

## SOME OBSERVATIONS ON LIVER FUNCTION TESTS IN DISEASES NOT PRIMARILY HEPATIC

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The liver is dependent upon a pure and unrestricted blood supply for the normal performance of its many functions. It is therefore not surprising that a proportion of abnormal results with a variety of liver function tests should have been recorded in conditions such as pneumonia (Curphey and Solomon, 1938), heart failure (Bernstein *et al.*, 1942; Chávez *et al.*, 1943), hyperthyroidism (Haines *et al.*, 1939; Lichtman, 1941; MacLagan and Rundle, 1940), rheumatoid arthritis (Rawls *et al.*, 1937), glandular fever (Kilham and Steigman, 1942; Davis *et al.*, 1945), after surgical operations (Boyce, 1941), and in therapeutic malaria (Fredericks and Hoffbauer, 1945; Kopp and Solomon, 1943).

Thus while the principal use of liver function tests is naturally in the investigation of gross liver disease, the interpretation of results is also sometimes dependent upon a knowledge of the reaction of the liver to disturbances of a more general nature. For example, the discovery of certain types of functional impairment in a patient with an enlarged liver would ordinarily suggest the probability of cirrhosis, but, if congestive heart failure were also present, then chronic passive congestion of the liver might well explain the findings. A further complication in the case of the flocculation tests is the uncertainty as to their mechanism. Recent work suggests that they depend principally upon changes in the serum gamma-globulin content (Kabat *et al.*, 1943; Moore *et al.*, 1945), and increases in this fraction are not confined to liver disease, but may occur in conditions such as rheumatic fever, aplastic anaemia, and peritonitis (Longworth *et al.*, 1939). Moreover, many circulating antibodies are known to occur principally in the gamma-globulin fraction (Enders, 1944).

The possibility of false positive reactions is therefore a real one, although this has not seriously interfered with the application of these tests to the study of liver disease in the case of cephalin-cholesterol (Hanger, 1939), the serum colloidal-gold (Gray, 1940; MacLagan, 1944a), and the thymol turbidity tests (MacLagan, 1944b). This type of interference appears, however, to be more common with the Takata-Ara reaction (Magath, 1940).

The above considerations suggested to us that it would be worth while to define more closely the nature and degree of the abnormalities likely to be encountered in certain conditions not primarily involving the liver, using the two flocculation tests—the serum colloidal-gold reaction and the thymol turbidity test—which we had found of particular value in the diagnosis of gross liver disease. Qualitative tests for urinary urobilin were also used as a confirmatory procedure.

### Methods

The serum colloidal-gold test was performed as previously described (MacLagan, 1944a). Normal serum gives a negative result with this test—recorded as 0 in the tables below—and 5+ is the strongest positive. The thymol turbidity test (MacLagan, 1944b) is expressed in units, normal limits being 0 to 4. Urine urobilin tests were made by spectroscopic examination with a direct-vision hand spectroscope, using a 1-in. (2.5-cm.) layer, after converting urobilinogen to urobilin by the addition of a few drops of iodine 1% solution and acetic acid. Particular care was taken to obtain fresh afternoon specimens and to mix any deposit thoroughly with the main bulk before testing.

### Material

The diseases chosen for the study were originally those in which liver involvement had been reported by others (e.g., heart failure, glandular fever, rheumatoid arthritis), but accidental positive findings led to the inclusion of malaria and infective endocarditis. The material is shown in detail in Table I, and

TABLE I.—Summary of 123 Cases Tested

Diagnosis	No. of Cases	% Gold-positive	% Thymol-positive
Malaria .. .. .	35	94	80
Rheumatoid arthritis .. .. .	34	76	38
Heart failure .. .. .	28	39	36
Glandular fever .. .. .	19	95	58
Subacute bacterial endocarditis	7	100	86

represents the admissions of the relevant types of illness to Ashford (formerly Staines) County Hospital during the last two years. In addition to these, 42 further cases shown in Table II were used as controls to exclude fever and joint

TABLE II.—42 Control Cases

Diagnosis	No. of Cases	No. Gold-positive	No. Thymol-positive
Pulmonary tuberculosis .. .. .	18	1	1
Acute articular rheumatism .. .. .	8	0	0
Infective arthritis .. .. .	6	3	0
Osteo-arthritis .. .. .	10	0	0

changes as possible contributing factors. The results in these control cases were mainly negative, the only positive reactors being one patient with pulmonary tuberculosis (gold, 3+; thymol, 7 units) and three with infective arthritis (all gold 1+, thymol-negative). It would appear, therefore, that the positive results recorded below are not directly related to pyrexia or joint involvement.

### Results

The results will be discussed under five headings.

1. *Congestive Cardiac Failure*.—Twenty-eight consecutive cases of marked congestive failure with gross oedema and hepatic enlargement were examined. The ages of the patients were from 25 to 70 years, and the aetiology of the failure was: rheumatic carditis, 10; arteriosclerotic hypertension, 11; coronary thrombosis, 3; pulmonary disease, 4. The results of the investigation are shown in Table III. Analysis of these

TABLE III.—Congestive Cardiac Failure (28 Cases)

Thymol Units	Gold Test						Totals
	0	1 +	2 +	3 +	4 +	5 +	
0-4 ..	17	0	0	0	1	0	18
5-7 ..	0	2	1	4	0	2	9
8-15 ..	0	0	0	0	0	1	1
Totals ..	17	2	1	4	1	3	28

39% gold-positive; 36% thymol-positive.

results showed no significant correlation between positive findings and size of liver, length of history, or aetiology of the failure. The only suggestive finding was in regard to prognosis, as death occurred in all the 8 markedly positive cases within four months, compared with 4 deaths out of the 17 negative cases in the same period. In 15 cases the urine was tested for urobilin, which was present in 8. All with positive flocculation reactions had urobilinuria, and it was present in 5 of those with negative reactions. The presence of urobilinuria therefore appears to be a more sensitive test of liver function in this condition. Of the two flocculation tests used, the gold and thymol seemed equally sensitive, being positive in 39% and 36% of cases respectively.

2. *Malaria*.—Thirty-five consecutive cases of relapsing benign tertian malaria in young soldiers were investigated. The diagnosis was confirmed in every case by the finding of *Plasmodium vivax* in the blood smear. The results are shown in Table IV. A very high proportion gave positive results, the gold being rather more sensitive than the thymol (94% and 80% respectively). Urobilinuria was present in 20 out of the 21

cases in which the test was carried out, but may well have been due in part to accompanying haemolysis. The temporary nature of the disturbance in this condition was shown by the repetition of the tests in 7 cases, in which a return to normal or nearly normal results was found after three weeks' treatment.

TABLE IV.—Malaria (35 Cases)

Thymol Units	Gold Test						Totals
	0	1 +	2 +	3 +	4 +	5 +	
0-4 ..	2	2	0	3	0	0	7
5-7 ..	0	2	5	4	4	0	15
8-15 ..	0	0	1	2	7	3	13
Totals ..	2	4	6	9	11	3	35

94% gold-positive; 80% thymol-positive.

3. *Glandular Fever*.—Nineteen cases of this condition were investigated, and the diagnosis was based in every instance on the clinical picture, the presence of abnormal mononuclear cells in the circulating blood, and a positive Paul-Bunnell reaction in a dilution of at least 1 in 64. The results are shown in Table V, where they are arranged in order of descending

TABLE V.—Glandular Fever (19 Cases)

Paul-Bunnell Titre	Thymol Units	Gold Test	Total Leucocytes per c.mm.	Atypical Mononuclears per c.mm.
2,048	14	5+	22,000	8,200
1,024	16	5+	15,000	5,200
1,024	16	3+	9,400	4,300
512	13	5+	14,800	5,400
256	12	3+	11,000	4,000
256	6	2+	15,000	3,200
256	5	2+	9,400	3,800
128	16	3+	13,600	4,100
128	7	4+	14,000	4,200
128	3	1+	9,000	700
128	5	2+	15,000	3,000
128	2	0	5,000	400
64	8	1+	17,000	1,400
64	2	1+	12,000	870
64	1	2+	9,000	280
64	4	1+	8,400	560
64	1	1+	13,000	900
64	2	1+	4,000	400
64	1	1+	7,000	950

95% gold-positive; 58% thymol-positive.

Paul-Bunnell titres. The thymol and gold reactions were both markedly positive in all the cases with high Paul-Bunnell readings and with an atypical mononuclear count of over 4,000. Urobilinuria was present in only one case, which was also clinically jaundiced.

This patient showed a very interesting correlation between clinical course and biochemical tests. She was an A.T.S. girl, aged 25, who came into hospital jaundiced and was thought at first to be a case of infective hepatitis with splenomegaly. Very few cervical glands were palpable and none elsewhere. Her blood count showed 15,000 leucocytes with 3,200 abnormal mononuclears, and the Paul-Bunnell test was positive in a dilution of 1 in 256. The liver function tests showed a thymol of 6 units, a colloidal gold of 2+, and a serum alkaline phosphatase of 25, with 6 units (Watson) of urobilin in the urine—results typical of infective jaundice. Her jaundice improved, but a week later axillary and inguinal glands became palpable and tender, her jaundice deepened once more, and the character of the reactions changed. The thymol was now 5 units, colloidal gold 0, and phosphatase 46, and urobilin was absent from the urine—results typical of obstructive jaundice. Recovery ensued. The course of the illness, correlated with the biochemical findings, suggests that the mechanism of jaundice in this condition may vary.

Thus Davis *et al.* (1945) have recently described, in necropsy material from a typical case of glandular fever, liver changes indistinguishable from those of infective hepatitis, and one of us has suggested that jaundice could occur in this condition from obstruction to the common bile duct by glandular enlargement in the porta hepatis (Carter, 1942). In the present case the initial jaundice was probably infective in origin and the exacerbation obstructive.

Taking the group as a whole, it appears that the flocculation tests are closely correlated with the abnormal mononuclear count and the Paul-Bunnell test in glandular fever.

4. *Subacute Bacterial Endocarditis*.—Seven proved cases of this condition, with a typical clinical picture, previous valvular

damage, embolic phenomena, and positive blood culture, were investigated. In addition, 5 other cases were examined in which the condition was suspected because of fever and evidence of endocarditis, although a negative blood culture persisted without embolic phenomena. In these 5 cases the diagnosis was eventually abandoned. The results are shown in Table VI. The

TABLE VI.—Subacute Bacterial Endocarditis (7 Cases)

Gold test ..	7 Proved Cases							5 Suspected Cases Subsequently Disproved				
	5+	5+	5+	5+	4+	3+	3+	1+	0	0	0	0
Thymol units ..	8	6	5	6	5	4	5	4	3	3	4	2

Of proved cases 100% were gold-positive and 86% thymol-positive.

almost uniformly positive results in the proved cases are striking in spite of their small number. Urobilinuria was present in only 2 out of these 7 cases. As a positive blood culture is not always easy to obtain quickly, the flocculation tests may have some diagnostic value in this condition and allow of treatment at the earliest opportunity. In the 5 negative suspected cases the tests were of considerable help, as illustrated by the following:

A man aged 30, with a rheumatic history, was admitted with irregular pyrexia, microcytic anaemia, tachycardia, and the signs of a mitral stenosis. He developed haematuria of a mild but persistent nature, a rising blood urea, and albuminuria without oedema. This condition subsided, and was followed by auricular fibrillation of paroxysmal type. The fever persisted and a cerebral embolism occurred. A confident diagnosis of bacterial endocarditis would have been clinically justified, but flocculation tests and blood culture remained negative. The patient slowly recovered with salicylates and iron, and was well eighteen months later except for his valvular defect, presumably having had a severe reactivation of his previous rheumatism. In one of the proved cases the patient appears to have recovered a year after his first treatment with penicillin, and there has been an interesting change in his flocculation reactions:

Mar. 13, 1945 .....	Thymol, 6 ; Gold, 5+
April 31, 1945 .....	Thymol, 5 ; Gold, 5+
June 30, 1945 .....	Thymol, 3 ; Gold, 1+
Oct. 30, 1945 .....	Thymol, 2 ; Gold, 0
Jan. 6, 1946 .....	Thymol, 2 ; Gold, 0

A comparison between the gold and thymol tests in the group shows a considerable difference in degree of sensitivity in favour of the gold, although the thymol reaction was just positive in 6 out of the 7 cases with positive gold reactions.

5. *Rheumatoid Type of Polyarthritis*.—This series consisted of 34 patients (30 women) who showed an atrophic type of polyarthritis ranging from the classical rheumatoid arthritis to a mild, fleeting, periarticular, inflammatory reaction with minimal radiological changes. The results are shown in Table VII. In

TABLE VII.—Rheumatoid Arthritis (34 Cases)

Thymol Units	Gold Test						Totals
	0	1+	2+	3+	4+	5+	
0-4 .. ..	8	5	2	4	1	1	21
5-7 .. ..	0	2	2	1	3	0	8
8-15 .. ..	0	0	0	0	1	4	5
Totals ..	8	7	4	5	5	5	34

76% gold-positive; 38% thymol-positive.

the whole group there was a marked difference between the gold test (76% positive) and the thymol test (38% positive). Urobilinuria was present in only 1 out of 19 cases tested. The series divides itself up into four classes:

(1) The very advanced long-standing typical rheumatoid arthritis with permanent deformity and gross generalized radiological changes. There were 4 of these, all with markedly positive colloidal gold reactions and very little change in the thymol turbidity test.

(2) The active classical rheumatoid arthritis, with involvement of wrists, fingers, knees, and feet, with spindling of fingers, atrophic radiological changes, and raised erythrocyte sedimentation rate. There were 16 in this group, with 1 man among them, and 4 of the cases showed Felty's syndrome with generalized superficial lymphatic glandular enlargement and splenomegaly; 11 of these had a markedly positive colloidal gold reaction, 3 were weakly positive, and 2 were negative.

(3) Ten cases with a history of fibrositic pains, occasional peripheral joint swellings, a minimal degree of finger-spindling, and some general osteoporosis around the affected joints, but no other joint changes. The sedimentation rate was raised in all, and a diagnosis of probable early rheumatoid arthritis was made. All these patients were between 20 and 30 years of age. Two showed a spondylitis with stiffness and pain, but no changes in radiological appearances of spinal or sacro-iliac joints. Two were associated with thyrotoxicosis. The colloidal-gold findings were: 1 markedly positive (3+), 1 positive (2+), and 8 weakly positive (1+).

(4) Four cases with atypical findings, usually fleeting joint pains and swellings, without spindling, with raised sedimentation rates, and radiological changes showing only a minimal bone rarefaction. All these had negative gold and thymol reactions.

In the whole group there was a rough though definite correlation between the gold test and the sedimentation rate, as shown in the accompanying Chart. It will be seen from this

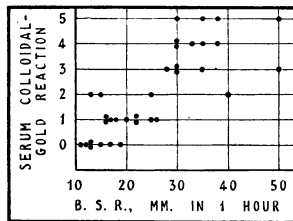


Chart showing the correlation between the serum colloidal-gold reaction and the sedimentation rate in 34 cases of rheumatoid arthritis

that all cases with 3+, 4+, or 5+ gold reaction had sedimentation rates over 25 mm. in 1 hour and that all cases with a negative gold reaction had rates below 20 mm.

Taking the rheumatoid group as a whole, it will be seen that the gold test was positive in 28 out of 30 cases in which the diagnosis was well established. The thymol test was much less sensitive, being positive in only 13 of these 30 cases. Urobilinuria was almost uniformly absent (18 out of 19 cases).

### Discussion

The results as a whole may be considered under three headings.

1. *Heart Failure.*—In this group the positive results are readily explicable on the basis of chronic passive congestion of the liver, and probably are mainly a reflection of this process. With the gold and thymol tests the proportion showing abnormal findings was nearly identical and was similar to that found with other tests by Chávez *et al.* (1943). The presence of urobilinuria in all the positive reactors serves as confirmation of the presence of liver dysfunction in these cases.

2. *Rheumatoid Arthritis.*—The balance of probability here is that the high proportion of positive results is not specifically related to liver pathology, since there was no urobilinuria and the published evidence of liver damage in this disease does not suggest such a degree of dysfunction as would explain the findings. It is possible that an antibody associated with the serum gamma-globulin fraction may be responsible for the flocculating power of patients with rheumatoid arthritis. It is noteworthy that there was a wide discrepancy between the two tests in this group (gold, 76% positive; thymol, 38% positive).

3. *Malaria, Glandular Fever, Infective Endocarditis.*—These conditions form an intermediate group in which there is some supporting evidence of liver disease, but in which it is doubtful whether this explains all the findings. Thus urobilinuria was constantly present in malaria, but may have been partly due to haemolysis; it was mainly absent in glandular fever and inconstant in infective endocarditis. There is histological evidence of hepatitis in malaria and in glandular fever (Lichtman, 1942; Davis *et al.*, 1945), and the latter is known to cause jaundice in a small proportion of cases. However, this jaundice may sometimes be due to enlarged glands in the portal fissure, as suggested by one of us (Carter, 1942) and as illustrated in the case above. In these three conditions the correlation between the tests also occupies an intermediate position (gold, 95% positive; thymol, 74% positive), being less close than in diseases with known liver damage, such as infective hepatitis (Maclagan, 1944b). It seems a reasonable inference

that in these three diseases both liver dysfunction and antibody formation contribute to the results, no doubt in varying degrees in different patients.

### Conclusion

In conclusion it may be stated that, while these flocculation tests have proved of great value in the investigation of primary diseases of the liver, positive results do certainly occur in certain conditions not usually regarded as liver diseases. This applies particularly to the gold test, the thymol test being rather more specifically related to liver pathology. Diseases such as heart failure, glandular fever, malaria, infective endocarditis, and rheumatoid arthritis must therefore be considered as possible alternative diagnoses in patients with suspected primary liver damage who have positive flocculation tests. Viewed from another angle, we have had some diagnostic help from the tests, particularly the gold test, in rheumatoid arthritis, glandular fever, infective endocarditis, and malaria. Negative results are unusual in these conditions and positive ones of some confirmatory value. The tests may possibly have some prognostic significance in heart failure.

### Summary

The serum colloidal-gold test and the thymol turbidity test have been performed in 35 cases of malaria, 34 of rheumatoid arthritis, 28 of congestive cardiac failure, 19 of glandular fever, and 7 of subacute bacterial endocarditis.

Some positive results occurred in each group, the proportion varying from 95% to 39% for the gold test, and from 80% to 36% for the thymol test. The two tests were equally sensitive in heart failure, but the gold test was more sensitive in other conditions, the difference being particularly great in rheumatoid arthritis.

In glandular fever there was a positive correlation between the gold test, the Paul-Bunnell test, and the abnormal mononuclear counts. In rheumatoid arthritis the gold test and the erythrocyte sedimentation rate were positively correlated.

The positive finding in heart failure probably resulted from chronic passive congestion of the liver. In the other conditions both liver damage and antibody formation may have contributed to the results.

The flocculation tests are not purely liver function tests, as they probably depend upon changes in the serum gamma-globulin fraction. They have proved of value as diagnostic aids both in primary liver diseases and in some of the above conditions, but the possibility of occasional interference between the two groups must be considered in interpreting the results.

We are much indebted to Dr. A. G. Signy for the haematological data and Paul-Bunnell tests. Part of the expenses of the work was defrayed by a grant to one of us (N. F. M.) from Westminster Hospital.

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The Scottish National Blood Transfusion Association states in its annual report that during the first three months of 1946 hospitals' demands for blood exceeded the amount given by volunteers. Requirements were met only by drawing on accumulated wartime reserves which it had not been necessary to expend during the war. As long as the war was on, an adequate number of volunteers were always ready to come forward to give blood. For peacetime conditions considerably more organized recruitment and propaganda will be necessary if the Association is to meet its commitments to the hospital services.