

# LIVER FUNCTION TESTS IN PRIMARY CARCINOMA OF THE LIVER IN THE SOUTH AFRICAN BANTU

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Primary carcinoma of the liver is a rare disease amongst Western population groups, irrespective of whether they live in Africa, America or Europe, but is a relatively common condition amongst the Bantu races of Africa and in certain Oriental races, where according to Berman<sup>1</sup> the incidence may be 40 times as frequent as in Western people. Primary carcinoma of the liver is the commonest form of malignancy encountered in Bantu males.<sup>1</sup>

It follows therefore that a clinical diagnosis is rarely made in the European, but even amongst the Bantu, where a clinical diagnosis of primary liver carcinoma is more readily made, difficulty in establishing such a diagnosis still exists, since primary carcinoma of the liver must be distinguished from many other conditions which give rise to hepatic enlargement.

I have carried out a large series of 'liver function tests' on suspected cases of primary carcinoma of the liver, with the hope that by careful selection of biochemical criteria it might be possible to establish a diagnosis of hepatoma more confidently, without resorting to liver biopsy. And here it must be borne in mind that even a liver aspiration

biopsy may not be conclusive, since it is obvious that a small fragment of liver tissue may fail to reveal the malignant growth.

## REVIEW OF LITERATURE

A review of the literature on the value of liver function tests in the diagnosis of primary carcinoma of the liver reflects conflicting opinions. Thus, Berman<sup>1</sup> in discussing laboratory aids in the diagnosis of primary carcinoma of the liver states that most liver function tests devised thus far are of doubtful value. 'I have seen', he says, 'many cases where the liver was practically replaced by carcinoma, and all liver function tests proved normal.' He attaches some importance to the van den Bergh test, especially as a means of detecting latent jaundice.

Stein<sup>2</sup> found the Takata-Ara reaction useful in differentiating between intra-hepatic and extra-hepatic carcinoma. In 6 cases of primary liver cancer he found the Takata reaction positive in 5, and the negative reaction occurred in spite of massive infiltration of almost all the

liver tissue. In 4 other anicteric cases of primary cancer of the liver the Takata reaction was positive in all.

Spellberg<sup>3</sup> states 'that liver function tests in primary carcinoma of the liver are variable, and depend to a great extent on the presence of an underlying cirrhosis'. If there is a marked derangement of the 'liver profile' the presence of cirrhosis is likely. He does state however that 2 tests are likely to be positive, in both primary and secondary malignant liver disease, viz. the bromsulphalein dye retention test and an elevated serum alkaline-phosphatase level.

In Lichtman's opinion<sup>4</sup> the bilirubin content is usually negative, the cephalin cholesterol test is strongly positive in primary carcinoma of the liver and negative in secondary carcinoma, and the thymol turbidity reaction is more uniformly negative in hepatic cancer. He states, too, that the alkaline phosphatase level may be normal in primary carcinoma which develop in cirrhotic livers.

Holley and Pierson<sup>5</sup> found that liver function tests as a rule were disappointing as a method of diagnosis of primary carcinoma of the liver. They state that damage must be extensive before any appreciable change could be detected in the function tests.

Spatt and Grayzel<sup>6</sup> state that the most consistent abnormal liver function tests in primary liver cancer were a raised icteric index, a raised alkaline phosphatase level and an abnormal bromsulphalein dye retention. The serum protein and serum cholesterol and the cephalin flocculation tests were not significant in the diagnosis.

Ricketts<sup>7</sup> discusses certain liver function tests in 2 cases of primary hepatoma of the liver. The one case was anicteric and had a slow-growing hepatic tumour of 8 years standing. The only abnormal tests were an elevated alkaline phosphatase test and abnormal bromsulphalein dye retention. The other cases was in a jaundiced Chinese subject, who had a very slightly elevated alkaline phosphatase level (13.5 units) and a slightly elevated serum-cholesterol level. All other liver function tests, including an albumin-globulin ratio, were normal in these 2 subjects.

Most of the authorities cited above base their data on European subjects and, as the biochemical pattern of the Bantu, especially that of serum protein and the commonly used flocculation and turbidity tests differ so markedly from that of the European, I decided to investigate whether, by carrying out a so-called 'battery of liver function tests', it would be possible to show a specific type of pattern in primary malignant disease of the liver, bearing in mind the connection between cirrhosis and primary carcinoma of the liver, and whether the coexistence of these two disease processes would not confuse the biochemical pattern.

#### MATERIAL AND METHODS

The investigation covered 37 cases of primary carcinoma of the liver in the African male Bantu. The diagnosis of all these cases was proved either by biopsy or by autopsy or by both. A 'battery of liver function tests' was carried out on serum from these cases on their admission to hospital. The biochemical tests and techniques used were as follows:

1. Thymol turbidity test (MacLagan)<sup>8</sup>
2. Thymol flocculation test (Neefe and Rheinhold)<sup>9</sup>
3. Colloidal-red test (Ducci)<sup>10</sup>
4. Cephalin-cholesterol flocculation test (Hanger)<sup>11</sup>
5. 'Takata-Ara' reaction (Ucko)<sup>12</sup>

6. Zinc-sulphate turbidity (Kunkel)<sup>13</sup>
7. Total lipid (Kunkel *et al.*)<sup>14</sup>
8. Alkaline phosphatase (King and Armstrong)<sup>15</sup>
9. Bilirubin, (a) direct, (b) total (Malloy and Evelyn)<sup>16</sup>
10. Pseudo-cholinesterase (Michel)<sup>17</sup>
11. Mucoprotein (Simkin *et al.*)<sup>18</sup>
12. Total cholesterol (Kaye)<sup>19</sup>
13. Free cholesterol (Kaye)<sup>19</sup>
14. Serum electrophoretic pattern
15. Bromsulphalein test (5 mg. of dye per kg. of body weight was injected intravenously and the percentage of dye retained at the end of 45 minutes was estimated). This test was performed in only 7 and 4 cases in the malignant and cirrhotic groups respectively.

The electrophoretic protein-analyses were carried out on the Antweiler micro-electrophoretic apparatus, with the use of a modified Michaelis buffer solution (pH 8.6).

TABLE I. LIVER FUNCTION TESTS IN BANTU SUBJECTS WITH PRIMARY CARCINOMA OF THE LIVER AND WITH CIRRHOSIS OF THE LIVER, AND IN NORMAL BECHUANA AND EUROPEAN SUBJECTS

	Primary carcinoma of the Liver, Bantu (37 cases)	Cirrhosis of Liver Bantu (20 cases)	Normal Bechuana African (50 cases)	Normal European Adult
Thymol Turbidity Test (units)	6.5	5	4	0.2
Thymol Flocculation Test	1.3+	2.5+	2.2+	neg.
Colloidal-red Test	2.7+	3.5+	3.6+	+ neg.
Cephalin-Cholesterol Test	2.3+	3+	2+	neg.
Takata Ara (Ucko)	2.1+	2.5+	1.5+	neg.
Zinc-sulphate Turbidity Test (units)	25	35	22	12.5
Total Lipids (mg/100 ml.)	541	450	490	600
Alkaline Phosphatase (K.A. units)	31.4	12.3	7.5	8
Bilirubin Total (mg/100 ml.)	3.8	0.9	0.5	up to 1.2
Cholinesterase ( $\Delta$ p H/hr.)	0.325	0.430	0.630	0.710
Mucoprotein (mg/100 ml.)*	205	70	110	80
Cholesterol Total (mg/100 ml.)	240	135	150	230
Cholesterol Esters (mg/100 ml.)	144	81	110	160
% Esters to Total	60	60	73	70
Total Protein (g/100 ml)†	7.3	7.8	7.7	7.1
% Albumin	29.2	33.0	42.7	55.0
% Alpha <sub>1</sub> globulin	3.9	3.0	3.8	2.0
% Alpha <sub>2</sub> globulin	13.1	7.2	9.4	6.5
% Beta globulin	16.9	12.2	12.6	15.5
% Gamma <sub>1</sub> globulin	5.5	6.2	5.5	4.5
% Gamma <sub>2</sub> globulin	31.4	38.4	26.0	16.5

\* Performed in 10 cirrhotic cases and 21 cases of carcinoma of the liver.

† Performed in 10 cirrhotic cases and 21 cases of carcinoma of the liver.

The bromsulphalein tests carried out on the 7 carcinoma and 4 cirrhotic cases are not included in this table (see text).

#### RESULTS AND COMMENTS

Table I shows the results of the liver function tests (mean values) carried out on the 37 cases of primary carcinoma of the liver. Mean results of similar tests carried out on a group of 50 'normal' Bechuana male subjects on their arrival in Johannesburg, on a group of 20 Bantu cirrhotic patients (proved by liver biopsy) and on a group of 100 healthy European adult subjects are also shown for comparison.

From the table, it is obvious that results on the so-called 'normal' Bantu differ markedly from his normal European counterpart.

#### Comments on Results

**Turbidity and Flocculation Tests.** The thymol turbidity test was slightly higher in the malignant than in the cirrhotic group, but probably not significantly so. There appears to be a disassociation between the thymol turbidity and the thymol flocculation tests in the malignant group. Usually, these two tests parallel each other closely, but in our malignant group there were 13 cases where the thymol turbidity test was high (as high as 15 units—average 8 units) with negative flocculation tests. The reverse is often found in residual hepatitis, where one may find a positive thymol flocculation test with a normal turbidity result, but our finding in malignant

liver disease is unusual. We have occasionally encountered similar findings in liver damage due to malignant extra-hepatic obstruction. Is there a substance in malignant sera which inhibits the thymol flocculation test? Whereas only 6 cases had a normal thymol turbidity test (2.5 units and lower), 25 cases showed normal thymol flocculation tests. The mean values for the colloidal-red, the cephalin-cholesterol flocculation tests and the Takata reaction did not differ significantly in the 2 groups of patients. In 5 cases of liver cancer the Takata reaction was negative.

The zinc-sulphate turbidity test was lower in the carcinoma than in the cirrhotic group, whilst the reverse applied to the serum-lipid level.

**Alkaline Phosphatase.** The mean level was definitely higher in the carcinoma group, 31.4 units, as compared with 12.3 units in the cirrhotic subjects. In the liver-cancer group, a normal level of 8 units or less was found in only 1 case, and in only 5 cases was the serum level below 13 units. In 22 cases a definite elevation of the phosphatase level was shown, with a low serum-bilirubin content (e.g. bilirubin 0.4 mg. per 100 ml., alkaline phosphatase 45 units).

**Bilirubin.** The mean serum-bilirubin level was 3.8 mg. per 100 ml. in the primary-carcinoma group as opposed to a mean level of 0.9 mg. per 100 ml. in the cirrhotic group. Of the carcinoma patients 17 were anicteric and 19 had a bilirubinaemia, which in the majority of patients was slight. Only 5 patients showed a bilirubin level of 10 mg. per 100 ml. or higher.

**Cholinesterase.** The level of serum pseudo-cholinesterase was slightly lower in the carcinoma group than in the cirrhotic group. Only one patient gave a normal result in the former group and one patient had a level of only 4% (100% =  $\Delta$  pH, 0.710).

**Mucoprotein.** The mucoprotein serum-content was significantly higher in the malignant than in the cirrhotic cases (205 mg. per 100 ml. as against 70 mg. per 100 ml.) Only one case of primary carcinoma of the liver had a normal mucoprotein level (75 mg. per 100 ml.)

**Cholesterol.** The cholesterol level in the hepatoma group was increased when compared with the cirrhotic group (240 mg. per 100 ml. as opposed to 135 mg. per 100 ml.) The cholesterol level in the malignant group approximated to the level found in healthy European subjects. The percentage of cholesterol esters to total cholesterol was the same in the malignant and cirrhotic groups, both showing a slightly decreased percentage of esterified cholesterol.

**Protein.** The total protein serum-content was virtually the same in the 2 groups. Significant features in the electrophoretic protein-analyses in the hepatoma group were an increased percentage of the  $\alpha_2$ -globulin fraction (in only one case was this level below 7% and in one case as high as 31.8%) and an increased percentage of the  $\beta$ -globulin fraction (in only one case was this fraction below 12% and in one case as high as 23.2%). The  $\gamma$ -globulin level in the malignant group was intermediate between that found in the cirrhotic patients and in the 'normal' Bantu. The albumin serum-content showed a slight reduction in the malignant group when compared with the cirrhotic group.

**Bromsulphalein Dye Retention.** As stated above, this test was only carried out on 7 cases of carcinoma of the liver and on 4 cases of cirrhosis of the liver. Abnormal

dye retention (more than 5%) was found in each of the 11 cases. The highest level of dye retention in the malignant groups was 53% and the lowest level was 6.0%.

#### DISCUSSION

The diagnostic biochemical features of primary carcinoma of the liver appear to be the following:

1. A disassociation between the thymol turbidity and flocculation tests.
2. A disassociation between the degree of bilirubinaemia and the serum alkaline-phosphatase level, especially the presence of a raised phosphatase level in the anicteric patient.
3. A raised serum-mucoprotein level and
4. A definite elevation of the  $\alpha_2$ - and  $\beta$ -globulin serum-fractions.

The disassociation between the thymol turbidity and flocculation tests is referred to above. The disparity between the degree of bilirubinaemia and the high phosphatase levels was a striking feature in our series of cases. Shay and Siple<sup>20</sup> suggest that this may reflect the sensitivity of the alkaline-phosphatase test to obstruction. The growth may obstruct enough bile canaliculi to cause an elevation of the phosphatase, but not enough to cause an increase in the serum bilirubin. Another hypothesis they put forward was that metastasis to the hilus nodes caused compression of the common duct sufficient to raise the pressure in the biliary tree to increase the blood-phosphatase level but not the serum-bilirubin level.

Gutman *et al.*<sup>21</sup> suggest that a rise in phosphatase levels in the absence of jaundice, may be dependent upon the excretion of bile in the urine and the impermeability of the human kidney to phosphatase excretion.

Bilirubinaemia was not a prominent feature in our series of cases. Of 36 cases 17 were anicteric, and in those showing bilirubinaemia the degree of jaundice was usually slight.

Holley and Pierson<sup>5</sup> stated that 4 out of 5 of their patients with primary carcinoma of the liver were jaundiced either at the time of admission or subsequently and that, though the jaundice was not intense, it was a fairly constant feature.

Likewise, Spatt and Grayzel<sup>6</sup> found a raised icteric index in 9 cases of primary carcinoma of the liver (average 84.1 units) and in only 2 cases was this index below 10 units. The icteric index in their group was high fairly consistently.

I have used the criteria of a raised alkaline-phosphatase level in the absence of jaundice as a useful aid in the diagnosis of malignancy, not necessarily confined to the liver. I have previously found this disassociation of the alkaline-phosphatase level and degree of bilirubinaemia in space-occupying tumours of the liver such as hydatid cyst, tuberculoma, amoebic abscess etc.

The level of serum mucoprotein was found by Greenspan *et al.*<sup>22</sup> to be elevated in 95% of patients with clinically evident enlargement of the liver due to neoplastic infiltration. Mucoprotein is a glucoprotein complex, probably related to changes in the  $\alpha$  globulins, which changes are not detected when electrophoretic analyses are carried out at an alkaline pH but only when performed at an acid pH.

The concentration of mucoprotein in the serum appears to represent the resultant of both intra-hepatic and extra-hepatic processes. Greenspan *et al.*<sup>22</sup> found a reduced mucoprotein level in infectious hepatitis and portal cirrhosis and a raised level in obstructive, inflammatory and neoplastic

diseases of the biliary system. I have found that, unless the cirrhosis or hepatitis is severe, it is more usual to find a low normal rather than a reduced mucoprotein level, but have not encountered elevated levels. In primary carcinoma of the liver, there is usually an accompanying cirrhosis, and the raised levels found in this series of malignant disease of the liver shows that the malignant process more than counteracts the damage to the parenchymal liver tissue. *In my opinion, the mucoprotein test is one of the best in the differential diagnosis between cirrhosis and malignant disease of the liver.*

A high cholesterol serum level favours a diagnosis of primary carcinoma of the liver rather than cirrhosis, but the range of serum-cholesterol levels was found to be very wide (86-580 mg. per 100 ml.) I therefore think that no importance can be attached to the serum-cholesterol level.

The low serum albumin probably reflects parenchymal liver impairment in both groups. Alpha-globulin levels are usually increased by inflammation and/or tissue destruction. Two competitive factors account for the wide range in distribution of the alpha globulins: the tendency for a rise with a drop in albumin and the tendency for a fall in liver-cell damage. It appears that the rise in the alpha<sub>2</sub>-globulin fraction in the carcinoma group reflects evidence of tissue destruction.

The beta-globulin fraction is concerned in the transport of lipids, and is elevated in most hepatobiliary diseases, especially those in which there is a concomitant rise in the serum lipids. The increased cholesterol and lipid levels in the carcinoma patients probably account for the raised beta-globulin fraction found in this group.

#### SUMMARY

1. 37 cases of proved primary carcinoma of the liver were subjected to a 'battery of liver function tests'. These results and results of similar tests performed on normal European and normal Bantu subjects and on patients suffering from cirrhosis of the liver are presented.

2. The following are diagnostic features in differentiating malignant liver disease from cirrhosis of the liver:

(i) A disassociation between the thymol turbidity and flocculation tests.

(ii) A disassociation between the serum bilirubin and alkaline phosphatase levels, especially a raised phosphatase level in the anicteric patient.

(iii) A raised serum-mucoprotein level.

(iv) Elevated alpha<sub>2</sub>- and beta-globulin fractions.

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