BRITISH MEDICAL JOURNAL

LONDON SATURDAY AUGUST 9 1947

LIVER FUNCTION TESTS IN THE DIAGNOSIS OF JAUNDICE

A REVIEW OF 200 CASES

BY

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It is now generally agreed that no one liver function test will give a satisfactory picture of the activities of an organ which has so many metabolic tasks to perform, and the present tendency, particularly in the United States, is to use a battery of tests in every case of suspected liver dysfunction. Thus Watson (1944) advocates eleven tests for jaundiced and seven for non-jaundiced patients; other workers have used similar combinations (Mateer *et al.*, 1942; Neeffe and Reinhold, 1946).

In this country few hospital laboratories have had the technical assistance required to follow these suggestions on any worth-while scale, and this has led to a rather conservative devotion to older tests such as the van den Bergh reaction, which has only a limited value in the differential diagnosis of jaundice. Thus while recent quantitative modifications of this test are certainly useful in distinguishing between retention and regurgitation jaundice (Watson, 1946; Gray, 1947), the older claims to distinguish toxic from obstructive jaundice by this means have been abandoned by experienced clinicians and pathologists. A recent report by Sherlock (1946) indicates that similar objections apply to the serum protein and cholesterol estimations and to the intravenous galactose tolerance and hippuric acid tests. Unfortunately this latter distinction—obstruction versus hepatitis—is a much more frequent diagnostic problem in jaundiced patients.

The present communication is concerned with two types of test—the serum alkaline phosphatase estimation and certain flocculation tests—which have been the subject of previous reports (MacLagan, 1944a, 1944b, 1944c, 1945). Although somewhat empirical in nature these tests have the advantage of technical simplicity and of being applicable to a single blood specimen. The combination has proved of particular value in distinguishing obstructive from non-obstructive jaundice, no doubt because the phosphatase shows a maximum deviation from normal in obstructive jaundice while the flocculation tests show maximum abnormality in hepatitis. It is hoped that they will be found useful by those who have lost faith in the older tests and have not been able to adopt the "battery" method of approach. While no combination of tests will give a definite diagnostic answer in every case of jaundice, the methods described here appear to be reliable in a high proportion of cases with a reasonably small expenditure of labour and materials.

Material

This consists of 200 consecutive jaundiced patients (serum bilirubin concentration over 2 mg. per 100 ml.) seen per-

sonally during the last four years in whom the cause of the jaundice was satisfactorily established by methods independent of the tests themselves, to the satisfaction of the clinician in charge of the case. Table I gives a detailed

TABLE I.—200 Cases of Jaundice

Diagnosis	Obstructive	Acute Hepatitis	Chronic Hepatitis	Haemolytic
No. of cases	56	118	15	11
Basis for diagnosis:				
Clinical	9	108	8	11
Operative	25	3	3	—
Necropsy	22	7	4	—
Age in years:				
Average	56.7	33.2	39.7	45
Range	24–76	17–71	10–58	22–64
Duration of jaundice (weeks):				
Average	6.5	1.4	53	29.7
Range	1–72	0.2–8	5–250	1–100
Serum bilirubin mg. per 100 ml.:				
Average	9.7	6.8	3.5	2.9
Range	2–24	2–25	2–8.3	2–8.1

analysis of the cases, on which the following comments may be made. The obstructive cases show, as would be expected, a higher average age and serum bilirubin than the other groups, and their average duration of jaundice (6.5 weeks) was intermediate between acute hepatitis on the one hand (1.4 weeks) and chronic hepatitis on the other (53 weeks). The obstruction was due to neoplasm in 37 cases, to gallstones in 17, and to lymphadenoma in 2. Diagnosis was by operation or necropsy in 47 of these 56 cases. The 118 cases of acute hepatitis were made up as follows: infective hepatitis (95), post-arsphenamine jaundice (13), jaundice with peritonitis (3), homologous serum jaundice (2), trinitrotoluene jaundice (2), glandular fever (1), leptospiral jaundice (1), eclampsia (1). There were six fatal cases of acute hepatic necrosis in the infective hepatitis group, and the patient with eclampsia also died. Chronic hepatitis cases were mainly of multilobular cirrhosis, but included five cases of chronic jaundice following acute hepatitis and one of Banti's syndrome. The haemolytic jaundice was classified as acholuric in four cases, post-transfusion in three, and of unknown aetiology in four.

Methods

1. *Serum Alkaline Phosphatase* (King and Armstrong, 1934).—Normal limits, 3–13 units. This method was latterly modified to use 0.2 ml. portions of serum, and the results were read on the King-Gallenkamp photo-electric colorimeter, but the original barbitone buffer was retained throughout.

2. *Serum Colloidal Gold Reaction*.—The buffer methods of MacLagan (1944a; 1946b). These two methods have been

shown to give the same results, but the later one employs an easier method of preparing the gold sol.

3. *Thymol Turbidity Test*.—The original method (MacLagan, 1944b) was used throughout except for a slight change in the turbidity standards noted below. This technique has now been used satisfactorily by a number of workers (Watson *et al.*, 1945; Cohn and Lidman, 1946; Havens and Marck, 1946; Maizels, 1946; Klatskin and Rappaport, 1947; Mawson, 1947), but as others have reported certain difficulties or modifications the following additional technical notes may be of interest.

(a) The pH of the thymol buffer was originally given as 7.8, but Volwiler (1946) found it to be 7.67 and adjusted it to 7.8 by the addition of more sodium barbitone, which would also have the undesirable effect of raising the ionic strength. Mateer *et al.* (1947), on the other hand, agreed with pH 7.8, but found their reagent too insensitive and adjusted the pH to 7.55. Some of these differences may well be due to difficulties inherent in the standardization of pH measurements, such as temperature effects and choice of check solutions. The value of pH 7.8 was obtained with the glass electrode (Marconi) at 20° C. using M/20 potassium hydrogen phthalate as a standard of reference with a value of 4.0, and is in fair agreement with the pK value of diethyl barbituric acid, given by Britton (1942) as 7.92. It should, however, be emphasized that the buffer was designed to have a salt-acid ratio of 2:3 rather than a pH of exactly 7.8. It is of course quite possible to increase the sensitivity of the reagent by lowering the pH, but data previously presented (MacLagan, 1944b) indicate that this would probably have the effect of diminishing the specificity of the reaction. In any case it would seem to be inadvisable to make any alterations in the buffer purely on the basis of the pH measurements, which are in general hardly accurate enough for this purpose unless very special precautions are taken. It is probably more important to check the purity of the reagents, as the possibility exists that certain brands of sodium barbitone may contain an excess of alkali and therefore yield an incorrect buffer. Thus 0.416 g. of the sodium barbitone should require exactly 20 ml. of 0.1 N hydrochloric acid for titration to methyl orange, and the barbitone should melt at $190 \pm 1^\circ \text{C.}$ (uncorrected). A rough check on the final pH with phenol red is a useful safeguard. Finally, an undue proportion of negative results such as those of Mateer *et al.* (1947) would obviously suggest the need for investigation, and as a rough guide the positives in a series of cases of epidemic infective hepatitis should amount to at least 90%. In the present work no readjustment of the buffer has been found necessary.

(b) Various photometric methods of measuring the turbidity have been published (Shank and Hoagland, 1946; Ley, Lewis, and Davidson, 1946), in which the measurements are standardized against barium sulphate suspensions. I have had difficulty in getting reproducible results with methods of this type, and in the present series visual comparison was adhered to throughout. The standards have, however, recently been made up from diluted normal serum of known protein content treated with three volumes of 3% sulphosalicylic acid. These appear to be as stable as the formazine standards and are simpler to renew and control. A similar recommendation to return to the simpler visual comparison was made by several speakers at the Conference on Liver Injury, New York, 1946.

(c) The normal limits, originally given as 0 to 4 units, have been criticized, and Neefe (1946) and Mateer *et al.* (1947) both consider values above 2 units as abnormal. This question seems to depend upon the choice of normal controls, and there is no doubt that completely healthy persons do give lower figures than those I obtained on "non-hepatic" hospital patients. Values between 2 and 4 units are relatively frequent in disease apparently unconnected with the liver, and it was in an effort to avoid these "false positive" results that the limits of 0 to 4 were suggested. However, if the test is being used for screening purposes on apparently healthy persons, the limits of 0 to 2 would no doubt be more appropriate. These considerations would not affect the arguments used in the present paper, in which an identical diagnostic significance has been accorded to negative and weakly positive flocculation tests.

4. *Thymol Flocculation Test*.—This is performed as an adjunct to the thymol turbidity test by allowing the tube to stand overnight and recording flocculation, as recommended by Neefe (1946) and MacLagan (1947). Some difficulty was experienced in grading the amount of flocculation consistently, and eventually the following procedure was adopted. If flocculation was *complete* the amount was related to the turbidity reading with the following convention:

Turbidity units	0-4	5-7	8-10	Over 10
Flocculation (if complete)	1+	2+	3+	4+

Any case showing *incomplete* flocculation was recorded as 1+, and absence of flocculation as 0 or negative.

Results

The results are shown in Figs. 1, 2, and 3. It will be seen that there is a clear general tendency for the obstruction cases to have high phosphatase values and negative flocculation reactions (top left-hand corner), while cases

FIG. 1.—Thymol turbidity test.

of hepatitis usually have positive flocculation reactions and normal or moderately raised phosphatase figures (bottom right-hand corner). The cases of haemolytic jaundice tend towards the bottom left-hand corners—that is, they have low phosphatase values with normal or weakly positive flocculation reactions. An intermediate zone contains cases of all types.

In interpreting the results it is of the greatest importance to distinguish between those of diagnostic value (unshaded in charts) and those which are equivocal and not of diagnostic value (shaded in charts). In this respect the following conclusions apply to all the 200 cases without exception:

1. All cases with phosphatase levels above 42 units were obstructive (26 cases). All with phosphatase levels below 15 units were non-obstructive (67 cases); it should be noted that all the 11 cases of haemolytic jaundice are included in this group.
2. All cases with strongly positive flocculation reactions were non-obstructive. These include: 92 cases with 2, 3, or 4+ thymol flocculation; 60 cases with 4 or 5+ gold reactions; and 55 cases with thymol turbidity over 8 units.

FIG. 2.—Thymol flocculation test.

3. All cases with negative or weakly positive flocculation reactions and, in addition, phosphatase levels above about 35 units were obstructive. These include: 38 cases with thymol flocculation 0 or 1+ and phosphatase above 35 units; 37 cases with thymol turbidity below 9 units and phosphatase above 35 units; and 34 cases with gold less than 4+ and phosphatase over 37 units.

4. All other combinations of results (shaded in charts) can occur in both types of jaundice and should therefore be regarded as equivocal except in so far as a raised phosphatase value is against a purely haemolytic type of jaundice. Nevertheless in cases where they approach the limits of these areas they can obviously be allowed a relative importance in diagnosis. For example, a jaundiced patient with negative flocculation reactions and a phosphatase of 33 units *might* have

hepatitis, but is much more likely to have biliary obstruction. Similarly, one with weakly positive flocculation reactions and a phosphatase of 18 units *might* have obstruction but is much more likely to have hepatitis.

The alkaline phosphatase results are in close agreement with those of Sherlock (1946), although expressed in a different form. Sherlock gives 30 units as a rough dividing-line between obstructive and non-obstructive cases, and this would also apply to the present series; such a statement, however, fails to express the equivocal nature of results in the region of 15–35 units, which may occur either in hepatitis or in biliary obstruction, and cannot therefore have decisive diagnostic value. The greater frequency of high phosphatase values in chronic as opposed to acute hepatitis is a finding which is well shown in the charts, and has been referred to elsewhere (MacLagan, 1944c).

The relative merits of the various tests are further illustrated in Table II, which shows the proportions of cases falling in the shaded and unshaded areas in Figs. 1, 2, and 3. It will be seen from this table that a combination of the

TABLE II.—Analysis of Results in 200 Cases of Jaundice

Test	No. Diagnosed	No. Equivocal
Phosphatase	93 (46.5%)	107
Thymol flocculation (T.F.)	92 (46%)	108
Colloidal gold (C.G.)	60 (30%)	140
Thymol turbidity (T.T.)	55 (27.5%)	145
Phosphatase and T.F.	158 (79%)	42
Phosphatase and T.T.	136 (68%)	64
Phosphatase and C.G.	131 (65.5%)	69

All cases in shaded area of Figs. 1, 2 and 3 have been counted as equivocal in this table.

thymol flocculation and phosphatase tests gives the best results, with definite diagnostic information in 79% of cases. The other two flocculation tests in conjunction with phosphatase give a somewhat lower proportion of successful results (68% for thymol turbidity and 65.5% for gold). As opposed to this the best results with any *one* test are 46.5% for the phosphatase and 46% for the thymol flocculation test, so that the advantage of combining the two types of test is evident.

The clinical application of the tests is further illustrated by the following two case reports.

Case Reports

Case 1: Hepatitis Simulating Biliary Obstruction.—A man aged 66 had been jaundiced for two weeks. This had been preceded by anorexia for some weeks following an attack of abdominal pain and vomiting ascribed to food-poisoning, and there had also been a patch of bronchopneumonia during this period with pyrexia to 103° F. (39.4° C.), which had been successfully treated with sulphamezathine. Abdominal pain had then reappeared followed by jaundice with dark urine and pale stools. There had been considerable loss of weight. On examination the liver was enlarged and tender. Cholecystography showed poor concentration of dye. The general picture was thought to be suggestive of malignant disease. The chemical findings were as follows: serum bilirubin, 8.7 mg. per 100 ml.; serum alkaline phosphatase, 17 units; thymol turbidity, 12 units; thymol flocculation, 4+; colloidal gold, 3+. These were strongly in favour of hepatitis, and the patient made a good recovery with medical treatment and has had no recurrence of symptoms.

Case 2: Biliary Obstruction Simulating Cirrhosis.—A woman of 43 had been jaundiced almost continuously, with some waxing and waning, for the past 18 months. The illness started with pyrexia and pain in the right shoulder-blade; the urine had been dark and the stools pale throughout. One year previously laparotomy had been performed at another hospital, but no obstruction to the biliary tract had been found. Cholecystectomy had been done and the common bile duct dilated. No improvement in symptoms had followed this operation, and

FIG. 3.—Serum colloidal gold reaction.

a diagnosis of hepatic cirrhosis had been made at that time. Clinical examination showed fairly deep jaundice in a well-preserved woman. The liver was enlarged and hard, and there was also clubbing of the fingers and toes. There was no x-ray evidence of calculi. The chemical findings were as follows: serum bilirubin, 10.8 mg. per 100 ml.; serum alkaline phosphatase, 36 units; thymol turbidity, 7 units; thymol flocculation, 1+; serum colloidal gold, 1+; galactose index, 135 (normal, 0-160). These results were strongly suggestive of obstructive jaundice, and a second operation disclosed the presence of a gallstone firmly imbedded in the bile ducts in the region of the head of the pancreas.

Discussion

In a recent article Ivy and Roth (1943) pose the question "Why do a liver function test?" and discuss the value of a variety of tests, not however including the thymol and gold tests. One reason for doing liver function tests is to distinguish obstructive from non-obstructive jaundice. While admitting the limitations stressed by these writers, it would appear from the results given above that for this special purpose the combination of the serum alkaline phosphatase estimation with the thymol or the colloidal gold test may be expected to provide a reliable answer in a high proportion of cases, the actual percentage varying from 65.5 to 79 according to the particular flocculation test used. The actual diagnostic value of these procedures is in practice rather greater than these figures would suggest, for two reasons. First, there is the inclusion of 13 cases of post-arsphenamine jaundice in the acute hepatitis group. These cases have a particular tendency to exhibit negative flocculation reactions (Maclagan, 1944a, 1944b, 1944c), and are in general easily eliminated on clinical grounds; if they are removed from the series the results, particularly with the thymol flocculation test, would be appreciably better. Secondly, although the cases in the shaded areas of Figs. 1, 2, and 3 have been recorded as failures in Table II, in many cases where the results approach the limits of these areas they were of some diagnostic help, as explained above. Nevertheless, it is true that certain combinations of results can occur in any type of jaundice, and there is probably no test or combination of tests which will give a diagnostic answer in every case.

As regards choice of test, thymol flocculation seems to be the best of the three flocculation tests tried. It is, however, known to be rather often negative in post-arsphenamine and homologous serum jaundice (Maclagan, 1947), and it is therefore desirable to retain the thymol turbidity test. The amount of flocculation can then be recorded more accurately as indicated above. The colloidal gold test has no advantage over the other two in the diagnosis of jaundice, but I retained it because of its greater sensitivity in conditions such as rheumatoid arthritis, malaria, and infective endocarditis (Carter and Maclagan, 1946). It is technically convenient to perform these tests together as they require the same small volume of serum (0.05 ml.).

Although all these tests are still partly empirical in character, it has recently been shown that the underlying chemical change in the case of the flocculation tests is probably a relative excess of gamma globulin in the serum (Gray and Barron, 1943; Kabat *et al.*, 1943; Maclagan and Bunn, 1947a, 1947b). Thus electrophoretically separated serum gamma globulin acts as a precipitating agent, and an absolute increase in this fraction has been demonstrated in hepatitis. Similarly normal serum albumin acts as an inhibitor, and this fraction is often reduced in hepatitis; moreover, hepatitis albumin is less effective as an inhibitor than normal albumin. The rise of the serum alkaline phosphatase in liver disease is, however, incompletely understood, having been ascribed to the phosphatase

content of the bile (Armstrong *et al.*, 1934) and to secondary interference with bone metabolism (Roberts, 1933). It seems more likely to me that this rise represents a positive secretory response of the liver cell to a variety of chemical or bacterial insults, of which biliary obstruction is the most powerful. It is somewhat remarkable that the liver cell damaged by biliary obstruction should fail so uniformly to produce those protein changes associated with positive flocculation tests, but such appears to be the case.

It is not of course suggested that the diagnostic criteria given above will hold generally without any exceptions, and in interpreting the results the possible presence of complicating diseases such as heart failure and rheumatoid arthritis should be borne in mind, as these conditions may produce positive flocculation reactions even in the absence of jaundice. However, the fact that no exception has occurred in these 200 cases is an encouraging feature. It is also obvious that the tests described will not elucidate every problem in hepatic pathology, and for special purposes other tests will also be required—for example, the serum protein estimation for prognosis (Higgins *et al.*, 1944), and recent modifications of the pigmentary tests for the diagnosis of non-icteric and pre-icteric hepatitis and haemolytic jaundice (Watson *et al.*, 1944; Pollock, 1945; Watson, 1946; Maclagan, 1946a; Neefe and Reinhold, 1946; Gray, 1947). Such tests are, however, of very limited value for distinguishing obstructive from non-obstructive jaundice, which is the special problem considered here, and for which the suggested combination of phosphatase and flocculation tests appears to represent an advance on previous chemical methods of approach.

Summary

The results obtained in 200 cases of jaundice with the serum alkaline phosphatase, thymol turbidity, thymol flocculation, and serum colloidal gold tests are analysed in relation to their diagnostic value.

The combination of phosphatase with one flocculation test will distinguish obstructive from non-obstructive jaundice in from 65 to 79% of cases. The thymol flocculation test is slightly the best of the three flocculation tests tried.

Diagnostic criteria are given for the various tests. Strongly positive flocculation reactions or phosphatase levels below 15 King-Armstrong units suggest non-obstructive jaundice; negative or weakly positive flocculation reactions with phosphatase levels above 35 units suggest biliary obstruction, and phosphatase levels above 42 units are also in favour of obstruction.

I am much indebted to the medical staffs of Westminster Hospital, Ashford County Hospital, Middlesex, and of E.M.S. Sector 7 for access to their patients and records; to Prof. R. J. V. Pulvertaft, Dr. Alan Morgan, and Dr. A. G. Signy for necropsy reports; and to Drs. J. Amor and J. Marshall for certain sera. Part of the expenses of the work was defrayed by a grant from Westminster Hospital.

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RELATIVE ORAL TOXICITY OF SOME THERAPEUTIC IRON PREPARATIONS

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Forbes (1947) and Thomson (1947) have recently described serious consequences in young children, including three deaths, following the unauthorized taking of ferrous sulphate tablets in excessive numbers. These and certain cases previously reported to us raised the general question of the toxicity of iron compounds when large doses were consumed orally, and also of the additional effect, if any, of the small amount of copper and manganese sulphates which were present in the tablets.

Examination of the literature failed to reveal earlier reports of ill effects from orally administered iron compounds, except for occasional references to slight alimentary disturbances. Further, and more surprisingly in view of the widespread use of iron compounds for the treatment of iron deficiency or "nutritional" anaemia, we have been unable to find any account of pharmacological investigations into the action of iron given by mouth, although its effects by injection have received some attention and it is accepted that serious ill effects may follow its absorption into the blood stream.

It therefore seemed desirable to test experimentally on several species of laboratory animal the effects of orally administering very large doses of some of the more commonly used iron preparations. For this purpose we chose ferrous sulphate, with and without the presence of the "trace" metals copper and manganese; ferrous carbonate in the form of Bland's pills; ferrous gluconate, because of the claims made for it by Reznikoff and Goebel (1937); ferric chloride, so as to include at least one preparation of ionizable ferric iron; and iron and ammonium citrate, widely used in compounded medicines, though nowadays largely replaced by ferrous sulphate for simple oral iron therapy—shown by many workers from Davidson (1933) onwards to be much more effective in haemopoiesis than the non-ionized "scale salts," when compared on an equivalent iron basis. Rabbits and guinea-pigs were used for studying the pathology and histology of any disturbances observed, and mice for the estimation of median lethal doses (LD50) with reasonable accuracy.

As a practical problem in human therapeutics we were really concerned with establishing the "therapeutic index" of ionizable iron—that is, the ratio of its median toxic dose to its median therapeutic dose. Here we encountered two difficulties, the one general and the other particular. First, it is ordinarily impossible to establish such an index for the action of a drug on the human organism, simply because data, unfortunately, are not as a rule available for the median toxic human dose. The pharmacologist therefore normally has recourse to establishing the therapeutic index of a drug on at least one species of laboratory animal, and preferably on more than one. If the value of this index is of roughly the same order for two or more species of laboratory animals it is reasonable to assume that the same value will apply to man; in general, experience bears out this assumption. However, even this line of approach was not available to us, for there is no species of animal—with the possible exception of the rat made anaemic on a milk diet—for which we have information about the median therapeutic dose of iron compounds.

We were consequently forced back on to the unsatisfactory step of relating toxic doses to the body weight of the animal used and arguing on the same basis for man. The objections to this line of reasoning, however, appear much less strong for iron compounds than they might for other drugs, for it will be seen that toxic effects—and, in particular, fatal results—were produced only by doses so immensely out of proportion to the animal's normal intake of iron as to make highly unlikely the ingestion of dangerous amounts by human subjects. The facts established by our experiments nevertheless make it clearly desirable to render access of infants and small children to iron preparations as difficult as possible. Such precautions would normally be taken with many of the common drugs to be found in the ordinary household—for example, quinine salts and acetylsalicylic acid—without in any way detracting from their value and importance in therapeutics.

Toxicity

The results obtained with the three species of animals used are summarized in the accompanying table. The

Table showing the Median Lethal Toxic Doses of Iron Compounds by the Oral Route

Compound	Iron in Preparation, Calculated as Fe	Dose per kg. of Body Weight					
		Rabbits		Guinea-pigs		Mice	
		Actual	Cal. as Fe	Actual	Cal. as Fe	Actual	Cal. as Fe
Ferrous sulphate crystals	20	3.0	0.6	1.5	0.3	4.5	0.9
Ferrous sulphate prep. with copper and manganese	24	3.0	0.72	1.25	0.3	4.1	1.0
Ferrous gluconate	16.6	3.5	0.58	2.1	0.35	6.6	1.1
Ferrous carbonate pills	12.5	17.8	2.22	16.0	2.0	31.0	3.8
Ferric chloride	34	1.2	0.4	0.6	0.2	1.5	0.5
Ferric and ammonium citrate	20	2.8	0.56	1.75	0.35	5.0	1.0

figures from tests on rabbits and guinea-pigs, owing to the relatively small numbers of such animals that can be used in toxicity tests, must be regarded as first approximations; the figures from the tests on mice have a greater accuracy, except those for iron and ammonium citrate, because with this product a great individual variation was found among the animals. Slopes of the regression curves, and the errors of estimating the LD50, which were of the order usually found in tests of this kind, were obtained by the graphical method of de Beer (1945).

It will be seen: (1) That the presence of small amounts of copper and manganese sulphates made no difference to