

Ascorbic acid deficiency in liver disease

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SUMMARY Leucocyte ascorbic acid (LAA) levels were measured in 138 patients with liver disease. Significantly reduced levels were found in 37 patients with alcoholic liver disease ($P < 0.01$) and 25 patients with primary biliary cirrhosis ($P < 0.05$). In the primary biliary cirrhosis patients, cholestyramine therapy was associated with significantly lower levels of the vitamin ($P < 0.05$). Liver ascorbic acid measured in Menghini needle biopsies in 20 patients was significantly correlated with LAA ($r = 0.807$, $P < 0.001$). No significant correlation was found between LAA and haematological indices, conventional liver function tests, or cholesterol levels in any group of patients. Patients with LAA levels below $100 \text{ nM}/10^8 \text{ WBC}$ had significantly higher antipyrine half-lives (mean = 28.3 h) than patients with LAA levels above this level (mean = 18.6 h) ($P < 0.05$). Delayed drug metabolism related to low LAA should be considered when drugs metabolised by the liver are prescribed for patients with alcoholic liver disease or primary biliary cirrhosis.

Ascorbic acid is a water soluble vitamin present in most plant foods especially citrus fruits, potatoes, and green vegetables. Dietary deficiency leads to scurvy and this discovery led to the classic work of Lind (1753) who showed that scurvy could be prevented in sailors by adding citrus fruits to their diet. It is now known that the vitamin is an organic acid whose properties are related to its facility to be oxidised reversibly to dehydroascorbic acid. Its physiological functions are varied and probably not fully worked out. It is generally believed that its oxidation-reduction system plays an important role in biological oxidations and reductions and in cellular respiration. La Du and Zannoni (1961) showed that it is involved in tyrosine metabolism. It is required for the conversion of folic acid to folinic acid. Goldberg (1963) showed that it is required for normal erythropoiesis. Ginter (1973) showed that guinea-pigs with chronic vitamin C deficiency had an impaired conversion of cholesterol to bile acids. Thus the effects of deficiency of the vitamin may be of considerable importance even although signs of scurvy are absent.

Several groups are known to have lower ascorbic acid levels than the general population. It is known that levels fall with age (Andrews and Brook, 1966). Women taking the contraceptive pill have slightly reduced levels (Horwitt *et al.*, 1975). O'Keane *et al.* (1972) showed that chronic alcoholics have reduced levels. The siderotic Bantu may develop scurvy and

it has been suggested that the iron overload may be a causal factor (Lynch *et al.*, 1967). Since alcoholism and hepatic siderosis are closely related to liver disease the present study was done to find out the prevalence of vitamin C deficiency in various types of liver disease and to investigate the possible pathophysiological effects in the deficient patients.

Methods

The 138 patients studied were all inpatients of the University Medical Unit, Royal Free Hospital, London. All suffered from liver disease and the numbers with each diagnosis are shown in Table 1. The control group consisted of members of staff and patients with non-hepatic disease not known to be related to ascorbic acid deficiency. The age range and sex distribution of the two groups were similar.

Leucocyte ascorbic acid measurement was done on each patient by the method of Denson and Bowers (1961). Ascorbic acid measurement was done on Menghini needle biopsies of the liver in 20 patients using a modification of the method of Denson and Bowers (1961) for leucocyte ascorbic acid. Biopsies of more than 10 mg were considered sufficient. The liver tissue was dried, weighed, and thoroughly homogenised in 1.3 ml 5% trichloroacetic acid. The homogenate was centrifuged at 3000 rpm for five minutes and 1 ml supernatant incubated for four hours with 0.3 ml 2.2% (w/v) 2,4-dinitrophenylhydrazine in 10 M H_2SO_4 as described in the leucocyte method. The results were expressed

Table 1 Leucocyte ascorbic acid levels (mean \pm SEM) in 138 patients with liver disease and 28 normal controls

Diagnosis	Number of patients	Leucocyte ascorbic acid nM/10 ⁸ WBC mean \pm SEM	Significance from control value
Control	28	161.8 \pm 6.8	—
Alcoholic liver disease	37	124.3 \pm 7.4	$P < 0.01$
Primary biliary cirrhosis	25	130.0 \pm 10.2	$P < 0.05$
Acute hepatitis	12	165.8 \pm 44.9	NS
Chronic active hepatitis	19	179.4 \pm 15.3	NS
Cryptogenic cirrhosis	13	190.8 \pm 24.4	NS
Extrahepatic cholestasis	13	140.2 \pm 22.1	NS
Extrahepatic portal hypertension	6	173.2 \pm 27.3	NS
Gilbert's disease	4	151.6 \pm 9.1	NS
Wilson's disease	3	189.6 \pm 48.3	NS
Drug-induced cholestasis	3	186.2 \pm 26.7	NS
Budd-Chiari syndrome	1	84.0	NS
Carcinoid syndrome	1	154.4	NS
Congenital hepatic fibrosis	1	144.2	NS

LAA = leucocyte ascorbic acid. Significance is by Student's *t* test between control and each group of patients.

in nM ascorbic acid/g liver tissue. Recovery experiments done with 10 to 20 μ g amounts of normal rat liver gave a mean recovery of 96% in 12 specimens.

Peripheral blood haemoglobin, mean corpuscular volume, leucocyte count, and platelet count were done on an electronic counter in the haematology department. Serum bilirubin, aspartate transaminase, cholesterol, iron, iron binding capacity, and folate were likewise done by conventional methods in the routine departments.

Plasma antipyrine (phenazone, BPC) half lives were done on 20 patients and four controls. No patient was receiving a drug known to induce liver enzymes and no control subject was receiving any drug at all. Each patient and control received 1.2 g antipyrine orally and blood samples were taken at approximately 2.5, four, six, eight, 12, and 24 hours. After separation of the plasma, the samples were stored at -20°C and the antipyrine concentration was measured in batches by the method of Brodie *et al.* (1949). The $T_{\frac{1}{2}}$ of antipyrine disappearance was calculated by regression analysis.

In the *in vitro* experiment to measure binding of cholestyramine to ascorbic acid, 50 mg cholestyramine were added to 1 ml amounts of aqueous solutions of ascorbic acid in 14.2 M, 28.4 M, and 56.8 M concentrations. The mixture was centrifuged at 3000 rpm for five minutes and the ascorbic acid content of the clear supernatant measured as in the leucocyte method.

The daily dietary intake of ascorbic acid was estimated in the patients with primary biliary cirrhosis according to the dietary equivalent tables in Davidson *et al.* (1972). The statistical methods

used were Student's *t* test and the coefficient of correlation.

Results

The mean LAA in the control group was 161.8 nM/10⁸ WBC (SEM \pm 6.8). Overall, the patients with liver disease had a mean of 149.9 nM/10⁸ WBC (\pm 7.4). The mean LAA (\pm SEM) for individual groups of patients is shown in Table 1. Significant reductions in LAA were found in 37 patients with alcoholic liver disease (124.3 \pm 7.4 nM/10⁸ WBC, $P < 0.01$) and 25 patients with primary biliary cirrhosis (130.0 \pm 10.2 nM/10⁸ WBC, $P < 0.05$). No other group differed significantly from control levels.

The 20 patients in whom liver ascorbic acid levels were measured had a mean LAA of 135.0 nM/10⁸ WBC and a mean liver level of 0.65 μ M/g tissue (Figure). A very highly significant correlation was found between the two measurements ($r = 0.81$, $P < 0.001$). The individual results and diagnoses are shown in Table 2.

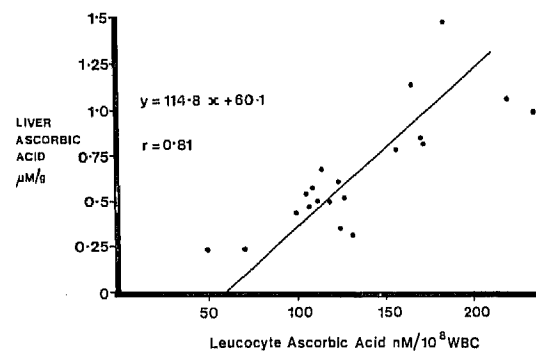


Figure Relationship between LAA and liver ascorbic acid in 20 patients with liver disease. LAA = leucocyte ascorbic acid.

No significant correlation was found between LAA and platelet count ($n = 86$, $r = 0.11$), serum iron ($n = 22$, $r = 0.05$), transferrin saturation ($n = 20$, $r = 0.01$), or serum folate ($n = 17$, $r = 0.44$). A significant negative correlation was present between LAA and mean corpuscular volume ($n = 95$, $r = -0.45$, $P < 0.01$). No significant correlation was found between LAA and serum cholesterol in 20 patients with alcoholic liver disease ($r = 0.02$) or in 20 patients with primary biliary cirrhosis ($r = -0.09$).

Of the 37 patients with alcoholic liver disease, 21 had LAA levels of less than 113 nM/10⁸ WBC. This

Table 2 Diagnosis, leucocyte ascorbic acid level, and liver ascorbic acid concentration in 20 patients with liver disease.

Diagnosis	LAA nM/10 ⁸ WBC	Liver ascorbic acid (μ M/g)
Alcoholic liver disease	131.2	0.34
Alcoholic liver disease	49.4	0.22
Alcoholic liver disease	233.9	0.99
Alcoholic liver disease	107.9	0.62
Alcoholic liver disease	127.7	0.51
Alcoholic liver disease	111.8	0.49
Alcoholic liver disease	106.7	0.47
Alcoholic liver disease	69.2	0.23
Alcoholic liver disease	124.9	0.34
Alcoholic liver disease	115.8	0.49
Alcoholic liver disease	156.7	0.78
Primary biliary cirrhosis	123.2	0.60
Primary biliary cirrhosis	105.0	0.54
Primary biliary cirrhosis	113.4	0.68
Primary biliary cirrhosis	98.8	0.43
Chronic active hepatitis	164.1	1.13
Chronic active hepatitis	219.7	1.06
Extrahepatic biliary obstruction	181.1	1.48
Extrahepatic biliary obstruction	170.3	0.82
Congestive cardiac failure	169.8	0.84

LAA = leucocyte ascorbic acid.

figure is 2 SD below the control mean and may be regarded as the lower limit of normal. No significant difference was found in serum bilirubin, serum aspartate transaminase, serum cholesterol, serum albumin, or prothrombin time between this group and the 16 patients with normal LAA levels. Eighteen of the 37 patients with alcoholic liver disease had biopsy evidence of hepatic cirrhosis. The mean LAA in these patients was 129.4 (\pm 17.6) nM/10⁸ WBC compared with 119.8 (\pm 15.3) nM/10⁸ WBC in the 19 patients with non-cirrhotic alcoholic liver disease. This difference is not significant.

LAA levels in the 25 patients with primary biliary cirrhosis did not show a significant correlation with conventional liver function tests. Ten of these patients had levels of less than 113 nM/10⁸ WBC. The mean plasma caeruloplasmin levels and mean daily dietary intakes of ascorbic acid are shown in Table 3. The patients with low ascorbic acid levels did not have dietary intakes that were significantly

different from those with high levels of the vitamin. Nine of the 25 patients with primary biliary cirrhosis were receiving cholestyramine therapy daily in a dose ranging from one to four sachets daily. The mean LAA in the group receiving cholestyramine was 106.2 (\pm 11.4) nM/10⁸ WBC, whereas the patients not receiving this drug had a mean LAA of 143.1 (\pm 16.5) nM/10⁸ WBC. This is a significant difference ($p < 0.05$).

When 50 mg cholestyramine were added *in vitro* to 1 ml amounts of aqueous solutions of ascorbic acid in 14.2 M, 28.4 M, and 56.8 M concentrations respectively, the mean recovery of ascorbic acid was 97.3%. This indicates that binding of ascorbic acid *in vitro* at the pH of the solution (4.5) did not take place.

Antipyrine half-life in four control subjects was 12.8 (\pm 0.9) h and in 20 patients with liver disease was 20.6 (\pm 3.8) h. There was no significant correlation with the LAA level. When Student's *t* test was performed on groups of patients above and below a given LAA value, however, the trend towards significance increased as the dividing level was reduced. Thus, when the group was divided at 142 nM/10⁸ WBC the *t* value was 0.88 ($p < 0.2$); at 113 nM/10⁸ WBC, the *t* value was 1.89 ($p < 0.1$); and at 100 nM/10⁸ WBC the *t* value was 2.40 ($p < 0.05$). The mean antipyrine half-life of the four patients with LAA levels less than 100 nM/10⁸ WBC was 28.3 h and of the 16 patients above this level was 18.6 h.

Discussion

The deficiency of vitamin C in patients with alcoholic liver disease substantiates the work of O'Keane *et al.* (1972). They showed that alcoholics, whether with or without liver disease, had significantly reduced levels which correlated with the dietary intake of the vitamin. Another possible explanation for the finding of reduced levels is the effect of siderosis. Lynch *et al.* (1967) showed an accelerated oxidative catabolism of ascorbic acid in siderotic Bantu patients. In the present study there was no correlation between LAA

Table 3 Plasma caeruloplasmin and dietary ascorbic acid intake in patients with primary biliary cirrhosis: A in the group as a whole; B in patients with LAA < 113 nM/10⁸ WBC; C in patients with LAA > 113 nM/10⁸ WBC.

Measurement	No of observations	A Mean in PBC group as a whole	B Mean in patients with LAA < 113 nM/10 ⁸ WBC	C Mean in patients with LAA > 113 nM/10 ⁸ WBC	Significance between B and C
Serum caeruloplasmin (mg/l)	9	499 \pm 47	531 \pm 76	474 \pm 63	NS
Daily dietary ascorbic acid (mg/day)	21	277 \pm 33	160 \pm 67	252 \pm 37	NS

LAA = leucocyte ascorbic acid.

levels and either serum iron or transferrin saturation. Neither of these is as accurate a measurement of siderosis as the serum ferritin or liver iron but it seems unlikely from our results that siderosis is a major cause of ascorbate deficiency in this group of patients.

The low levels of LAA in primary biliary cirrhosis were unexpected and three possible explanations were studied. Firstly, the diet might be deficient. Dietary ascorbic acid was lower in the deficient group but not significantly so, and this led us to look at alternative causes. Secondly, we considered the suggestion of Briggs and Briggs (1972) that women on the contraceptive pill have lower ascorbate levels because of the ascorbate reductase activity of caeruloplasmin (Niedermeier *et al.*, 1967). However, no correlation could be found between LAA levels and caeruloplasmin levels, which suggested that this was not an important cause of the LAA deficiency. The third possibility was that cholestyramine, which nine of the patients were taking because of itching, was binding ascorbate in the gut. This hypothesis appeared to be supported by the finding of significantly lower LAA levels (mean = 106.2 nM/10⁸ WBC) in the group receiving cholestyramine in comparison with the other primary biliary cirrhotic patients (mean 143.1 nM/10⁸ WBC). West and Lloyd (1975) showed that cholestyramine was associated with a reduction in serum folate levels in children and it seems likely that ascorbic acid is similarly affected by the ion exchange resin. The binding could not be confirmed *in vitro* but this may be due to failure to reproduce the ion exchange conditions which exist in the small intestine.

The hepatic levels of ascorbic acid had a significant correlation with leucocyte levels of the vitamin. This is of some importance, since many of the metabolic actions of ascorbic acid take place in the liver. Since liver levels cannot be easily measured, it is useful to know that they are fairly closely reflected in the peripheral blood.

The possible effects of ascorbic acid deficiency are so varied that only some of them could be considered. Since the conversion of folic acid to folinic acid is facilitated by ascorbic acid, a macrocytic anaemia might be expected to result from deficiency of the vitamin and, indeed, Goldberg (1963) showed that this is so. In our patients there was a significant correlation between LAA and mean corpuscular volume, but the data were not sufficient to show whether this was a separate effect of ascorbic acid deficiency or due to concomitant folate deficiency. Although the folate levels did not correlate in the small number of patients tested, there was a suggestive trend ($r = 0.447$, $P < 0.1$) which probably

reflects the partly shared dietary origin of the two substances.

The relationship between serum cholesterol and LAA was first shown by Myasnikov (1950). Since then, there have been several conflicting reports but most authors have found a reduction in cholesterol levels when high doses of ascorbic acid are given to normal subjects (Sokoloff *et al.*, 1967; Ginter *et al.*, 1970). Ginter (1973) showed that conversion of cholesterol to bile salts is delayed in scorbutic guinea pigs and he has suggested that ascorbic acid may be necessary for the formation of bile salt micelles which keep cholesterol dissolved in bile. Thus, ascorbic acid deficiency might enhance gall-stone formation. Pedersen (1975) has shown that ascorbic acid in high doses has no effect on biliary lipid composition in normal subjects, but a similar study on patients who are deficient in ascorbic acid might be more rewarding. In the present study there was no correlation between LAA and serum cholesterol in any group but, in view of the effects of cholestasis on serum cholesterol in liver disease, this absence of correlation is not necessarily meaningful.

Delayed drug metabolism has been a known feature of both ascorbic acid deficiency and of impaired liver function for several years. Richards (1941) showed that pentobarbitol sleeping time was prolonged in scorbutic guinea pigs compared with normal controls. Zannoni *et al.* (1972) showed significant impairment of drug oxidation when hepatic microsomal ascorbic acid had reached 30% of control values in guinea pigs. Dow *et al.* (1975) found a significant correlation between LAA and alcohol dehydrogenase in patients with liver disease. Several authors have demonstrated the prolonged elimination of drugs in liver disease (Levi *et al.*, 1968; Mawer *et al.*, 1972; Hvidberg *et al.*, 1974; Klotz *et al.*, 1974). In some of these reports significant correlations have been found with serum albumin levels, while in others this relationship has not been found. Branch *et al.* (1973) showed prolonged antipyrine half lives in patients with liver disease. In this study we have investigated the possibility that ascorbic acid deficiency is a causal factor in the prolonged drug metabolism of liver disease. From our results it seems that LAA levels below 100 nM/10⁸ WBC are likely to be associated with increased drug half life, although prolonged half-life in patients with normal LAA levels suggests that ascorbic acid is only one of several factors involved.

The possibility of ascorbic acid deficiency should be considered in patients with alcoholic liver disease and primary biliary cirrhosis, especially when the diet is poor or when cholestyramine is prescribed.

When ascorbic acid deficiency is diagnosed, drugs metabolised by hepatic enzymes should be prescribed with caution and possibly in lower dosage until the deficiency is corrected.

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