Reassessment of changes in leucocyte and serum ascorbic acid after acute myocardial infarction

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After an acute myocardial infarction, there is an apparent acute fall in leucocyte ascorbic acid associated with an acute rise in white blood cells and serum cortisol. The apparent fall in leucocyte ascorbic acid is the result of the granulocytosis which occurs after the infarction. Estimations of ascorbic acid disclose that the granulocyte contains approximately half the ascorbic acid of the lymphocyte. When the granulocytosis subsides, the new population of white blood cells is depleted of ascorbic acid for at least 56 days, reflecting tissue desaturation which can be corrected by ascorbic acid supplements. Tissue desaturation is also reflected in subnormal serum ascorbic acid levels which persist also unless ascorbic acid supplements are given. Observations on normal subjects given infusions of tetracosactrin (Synacthen) show that adrenal stimulation can produce a similar rise in white blood cells and an apparent fall in leucocyte ascorbic acid concentration with the exception that the serum ascorbic acid remains unaltered. Therefore, while adrenal stimulation can mimic 'stress' with regard to the changes in the white blood cells, tissue depletion of ascorbic acid as reflected in the white blood cells and serum after a myocardial infarction requires a focus of damaged tissue.

It has previously been shown that after infection by the common cold virus (Hume and Weyers, 1973) or after an acute myocardial infarction (Hume *et al.*, 1972) there is a profound fall in leucocyte ascorbic acid within hours of the onset of symptoms, and that the return of leucocyte ascorbic acid towards normal levels occurs only after the cessation of the acute symptoms. Furthermore, it has been shown that administration of ascorbic acid to patients before infection with the common cold virus does not prevent this fall in leucocyte ascorbic acid (Hume and Weyers, 1973).

The leucocytosis of myocardial infarction is significantly correlated with an increase in serum cortisol (Bailey *et al.*, 1967). Because the administration of cortisone can itself cause a leucocytosis, it has been suggested that the fall in leucocyte ascorbic acid after infarction may be controlled by the adrenal gland (Hume *et al.*, 1972).

It seems important, therefore, to reassess these changes in patients who have sustained an acute myocardial infarction, to try to determine the role of the adrenal gland, and to explain the apparently obligatory fall in leucocyte ascorbic acid. Opportunity was also taken to observe the changes in

serum ascorbic acid and the effect of ascorbic acid supplements on these changes.

Patients and methods

All the patients and volunteers studied gave their informed consent.

MYOCARDIAL INFARCTION PATIENTS

Twelve patients (7 men with a mean age of 56.4 ± 7.3 years and 5 women with a mean age of 61.2 ± 10.3 years) who had sustained an acute myocardial infarction were, within 24 hours of admission to hospital, randomly allocated to either a supplemented or unsupplemented group. Both groups had routine intravenous infusions set up on admission. The patients who were allocated to the supplemented group were given by this route 1 g ascorbic acid every 4 hours for 48 hours after which they were started on 1 g ascorbic acid orally twice daily. The unsupplemented group received a placebo tablet twice daily starting 48 hours after admission. Venous blood samples were taken for the estimation of leucocyte ascorbic acid, serum ascorbic acid, plasma cortisol, and total white blood cell count within 12 hours of admission,

at 9.00 a.m. thereafter on the following 5 consecutive days, on the 9th day, and then on the 56th day.

TETRACOSACTRIN INFUSION STUDY
Six healthy volunteer subjects were infused over a period of 4 hours with tetracosactrin 0·5 mg diluted in 500 ml normal saline. Venous blood samples were taken for estimation of leucocyte ascorbic acid, serum ascorbic acid, plasma cortisol, and total white blood cell count before starting the infusion, and at 2 hours, 4 hours, 5 hours, and 6 hours thereafter. The same group of volunteers then received 1 g ascorbic acid twice daily for 2 weeks. The infusion was then repeated in the same manner and the same observations made.

ASCORBIC ACID CONTENT OF WHITE BLOOD CELLS
Buffy layer samples were obtained from 11 normal subjects and the ascorbic acid content of the granulocytes, lymphocytes, and platelets was estimated separately. The different cells and platelets were separated by the method based on Kissmeyer-Neilsen and Kjerbye (1967).

LEUCOCYTE ASCORBIC ACID
Leucocyte ascorbic acid was measured by the method of Bessey *et al.* (1947) as modified by Denson and Bowers (1961), which gives a mean normal value (24 observations) of 138 ± 49 nmol/10^8WBC.

SERUM ASCORBIC ACID
Serum ascorbic acid was measured by the method of Denson and Bowers (1961) which gives a mean normal value (24 observations) of 52 ± 23 μmol/l.

PLASMA CORTISOL
Plasma cortisol (11-hydroxycorticoids) was measured by the method of Mattingly (1962) which gives a normal range of 140 to 390 nmol/l.

TOTAL WHITE BLOOD CELL COUNT
Total white blood cell counts were performed by a Coulter Counter, normal range 4 to 10×10^9/l.

Results

MYOCARDIAL INFARCTION PATIENTS
Unsupplemented group
The mean values of the observations on 6 patients are shown in Fig. 1. The peak white blood cell count ($14 \cdot 1 \pm 5 \cdot 8 \times 10^9$/l) is associated with a profound fall in the leucocyte ascorbic acid to 35 ± 11 nmol/

10^8WBC and in the serum ascorbic acid to 19 ± 17 μmol/l, levels that are well below the normal range (138 ± 49 nmol/10^8WBC, 52 ± 23 nmol/l, respectively), with a peak rise in the plasma cortisol level to 572 ± 147 nmol/l (normal plasma cortisol level being 140 to 390 nmol/l). The leucocyte ascorbic acid and the serum ascorbic acid levels remained subnormal throughout the period of the 56 days of the study. In some instances the serum ascorbic acid was unrecordable. The leucocyte ascorbic acid started to rise when the white blood cell count began to return to normal and this coincided with the decline in the cortisol level.

Supplemented group
The mean values of the observations on 6 patients are shown in Fig. 2. The pre-ascorbic acid infusion levels of leucocyte ascorbic acid (42 ± 18 nmol/10^8WBC), serum ascorbic acid (13 ± 12 μmol/l), plasma cortisol (728 ± 452 nmol/l), and the white blood cell count ($12 \cdot 2 \pm 2 \cdot 5 \times 10^9$/l) were similar to the unsupplemented group. However, on this occasion, the serum ascorbic acid rose to high normal levels (139 ± 100 μmol) within 48 hours and remained so throughout the period of study as indeed did the leucocyte ascorbic acid levels (201 ± 61 nmol/10^8WBC) after the white blood cell count had returned to normal.

TETRACOSACTRIN INFUSION STUDY
The mean values of the observations on 6 subjects infused with tetracosactrin are shown in Fig. 3.
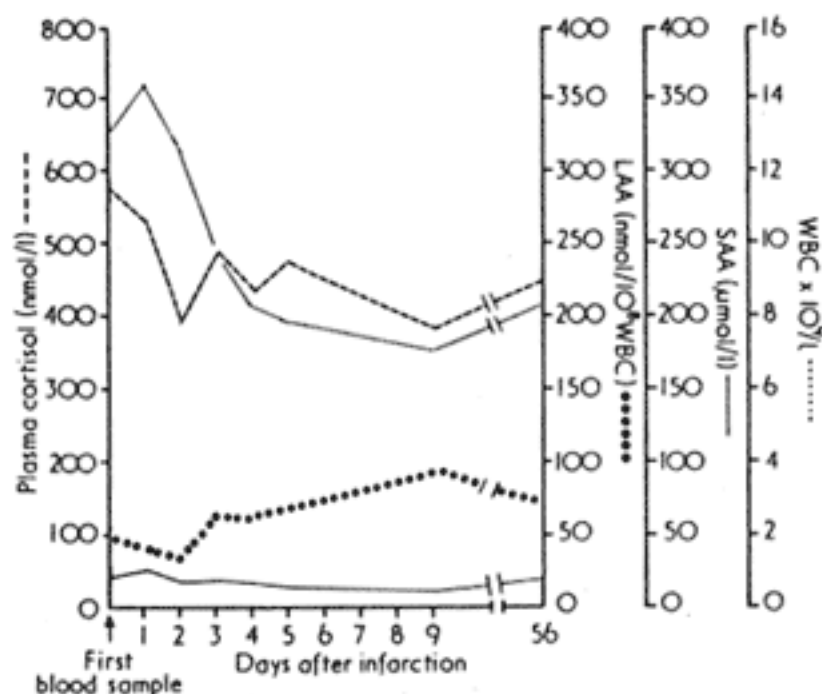

Fig. 1 *Acute myocardial infarction. Mean values of 6 patients unsupplemented with ascorbic acid. LAA, leucocyte ascorbic acid; SAA, serum ascorbic acid; WBC, white blood cells.*

It can be seen that the pattern is not dissimilar to that after an acute myocardial infarction, namely the plasma cortisol level rose steeply to a peak (1876 ± 401 nmol/l) and there was a corresponding rise in the white blood cell count ($11 \cdot 7 \pm 1 \cdot 9 \times 10^9$/l) associated with a corresponding fall in the leucocyte ascorbic acid (67 ± 37 nmol/10^8WBC) to levels outwith the normal range. However, on this occasion, there was no change in the serum ascorbic acid levels during the study period. Supplements of ascorbic acid 1 g twice daily for two weeks did not change this pattern (Fig. 4) though the initial leucocyte ascorbic acid (206 ± 82 nmol/10^8WBC) and serum ascorbic acid (147 ± 40 μmol/l) were set at a much higher level. The serum ascorbic acid, on this occasion, fell to 99 ± 22 μmol/l. It should be noted that the fall in the serum ascorbic acid, which did not reach significant levels in the supplemented group, is a reflection of the renal excretion of the ascorbic acid which the subjects had ingested before the test. Studies on 4 subjects showed that tetracosactrin did not alter the renal handling of ascorbic acid.

ASCORBIC ACID CONTENT OF GRANULOCYTES, LYMPHOCYTES, AND PLATELETS

The ascorbic acid content of the granulocytes, lymphocytes, and platelets obtained from 11 normal subjects is shown in the Table. It was found that there was significantly less ascorbic acid in the granulocytes (1323 ± 949 nmol/l) compared with the lymphocytes (2449 ± 910 nmol/l), the ratio being $1 : 1 \cdot 9$ ($t = 2 \cdot 8390$, $P < 0 \cdot 01$), and that the

ascorbic acid content of the platelets (2078 ± 875 nmol/l) was intermediate between the granulocytes and the lymphocytes.
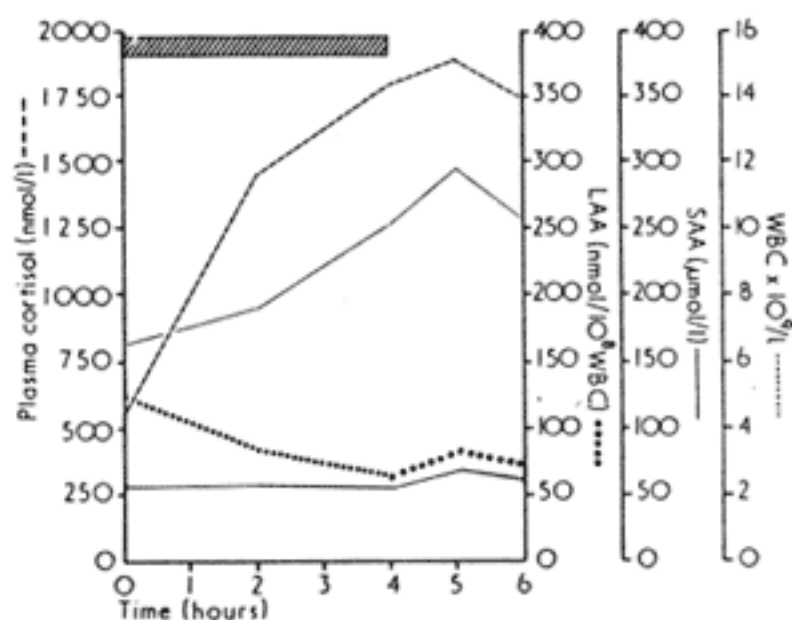
Fig. 3   *Normal subjects infused with tetracosactrin. Mean values of 6 responses, unsupplemented with ascorbic acid. Cross-hatched area represents duration of infusion. LAA, leucocyte ascorbic acid; SAA, serum ascorbic acid; WBC, white blood cells.*
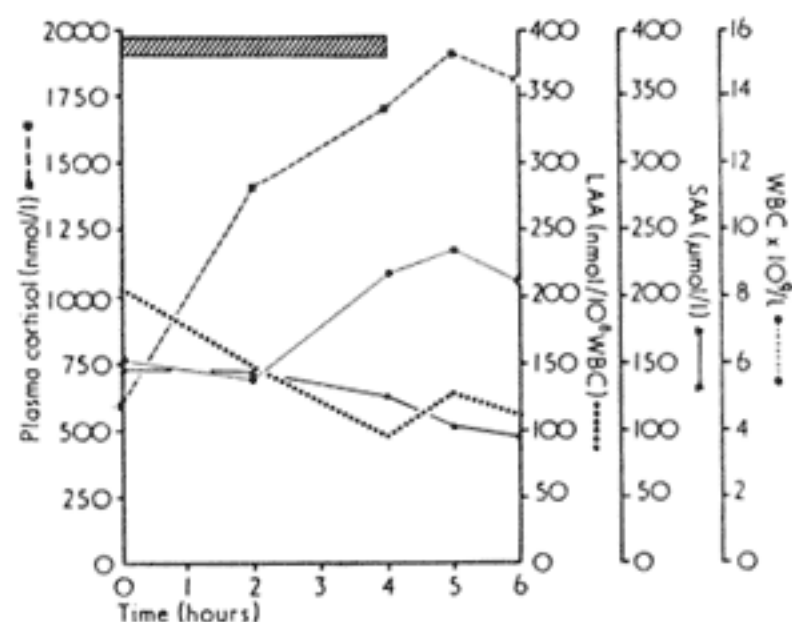
Fig. 4   *Normal subjects infused with tetracosactrin. Mean values of 6 responses, supplemented with ascorbic acid. Cross-hatched area represents duration of infusion. LAA, leucocyte ascorbic acid; SAA, serum ascorbic acid; WBC, white blood cells.*
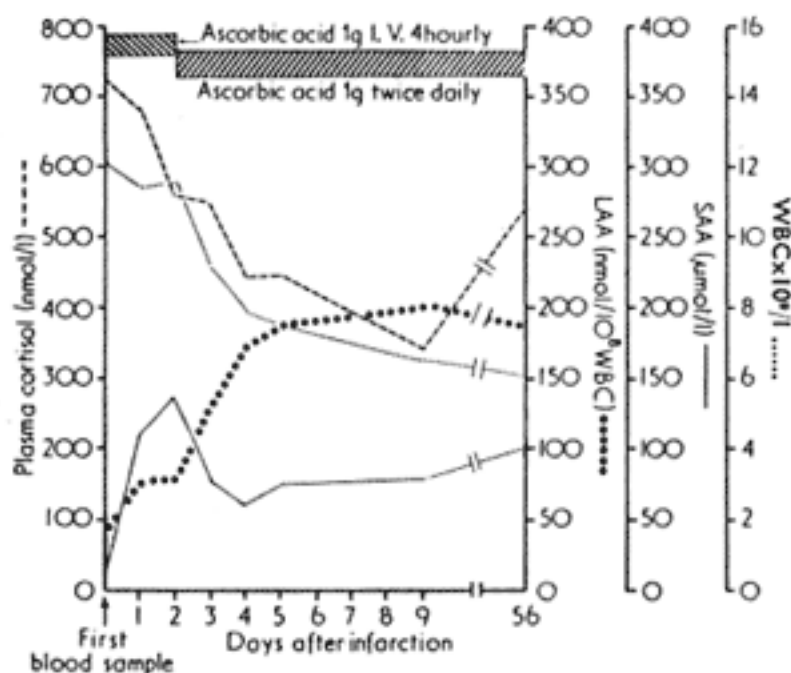
Fig. 2   *Acute myocardial infarction. Mean values of 6 patients supplemented with ascorbic acid. LAA, leucocyte ascorbic acid; SAA, serum ascorbic acid; WBC, white blood cells.*

Table   *Ascorbic acid content of white blood cells and platelets*

	Buffy layer (nmol/l)	White blood cells (nmol/l)	Granulocytes (nmol/l)	Lymphocytes (nmol/l)	Platelets (nmol/l)
Mean n = 11	5832·5 ±1279·5	3761·6 ±976·5	1323·2 ±949·0	2448·8 ±909·8	2077·6 ±874·5

Discussion

These results confirm the previous observation that after an acute myocardial infarction there is an acute fall in leucocyte ascorbic acid associated with an acute rise in the total white blood cell count (Hume *et al.*, 1972). Both these events can be simulated by adrenal stimulation by tetracosactrin in normal subjects. It is recognised that cortisone can stimulate the bone marrow to produce more granulocytes and that cortisone and adrenaline can mobilise the granulocytes from the marginated pool (Vincent, 1974), that is, the granulocytes which are adherent to venules and trapped in capillaries in the lungs, spleen, and bone marrow, etc. It seems, therefore, that the rise in the white blood cells and the fall in the leucocyte ascorbic acid is related to the output of cortisol by the adrenal gland. A significant correlation between the white blood cell count and the cortisol level after a myocardial infarction has previously been described (Bailey *et al.*, 1967). Furthermore this fall in leucocyte ascorbic acid cannot be prevented by ascorbic acid supplements of 1 g per day in the case of infection by the common cold virus (Hume and Weyers, 1973) or by 2 g per day before tetracosactrin stimulation. The studies on the ascorbic acid content of granulocytes (1323 ±949 nmol/l), lymphocytes (2449 ±910 nmol/l), and platelets (2078 ±875 nmol/l) suggest that the simple explanation for this obligatory fall in leucocyte ascorbic acid after a 'stressful' event is that the circulating white blood cell pool is being augmented by granulocytes and depleted of lymphocytes. Differential counts carried out on 6 patients who had sustained a myocardial infarction showed that at the peak white blood cell count the granulocytes had risen by 93·6 per cent from a mean normal level and the lymphocytes had dropped by 15·2 per cent from a mean normal level. Thus, the total white blood cell pool is being augmented by granulocytes which have approximately half the amount of ascorbic acid in them as the lymphocytes. While the initial apparent fall in leucocyte ascorbic acid after an acute myocardial infarction seems to be a reflection of the granulocytosis, the eventual return of leucocyte ascorbic acid to normal when the acute stress has subsided is dependent on an 'adequate' supply of ascorbic acid. The combination of damaged heart muscle and the granulocytosis results in tissue depletion of ascorbic acid which is reflected in the fact that the new population of white blood cells which emerge after the granulocytosis has a reduced content of ascorbic acid, and this may persist for at least 56 days unless supplements of ascorbic acid are given.

Tissue depletion of ascorbic acid is also reflected in the very low serum ascorbic acid levels which persisted throughout the period of study in the unsupplemented group. This was in spite of a mean intake of 49 ±30 mg/day of ascorbic acid as determined by diet histories. This depletion of serum ascorbic acid is presumably dependent on the presence of a 'target site' such as damaged heart muscle and is not apparently produced as a result of adrenal stimulation. The failure of adrenal stimulation to influence the serum ascorbic acid is clearly illustrated in the subjects who received infusions of tetracosactrin, while the demand of the damaged myocardium for excess ascorbic acid has previously been shown (Hume *et al.*, 1972). Further support for the hypothesis that a 'target site' is necessary before the serum ascorbic acid will fall is the observation made on 31 professional footballers that after a period of severe strenuous exercise there is a rise in the white blood cell count and a fall in the leucocyte ascorbic acid (Boddy *et al.*, 1974), but there was no significant change in the serum ascorbic acid levels which were 80 ±28 μmol/l before and 97 ±34 μmol/l after the exercise ($t = 1·8574$, $P > 0·05$) (unpublished observation).

While it is well recognised that only small amounts of ascorbic acid, probably less than 10 mg per day, are required to prevent scurvy (Krebs, 1953), it has to be questioned whether this is the optimum intake for normal health and for combating disease and the stress of disease. The present study has shown that ascorbic acid deficiency exists for a long time after an acute myocardial infarction, at least 56 days, and while obvious scurvy has not been observed in these subjects, it is unlikely that this is a satisfactory situation in view of the involvement of ascorbic acid in tissue repair and cell metabolism (Birch and Parker, 1974). It is clear that further study is necessary to determine the optimum daily requirements of ascorbic acid and whether supplements of ascorbic acid after an acute myocardial infarction would contribute to the welfare of such patients.

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