

## Reduced leucocyte zinc in liver disease\*

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**SUMMARY** The zinc content of peripheral blood leucocytes has been measured in normal controls and in three groups of patients with liver disease. A significant reduction in leucocyte zinc, but not erythrocyte zinc, was observed in patients with primary biliary cirrhosis, alcoholic cirrhosis, and active chronic hepatitis. It is suggested that the nucleated tissues of some patients with liver disease are therefore zinc deficient, and that leucocyte zinc may prove of value in the assessment of the zinc status of such patients.

Reduced plasma zinc levels and increased urinary zinc excretion have frequently been reported in patients with cirrhosis, and it has therefore been suggested that they are zinc deficient.<sup>1,2</sup> It is unwise, however, to extrapolate from plasma concentration to the levels of zinc in tissues, as the zinc in plasma accounts for less than 1% of total body zinc, most of which is intracellular or associated with bone.<sup>3,4</sup> Furthermore, 60% of plasma zinc is bound to albumin, which is itself often reduced in liver disease.<sup>5,6</sup> The zinc content of an appropriate nucleated tissue might therefore more closely reflect total body zinc, but which tissue is appropriate? Measurement of liver biopsy specimens is complicated by their increased content of fibrous tissue in liver disease, while muscle biopsies are not routinely available.<sup>7</sup> Neither can be readily repeated at intervals for reassessment of tissue zinc status.

Blood cells are a 'tissue' with the unique advantage of being easily sampled for repeated study. Erythrocytes have no nuclei and are metabolically highly specialised<sup>8</sup> and previous reports of their zinc content in liver disease have been inconsistent.<sup>8,9</sup> Leucocytes, however, are nucleated, and might better reflect the tissue environment; in a single report their zinc content was found to be reduced in patients with alcoholic liver disease.<sup>9</sup>

We have, therefore, studied the zinc content of leucocytes and erythrocytes and the concentrations

of zinc in plasma and urine in patients with three types of liver disease, and compared them with measurements in control subjects.

### Methods

#### PATIENTS

Twenty-seven control subjects, consisting of laboratory staff and patients admitted for minor surgical operations, and 46 patients with histologically proven liver disease were studied. The patients were classified into primary biliary cirrhosis (n=11), alcoholic cirrhosis (n=21) and active chronic hepatitis (n=14). All patients gave informed consent and the study was approved by the hospital ethical committee.

#### TECHNIQUES

A 50 ml blood sample was taken into an heparinised plastic syringe and leucocytes prepared by the method of dextran sedimentation.<sup>10</sup> The leucocyte pellet was finally resuspended in ice cold isotonic MgCl<sub>2</sub> (99 mmol/l), transferred to a weighed polyethylene lay flat tube and centrifuged at 0°C for three minutes at 250g. The supernatant was discarded and the cell pellet wiped, and dried at 100°C to constant weight. An aliquot of blood was centrifuged and the plasma retained. The packed erythrocytes were resuspended and washed in ice cold MgCl<sub>2</sub> and a sample was prepared as for leucocytes. The zinc content of both leucocytes and erythrocytes were determined by atomic absorption spectrometry (Instrumentation Laboratory 251) after extraction with 1.0 mol/l HCl. Leucocyte and erythrocyte zinc levels have been expressed per unit dry weight (DW) in view of the well known

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changes in cell water that may accompany liver disease in at least the leucocyte.<sup>11</sup> The use of dry weight also corrects for any change in the peripheral cell count. Twenty-four hour urine samples were collected into precleaned polyethylene containers and the zinc content of both urine and plasma was measured in aliquots diluted 1 in 10 with 80 mmol/l HNO<sub>3</sub>.

Plasma albumin was measured by the bromocresol-green method.<sup>12</sup>

Accuracy of the analytical technique in plasma and urine was tested by the method of standard additions. A mean accuracy of 101% was observed throughout the range of values and the coefficient of variation of repeated dilutions of urine or plasma was 2.7%. The coefficients of variation of leucocyte zinc measurements were determined when quadruplicate specimens were prepared from single blood sample and ranged from 2.2 to 5.0% in four experiments. The extraction of zinc from dried material was determined in erythrocytes and leucocytes which had been incubated with radioactive zinc (<sup>65</sup>Zn) for four and one hours respectively. The radioactivity of the dried pellet was compared with that of the extraction fluid. Mean recovery of <sup>65</sup>Zn was 106% with a coefficient of variation of 1.8% in erythrocytes and 95.5% with a coefficient of variation of 9.8% in leucocytes.

Student's *t* test was used to examine the difference between group means.

## Results

All three groups of patients with liver disease had significantly lower mean values for leucocyte zinc content than controls ( $P < 0.01$ ). As shown in the Figure, leucocyte zinc content in patients with active chronic hepatitis was closely grouped, whereas there was a larger variation in patients with alcoholic and primary biliary cirrhosis. The values were normally distributed in all groups. Five patients with primary biliary cirrhosis had leucocyte zinc levels within the normal range and these patients were considered on clinical and

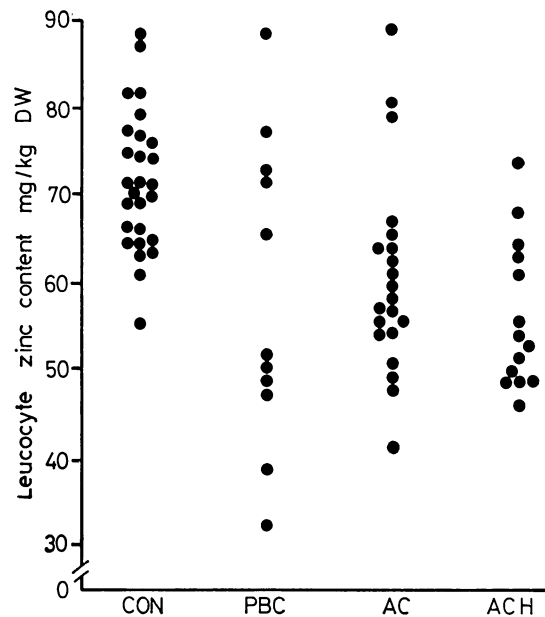


Figure Leucocyte zinc content in control subjects and patients with liver disease. Con: controls. PBC: primary biliary cirrhosis. AC: alcoholic cirrhosis. ACH: active chronic hepatitis.

histological grounds to have mild disease, while the remaining six had markedly depressed leucocyte zinc and were clinically more ill, in three cases with ascites. No such relationship between clinical state and leucocyte zinc was seen in the patients with alcoholic cirrhosis or active chronic hepatitis, although in the group with alcoholic cirrhosis there was an impression that the more severely ill patients had the lowest values for leucocyte zinc.

No significant change in erythrocyte zinc was observed in any patient group. The mean plasma albumin concentration was significantly lower in all patient groups compared with the control subjects (Table). The mean plasma zinc concentration was significantly reduced in the biliary cirrhosis and alcoholic groups only (Table). The mean 24 hour

Table Plasma albumin, and plasma urinary, erythrocyte, and leucocyte zinc in control subjects and patients with liver disease

Patient group	Plasma albumin (g/l)	Plasma zinc ( $\mu$ g/ml)	Urinary zinc ( $\mu$ g/24h)	Erythrocyte zinc (mg/kg DW)	Leucocyte zinc (mg/kg DW)
Control subjects	43.3 $\pm$ 0.8 (27)	0.90 $\pm$ 0.03 (27)	663 $\pm$ 49 (18)	38.7 $\pm$ 1.4 (27)	71.9 $\pm$ 1.6 (27)
Alcoholic cirrhosis	33.1 $\pm$ 0.9 (20)†	0.64 $\pm$ 0.06 (20)†	1400 $\pm$ 138 (6)*	40.2 $\pm$ 2.2 (21)	60.8 $\pm$ 2.5 (21)†
Primary biliary cirrhosis	34.4 $\pm$ 1.6 (11)†	0.70 $\pm$ 0.03 (11)*	643 $\pm$ 113 (8)	38.1 $\pm$ 3.3 (10)	58.8 $\pm$ 5.3 (11)*
Active chronic hepatitis	38.9 $\pm$ 1.6 (14)*	0.78 $\pm$ 0.07 (13)	—	40.1 $\pm$ 2.2 (8)	56.4 $\pm$ 2.3 (14)†

Values are the mean  $\pm$  SEM, numbers of patients in parentheses.

\*  $P < 0.01$ .

†  $P < 0.001$ .

DW = dry weight.

urinary loss of zinc was significantly increased in patients with alcoholic cirrhosis, but not those with primary biliary cirrhosis (Table); urinary zinc was not measured in the active chronic hepatitis group.

Leucocyte zinc did not correlate with erythrocyte zinc, plasma, or urinary zinc, nor with plasma albumin. Nor was there a correlation between plasma albumin and plasma zinc within any group, but when the groups were combined a weak but significant correlation was found ( $r=0.51$ ,  $n=63$ ,  $P<0.001$ ).

## Discussion

The methods available for the assessment of zinc deficiency in man are unsatisfactory. The plasma pool of zinc is extremely small in relation to the total body pool, and is greatly influenced by changes in the concentration of the various plasma proteins to which it is bound. Biopsies of tissues such as liver and muscle allow a more direct estimation of the intracellular content of zinc, but specimens are seldom available. The problems associated with the interpretation of the zinc content of liver biopsy specimens have been mentioned.

In these studies we have measured erythrocyte and leucocyte zinc contents, plasma zinc, and albumin concentrations in a single blood sample. In none of the patient groups did the mean erythrocyte zinc differ from the control values, confirming previous reports that erythrocyte zinc does not change consistently in liver disease.<sup>9,8</sup> However, the results of leucocyte zinc content are of greater interest. In the three well-defined types of liver disease studied, leucocyte zinc was reduced by about 25%, although the pattern varied. Patients with active chronic hepatitis had the lowest mean values and showed little variability. In patients with alcoholic and primary biliary cirrhosis a much wider range of values for leucocyte zinc was seen.

As the majority of plasma zinc is bound to albumin and prealbumin, the weak correlation between the two values is surprising. However, similar findings have been reported previously, and it has been suggested that the capacity of albumin to bind zinc is altered in patients with cirrhosis.<sup>5,6,13,14</sup> Further, there may be alterations of other plasma constituents such as  $\alpha_2$  macroglobulin to which zinc is bound.<sup>13</sup>

The urinary zinc excretion in alcoholic and primary biliary cirrhosis is clearly different. Patients with alcoholic cirrhosis demonstrated a greatly increased zinc excretion, while zinc losses from those with primary biliary cirrhosis were normal, indicating that a high level of urinary zinc loss is not necessary for a reduction in leucocyte zinc content. The cause

of the increased urinary excretion of zinc in patients with alcoholic cirrhosis is, however, not explained.

These studies demonstrate that a reduced leucocyte zinc content is common in patients with liver disease and lends support to the suggestion that some of these patients are tissue zinc deficient. Leucocyte zinc can be lowered by experimental zinc deficiency in man,<sup>15</sup> and it may be that the leucocyte represents a useful tissue in which zinc status may be measured.

The leucocyte is not directly involved in liver damage, it has a rapid turnover rate, and samples can be safely and repeatedly obtained in the same patient, thus allowing changes in zinc status to be followed throughout the course of the disease and its treatment. It will be important to compare leucocyte zinc with concentrations in less easily obtained tissues, such as muscle and bone, which are also rich in zinc but whose zinc turnover is probably greatly different.

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