THE EFFECT OF SUPPLEMENTARY VITAMIN C AND IRON ON HAEMOPOIESIS IN OLD AGE

by

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ABSTRACT

Supplementary Vitamin C (500 mg daily), slow release iron (105 mg elemental iron daily), or the two forms of medication in combination were administered daily by mouth to three groups of male and three groups of female geriatric subjects. Leucocyte ascorbic acid concentrations and haemoglobin levels were measured in all the subjects at intervals during the fourteen week period of the investigation.

Administration of Vitamin C with or without iron caused significant elevations in leucocyte ascorbic acid concentrations in both sexes within one week. These concentrations diminished in both sexes during the sixth week. Additional iron in the males caused a progressive reduction in the ascorbic acid concentrations. In the females it was associated with a reduction and subsequent increase in ascorbic acid concentrations. Iron therefore creates a greater demand for Vitamin C in elderly females. In the males, iron alone, and iron with Vitamin C, caused elevations in the haemoglobin during the trial period. In the females all three types of medication caused an elevation in haemoglobin levels but the most consistent and uniform rise occurred in the group receiving iron with Vitamin C.

In both sexes, changes in ascorbic acid metabolism preceded or were concomitant with, changes in haemoglobin formation. A significant degree of haemopoietic instability developed in the males during the sixth week and in the females during the eighth week of supplementation. Supplementation with iron and Vitamin C together in males can produce a new improved and stable relationship between ascorbic acid and haemoglobin in ten weeks. Daily supplements of iron and Vitamin C together, while able to produce raised haemoglobin levels in females, do not result in an improved and stable relationship between these elements in the blood within fourteen weeks.

INTRODUCTION

The assumption is generally made that deficiency of iron intake among normal subjects limits the attainment of an optimal haemoglobin level (1). However there is evidence that Vitamin C can alter iron absorption (2), affect its transport (3), and performs a function in its tissue release (4). Dietary intake of Vitamin C in the elderly is often low (5-8) and it seems possible that the hitherto unexplained variability in the response of anaemic and elderly people to iron administration, and often reported failure of success with iron therapy (1) could be attributable to an underlying Vitamin C deficiency which limits optimal haemoglobin formation. The effect produced by combined therapy with Vitamin C and iron (9) supports the suggestion that such medication can produce haemopoiesis in anaemic patients. In the present investigation therefore the response of combined therapy with Vitamin C and iron in combination has been compared with the effects of administration of iron or Vitamin C individually during a fourteen week period in healthy elderly subjects.

METHODS

The geriatric subjects (M2) were drawn from the wing of a large hospital in Dublin (Table 1). They had all been in hospital for at

TABLE 1

NUMBERS AND AGES OF SUBJECTS (M2) IN THE MALE AND FEMALE GROUPS. DAILY SUPPLEMENTATION WITH IRON AND VITAMIN C TO EACH GROUP

GROUP		NUMBERS OF SUBJECTS		MEAN AGES	
		Male	Females	Male	Females
Fe	Ferrogradumet 105 mg Slow Release Iron	7	8	68	73
C	Vitamin C 500 mg ascorbic acid	5	8	79	73
FeC	Ferrograd C 105 mg Fe + 500 mg Vit C	5	14	68	75

least three months. Hospital records showed that haemoglobin levels had remained stable during this period in comparison with those found at the beginning of the investigation. The old people were healthy and normal for their age but were not able to take care of themselves adequately at home. They cared for themselves in hospital but all their

meals were supplied through the hospital kitchen. The content of the diets supplied through the kitchen did not vary during the course of the investigation. Particular attention was directed towards the possibility of temporary variation in the dietary intake of individuals during the period of the trial. Individual dietary variation was not observed at any time. There was no alteration in environmental conditions or routine of the subjects during the trial, apart from differences in the types of drug therapy administered to the groups, which might have given rise to any abnormal stress. The subjects were selected for the investigation during September 1969 and divided into three groups of males and females, each group containing 15 subjects. They received a supply of tablets every fortnight and ward orderlies ensured that the medication was taken daily. Two groups, one from each sex (C groups), received Vitamin C tablets (Abbott) containing 500 mg ascorbic acid daily; two groups (Fe groups) each received a Ferrogradumet tablet (Abbott) daily, containing 525 mg ferrous sulphate in slow-release form equivalent to 105 mg of elemental iron; and the last two groups (FeC groups) each received a tablet of Ferrograd C (Abbott) daily, containing 525 mg ferrous sulphate in slow-release form, together with 500 mg ascorbic acid. Administration of the tablets commenced at the beginning of October after control samples of blood had been removed for analysis between 0930 hours and 1200 hours during the last week of September. The investigation was stopped in the middle of January, 1970, when all the subjects had received medication for fourteen weeks. During the course of the trial a number of subjects dropped out or were excluded for various reasons. Only subjects who had completed the whole trial were included in the final results.

Leucocyte ascorbic acid concentrations were estimated by the method of Denson and Bowers (10) using a Coulter Counter for the leucocyte counts. Leucocyte counts and ascorbic acid estimations were carried out in duplicate. Good correlation was obtained between the duplicates (11). One ml. of blood was placed in a sequestrene tube for haemoglobin estimations by the oxyhaemoglobin method using a Beckman spectrophotometer (12).

RESULTS

Haemoglobin levels, and leucocyte ascorbic acid concentrations, were measured at one week, and later at two week intervals. Individual values for each period and overall means and standard deviations throughout the period of supplementation are shown for the leucocyte ascorbic acid concentrations and haemoglobin levels in Table 2.

Leucocyte Ascorbic Acid Concentrations

Supplementary Vitamin C daily, with or without iron, caused a significant elevation in leucocyte ascorbic acid concentrations at the end of the first week of administration in both sexes. The mean value for the ascorbic acid concentrations during the entire period of supplementation was greatest in the female C group, and least in the

TABLE II

HAEMOGLOBIN LEVELS AND LEUCOCYTE ASCORBIC ACID CONCENTRATIONS IN THE DIFFERENT GROUPS DURING THE COURSE OF THE TRIAL. HAEMOGLOBIN: g/100 ml BLOOD. LEUCOCYTE ASCORBIC ACID CONCENTRATION: ug/10^8 CELLS

MALES

	Fe Group		C Group		FeC Group	
Week	Haemoglobin	Leuc.AA	Haemoglobin	Leuc.AA	Haemoglobin	Leuc.AA
0	14.7±1.6	26.0±17.1	15.1±1.7	24.6±11.7	14.2±1.0	21.5±11.8
1	14.3±1.9	27.1±16.1	14.3±0.9	40.1±19.8	14.3±0.7	34.6±15.0
2	14.9±1.0	25.5±7.1	15.3±1.5	43.6±8.2	14.9±0.8	46.3±14.9
4	15.0±1.3	23.8±5.8	14.9±1.5	39.1±3.6	14.8±1.1	40.1±6.6
6	15.4±2.1	22.8±12.0	15.3±1.3	31.0±7.6	16.5±2.4	37.8±11.3
8	15.1±1.2	22.6±6.8	14.8±0.8	40.3±9.6	15.1±0.7	40.8±15.4
10	15.3±2.0	23.8±9.0	14.7±1.0	45.0±13.0	15.3±1.0	39.8±16.8
14	15.8±1.2	21.8±3.7	15.3±1.0	47.0±1.9	15.5±1.2	41.6±20.3
Mean 1-14	15.0±1.5	24.3±10.4	15.0±1.2	38.6±12.2	15.1±1.3	37.5±14.2

FEMALES

0	13.4±1.3	24.1±9.3	13.1±1.0	25.4±13.8	13.8±0.9	21.9±11.5
1	13.5±0.6	23.3±9.2	13.5±0.7	49.5±10.8	14.0±1.6	41.4±8.7
2	14.4±1.0	29.0±9.5	13.4±1.4	45.6±17.3	14.1±1.0	44.2±9.1
4	14.4±0.4	22.8±7.5	14.1±0.9	44.6±9.1	14.5±1.4	40.0±6.5
6	15.1±0.8	15.0±6.1	14.0±0.7	37.4±13.4	14.1±0.4	32.8±5.3
8	15.7±1.4	20.8±6.2	14.6±1.4	45.9±9.0	14.7±1.2	32.9±8.1
10	14.2±0.7	21.4±8.5	13.7±1.4	50.8±11.3	14.4±0.7	36.6±3.5
14	15.2±1.4	32.0±24.0	14.3±1.2	49.9±18.6	15.3±1.7	34.9±9.8
Mean 1-14	14.4±1.2	23.5±11.0	13.9±1.1	43.2±14.9	14.3±1.2	35.2±10.8

female FeC group. The difference between these values was statistically significant ($p < 0.05$). The overall ascorbic acid values for the two male groups were similar, and lay between those for the two female groups (Table 2).

Leucocyte ascorbic acid concentrations fluctuated throughout the course of the trial showing an initial rise during the first and second weeks of administration in all the groups (Fig.1). The male FeC group had the largest, and the male Fe group had very small peaks. Ascorbic acid concentrations suddenly fell during the sixth week in all except the male Fe group in which there was a general tendency to decline throughout the trial. The extent of this fall, and the rate of recovery, were characteristically different in the other five groups. The fall was least in the male FeC group. Its ascorbic acid then rose during the eighth week to a concentration which was maintained during the remainder of the trial. The fall was greatest in the males receiving Vitamin C alone. The concentration did not attain the original peak value again until the end of the trial in this group. The final concentrations in the two male groups were similar at the end of the trial. The female FeC group never recovered from the fall in ascorbic acid concentrations which occurred during the sixth week (Fig. 1b). However, ascorbic acid concentrations resumed their initial peak values after four weeks in the C group. These values were then consistently maintained. The female group which received iron alone showed comparable fluctuations in ascorbic acid concentrations to the other two female groups though the absolute ascorbic acid concentrations were consistently lower throughout the course of the trial. Complete recovery from the fall in the sixth week was delayed for eight weeks in this group. However the ascorbic acid concentration finally did exceed the initial peak which had occurred during the second week.

Haemoglobin Levels

At the end of fourteen weeks, supplementation resulted in an overall mean elevation of the haemoglobin in all groups except the males who received Vitamin C alone (Table 2). During the course of the trial haemoglobin levels fluctuated considerably (Fig. 2). The two male groups receiving iron, or Vitamin C alone, showed a fall in haemoglobin level during the first week (Fig. 2a). The haemoglobin rose fairly consistently during the first four weeks of the trial only in the male FeC group. All the male groups showed peak haemoglobin concentrations during the sixth week. During the following two weeks the haemoglobin level fell significantly in the FeC group. Thereafter the haemoglobin increased during the remainder of the trial in this group. In the other two male groups the peak elevation was considerably less during the sixth week. The Fe group showed a consistent increase from the eighth to the fourteenth weeks of the trial so that a rise in haemoglobin level was evident in this group at the end of the trial. Throughout the course of the trial haemoglobin levels merely fluctuated round the initial pre-supplementation value in the C group.

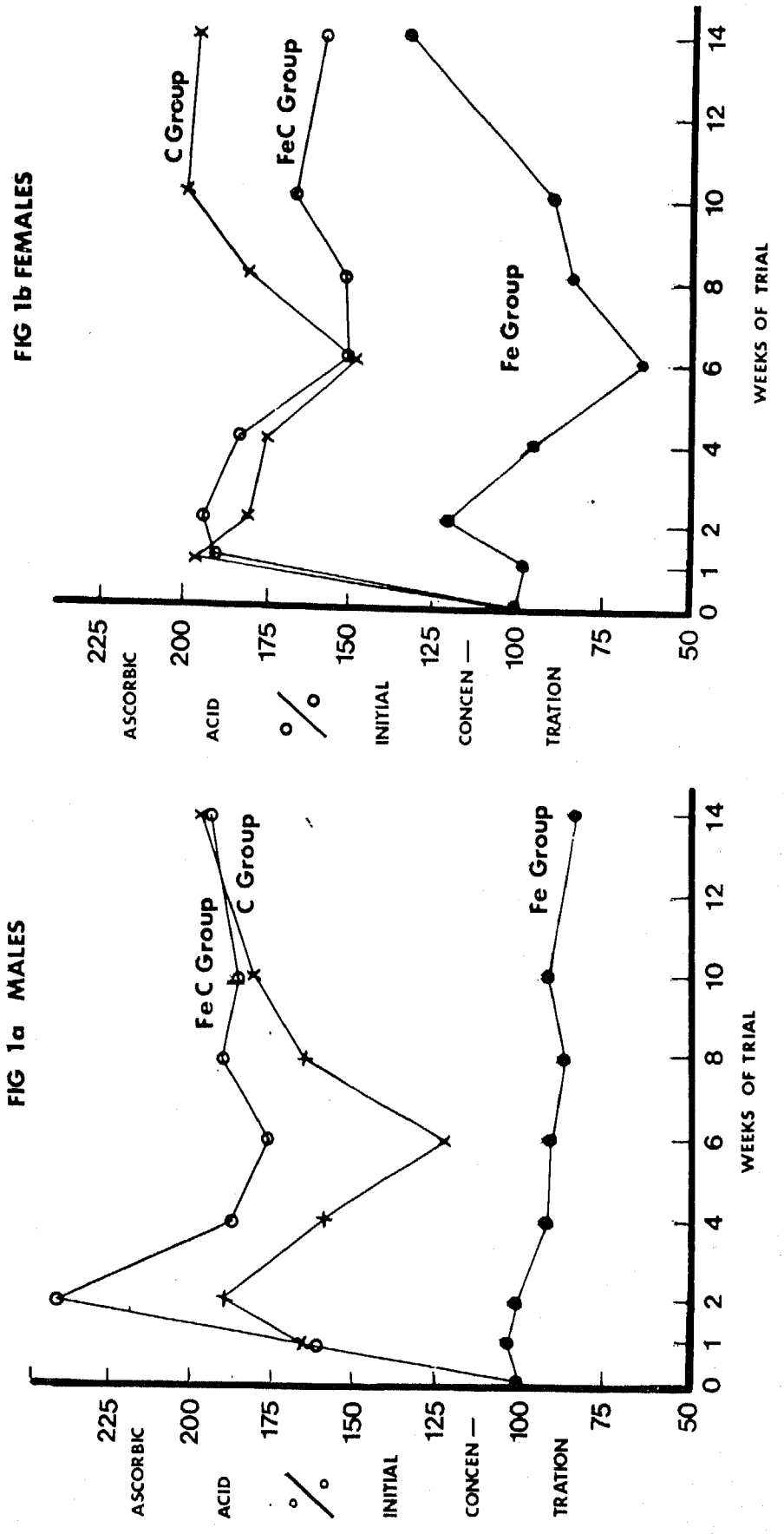

Figure 1. Per cent initial ascorbic acid concentrations in the leucocytes in the groups receiving iron alone (Fe Group o-o), Vitamin C alone (C Group x-x), and iron with Vitamin C in combination (FeC Group o-o).

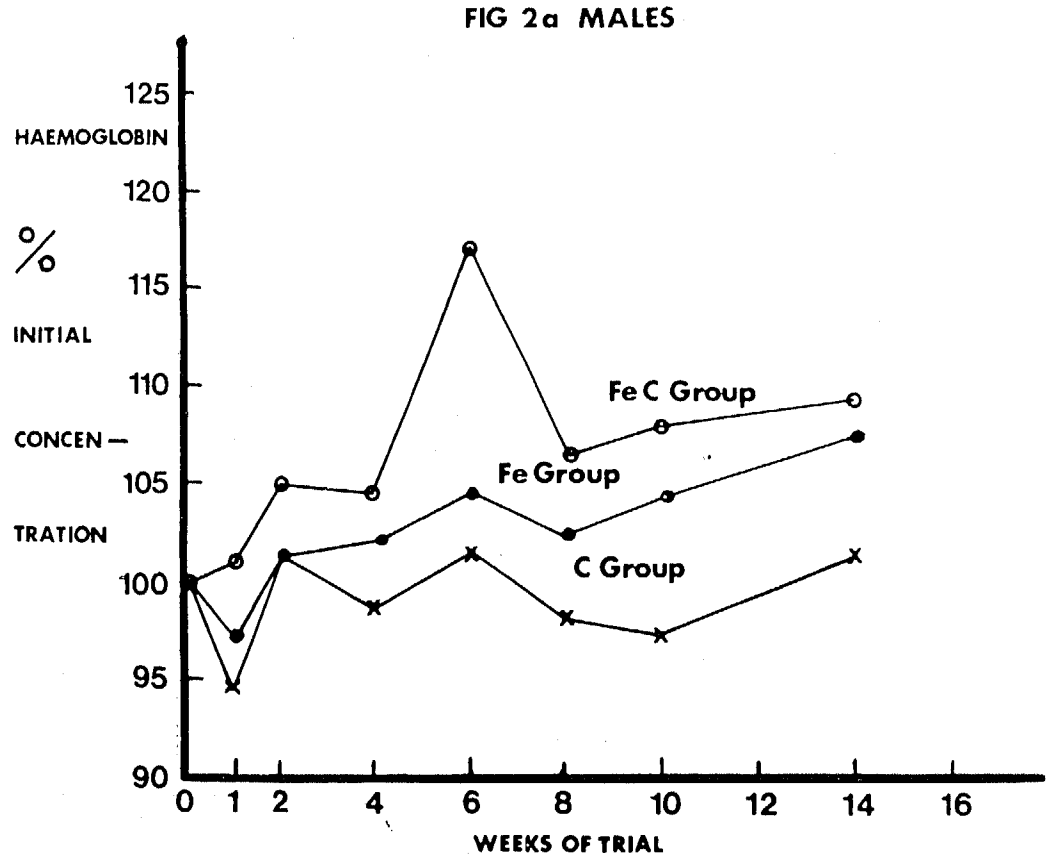

Figure 2a. Per cent initial haemoglobin levels for the groups receiving iron alone (Fe group ●-●), Vitamin C alone (C group x-x) and iron with Vitamin C in combination (FeC group o-o).

Haemoglobin rose to a peak level in all the female groups during the eighth week of the trial (Fig. 2b). Up to the eighth week the rise in haemoglobin level was fastest and most consistent in the Fe group. The groups receiving Vitamin C alone, or Vitamin C with iron, showed small falls in haemoglobin levels during the sixth week before reaching their peak levels in the eighth week. All the groups exhibited a sharp fall in haemoglobin levels during the tenth week. This was followed by a consistent and almost parallel rise during the last two weeks of the trial so that haemoglobin levels were similar in all the female groups at the end of the fourteenth week. During the course of the trial the FeC group manifested the most regular increase, and the Fe group showed the widest fluctuations in haemoglobin levels.

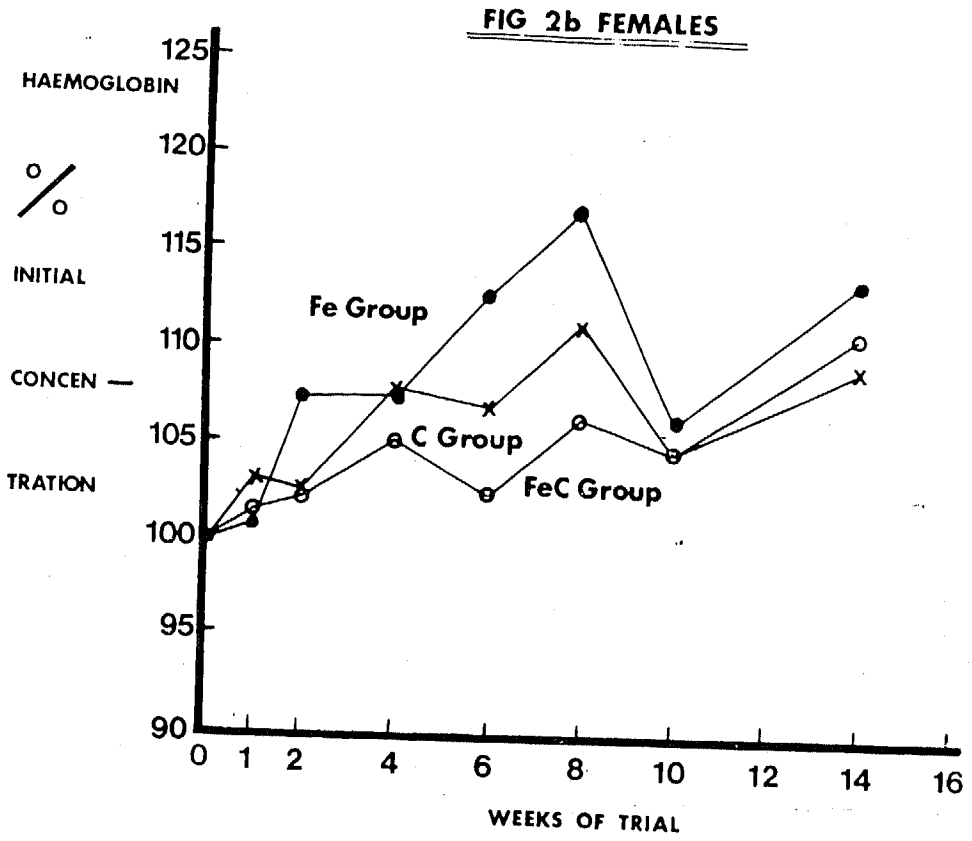

Figure 2b. Per cent initial haemoglobin levels for the groups receiving iron alone (Fe group •-•), Vitamin C alone (C group x-x) and iron with Vitamin C in combination (FeC group o-o).

DISCUSSION

Administration of 500 mg of Vitamin C daily with or without iron caused a significant elevation in leucocyte ascorbic acid concentrations in both sexes within one week. The mean values for the ascorbic acid concentrations during the period of Vitamin C supplementation were significantly elevated in all four groups. In the two male groups the mean values for the ascorbic acid were similar. Among the males therefore additional supplementation with iron did not influence the overall ascorbic acid concentrations in the leucocytes. However, the overall ascorbic acid concentrations in the two female groups were significantly different during the period of supplementation. The females receiving supplementary iron with the Vitamin C had the lower leucocyte ascorbic acid concentrations. There was no significant difference between the overall ascorbic acid concentrations in the males and females who did not receive iron. It can therefore be concluded that administration of iron results in a greater demand for Vitamin C in elderly females than in elderly males.

In both sexes leucocyte ascorbic acid reached a peak concentration during the first and second week with all forms of supplementation. The demand for ascorbic acid became evident six weeks after commencing each type of supplementation except in the case of the males receiving iron alone. The heights of the peaks, and the extents and rates at which the demands for ascorbic acid were satisfied, demonstrate the difference between the sexes in ascorbic acid saturation and metabolism, and illustrate the important interdependence of iron and Vitamin C for haemoglobin formation in apparently healthy elderly people. These observations can be explained on the assumption that iron administered by mouth, after absorption in the presence of alimentary ascorbic acid, is used normally by the marrow, but that ascorbic acid is necessary for the mobilisation of iron from the tissues (13). Among the males the ascorbic acid peak was greatest and the demand for ascorbic acid was least in the FeC group. In this group, therefore, the demand for ascorbic acid for the absorption, and mobilisation and use, of iron for haemoglobin formation, was relatively small. In consequence the initial peak was largest in this group and saturation of body stores with ascorbic acid was achieved relatively quickly and easily. In the group receiving Vitamin C alone, the peak was smaller and the demand for Vitamin C was larger. This is understandable because ascorbic acid was in constant demand for absorption, mobilisation, and use of all available iron in this group. Saturation of body stores was achieved temporarily at the beginning of the trial, and again at the end of the trial. In the group receiving iron alone, ascorbic acid was in constant and critical demand. The leucocyte concentrations at the beginning of the trial progressively decreased, and haemoglobin formation was relatively slow. In this male group alone no supply of ascorbic acid was available to meet the demand at six weeks.

Haemoglobin formation and disappearance differed between the sexes in response to the supplementation. Among the males the absence of a fall in haemoglobin level during the first week in the FeC group, and its presence in the other two groups, indicates the necessity of iron and Vitamin C in order to prevent a reduction in haemoglobin level when the stable haemopoietic state is initially being altered. A similar fall in haemoglobin level has been reported during the first week in patients receiving total dose iron infustions with iron dextran (14). A reduction in haemoglobin level has also been demonstrated during the fourth week in patients receiving daily ferrous sulphate therapy (15). This did not occur in the patients receiving ferrous carbonate with ascorbic acid. These observations support the present findings. They indicate the necessity for combined therapy with iron and Vitamin C in order to produce a consistent and uniform increase in haemopoiesis. The increase in haemoglobin during the sixth week, which was greatest in the FeC group, suggests that a haemopoietic surplus occurred during the sixth week. Haemopoiesis suddenly stopped when the ascorbic acid was 91% in the Fe group, 122% in the C group, and 176% in the FeC group, of the initial male values. The surplus was dependent on the availability of iron and Vitamin C

together, but the Fe group never subsequently exceeded an ascorbic acid percentage of 91%, whereas the percentage in the C group progressively increased from the eighth week onwards. The FeC group maintained a stable ascorbic acid value of about 190% of the initial stable concentration, and resumed normal haemoglobin formation. Haemopoiesis did not occur in the C group because adequate iron was not available to enable haemopoiesis to take place in the presence of the excess ascorbic acid. Throughout the trial haemopoiesis was greatest and most consistent in the FeC group because iron and Vitamin C were available in adequate quantities to ensure controlled haemoglobin formation.

Among the females a small reduction in leucocyte ascorbic acid appeared during the first week in the Fe group. The ascorbic acid concentration increased during the second week as available ascorbic acid was mobilised from body stores and passed into the labile ascorbic acid stores in the leucocytes (16). The available ascorbic acid in the blood diminished after the second week as metabolic requirements for Vitamin C increased in response to the continued iron stress. The rise in blood ascorbic acid concentrations occurred at the same time in the women as the comparable increase takes place in female guinea-pigs on a scorbutogenic diet (17). The lowest value for the leucocyte ascorbic acid, namely 62% of the initial value, was attained in the women approximately two weeks later than the lowest levels for plasma ascorbic acid are attained by female guinea-pigs surviving a scorbutogenic diet. As in the surviving female guinea-pigs in which a readjustment of ascorbic acid metabolism takes place in response to the stress of the scorbutogenic diet, an increase in leucocyte ascorbic acid concentrations occurred in the women in response to the metabolic requirements for ascorbic acid induced by the iron administration. Two weeks after this metabolic readjustment began to occur in the women, a haemopoietic surplus appeared, and haemoglobin synthesis became temporarily arrested. In the FeC and C groups qualitatively similar changes in leucocyte ascorbic acid appeared during the first six weeks to those observed in the Fe group. However the metabolic readjustment in the FeC group was made less apparent by the presence of the exogenous Vitamin C. The most continuous and regular haemopoiesis took place in this group in consequence of the combined effect of the exogenous Vitamin C and the metabolic readjustment of ascorbic acid metabolism induced by the iron stress. A limited increase in haemopoiesis occurred in the C group between the first and second weeks when the ascorbic acid was being used to mobilise tissue iron for haemoglobin synthesis (13). The metabolic readjustment of ascorbic acid metabolism followed at the end of the sixth week, accounting for the increase in leucocyte ascorbic acid concentrations between the sixth and tenth weeks. Further haemoglobin synthesis was then limited in comparison with that which occurred in the FeC group, owing to lack of available iron.

The sex related difference in the utilisation of Vitamin C in humans provides the explanation for the different responses of the sexes to supplementation (16). This can be analysed by relating

haemoglobin levels and ascorbic acid concentrations. The males were able to carry out adequate haemopoiesis when they were receiving the full supplement of 105 mg of iron and 500 mg of Vitamin C daily. Lack of either of these haemopoietic factors prevented a complete haemopoietic response in males and resulted in the development of an unstable haemopoietic state and interference with optimal haemoglobin formation (18). Females, in contrast to males, have some capacity to compensate for lack of exogenous Vitamin C during iron therapy by making adjustments in their ascorbic acid metabolism at the end of the sixth week. Administration of iron alone necessitates a more radical readjustment than is required when exogenous Vitamin C is administered with the iron. The haemopoietic response is limited when Vitamin C alone is administered because of lack of easily available iron after the second week. Administration of iron and Vitamin C simultaneously in a ratio of 1 to 5 enables females to make a progressive haemopoietic response. However this combination does not relieve them of the necessity to carry out some metabolic readjustment of their ascorbic acid metabolism at the end of the sixth week. Measurement of the response of the red blood cells alone does not provide adequate information about the state of haemopoietic stability in an individual during administration of iron therapy (16).

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