	10	13
Extrahepatic obstructive jaundice	20	18	3	4	11

FIG. 1. Results of Jirgl's flocculation test in the various groups of patients studied.

secondary biliary cirrhosis. There was a history of upper abdominal pain to which little attention had been paid and the serum alkaline phosphatase level had always been considerably elevated. Another patient is currently in the ward with a suspected hepatoma. Three other cirrhotic patients in whom a definite hepatoma was proved at necropsy also had a positive test (Table II). The cause of the positive reaction in these patients with a hepatoma was uncertain. Although all three had widespread growth at necropsy the intrahepatic bile ducts were not dilated. Two were markedly jaundiced (serum total bilirubin level > 20 mg. per 100 ml.) and the third only moderately so. Considering the group of cirrhotic patients as a whole there was no correlation between the height of the serum bilirubin or alkaline phosphatase levels and a positive Jirgl flocculation. It is to be noted that all the patients with that particularly active variety of cirrhosis termed 'juvenile' cirrhosis or lupoid hepatitis (Bearn, Kunkel, and Slater, 1956) had consistently negative tests.

INTRAHEPATIC OBSTRUCTIVE JAUNDICE Twenty-four of the 25 patients in this group had a flocculation reaction. In 23 of these the reaction was recorded as ++ or +++. The one patient with a negative result had a short history consistent with primary biliary cirrhosis. She was only slightly jaundiced (serum total bilirubin level 1.2 mg. per 100 ml.) although the alkaline phosphatase level was considerably raised (45 K.-A. units). The liver biopsy showed an expanding portal tract fibrosis with cellular infiltration and there seems to be little doubt about the diagnosis.

There was no correlation between the degree of flocculation recorded and the height of the serum bilirubin or alkaline phosphatase level (Figs. 2 and 3).

EXTRAHEPATIC OBSTRUCTIVE JAUNDICE In 18 of the 20 cases the sera gave a positive reaction and in over a half of these the reaction was strongly positive (+++). As in the patients with intrahepatic obstructive jaundice there was no correlation with the serum bilirubin or alkaline phosphatase level (Figs. 2 and 3). The three with a weakly positive test (+) had comparable serum bilirubin levels to those with strongly positive results (+++) but it may be significant that they had been jaundiced for only a short time before admission. There was also no apparent relation between the result of the test and the cause of the obstruction. The number of patients with obstruction due to gallstones or stricture was relatively small but strongly positive reactions were obtained in these as well as in the cases with complete obstruction due to carcinoma of the pancreas or bile ducts.

The two patients with a negative result had partial bile duct obstruction. Their serum total bilirubin levels at the time of testing were 2.0 and 2.2 mg. per 100 ml. respectively.

Serial tests were performed in seven of these patients at varying times during the first two weeks after surgical relief of the obstruction (Table III). The reaction became negative in four. The others continued to have a positive reaction though it is to be noted that one was tested only once at three days and that the reaction was less strongly positive in all three, being recorded as +, whereas before operation it was ++ or +++.

The reaction appeared to decrease in intensity very rapidly after surgery as can be seen from the findings in the cases examined one to four days after operation (Table III). This occurred even though there had been little fall in the serum bilirubin level. The change in reaction appeared to be entirely

FIG. 2. Relation between degree of flocculation reaction and the serum total bilirubin level in intra- and extrahepatic obstructive jaundice.

FIG. 3. Relation between degree of flocculation reaction and the serum alkaline phosphatase level in intra- and extrahepatic obstructive jaundice.

dependent upon the relief of the obstruction, for in none of the patients with carcinomatous obstruction was the growth resected, a palliative by-pass operation only being possible.

CONGENITAL HYPERBILIRUBINAEMIA None of the four cases tested gave a positive reaction. This finding was of particular interest in the three patients with conjugated hyperbilirubinaemia. One of these had the classical Dubin-Johnson syndrome with black pigment in the liver biopsy and a persistently raised serum total bilirubin level of 6.5 mg. per 100 ml. The other two had had recurrent attacks of an obstruc-

tive-like jaundice and appeared to be similar in all respects to the cases described by Tygstrup (1960) and by Summerskill and Walshe (1959) who termed the condition 'benign recurrent intrahepatic obstructive jaundice'. One of the patients was tested at a time when he was deeply jaundiced, with a serum total bilirubin level of 13.0 mg per 100 ml., but the Jirgl reaction was consistently negative.

HAEMOLYTIC JAUNDICE All four cases studied had an acquired haemolytic anaemia. The flocculation test was negative.

TABLE III
RESULTS OF JIRGL'S FLOCCULATION REACTION IN SEVEN PATIENTS EXAMINED BEFORE AND AFTER SURGICAL RELIEF OF OBSTRUCTION

Case	Diagnosis	Pre-operative	Post-operative	
			1 to 4 Days	4 to 14 Days
S.Z.	Carcinoma of pancreas	+++ (7) ¹		-ve (2)
M.D.	Carcinoma of pancreas	+++ (54)		+
C.G.	Carcinoma of pancreas	++ (18)	+	-ve (13)
W.C.	Carcinoma of pancreas	++ (20)	+	+
N.I.	Carcinoma of pancreas	+	-ve (9)	
T.C.	Carcinoma of hepatic duct	+++ (17)	+	
D.M.	Stricture of common bile duct	+++ (6)		-ve (2)

¹Corresponding serum total bilirubin levels in mg. per 100 ml. are given in brackets.

DISCUSSION

It is clear from these findings and those of previous workers that a high percentage of cases of extrahepatic obstructive jaundice have a strongly positive flocculation reaction. This was present in 90% of our cases. An occasional negative result may, however, be found, particularly in patients with partial bile duct obstruction. Perhaps one of the most important findings in the present study was the consistently positive reaction found in cases of chronic intrahepatic cholestasis. Many patients with so-called 'primary biliary cirrhosis' do not have clinical or histological evidence of cirrhosis during the early stages of the disease and the distinction of this condition from surgically remediable extrahepatic obstruction is one of the most difficult problems in differential diagnosis in liver disease. Jaundice due to chlorpromazine may also be extremely difficult to distinguish from surgical obstruction. Jirgl's flocculation reaction is of no help here and both our cases had strongly positive reactions. It does appear to be of value, however, in the diagnosis of the 'cholestatic' variety of acute infective hepatitis. Here again the jaundice may show all the features of obstruction. Only one of the three cases studied had a flocculation reaction and this was only weakly positive (+). Daikos and his colleagues (1959) also describe a further case of what is termed 'cholangiolitic' hepatitis which gave a negative result. Both in this and in previous series the test has been uniformly negative in the ordinary variety of infective hepatitis even though the patient may be deeply jaundiced.

The distinction of portal cirrhosis from bile duct obstruction is usually straightforward. The Jirgl reaction is nevertheless of some value, for the finding of a positive result in a patient who appears to have otherwise typical portal cirrhosis should arouse suspicion either that the diagnosis is wrong or that the patient has developed a hepatoma.

A particularly interesting finding was the negative result in the two cases of recurrent conjugated hyperbilirubinaemia. This rare condition, which is possibly of congenital origin, is characterized by recurrent attacks of jaundice which start with intense itching and show all the features of extrahepatic obstruction. The condition usually remits spontaneously although the jaundice may last two to three months. All the reported cases have been explored surgically (Summerskill and Walshe, 1959; Tygstrup, 1960). The finding of a negative Jirgl test in a patient whose history and findings are otherwise consistent with this diagnosis may save an unnecessary laparotomy though it is clear that more cases have to be studied to confirm this finding. It is of interest that recent studies of bromsulphthalein uptake and excretion in

this condition (Williams, Billing, and Sherlock, 1963) have shown a marked decrease in both storage capacity and excretory transport maximum which is similar to the changes found in infective hepatitis during the acute stage (Williams, Preisig, Sweeting, and Bradley, 1963). In intrahepatic cholestasis the storage capacity is usually normal and the predominant defect is in excretion. The negative Jirgl reaction is therefore consistent with these findings.

The very rapid return of the flocculation reaction to normal after surgical relief of extrahepatic obstructive jaundice, at a time when the serum bilirubin and alkaline phosphatase levels are still considerably raised, is of interest in relation to the underlying mechanism of the flocculation reaction. It suggests that the flocculation is dependent on a factor normally present in bile which is retained in the serum in obstructive jaundice. It seems more likely that the flocculation is due to this rather than to some complex interprotein reaction as in the other flocculation tests. Shimaoka and Firat (1962) have also produced evidence in favour of this view. They obtained a positive flocculation reaction with a sample of gall-bladder bile obtained at operation from a patient whose liver function was normal and whose serum reaction was negative. They also obtained positive flocculation tests in bile obtained by T-tube drainage from two patients after surgical relief of obstructive jaundice, again at a time when the serum reaction had become negative. Although Jirgl (1957) discovered the reaction while determining blood mucoprotein, the responsible factor is not a mucoprotein. Sera remain positive after extraction with ether or chloroform. Shimaoka and Firat (1962) attempted to characterize the positively reacting substance but all the substances they have tested so far, including albumin, γ globulin, cholesterol, taurocholic and glycocholic acids, have given negative results.

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