LABORATORY TESTS IN THE DIAGNOSIS OF LIVER DISEASE

A REPORT ON THREE PROCEDURES

BY

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Two important trends in recent work on liver function tests are noticeable at the present time. The first is a broad division between those which test known or accepted functions of the liver and certain empirical procedures which do not test known functions but which are obviously correlated either with certain types of liver damage or with certain diseases in which liver damage is prominent. Among the former the most important are the serum bilirubin estimation, galactose and laevulose tolerance tests, serum protein and urinary urobilin estimations, and the hippuric acid test. The latter include estimation of the serum alkaline phosphatase and choline-esterase activity and the flocculation tests (i.e., the Takata-Ara reaction, the serum colloidal gold reaction, and the cephalin-cholesterol test). The flocculation tests are thought to depend upon an increase in the gamma globulin content of the serum, and the hepatic origin of this protein has not been proved. Nevertheless positive results occur principally in diseases in which liver damage is known to be present. The former tests, with certain exceptions, tend to give similar results in all types of liver damage, while some of the latter show striking differences and therefore promise more diagnostic help.

The second trend is the increased sensitivity of most of the tests as compared with those in use 15 years ago. While the earlier complaints of negative results in cases of known hepatic insufficiency have largely disappeared, we are now faced with the opposite finding of a proportion of positive results in a variety of diseases not primarily affecting the liver, such as pneumonia (Curphey and Solomon, 1938), hyperthyroidism (Althausen and Wever, 1937; Haines, 1939; MacLagan and Rundle, 1940), rheumatoid arthritis (Rawls *et al.*, 1937), following surgical operations (Boyce, 1941), and in scarlet fever, diphtheria, and tuberculous meningitis (Meneghelo and Drinberg, 1943). It is hardly surprising that liver function should be impaired in general intoxications of this type, but it complicates the question of interpretation since we need to distinguish between functional impairment and structural damage.

The three procedures described here were originally chosen because they appeared to give the maximum chance of differentiating toxic from obstructive jaundice, but they happen to include one example from each of the three main groups—i.e., a flocculation test, an enzyme test, and a purely functional test. This selection is not intended to preclude the use of other tests such as the serum bilirubin and urinary urobilin estimations, which are both of particular value in patients not obviously jaundiced and the first of which is indispensable as a record of the intensity of jaundice when present. The serum protein estimation and the hippuric acid tests also have their special merits, but on the whole their value has been greater in prognosis than in diagnosis (Foley *et al.*, 1937; Higgins *et al.*, 1944; Quick, 1933, 1939; Boyce, 1941).

I. Serum Colloidal Gold Reaction

Introduced by Gray in 1940, this appears at present to be the best of the flocculation tests from the diagnostic standpoint. It does not test any known function of the liver and should be regarded as an indicator of disturbed liver metabolism rather than a function test. The results reported here were obtained by a modified method, described elsewhere (MacLagan, 1944a),

in which 0.05 ml. of serum, 0.5 ml. of special buffer solution at pH 7.8, and 2.5 ml. of gold sol prepared by Patterson's (1931) method are mixed in a single tube. 5 represents complete precipitation of the sol and is the strongest positive, while 0 represents no precipitation and is a negative or normal result. The reaction apparently depends upon an excess of gamma globulin in the serum (Gray and Barron, 1943; Kabat *et al.*, 1943); it is thus not directly dependent upon either the total globulin or the albumin-globulin ratio. This was shown by Gray (1940), but also holds for the modified technique used here (Table I).

TABLE I.—Serum Proteins (G.%) and Gold Reaction in 24 Cases

Case No.	Gold (No.)	Albumin (Normal 4.0-5.5)	Globulin (Normal 1.5-3.0)	Total (Normal 6.0-8.0)	Diagnosis
1	5	2.4	7.8	10.2	Infective hepatitis
2	5	4.0	2.9	6.9	" "
3	5	3.7	2.7	6.4	" "
4	5	4.3	2.2	6.5	" "
5	4	3.9	3.2	7.1	" "
6	5	2.9	3.0	5.9	" "
7	5	3.3	3.6	6.9	" "
8	3	3.7	2.8	6.5	" "
9	0	3.9	2.2	6.1	" "
10	4	3.0	3.2	6.3	Arsenical jaundice
11	5	2.5	5.5	8.0	Subacute hepatitis*
12	5	3.9	2.3	6.2	" "
13	5	1.4	6.4	8.2	" "
14	5	2.1	4.0	6.1	Cirrhosis
15	5	2.5	3.2	5.7	" "
16	5	2.3	4.5	6.8	" "
17	5	3.4	2.2	5.6	Bacterial endocarditis
18	5	1.7	2.6	4.3	" "
19	0	1.8	1.6	3.4	Nephritis
20	0	1.8	2.0	3.8	" "
21	0	2.5	1.6	4.1	" "
22	0	4.6	2.2	6.8	" "
23	0	3.3	2.4	5.7	" "
24	0	3.6	3.2	6.8	Biliary obstruction, recurrent

* Subacute hepatitis is used here to indicate hepatitis with jaundice of more than two months' duration (Higgins *et al.*, 1944) but without ascites. The dividing line between this condition and cirrhosis is difficult to draw, and the biochemical findings are very similar in the two.

It will be seen from Table I that, although there is some correlation with the total serum globulin, nearly half the cases with strongly positive gold reactions had normal globulin figures, and one case of recurrent biliary obstruction had a raised globulin and a negative gold reaction. The inversion of the albumin-globulin ratio in nephritis was associated with negative gold tests. These protein estimations were performed by the manometric method of Peters and van Slyke (1932), using the selenate digestion mixture of Beaumont and Dodds (1941). The globulins were precipitated with 22.5% sodium sulphate.

Table II gives the serum colloidal gold reactions in 319 cases of liver disease. It will be seen that the test is usually positive in infective hepatitis (93%) and in cirrhosis, and is negative in jaundice due to biliary obstruction (93%). It usually remains negative in obstructive jaundice in spite of increasing liver damage as shown by other tests—e.g., the galactose index—and is therefore of value in distinguishing this condition from the first two. A strongly positive gold reaction (4 or 5+) has not been seen in obstructive jaundice, and is thus of more help in this connexion than a negative result, which does not absolutely exclude infective hepatitis. The results in infective hepatitis are usually maximal at the onset of jaundice and

become negative after 3 to 6 weeks, although sometimes persisting much longer. In cirrhosis there is little change from month to month, so that repeated observations help to decide between these two. In arsenical jaundice—i.e., jaundice

TABLE II.—Serum Colloidal Gold Reaction in 319 Cases

	Precipitation Number						Totals	% Pos.
	5	4	3	2	1	0		
Obstructive jaundice	0	0	0	3	0	39	42	7
Infective hepatitis	84	24	22	10	7	11	158	93
Cirrhosis and subacute hepatitis ..	12	0	0	0	0	0	12	100
Arsenical jaundice	5	5	4	6	7	35	62	44
Weil's disease (jaundiced) ..	0	0	3	2	3	11	19	42
Weil's disease (not jaundiced) ..	0	1	1	1	1	10	14	29
Toxic jaundice	0	0	0	1	0	2	3	7
Amyloidosis	0	0	0	1	1	0	2	7
Liver abscess	0	0	0	0	0	2	2	

occurring during antisyphilitic treatment—and also in Weil's disease negative results are frequent (about 60%) and make the distinction from biliary obstruction more difficult, but fortunately this is rarely a clinical problem. The rather striking difference between the infective and the arsenical cases has been noted elsewhere (MacLagan, 1944b), and suggests a probable aetiological difference in the two groups. In liver abscess and in amyloidosis results have been mainly negative.

As with most other liver function tests some positive results occur in heart failure, in severe chronic anaemia, and in various acute and chronic infections, of which the following are the most important: glandular fever, atypical pneumonia, infective endocarditis, advanced pulmonary tuberculosis, and rheumatoid arthritis (MacLagan, 1944a). In this group the liver involvement was obviously only an incident in the primary disease, but such conditions might complicate obstructive jaundice, when due allowance would have to be made. In practice they can usually be eliminated without difficulty by other means.

Finally, it should perhaps be emphasized that the results given here are not the same as those obtained with Gray's (1940) original technique, which has been adversely criticized by Mateer *et al.* (1942). The technical details referred to above must be followed exactly if comparable results are to be obtained.

II. Serum Alkaline Phosphatase

This estimation, like the gold reaction, does not test any known function of the liver, and raised values are found in infancy and in a variety of bone diseases. In spite of this the test has been much used in jaundice since Roberts's (1933) observation that the level is usually higher in obstructive than in other types of jaundice. The 136 results in Table III were obtained by the method of King and Armstrong (1934).

TABLE III.—Serum Alkaline Phosphatase in 136 Cases

	Range of Values (Normal 3–13 Units)							Totals
	0–13	14–24	25–35	36–45	46–55	56–80		
Obstructive jaundice	0	3	7	13	11	9	43	
Infective hepatitis	6	35	6	0	0	0	47	
Cirrhosis and subacute hepatitis ..	0	6	3	2	2	1	14	
Arsenical jaundice	8	12	2	0	0	0	22	
Toxic jaundice	1	3	0	0	0	0	4	
Amyloid disease	0	0	2	1	0	0	3	
Liver abscess	0	0	2	0	1	0	3	

It will be seen that the test is of some value in distinguishing biliary obstruction on the one hand—usually over 35 units—from infective hepatitis and arsenical jaundice on the other—usually below 25 units and may be within normal limits. However, 15 of these cases fall into the doubtful range (25–35 units), and 3 of the obstructive cases were below 25 units. This is about the same degree of overlap reported by others (Gutman *et al.*, 1940; Giordano *et al.*, 1939; Higgins *et al.*, 1944). If, however, cirrhosis has to be considered the test is of much less value, as figures over 35 are frequent in this condition. In liver abscess and in amyloid disease a raised phosphatase is often present, and may be the only biochemical indication of disturbed liver metabolism.

III. Galactose Index

This index (MacLagan, 1940) is the sum of the four blood-galactose values at 1/2, 1, 1½, and 2 hours after the oral

administration of 40 g. of the sugar. The estimations were performed mainly on capillary blood as previously described, the results being read from the table given below (see Appendix). This table is based on figures given in the reference cited above and does not involve any alteration in the method. The normal limits for the galactose index, based on 50 normal subjects, are 0–160 (0–110 for male medical students). The test is contraindicated by nausea or vomiting from any cause.

TABLE IV.—Galactose Index in 145 Cases

	Range of Values (Normal 0–160)						Totals
	0–160	161–200	201–300	301–400	401–500	501–600	
Obstructive jaundice	23	3	7	4	2	0	39
Infective hepatitis	4	5	15	8	3	1	36
Cirrhosis and subacute hepatitis ..	0	1	8	2	2	0	13
Arsenical jaundice	0	1	2	6	1	2	12
Toxic jaundice	0	0	2	1	1	0	4
Hyperthyroidism (from MacLagan and Rundle, 1940)	11	7	16	4	3	0	41

It will be seen from Table IV that the index is nearly always above normal in infective hepatitis, arsenical jaundice, and cirrhosis, but is frequently normal in jaundice due to biliary obstruction. Considerable impairment of function can, however, be demonstrated in certain cases of obstructive jaundice, particularly after the first three weeks, in febrile or cachectic patients, and after repeated attacks of obstruction (MacLagan, 1941). The only normal results in the toxic and infective group were in 4 cases of infective hepatitis in young adults from the Services, a class of patient in which liver function is no doubt above the average. The test is therefore of some value in distinguishing these two groups if interpreted with due regard to clinical data. A normal result in an elderly jaundiced patient is of particular diagnostic value and has so far been seen only in obstructive jaundice. It is also useful as an extra aid in cases in which the gold and phosphatase tests are equivocal and for assessing the degree of liver damage when present.

The test has also been much used in hyperthyroidism, in which there is impaired tolerance in a high proportion of cases (Althausen *et al.*, 1940; MacLagan and Rundle, 1940; Barnes and King, 1943; and Table IV). Some workers have found it as sensitive as the B.M.R. in diagnosis, although Rundle and I got values decisively above normal in only 75% of our cases; it is agreed that the test is less affected by iodine therapy than is the B.M.R. There is some difference of opinion as to the mechanism involved, since both liver damage and increased intestinal absorption have to be considered. Most recent articles express the view that both factors contribute to the results in hyperthyroidism (Lichtman, 1941; Grauer *et al.*, 1942; Rosenkrantz *et al.*, 1942).

Discussion

It is now generally agreed that a combination of liver function tests yields more information than any one test, and this is particularly true of those described here. Liver function tests are useful in three principal types of case.

1. *Suspected Liver Disease in Non-jaundiced Patients.*—The three tests appear to be sensitive enough to detect cirrhosis, and in common with others they also indicate hepatic involvement in a variety of other diseases such as hyperthyroidism (galactose index), heart failure and various infections (serum colloidal gold reaction), and in liver abscess and amyloidosis (serum alkaline phosphatase). In this group the pigmentary tests—serum bilirubin and urinary urobilin estimations—reach their maximum degree of usefulness and should not be omitted, but they may sometimes be completely negative in cases with known liver damage.

2. *Jaundice of Known Origin.*—The galactose index will reveal liver damage in late biliary obstruction, the other two tests being of no value in this respect. Either the gold reaction or the galactose index may be used to follow the course of a hepatitis, and will often give positive results for some weeks or months after the disappearance of clinical or latent jaundice. In cirrhosis and in subacute hepatitis all three tests have been positive, and the galactose index appeared to have some correlation with clinical severity, so far as could be judged from this small series (13 cases).

3. *Jaundice of Unknown Origin.*—Haemolytic jaundice is usually diagnosed by clinical and haematological methods, and will not be considered here. The way in which these tests can help in the more difficult task of distinguishing obstructive from toxic and

infective jaundice is indicated in Table V, which shows the typical or average results in the types most commonly encountered.

TABLE V.—“Typical” Results with 3 Tests in Jaundiced Patients

	Gold	Phosphatase	Galactose Index
Obstructive	Negative	Over 35	Frequently normal
Infective hepatitis	Positive	Under 25	Raised
Cirrhosis and subacute hepatitis	Positive	Raised	Raised
Arsenical	44% pos.	Under 25	Raised
Weil's disease	42% pos.	?	?

The interpretation of the results in these cases must always depend partly upon clinical data, and can never be completely rule of thumb. Nevertheless there are many instances in which a slight acquaintance with the history permits an almost certain diagnosis on the basis of the tests. Considering the gold and phosphatase tests first, three particular combinations appear to have diagnostic significance: (A) A negative gold reaction with phosphatase above 35 units suggests biliary obstruction. (B) A positive gold reaction with phosphatase below 25 suggests parenchymatous hepatic disease and absence of biliary obstruction. (C) A strongly positive gold reaction (4 or 5+) has not been seen in obstructive jaundice and appears to be diagnostic with any phosphatase level. In such cases a phosphatase above 35 suggests cirrhosis rather than acute hepatitis.

It will be evident from Table V that the chances of making the distinction are greater in infective hepatitis and cirrhosis than they are in arsenical jaundice and Weil's disease, but fortunately the two latter conditions do not usually present great diagnostic difficulties. When the gold reaction is positive possible complicating diseases must be considered, but these rarely cause serious trouble in interpretation.

The constancy of these findings is illustrated in Table VI, which includes all jaundiced patients on whom both tests were

TABLE VI.—Combination of Two Tests in 100 Jaundiced Patients

	1	2	3	4	Totals
Gold	Negative	Negative	Positive ⁴	Positive	
Phosphatase	Over 35	Under 35	Over 25	Under 25	
Obstructive jaundice ¹	22	9	1 (1+)	0	32
Infective hepatitis	0	7	1 (5+)	28	36
Cirrhosis and subacute hepatitis ²	0	0	4 (All 5+)	6	10
Arsenical jaundice	0	6	1 (5+)	12	19
Toxic jaundice ³	0	2	0	1	3
Totals	22	24	7	47	100

¹ Due to pancreatic carcinoma in 14, gall-stones in 9, hepatic metastases in 6, and other causes in 3. Diagnosis established by necropsy in 15, laparotomy in 15, and by other methods in 2.

² Diagnosis established by necropsy in 5 cases, biopsy in 2, and clinically in 3.

³ Due to T.N.T. in 2 cases and to sulphapyridine in 1.

⁴ Figures in brackets indicate strength of gold reaction.

done. It will be seen that out of these 100 cases 75 fall into group A, B, or C (columns 1, 3, and 4), and in all of these the findings were in keeping with the final diagnosis. The galactose test was confirmatory in 26, omitted in 41, and atypical in 8. The atypical galactose tests were 4 cases of biliary obstruction with secondary liver damage and the 4 cases of infective hepatitis in young adults referred to above.

In the remaining 25 cases (column 2 and one case in column 3) the results did not always give a definite answer, but in some instances the galactose test provided further information. They may be classified as follows: (1) Three cases of obstructive jaundice with negative gold reactions and phosphatases of 16, 25, and 29, and galactose indices of 118, 126, and 114 respectively. In view of the ages of the patients—72, 62, and 40—the normal galactose tests here were felt to be strongly in favour of what proved to be the correct diagnosis. (2) A group of 22 cases with negative or weakly positive gold reactions in which the findings were conflicting and of little diagnostic value. These consist mainly of obstructive and arsenical jaundice, but include some cases of infective hepatitis and toxic jaundice. The galactose test was omitted in 18 of these, and in some it would probably have helped. In two of the obstructive cases a later repetition of the tests showed a rise of the phosphatase to a diagnostic level.

The final assessment of the three tests in these 100 jaundiced patients is therefore that the distinction between biliary obstruction

and generalized liver disease could be made in at least 78, which include about the same proportion of the obstructive and of the non-obstructive cases. It is not, of course, suggested that these rules of interpretation will be infallible, but since no exception to them has been observed in this series they appear to be reliable enough to form a useful diagnostic guide.

As a matter of practical convenience it is easier to do the gold and phosphatase tests first, and to reserve the galactose for cases in which the first two are atypical or in which the degree of liver damage is of interest. The galactose index has, however, a special place in the investigation of hyperthyroidism.

Summary

The clinical value of laboratory tests in the diagnosis of liver disease has been reviewed with special reference to the serum colloidal gold reaction (319 cases), the serum alkaline phosphatase (136 cases), and the galactose index (145 cases).

The first two tests, taken together, often give valuable diagnostic information when considered in conjunction with clinical data. A jaundiced patient with a negative gold reaction and a phosphatase above 35 King-Armstrong units probably has biliary obstruction; one with a positive gold reaction and a phosphatase below 25 units probably has not. A 4 or 5+ gold reaction is against biliary obstruction whatever the phosphatase level.

The galactose index is useful as a confirmatory test in special cases and for assessing the degree of liver damage when present. It is also of value in the diagnosis of hyperthyroidism.

The results given indicate that out of 100 jaundiced patients the distinction between biliary obstruction and generalized liver-disease could be made in 75 cases with the gold and phosphatase tests, and in a still higher proportion if the galactose index was included.

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APPENDIX

Table for Blood Galactose Method of MacLagan (1940)

ML. of 0.002 N sodium thiosulphate (blank minus unknown)										
	0.0	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45
Mg. of galactose per 100 ml. of blood										
0.0	0	10	17	21	24	27	29	33	35	37
0.5	40	43	46	48	51	54	57	59	62	65
1.0	67	70	72	75	78	80	83	85	88	90
1.5	94	97	99	101	104	107	110	112	115	118
2.0	121	124	127	130	132	135	138	140	143	146
2.5	149	151	154	156	159	162	165	167	170	173
3.0	176	178	181	183	185	187	190	192	194	196
3.5	199	201	203	205	208	210	212	215	217	219

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