LIVER-FUNCTION TESTS IN PRIMARY CANCER OF THE LIVER IN THE BANTU*

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The South African Primary Liver Cancer Research Group was established in March 1965 for the purpose of investigating all aspects of this disease in male Bantu mine-workers, in whom it is a relatively common condition.[1]

The world distribution of this cancer is variable, being uncommon in western countries, but common in Africa south of the Sahara. In Africa it occurs not only in the Bantu but in all people of Negro stock. It is 6 times more common in Portuguese East Africans (88·3/100,000 *per annum*) than it is in South African Bantu (14·8/100,000 *per annum*).[2] This contrasts with a lower incidence (3·2/100,000 *per annum*) in the American Negro population.[3] The variable incidence suggests increased susceptibility, or the possibility of environmental aetiological factors.

A review of the literature on the value of liver-function tests in the diagnosis of primary cancer of the liver reflects conflicting opinions, and it is generally stated that most liver-function tests are of no value or of only doubtful value.

The apparent fundamental difference in the development of primary cancer of the liver in western and non-western populations—e.g. the necessity for pre-existing liver disease in the western populations—to a large degree confuses the comparison of results from different sources. Most detailed reports have come from western communities.

Holley and Pierson[4] stated that damage must be extensive before any appreciable change could be detected in liver-function tests in cases of primary liver cancer.

Spatt and Grayzel[5] stated 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, serum cholesterol and the cephalin flocculation tests were not of use in diagnosis.

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In Lichtman's opinion[6] the bilirubin content was usually normal and the cephalin cholesterol test was strongly positive in primary cancer of the liver and negative in secondary cancer. The thymol turbidity reaction was more uniformly negative in hepatic cancer. The alkaline phosphatase level might be normal in primary cancers which develop in cirrhotic livers.

Spellberg[7] stated that liver-function tests in primary cancer of the liver were variable and depended on the presence of underlying cirrhosis. If there was a marked derangement of liver-function tests the presence of cirrhosis was likely. Two tests, however, were likely to be disturbed in both primary and secondary malignant liver disease, viz. the bromsulphalein dye-retention test and the serum alkaline-phosphatase concentration.

Popper and Schaffner[8] stated that two findings were suggestive of primary carcinoma, but their absence did not exclude this diagnosis, viz. an elevation of serum alkaline phosphatase out of proportion to the degree of jaundice and an increase of serum mucoprotein to high levels during the course of cirrhosis, since the levels were low in uncomplicated cirrhosis, even with jaundice.

It has been shown[9] that the main diagnostic features in differentiating malignant liver disease from cirrhosis of the liver are a dissociation between the thymol turbidity and flocculation tests, a dissociation between the serum bilirubin and alkaline phosphatase levels (especially a raised alkaline phosphatase level in an anicteric patient), a raised serum mucoprotein level, elevated alpha$_2$- and beta-globulin fractions and a high serum cholesterol level.

Lin Chao *et al.*[10] stated that, owing to the reserve power and regenerative capacity of the liver, and to the fact that the lesion in primary cancer of the liver was often localized, liver-function tests were of little value in the early diagnosis of primary cancer of the liver. In view of the high incidence of co-existent hepatic cirrhosis and primary hepatic cancer, positive results of liver-function tests obtained could have been due to cirrhosis rather

than to cancer. In their opinion, increased activity of serum alkaline phosphatase in the absence of jaundice was of diagnostic value (in 130 cases, 54·6% exceeded 15 KA units).

Saragoga *et al.*[11] reported on the biochemistry of 7 cases of primary carcinoma and regarded a high level of alkaline phosphatase in the presence of low serum bilirubin as an important diagnostic aid. This observation has also been reported by others.[12-15] These two tests and the consistently high level of the transaminases (SGOT/SGPT ratio always greater than 1) could be regarded as the biochemical expression of Brem's concept of an 'expanding, space-occupying lesion'.[16]

Kay,[17] in a review of 96 patients with primary hepatic cancer, found that although the results of liver-function tests were usually abnormal, they were not specific. In the absence of non-hepatic disease the most specific test was the serum alkaline phosphatase, especially if very elevated.

Sherlock[18] found the serum alkaline phosphatase, transaminase, alpha$_2$-globulin, aldolase and bromsulphalein dye retention to be increased in primary liver cancer.

Recently it has been shown that the presence of alpha-feto-protein (AFP) is a reliable indicator of the presence of primary cancer of the liver and that the proportion of cases that show a positive result ranges from 50 to 79%.[19-22]

MATERIAL AND METHODS

Beginning in March 1965, Bantu mineworkers of the Witwatersrand and Orange Free State goldmines were referred to the clinical unit at City Deep Mine Hospital, Johannesburg, from other mine hospitals, for the investigation of hepatomegaly suspected, on clinical grounds only, of being due to primary liver cancer. Occasionally cases were diagnosed incidentally at laparotomy, e.g. for an 'acute abdomen'. Some cases resembled amoebic liver disease and were initially misdiagnosed until biopsied and perhaps even treated. Because these measures could possibly vitiate the results of liver-function tests performed on admission to the unit, these cases were excluded from this report.

Many patients arriving at the unit were clearly typical cases of primary carcinoma of the liver—frank cancer type 1.[23] After the diagnosis was made by histological examination of a liver biopsy, patients were accepted for study.

The first 200 consecutive patients with hepatomegaly comprise the series that we are reporting.

There were 63 cases, negative for primary cancer of the liver by repeated biopsies. Liver-function tests were not carried out in 2, leaving 61 cases, designated as the 'non-cancer' group. Since these cases were often lost to follow-up and no final diagnosis could be made, the possibility of some of these cases having primary liver cancer could not be excluded. In fact, retrospective testing of sera from these cases revealed a positive AFP test in 5 of these cases.

Primary extrahepatic malignancy was found in 6 cases; and in a further 6 cases the diagnosis remains in dispute histologically, but a positive AFP test was found in 5. A definite histological diagnosis was made in 125 cases. Of the 125 cases proved histologically, 17 were excluded on the grounds of having had previous treatment, biopsy or laparotomy, and 15 were repatriated. In the remaining 93 patients the diagnosis was proved by postmortem examination and it is these unequivocal cases which were analysed further for this report. Subsequent analysis has, however, revealed that, even if the biopsy-positive and AFP-positive cases are included, the conclusions we reach are the same.

Tables I and II depict the tribal composition and age distribution of the cancer and non-cancer groups. The provisional clinical diagnosis of the non-cancer group is shown in Table III.

There was sufficient material to group 90 cases into cirrhotic (48 cases) and non-cirrhotic categories (30 cases). In 12 cases there was some doubt as to classification. They had no naked-eye cirrhosis of the liver, and sections taken at a distance from the tumour failed to show more than a few foci of subcapsular nodular regeneration. A cirrhotic liver was defined as one showing deformity of the normal lobular architecture as the result of both excessive fibrous tissue formation and nodular parenchymal hyperplasia or regeneration. This definition excluded hepatofibrosis and nodular regeneration without

TABLE I. TRIBAL COMPOSITION

Tribe	*Cancer*	*Non-cancer*
South African Bantu		
Zulu	2	3
Xhosa	5	10
Hlubi	1	1
Pondo	1	8
Pedi	1	2
Baca	1	2
Total	11	26
Portuguese East Africans		
Shangaan	54	19
Chopi	8	1
Nyembaan	10	1
Tonga	1	1
Total	73	22
Other tribes		
Angola	0	3
Basuto	3	1
Nyasa (Malawi)	1	6
Chewa (Malawi)	1	0
Mlubali (Angola)	1	0
Mbunda (Southern Rhodesia)	1	0
Bechuana	2	1
Rotsi	0	2
Total	9	13
	93	61

There were no Transvaal Shangaans and no South African Basuto in the series.

TABLE II. AGE DISTRIBUTION

Age (years)	*Cancer*	*Non-cancer*
18 - 20	7	5
21 - 30	40	18
31 - 40	32	14
41 - 50	11	14
51 - 60	3	9
61 and over	0	1
Mean age	31·8	37·4

TABLE III. NON-CANCER GROUP (CLINICAL DIAGNOSIS)

Diagnosis	*Total*
Cirrhosis	30
Miliary tuberculosis	8
Congestive cardiac failure	6
Amoebic hepatitis	4
Tuberculosis (3 peritonitis)	4
Infective hepatitis	2
? Carcinoma of the pancreas	1
Dysentery (bacterial)	1
Hydatid cyst	1
Liver abscess	1
Loeffler's syndrome	1
Ruptured rectus muscle	1
Typhoid	1
Total	61

fibrosis. True cirrhoses are generalized throughout the liver.[24] According to the above definition these 12 cases should be grouped with the non-cirrhotic cases, but they have been grouped separately for the purpose of this study.

Forty-seven per cent of our cancer cases (if we include cases with focal cirrhosis as non-cirrhotics) were therefore not associated with cirrhosis of the liver. These figures are in contrast to those of Higginson,[25] Thompson[26] and Davies[27] who found that in their series cancer of the liver occurred in non-cirrhotic livers in 14%, 21% and 11% of cases. Prates,[2] in 399 autopsy cases of liver cancer in the Portuguese East African, showed that 87% were associated with cirrhosis.

In the cancer group 41% had evidence of bilharziasis on postmortem examination and 63% on clinical testing. In the non-cancer group 53% were positive for bilharziasis on clinical grounds.

In this series the ratio between Portuguese East African and South African Bantu autopsies was 6·6 : 1. This was similar to the ratio obtained in autopsy material by Berman,[28] who found that primary cancer of the liver was almost 6 times more frequent in the Portuguese East African than it was in the South African Bantu. Rate studies in the Bantu population of Johannesburg and in Lourenço Marques have confirmed this increased incidence.[2,3]

The biochemical tests carried out on the patients admitted to the study are listed in Table IV.

RESULTS

Tables VA-VD show the means, standard deviations and statistical analyses of the cancer and non-cancer groups, and cases with and without cirrhosis. Tables VIA-VID show the percentage of abnormal results in the groups studied. The 'Student's T' test was calculated by standard methods with degrees of freedom equal to (total cases -2); and the probability (p) of two sets of data, e.g. (A) and (B + C), being significantly different, was obtained from standard tables. For (e.g. 60 degrees of freedom) if the result of 'Student's T' $>2{\cdot}39$ then $p<0{\cdot}01$ (highly significant) and if $2{\cdot}39 >$ 'Student's T' results $> 1{\cdot}67$ then $0{\cdot}05 > p > 0{\cdot}01$ (possibly significant).

DISCUSSION

The biochemical tests carried out in the proved cancer group showed in practically every instance marked deviations from the normal range. This also applied to the non-cancer group, but not to the same degree. There was, however, in both groups a very wide scatter in most results which was reflected in the high standard deviations (Tables VA-VD).

When the cancer and the non-cancer groups were compared the striking feature was the marked elevation in the enzyme levels in the cancer group. The SGOT, SGOT/SGPT ratio, LDH, alpha-hydroxybutyric dehydro-

TABLE IV. BIOCHEMICAL TESTS CARRIED OUT

Tests	*Normal values*
Thymol turbidity (MacLagan)[28]	0–2 units
Thymol flocculation (Neefe and Reinhold)[29]	Neg.
Colloidal red (Ducci)[30]	Neg.
Cephalin cholesterol flocculation (Hanger)[31]	Neg.
Takata Ara (Ucko)[32]	Neg.
Zinc sulphate turbidity (Kunkel)[33]	Up to 12·5 units
Alkaline phosphatase (King and Armstrong)[34]	4–13 units
Bilirubin (Malloy and Evelyn)[35]	Up to 1·2 mg./100 ml.
Pseudocholinesterase (Michel)[36]	80–120% of normal activity
Mucoprotein (Natelson)[37]	25–125 mg./100 ml.
Cholesterol (Sperry and Webb[38])	Up to 230 mg./100 ml.
Percentage esterified cholesterol (Sperry and Webb[38])	66–72%
Glutamic oxaloacetic transaminase (SGOT) (Reitman and Frankel[39])	2–35 units
Glutamic pyruvic transaminase (SGPT) (Reitman and Frankel[39])	2–35 units
Alpha-hydroxybutyric dehydrogenase (HBD) (Elliott and Wilkinson)[40]	70–180 units
Lactic dehydrogenase (LDH) (Wroblewski and LaDue)[41]	100–280 units
Lactic dehydrogenase isoenzyme fraction V (modified Raboo)[42]	6–8%
Aldolase (modified Boehringer kit)	9–25 units
Isocitric dehydrogenase (ICD) (Bowers)[43]	2–7 units
Leucine amino-peptidase (LAP) (Goldbarg and Rutenberg)[44]	180–350 units
Glutathione reductase (GR) (Kerppola, Nikkila and Pitkanen)[45]	25–54 units
5'nucleotidase (King and Wootton)[46]	3–11 units
Total protein (Biuret)[47]	6–8 G/100 ml.
	% / *G/100 ml.*
Albumin	50–66 / 3·20–4·80
Alpha$_1$-globulin	2–5 / 0·17–0·33
Alpha$_2$-globulin	5–11 / 0·40–0·96
Beta-globulin	8–16 / 0·50–1·20
Gammaglobulin	14–30 / 1·10–2·20
(Electrophoresis was carried out using the Beckman microzone apparatus.)	
C-reactive protein (CRP kit)	Neg.

TABLE VA. MEANS, STANDARD DEVIATIONS AND STATISTICAL ANALYSES (FLOCCULATION TESTS)

Cases	*Proved cancer (93 cases)*	*'p'*	*Non-cancer (61 cases)*	*Cirrhosis (48 cases)*	*'p'*	*No cirrhosis (30 cases)*	*Focal cirrhosis (12 cases)*
Thymol turbidity (units)	5·6 ± 3·6	HS	3·9 ± 2·4	6·0 ± 3·4	NS	4·9 ± 3·7	5·0 ± 3·7
Thymol flocculation	1·8 ± 1·8	NS	1·4 ± 1·6	2·1 ± 1·8	PS	1·4 ± 1·6	1·7 ± 1·8
Colloidal red	3·2 ± 1·4	NS	3·1 ± 1·4	3·6 ± 0·9	HS	2·8 ± 1·7	2·7 ± 1·7
Cephalin cholesterol	2·4 ± 1·8	NS	3·9 ± 2·4	2·6 ± 1·7	PS	3·2 ± 1·8	2·5 ± 1·7
Takata Ara reaction	1·9 ± 1·0	NS	1·7 ± 1·1	2·1 ± 1·0	PS	1·7 ± 1·0	1·6 ± 0·9
Zinc sulphate turbidity (units)	36·5 ± 16·2	PS	32·1 ± 12·1	40·6 ± 17·9	HS	30·7 ± 13·2	33·7 ± 11·8

HS $p < ·01$
PS $0·01 < p < 0·05$
NS $p > 0·05$

TABLE VB. MEANS, STANDARD DEVIATIONS AND STATISTICAL ANALYSES (ENZYMES)

	Proved cancer (93 cases)	*'p'*	*Non-cancer (61 cases)*	*Cirrhosis (48 cases)*	*'p'*	*No cirrhosis (30 cases)*	*Focal cirrhosis (12 cases)*
Alkaline phosphatase (units)	27·8 ± 15·4	PS	20·9 ± 24·5	25·6 ± 14·7	NS	26·5 ± 12·5	37·9 ± 20·2
SGOT (units)	195·5 ± 215·2	HS	77·5 ± 114·7	182·6 ± 153·1	NS	219·5 ± 315·4	177·2 ± 96·0
SGPT (units)	70·2 ± 93·7	NS	60·1 ± 99·3	53·7 ± 24·7	PS	103·9 ± 154·6	58·2 ± 39·6
SGOT/SGPT ratio	2·8 ± 1·8	HS	1·3 ± 0·7	3·3 ± 1·6	HS	2·1 ± 1·7	3·0 ± 1·2
LDH (units)	1,099·9 ± 1,043·4	HS	395·4 ± 207·4	1,100·3 ± 1,140·7	NS	1,049·0 ± 838·5	906·7 ± 449·9
HBD (units)	660·9 ± 715·9	HS	251·3 ± 130·1	648·7 ± 733·3	NS	646·6 ± 572·7	500·0 ± 269·0
LDH/HBD ratio	1·7 ± 0·5	NS	1·6 ± 0·6	1·7 ± 0·4	NS	1·6 ± 0·3	1·8 ± 0·2
% LDH isoenzyme fraction V	28·6 ± 14·5	HS	16·5 ± 9·1	26·4 ± 14·4	NS	30·4 ± 14·3	31·2 ± 10·0
Aldolase (units)	61·4 ± 45·1	HS	32·7 ± 13·7	55·7 ± 32·2	NS	52·0 ± 25·4	67·5 ± 25·9
ICD (units)	15·1 ± 9·6	HS	5·9 ± 3·1	13·9 ± 8·7	NS	16·2 ± 11·1	16·3 ± 10·2
LAP (units)	623·0 ± 431·3	HS	378·7 ± 226·4	484·4 ± 262·6	HS	763·3 ± 611·6	719·0 ± 292·4
5′nucleotidase (units)	30·7 ± 19·7	HS	19·0 ± 30·2	27·9 ± 19·4	NS	27·2 ± 15·9	46·9 ± 26·5
GR (units)	150·1 ± 92·8	HS	70·3 ± 38·2	135·0 ± 73·8	NS	150·9 ± 123·2	46·3 ± 63·7
Cholinesterase % of normal	50·3 ± 22·7	NS	55·8 ± 25·6	49·8 ± 23·8	NS	52·5 ± 23·5	44·5 ± 16·3

TABLE VC. MEANS, STANDARD DEVIATIONS AND STATISTICAL ANALYSES (PROTEINS)

	Proved cancer (93 cases)	*'p'*	*Non-cancer (61 cases)*	*Cirrhosis (48 cases)*	*'p'*	*No cirrhosis (30 cases)*	*Focal cirrhosis (12 cases)*
Total protein (G/100 ml.)	8·2 ± 0·9	NS	8·1 ± 0·8	8·2 ± 0·9	NS	8·0 ± 0·9	8·2 ± 0·8
Albumin %	39·5 ± 6·1	NS	41·3 ± 8·9	39·0 ± 6·1	NS	40·3 ± 6·4	38·6 ± 5·1
Albumin (G/100 ml.)	3·2 ± 0·6	NS	3·3 ± 0·8	3·2 ± 0·6	NS	3·2 ± 0·6	3·1 ± 0·4
Alpha$_1$-globulin %	6·0 ± 1·7	HS	4·8 ± 1·4	6·0 ± 1·6	NS	5·9 ± 1·5	6·4 ± 2·5
Alpha$_1$-globulin (G/100 ml.)	0·5 ± 0·1	HS	0·4 ± 0·1	0·5 ± 0·1	NS	0·5 ± 0·1	0·5 ± 0·2
Alpha$_2$-globulin %	9·8 ± 2·2	NS	9·8 ± 2·1	9·5 ± 1·9	PS	10·4 ± 2·4	9·9 ± 2·2
Alpha$_2$-globulin (G/100 ml.)	0·8 ± 0·2	NS	0·8 ± 0·1	0·8 ± 0·2	NS	0·8 ± 0·2	0·8 ± 0·2
Beta-globulin %	12·3 ± 2·2	NS	11·6 ± 2·3	11·8 ± 2·0	NS	12·3 ± 1·7	12·6 ± 3·1
Beta-globulin (G/100 ml.)	1·0 ± 0·2	NS	0·9 ± 0·2	1·0 ± 0·2	NS	1·0 ± 0·2	1·0 ± 0·3
Gammaglobulin %	32·6 ± 7·0	NS	32·3 ± 8·7	33·7 ± 7·1	NS	31·0 ± 5·8	32·5 ± 6·7
Gammaglobulin (G/100 ml.)	2·7 ± 0·9	NS	2·6 ± 0·8	2·8 ± 0·8	PS	2·5 ± 0·7	2·7 ± 0·6
Mucoprotein (mg./100 ml.)	178·0 ± 69·3	NS	177·7 ± 95·4	172·1 ± 67·6	NS	186·9 ± 67·1	182·2 ± 82·5
C-reactive protein	2·5 ± 1·4	HS	1·2 ± 1·5	2·6 ± 1·3	NS	2·3 ± 1·6	2·1 ± 1·5

TABLE VD. MEANS, STANDARD DEVIATIONS AND STATISTICAL ANALYSES (BILIRUBIN AND CHOLESTEROL)

	Proved cancer (93 cases)	*'p'*	*Non-cancer (61 cases)*	*Cirrhosis (48 cases)*	*'p'*	*No cirrhosis (30 cases)*	*Focal cirrhosis (12 cases)*
Total bilirubin (mg./100 ml.)	2·5 ± 4·3	NS	1·9 ± 4·3	2·3 ± 2·8	NS	3·3 ± 6·6	1·6 ± 0·9
Cholesterol (mg./100 ml.)	170·0 ± 74·5	HS	132·7 ± 67·5	160·5 ± 71·5	NS	172·2 ± 73·5	173·1 ± 68·8
Esterified cholesterol %	53·2 ± 15·4	HS	60·7 ± 11·7	52·3 ± 15·3	NS	53·2 ± 16·6	53·8 ± 11·6

TABLE VIA. PERCENTAGE OF ABNORMAL TESTS (FLOCCULATION TESTS)

	Proved cancer (93 cases)	*Non-cancer (61 cases)*	*Cirrhosis (48 cases)*	*No cirrhosis (30 cases)*	*Focal cirrhosis (12 cases)*
Thymol turbidity (units)	77	73	87	67	58
Thymol flocculation	56	47	64	47	50
Colloidal red	88	88	98	76	75
Cephalin cholesterol	68	62	73	57	75
Takata Ara reaction	86	81	87	83	83
Zinc sulphate turbidity (units)	96	95	100	90	100

TABLE VIB. PERCENTAGE OF ABNORMAL TESTS (ENZYMES)

	Proved cancer (93 cases)	*Non-cancer (61 cases)*	*Cirrhosis (48 cases)*	*No cirrhosis (30 cases)*	*Focal cirrhosis (12 cases)*
Alkaline phosphatase (units)	88	49	85	90	91
SGOT (units)	97	69	96	100	100
SGPT (units)	73	57	71	73	75
SGOT/SGPT ratio	89	43	89	83	91
LDH (units)	97	62	98	100	100
HBD (units)	95	65	98	100	100
LDH/HBD ratio	58	41	60	60	67
% LDH isoenzyme fraction V	95	97	96	97	100
Aldolase (units)	90	65	89	87	100
ICD (units)	84	21	79	90	91
LAP (units)	75	39	66	83	91
5′nucleotidase (units)	87	52	79	93	100
GR (units)	95	61	91	100	100
Cholinesterase % of normal	89	75	87	87	100

TABLE VIC. PERCENTAGE OF ABNORMAL TESTS (PROTEINS)

	Proved cancer (93 cases)	*Non-cancer (61 cases)*	*Cirrhosis (48 cases)*	*No cirrhosis (30 cases)*	*Focal cirrhosis (12 cases)*
Total protein above 8·0 G/100 ml.	51	56	56	43	58
Albumin below 50%	98	82	98	93	100
Albumin below 3·2 G/100 ml.	58	39	63	53	50
Alpha$_1$-globulin above 5%	72	36	68	73	75
Alpha$_1$-globulin above 0·4 G/100 ml.	43	21	48	36	41
Alpha$_2$-globulin above 11%	32	28	23	40	41
Alpha$_2$-globulin above 0·96 G/100 ml.	16	18	13	23	9
Beta-globulin above 16%	6	3	4	0	16
Beta-globulin above 1·2 G/100 ml.	18	8	10	14	50
Gammaglobulin above 30%	63	52	63	57	75
Gammaglobulin above 2·2 G/100 ml.	73	64	77	70	67
Mucoprotein above 150 mg./100 ml.	76	63	75	84	75
C-reactive protein + and over	91	52	96	80	83
Alpha-feto-protein					
Initial* positive	75	8†	81	53	100
Eventual** positive	82		89	70	100

*On admission
**Final status
†See text

TABLE VID. PERCENTAGE OF ABNORMAL TESTS (BILIRUBIN AND CHOLESTEROL)

	Proved cancer (93 cases)	*Non-cancer (61 cases)*	*Cirrhosis (48 cases)*	*No cirrhosis (30 cases)*	*Focal cirrhosis (12 cases)*
Total bilirubin (mg./100 ml.)	46	19	54	30	50
Cholesterol (mg./100 ml.)	54	29	54	53	50
Esterified cholesterol %	80	59	77	83	75

genase, LDH isoenzyme fraction V (liver fraction), aldolase, isocitric dehydrogenase, leucine amino-peptidase, 5′nucleotidase and glutathione reductase were all significantly higher ($p = <0{\cdot}01$) in the cancer cases. The mean SGPT and cholinesterase levels were not as significantly different (although an increased percentage showed abnormalities) as was the lactic dehydrogenase/alpha-hydroxybutyric dehydrogenase ratio. This was in keeping with the observation of Elliott and Wilkinson, who found high ratios of lactic dehydrogenase/alpha-hydroxybutyric dehydrogenase in acute liver disease, but normal ratios in chronic liver conditions.[40]

The mean alkaline phosphatase level was only slightly higher in the cancer group (possibly significant), although a higher percentage of cases showed raised levels (88% *vs.* 49%). In the cancer group 54% were non-icteric (84% of whom had a raised alkaline phosphatase level), while in the non-cancer group 81% were non-icteric (49% of whom had a raised alkaline phosphatase level). This confirms a previous report by one of us (I.B.) that a raised alkaline phosphatase level in the absence of jaundice is a useful aid in the diagnosis of malignancy and space-occupying lesions of the liver.[9] This observation is similar to that of Jose *et al.*[48] who stated that elevated levels of alkaline phophatase, which continued to mount during the period of observation, were valuable clues to the diagnosis of space-occupying lesions of the liver in the absence of bone disease or jaundice.

The significant increase in the SGOT level in the cancer group, without significant differences in the SGPT level, is reflected by a significantly higher SGOT/SGPT ratio in the cancer group. SGOT has cytoplasmic and mitochondrial components, while the SGPT is wholly cytoplasmic. Severe liver cell damage is necessary for the mitochondrial enzyme to pass into the serum, and for this reason the ratio in the cancer group was probably higher than in the non-cancer group.[49]

Isocitric dehydrogenase was significantly elevated in the cancer group. Elevated levels usually occur during the early stages of infective hepatitis, and normal levels are encountered in most cases of obstructive jaundice irrespective of causation.[50] Raised levels occurred, according to Okumura and Spellberg,[51] in some patients with hepatic metastatic tumours, but the values could be normal

with small metastases. Patients with cirrhosis had normal or only slightly elevated levels. The degree of serum elevation seemed to depend on the size of the metastatic tumour. Sterkel *et al.*[52] studied isocitric dehydrogenase levels in 49 patients with extensive malignancy. Approximately one-half of the 21 patients with liver metastases exhibited relatively small elevations of isocitric dehydrogenase. In the group of 28 cases without liver metastases, no abnormal levels were noted.

The mean aldolase level was significantly higher in the cancer group, and 90% of the cancer group had raised levels. Serum aldolase levels are markedly increased in the early stages of acute hepatitis, but in other liver diseases, including cirrhosis and obstructive jaundice, the serum aldolase level is usually within normal limits or only slightly elevated.[53] Sibley and Lehninger[54] found raised levels in 20% of 104 cancer patients. There was no correlation with clinical findings. The presence of actively regenerating liver did not cause increased aldolase levels. Surgical removal of the tumours caused a prompt decline of the serum aldolase to normal levels. Warburg and Christian,[55] on the other hand, found no significant increase over normal values in serum of patients with cancer. They concluded that serum aldolase levels were not of diagnostic usefulness in human cancer.

Blostein and Rutter have shown that two types of aldolases occurred in mammalian tissue—aldolase A, which was specific for fructose-1, 6-diphosphate (FDP), and aldolase B, which reacted equally with FDP and fructose-1-phosphate (F1P). Foetal liver contains both aldolases, and hence shows an FDP/F1P activity ratio of 5, compared with a ratio of 1 in the adult. Liver cancer tissues have been shown to revert to the foetal condition, giving ratios of 2·2 - 13·0.[56,57]

One of us (I.B.) studied the sera of 11 normal subjects who exhibited activities with FDP of 2·1 ± 0·8 units, F1P 0·7 ± 0·4 units, and FDP/F1P ratios of 3·5 ± 1·8. The sera of 7 subjects suffering from liver cancer gave activities with FDP of 8·7 ± 4·5 units, with F1P 2·3 ± 1·2 units, and FDP/F1P ratios of 4·9 ± 4·2. The differences between these ratios were not significant, hence it was felt that the technique could not be used as a diagnostic test for liver cancer. The mean FDP aldolase activity of cancerous cases was, however, significantly raised ($p<0·005$). FDP aldolase activities were also measured on a larger series of specimens. Activities of 3·1 ± 0·9, 5·7 ± 3·3 and 3·0 ± 1·1 units were obtained for 42 normals, 40 cancer cases and 27 miscellaneous non-cancer liver disease respectively. The difference between the mean non-cancer and cancer values was highly significant ($p<0·001$). This was a retrospective study and the 40 cancer cases studied were from patients in this study.[58]

The thymol and zinc sulphate turbidity tests were significantly higher in the cancer group.

The mean bilirubin levels were only slightly raised in the two groups, and there was no significant difference. However, 54% of the cancer group and 81% of the non-cancer group were anicteric, emphasizing that marked bilirubinaemia is not a prominent feature of malignant hepatoma.

The cholesterol level was significantly higher in the cancer group and the percentage of esterified to total cholesterol was significantly lower. We have previously mentioned that a high cholesterol level favours a diagnosis of primary cancer of the liver, but since the range of levels is very wide, no importance can be attached to individual results.[9]

Apart from a significantly increased level of the alpha$_1$-globulin, there were no significant differences in the total protein, albumin, alpha$_2$-, beta- and gammaglobulin levels.

A higher percentage of low albumin levels (below 3·2 G/100 ml.) was found in the cancer group (58% *vs.* 39%). The cancer group had a raised alpha$_1$-globulin level (43% *vs.* 21%) compared with the non-cancer group.

A higher percentage of cases in the cancer group had raised beta-globulin levels than in the non-cancer group (18 *vs.* 8), while there was little difference in the percentage of cases showing raised gammaglobulin levels in the two groups (73 *vs.* 64).

The C-reactive protein was statistically higher in the malignant group and there was a higher percentage of abnormal results (91% *vs.* 52%).

There was no significant difference in the mean serum mucoprotein level. This was in contrast to our previous study that compared mucoprotein levels in primary cancer of the liver with portal cirrhosis of the liver in the Bantu. There was a significant difference in these two groups. In the present series we compared cases of primary cancer of the liver with a heterogenous group of cases with a similar clinical presentation to primary liver cancer, and bearing in mind that some of the non-cancer group had 'space-occupying lesions' of the liver, and infections, it is not surprising that the mucoprotein levels were similar in the two groups.

When the cases with cirrhosis and without cirrhosis were compared (those with histological evidence of focal cirrhosis were omitted) there were possibly significant differences in the SGPT level, and highly significant differences in the SGOT/SGPT ratio and in the leucine aminopeptidase levels. The only significant difference in the protein pattern was a slightly increased gammaglobulin level in the cirrhotic group. The thymol flocculation, colloidal red, the Takata Ara reaction and the zinc sulphate turbidity tests were all significantly increased in the cirrhotic group.

The bilirubin, cholesterol (total and esterified) and alkaline phosphatase levels were not significantly different in the two groups.

The cases have also been subdivided according to the presence of pulmonary metastases on admission, later proved at postmortem examination (23 cases), or as to whether metastases were not present at postmortem (41 cases). The only significant differences between these two groups were increases in the LDH, alpha-hydroxybutyric dehydrogenase and total protein in the metastatic group.

The AFP (quantitative)[59] test was positive initially in 75·2% and eventually in 82% of the cancer group. This agrees with our positivity rate of 76% in a larger series. There were 5 cases in the 'non-proved' group with a positive AFP test but in whom no final diagnosis was made. These cases were lost to follow-up as the AFP test was strictly retrospective, and these cases may well have had primary cancer of the liver.

A detailed analysis of the relationship between all the biochemical parameters and the presence or the concen-

tration of AFP revealed no striking correlations. This was not altogether surprising, as none of the biochemical tests was of such definite discriminatory value as the AFP test.

However, by dividing our cases into groups (alpha-feto-protein-absent, less than 20 mg./100 ml. and more than 20 mg./100 ml.), we were able to establish definite gradients.[60] The mucoprotein, C-reactive protein and alpha$_2$-globulin decreased (each with a significance level of at least $p<0.05$) as the AFP rose. Serum cholesterol and total protein increased. Scrutiny of the data did not enable us to attribute this gradient to a greater degree of liver damage or cirrhosis in the AFP-positive groups. The usual biochemical indicators of cirrhosis, even in the presence of primary cancer of the liver (Tables VA - VD), were observed when the cirrhotic and non-cirrhotic groups were compared. This was not the case with the three AFP groups, although there is a suggestion that initially the non-cirrhotic group had fewer AFP-positive cases (53% *vs.* 70%) although the absolute mean value of AFP was not different.

Eventual survival time (from the date of the first symptom) was compared with all our parameters on admission. The cases were grouped: 30, 60, 90, 120, <200, >200 days. In the first three groups there was a significant gradient for zinc sulphate turbidity, SGOT and SGPT, which were higher in the shorter survivals, otherwise the results were similar for the rest of the groups. There was a possibly significant gradient for all the groups for gammaglobulin, the value being higher in short survivals.

It appears that there are no biochemical tests that can be considered specific enough to be diagnostic of primary cancer of the liver. A combination of tests such as markedly elevated enzyme levels, a raised cholesterol level, a dissociation between the alkaline phosphatase and bilirubin level (particularly a raised alkaline phosphatase level in the absence of jaundice), may be used as possible presumptive evidence of primary liver cancer. The only test that affords a specific diagnosis of primary cancer of the liver, in approximately 82% of cases, is the AFP test. AFP has not been found in the sera of several thousand healthy individuals tested, nor in patients with a wide variety of cancers other than hepatocellular carcinoma (except for positive results in a few cases of neoplasms of embryonal character) even when the liver contained metastases.[20]

SUMMARY

A wide range of biochemical liver-function tests were carried out on an unbroken series of Bantu mineworkers of the Witwatersrand and Orange Free State goldmines suspected of having primary cancer of the liver. There were 93 cases, proved at postmortem examination, of primary cancer of the liver. These cases were further categorized into cirrhotic, focal cirrhotic and non-cirrhotic groups.

There were 61 cases which were negative for cancer by repeated liver biopsies. The biochemical liver-function tests of these groups were compared. No single biochemical test was considered specific enough to be diagnostic of primary cancer of the liver, but a combination of tests could be used as possible presumptive evidence.

The only test that affords a specific diagnosis of cancer of the liver is the alpha-feto-protein test which is positive in approximately 82% of cases.

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