Ascorbic acid status in iron-deficiency anaemia

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SYNOPSIS  Leucocyte ascorbic acid concentration declines with age. Patients with iron-deficiency anaemia have higher concentrations than normal while those with iron overload have a reduced concentration. It is suggested that these phenomena may be a result of reduced ascorbate catabolism in iron-deficiency anaemia and they provide support for the suggestion that the amount of iron in the tissues may be an important factor in determining ascorbic acid utilization.

It has been suggested that ascorbic acid may be involved in the release of iron from ferritin (Mazur, Baez, and Shorr, 1955) and in iron uptake by ferritin from the plasma (Mazur, Green, and Carleton, 1960). Further evidence that ascorbic acid is important as an intermediary in iron metabolism is its ability to promote the availability of storage iron for chelation by desferrioxamine (Wapnick, Lynch, Charlton, Seftel, and Bothwell, 1969) and to increase serum iron levels in subjects with iron overload (Wapnick, Bothwell, and Seftel, 1970). Lynch, Seftel, Torrance, Charlton, and Bothwell (1967) have suggested that iron overload may result in an accelerated catabolism of ascorbic acid and thus contribute to the scurvy state found in many siderotic Bantu. If ascorbic acid status is affected by the magnitude of iron turnover in the body then iron-deficient subjects, in whom ascorbic acid utilization for this purpose would be reduced to a minimal level, might be expected to show higher levels of ascorbic acid in the tissues. The present study investigates this possibility by assessing ascorbic acid status in normal, iron-deficient, and iron-loaded subjects. Leucocyte concentrations of ascorbic acid have been used as an index of vitamin status and plasma levels have also been measured.

Subjects

Forty-two normal subjects aged between 18 and 87 years old were examined. There were 20 men and 22 women. Their haemoglobin concentrations were within the range 12·2 to 16·6 g per 100 ml (mean ± SE, 14·7 ± 0·17). Serum iron concentration in these subjects was between 65 and 190 μg per 100 ml (mean ± SE, 118·3 ± 5·95). Twenty-one subjects with iron-deficiency anaemia aged between 17 and 79 years were examined. Haemoglobin concentrations were between 5·3 and 11·5 g per 100 ml (mean ± SE, 8·5 ± 0·40). Serum iron levels were between 10 and 60 μg per 100 ml (mean ± SE, 22·1 ± 2·11). Nine patients with iron overload aged 33 to 77 years old were examined. Two of these were cases of primary haemochromatosis and seven were cases of hypoplastic anaemia with secondary haemosiderosis. Haemoglobin concentrations in this group ranged between 4·4 and 13·2 g per 100 ml (mean ± SE, 9·8 ± 1·72). Serum iron concentrations were between 165 and 300 μg per 100 ml except in one case with a serum iron level of 60 μg per 100 ml. In this particular instance the total iron-binding capacity was 90 μg per 100 ml. Confirmation of the iron-loaded state of these patients was obtained by visual examination of bone marrow sections stained with Perls' reagent and by estimation of the urinary iron excretion after two injections of 500 mg desferrioxamine one hour apart. The 24-hour urinary iron excretion following these injections ranged from 1·8 to 23·0 mg, the normal range being 0·5 to 1·5 mg.

Methods

Standard haematological methods were used (Dacie and Lewis, 1968). Plasma ascorbic acid concentration was measured using dichlorophenolindophenol (Varley, 1962). Leucocyte ascorbic acid levels were measured by the method of Denson and Bowers (1961). The precipitation of plasma protein and the separation of supernatant was carried out immediately following venepuncture and the separation of white cells by sedimentation was also done im-
mediately. Serum iron concentrations were determined by the method of Young and Hicks (1965) and the total iron-binding capacity of serum by the method of Ramsay (1957). Urinary iron was measured by the method of Tennant and Greenman (1969). All samples of blood were taken between 9 am and 11 am. None of the subjects were taking haematinics or vitamin preparations.

**Results**

In normal subjects plasma ascorbic acid concentration showed a negative correlation with age \( r = -0.56; P < 0.001 \). Leucocyte ascorbic acid concentration showed a similar correlation (Fig. 1) \( r = -0.48; P < 0.001 \). There was a correlation of plasma and leucocyte ascorbic acid concentrations in the same subjects \( r = 0.68; P < 0.001 \). No significant sex difference in ascorbic acid levels was found.

In patients with iron-deficiency anaemia there was no correlation of either plasma or leucocyte ascorbic acid concentrations with age and no correlation between plasma and white cell concentrations in the same subjects. Leucocyte concentrations were generally higher than in normal subjects of a similar age (Fig. 2). The mean (± SE) leucocyte concentration of ascorbic acid in iron-deficient patients (55.38 ± 4.26 µg per 10⁸ cells) was significantly higher than the mean value in normal subjects corrected for age to 20 years which was 38.57 ± 1.63 µg per 10⁸ cells \( (P < 0.001) \). The mean (± SE) plasma ascorbic acid level in normal subjects corrected for age to 20 years was 0.83 ± 0.154 mg per 100 ml which was not significantly different from the mean concentration of 0.59 ± 0.07 mg per 100 ml in patients with iron deficiency.

The patients with iron overload were mainly older than the normal group and there was no apparent age correlation in these subjects. Both plasma and leucocyte ascorbic acid levels were lower than in normal subjects of a similar age. The mean (± SE) plasma level of 0.26 ± 0.077 mg per 100 ml was significantly lower than in normal subjects corrected for age to 20 years \( (P < 0.005) \). The mean leucocyte ascorbic acid concentration of 22.22 ± 2.99 µg per 10⁸ leucocytes was also significantly lower than the corresponding normal value corrected for age \( (P < 0.001) \). There is a significant correlation in the iron-loaded subjects between plasma and leucocyte ascorbic acid levels \( (r = 0.71, P < 0.025) \). Leucocyte
Ascorbic acid levels are shown in Figure 2.

There was no correlation between age and serum iron concentration or transferrin saturation in normal subjects. When all three groups of subjects are considered together those with low serum iron concentrations tend to have higher levels of leucocyte ascorbic acid and those with high serum iron concentrations tend to have low levels of leucocyte ascorbic acid (Fig. 3) \( r = 0.52; p < 0.001 \). A significant correlation between these two parameters is also found when only normal subjects and those with iron deficiency are considered \( r = -0.50; p < 0.001 \). There was no significant correlation between serum iron and plasma ascorbic acid concentrations.

**Discussion**

Leucocyte ascorbic acid concentration is a sensitive index of ascorbic acid status. In guinea pigs leucocyte concentration closely parallels tissue saturation (Chevillard and Hamon, 1943) and in human subjects who develop ascorbic acid deficiency clinical scurvy occurs shortly after leucocyte levels have been depleted (Bartley, Krebs, and O’Brien, 1953).

Ascorbic acid in the plasma is rapidly taken up by leucocytes against a concentration gradient (Denson and Bowers, 1961) and when leucocyte concentrations are low both body stores and circulating plasma levels have been reduced to a minimum. The leucocytes provide a better index of tissue ascorbic acid saturation than the plasma, and special attention has been given to leucocyte concentrations in the present study.

Brook and Grimshaw (1968) found a significant fall in plasma ascorbic acid concentration with age in both sexes. Our results show a similar regression in normal individuals but this is not evident in subjects with iron-deficiency anaemia. We also found a significant decrease in leucocyte ascorbic acid concentration with age in normal subjects though not in patients with iron deficiency. In iron-deficient patients leucocyte ascorbic acid concentrations are significantly increased. Patients with iron overload have decreased leucocyte concentrations but there is no apparent relationship to the amount of stored iron as indicated by the iron excretion after desferrioxamine.

In nine of the present patients with iron-deficiency anaemia it was possible to re-estimate leucocyte...
ascorbin acid concentrations following a four to six weeks' course of oral iron therapy. It was not possible to standardize treatment rigidly but all cases showed a haematological response. Eight of the nine cases showed a 10 to 54% decrease in leucocyte ascorbic acid. In one case there was a 4% increase. Repeated estimations carried out in two subjects suggest that the fall in leucocyte ascorbic acid levels does not occur immediately after the institution of treatment but takes place gradually after about two weeks and may be related to the re-establishment of iron stores.

The transfer of iron from plasma to tissue ferritin is facilitated by the presence of ascorbic acid (Mazur et al, 1960). The release of iron from ferritin is thought to be associated with its reduction from the ferric to the ferrous state and ascorbic acid is also involved in this process (Mazur et al, 1955). Studies in vitro have shown that ascorbic acid may undergo irreversible oxidation when large amounts of ferritin iron are present. Lynch et al (1967) showed that in Bantu with gross iron overload urinary ascorbic acid excretion was reduced and plasma clearance of an intravenous dose of ascorbic acid was more rapid than in normal subjects, the findings being consistent with a scorbetic state. Oxalic acid is a breakdown product of ascorbic acid and in siderotic subjects there was a higher excretion of this metabolite than in normal subjects. Evidence of vitamin C depletion and increased utilization remained after a prolonged course of parenteral ascorbic acid. It was concluded by these workers that the vitamin was being catabolized at an increased rate.

It appears that ascorbic acid is involved whenever there is an exchange of iron between the storage compartment and the other metabolic compartments in the body and its utilization is likely to be largely determined by the magnitude of ferritin iron turnover. In iron-deficiency anaemia storage iron is absent, transferrin-bound iron is reduced in amount, and it is unlikely that any of the residual iron in the body will be diverted to ferritin formation. In the present iron-deficient subjects the high concentrations of ascorbic acid present could be the result of a reduced catabolic rate and they provide supporting evidence for the suggestion that the amount of iron present in the tissues may be an important factor in determining ascorbic acid utilization.

References