The Anaemia of Scurvy*

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The haematologic findings in five patients with scurvy are presented. Four had hypercellular bone marrows which, although predominantly macronormoplastic, showed some megaloblastic change. Complete clinical and haematologic remission was effected by the administration of ascorbic acid alone in five of the six episodes of scurvy.

Evidence of the effect of ascorbic acid deficiency on folate metabolism is presented. It is suggested that this is the primary mechanism whereby anaemia develops in scurvy.

In 1953 Lind [*1*] described the clinical features of scurvy and observed the associated pallor. The first detailed account of the anaemia of scurvy was given by Mettier, Minot and Townsend [*2*] in 1930. Since then it has been recorded in the majority of patients [*3–6*]. The anaemia has been attributed to many factors: haemorrhage into the tissues [*7*], intravascular haemolysis [*6,8,9*], blood loss via the alimentary tract, the coexistence of dietary deficiencies such as liver factor and iron [*10*], and finally deficiency of ascorbic acid adversely affecting erythropoiesis [*2,11,12*] and the enzymes concerned with red cell and haemoglobin metabolism [*13*]. In support of this last hypothesis, Nichol and Welch [*14*] suggested that ascorbic acid is required for the conversion of folic acid to folinic acid. Brown [*15*] considered that there is a disordered utilisation of the pteroylglutamate complex, and Cox and associates [*16*] showed that vitamin C therapy in scurvy produced changes in the metabolism of pteroylglutamates.

In this paper further evidence is presented of a metabolic relationship between vitamin C and the folates which does not appear to involve the conversion of folic acid to folinic acid. Observations have been made of five patients with severe scurvy, one of whom was again studied in relapse two years after the first episode.

CLINICAL MATERIAL

One patient (Case 1) was a recluse and one (Case 2) was a crank with an excessive intake of beer. Two patients (Cases 3 and 4) took scorbutogenic diets to avoid the diarrhoea associated with jejunal diverticulosis and diverticulitis coli, respectively. One patient (Case 5) was unemployed for eight months and changed his diet for economic reasons during this time.

The diets of these patients were devoid of fruit and vegetables and consisted of bread, butter, margarine, biscuits and tea, and occasionally cooked meat, bacon, fried fish and potatoes. The diet of one patient (Case 5) included two or three fried eggs daily.

METHOD OF INVESTIGATION

From the time of admission, all the patients were maintained on a diet devoid of vitamin C and low in pteroylglutamates. This consisted of toasted bread, biscuits, margarine, rice, white fish, bacon, ham, cheese, reconstituted potato and turnips. After cooking, all meals were further devitaminised by being kept warm for a prolonged period. In all patients the manifestations of scurvy increased while they were on this diet. A slightly less rigid diet produced no haematologic improvement in three patients with folic acid-deficient megaloblastic anaemia in whom no gastrointestinal disease was detected despite full investigation.

A complete haematologic investigation was made of each patient, together with determination of the serum proteins and the seromucoids [*17*]. An assessment of haemolysis was made from the levels in the serum of bilirubin, haptoglobin and methaemalbumin [*18*]. Vitamin B_{12} metabolism was studied by assaying the serum vitamin B_{12} level with Lactobacillus leichmannii [*19*] modified by the addition of cyanide to the extraction medium [*20*], by estimating the urinary excretion of methylmalonic acid in 24 hours [*21*], and by the absorption of radioactive vitamin B_{12} [*22*]. Pteroylglutamate metabolism was studied by

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TABLE I
PRINCIPAL LABORATORY FINDINGS

Laboratory Data	Normal Values	Case 1 F, 59	Case 2A F, 47	Case 2B F, 49	Case 3 M, 65	Case 4 F, 78	Case 5 M, 42
Red blood cells (× 10^6/ cu. mm.)	...	1.73	3.52	3.11	2.13	1.91	1.80
Hemoglobin (gm. %)	...	6.0	11.6	10.4	6.3	8.3	5.5
White blood cells (per cu. mm.)	...	3,800	9,800	5,200	5,500	3,900	7,500
Mean corpuscular volume (cu. μ)	...	110	103	108	102	136	94
Mean corpuscular hemoglobin concentration (%)	...	32	32.5	31	32.5	32	32
Platelets (per cu. mm.)	150–400,000	252,000	195,000	206,000	464,000	155,000	195,000
Thromboplastin generation test	...	Normal	Normal	Normal	Normal	Normal	Normal
Prothrombin (% normal)	...	63	80	63	100	63	100
Bleeding time (min.)	...	3	4	4½	3½	2	8
Clotting time (min.)	...	10½	9	9	8	9	6
Tourniquet test	...	+	+	+	+	+	-ve
Clot retraction	...	Poor	Normal	Normal	Normal	Normal	Normal
Serum albumin (gm./ 100 ml.)	...	3.9	3.2	3.6	2.4	4.0	2.8
Serum globulin (gm./ 100 ml.)	...	3.6	4.0	2.9	3.6	3.5	3.2
Seromucoids (mg./100 ml.)	55–105	256	209	302	195	160	325
Reticulocytes (%)	...	4.6 to 7.2	2.0 to 3.8	2.6 to 5.8	3.6 to 2.8	4.0 to 5.8	4.0 to 4.0
Serum bilirubin (mg./ 100 ml.)	...	1.3	0.6	1.0	1.2	0.8	0.7
Haptoglobin	...	Absent	Normal	Normal	Reduced	...	Normal
Methaemalbumin	...	2+	1+	1+	2+	...	Nil
Serum vitamin B_{12} (μμg./ml.)	>140	145	250	270	265	215	170
Methylmalonic acid, urinary excretion (mg./24 hr.)	1–3.1	...	...	...	1.2	3.0	1.8
Serum (L. casei) (mμg./ml.)	3.0–15.0	5.2	...	7.1	1.8	6.1	2.9
Blood (L. casei) (mμg/ml.)	36–120	63	...	38	...	14.8	24.0
Blood (Strep. faecalis) (mμg./ml.)	2–40	2.6	...	10.2	...	12.0	4.2
FIGLU (mg./hr.)	<7	10.4	3+	11.8	4+	...	17.3
Serum Fe (μg. %)	40–120	48	37	176	35	180	35
TIBC (μg. %)	250–420	301	...	294	367	345	226

assaying the serum with Lactobacillus casei [*23*], heparinised blood with L. casei and Streptococcus faecalis [*24*] and estimating the urinary excretion of formiminoglutamic acid (FIGLU) 3 to 8 hours after a 15 gm. loading dose of histidine [*25*]. The 24-hour urinary excretion of folates and Leuconostoc citrovorum (Pediococcus cervicis) activity was determined, the urine being collected in bottles containing toluene, 5 gm. sodium bicarbonate and sodium ascorbate. Iron metabolism was studied by estimating the serum iron [*26a*] and total iron-binding capacity [*26b*].

In three patients (Cases 2A, 3 and 4) the effect of folinic acid was observed prior to treatment with ascorbic acid. Following the baseline observations, all patients were given an injection of 250 mg. sodium ascorbate intravenously. In Cases 1, 2A and 2B, 3 and 4 the treatment was continued with ascorbic acid, 200 mg. three times daily. In Case 5 the effect of the intravenously administered ascorbate alone was assessed. The effect of ascorbic acid therapy on each patient was subsequently studied, using the methods already indicated.

RESULTS

Initial Findings in Scurvy. Table 1 shows the initial findings. Tests for the presence of occult

TABLE II
URINARY FOLATE EXCRETION

Subjects			L. casei (μg. 124 hr.)	Strep. faecalis (μg. 124 hr.)	Leuconostoc Citrovorum
		Control Subjects			
Normal subjects	1	3	97.2	6.4	2.3
	2	3	40.0	7.8	3.2
	3	3	26.4	3.2	1.6
Hospital patients	1	2	47.0	10.8	1.5
	2	2	38.0	6.4	1.2
	3	2	29.1	3.9	1.3
	11	2	26.5	3.2	1.2
Total	7	17	...	...	...
Mean	...	...	45.6 ± 8.0	5.9 ± 2.4	1.9 ± 0.81
Patients with folic acid deficiency	1	5	8.9	6.5	1.5
	2	2	7.5	4.1	1.2
Total	2	7	...	...	...
Mean	...	...	8.2	5.8	1.4
		Scorbutic Subjects			
	1	4	38.5	27.6	5.5
	2B	1	35.1	23.7	3.2
	4	2	26.3	22.6	1.6
	5	1	35.42	30.12	3.42
Total	4	8	...	...	...
Mean	...	...	35.3 ± 12.4	27.0 ± 11.9	3.83 ± 2.6

blood in the faeces were consistently negative in all patients. Three patients (Cases 2, 3 and 5) had steatorrhoea, excreting 7.7, 8.6, and 11.5 gm. fat daily, respectively. Jejunal biopsy specimens showed only minor villous abnormalities. Although marrow examinations on admission showed some evidence of megaloblastic change in all cases, the erythropoiesis in each instance was predominantly normoblastic or macronormoblastic. In all marrows occasional giant metamyelocytes were seen. The marrows were obviously hypercellular, apart from Case 5 which was hypocellular from three sites. There was no evidence of either a deficiency or a disturbed metabolism of vitamin B_{12}, the serum vitamin B_{12} levels and the urinary excretion of methylmalonic acid being within the normal range. In two patients tested in the scorbutic state, vitamin B_{12} absorption was normal. The serum folate level (L. casei) was subnormal only in Cases 3 and 5 but the FIGLU excretion was increased in five scorbutic episodes. Serum iron was low in Cases 2A, 3 and 5. The 24-hour urinary excretion of folates assayed with L. casei (L. casei activity), Strep. faecalis (Strep. faecalis activity) and Leuconostoc citrovorum (citrovorum activity) in patients with scurvy, normal subjects, hospital patients and two patients with megaloblastic anaemia due to folic acid deficiency are compared in Table II. In scurvy there was an increased excretion of material with Strep. faecalis activity, whereas that with L. casei or citrovorum activity was normal.

Effect of Folinic Acid in Scurvy. The administration of 5 mg. folinic acid produced no improvement in the haematologic or scorbutic state of Cases 2A, 3 and 4. In Case 4 the serum iron level fell from 180 to 101 μg. per 100 ml. after two days. On the fourth day this patient's general condition deteriorated rapidly and ascorbic acid therapy was therefore commenced. In the other two patients the blood count fell gradually and the clinical manifestations of scurvy persisted during the eight days of observation. Nevertheless the increased excretion of FIGLU returned to normal in Case 3 but persisted in Case 2A. However, in this case the patient did have a fatty hepatosis presumably associated with her excessive intake of beer.

Effect of Ascorbic Acid. Ascorbic acid produced a striking improvement in well-being, gradual disappearance of the clinical manifestations of scurvy and a haematologic remission in Cases 1, 2A, 2B and 4. The response was equally satis-

factory in Case 5 during the twenty-eight days following the single intravenous injection of 250 mg. sodium ascorbate. His bone marrow, although initially hypocellular, was hypercellular three days after vitamin C therapy but still showed a macronormoblastic picture with occasional megaloblasts (Fig. 1).

None of these patients required folic acid therapy in addition, but two patients (Case 2A and 4) had received folinic acid prior to treatment with ascorbic acid. The excretion of FIGLU was unchanged by ascorbic therapy in Case 1 but fell from 11.8 to 5.4 mg. per hour in Case 2B and from 17.3 to 6.4 mg. per hour seven days after therapy in Case 5.

Iron therapy was not necessary to achieve normal blood counts, despite the presence of low serum iron values in three patients. Following

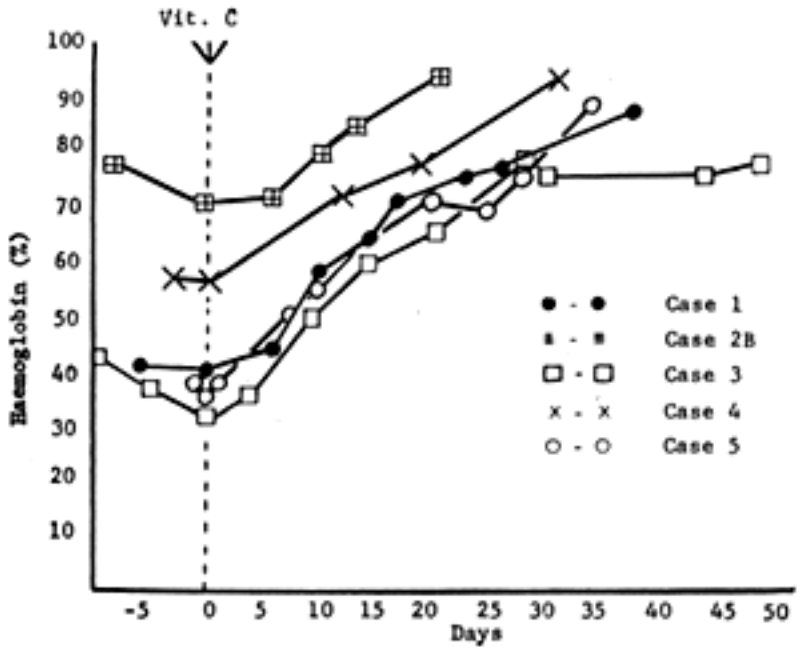

FIG. 1. Effect of ascorbic acid on haemoglobin levels of five patients with scurvy.

ascorbic acid only one patient (Case 4) showed an initial fall in the level of serum iron; the other patients showed either an increase (Cases 2A and 5) or no significant change (Cases 1, 2B and 3). The serum total iron-binding capacity remained constant or rose slightly.

Ascorbic acid therapy produced an initial transient fall in serum vitamin B_{12} levels which was followed by a gradual increase on resumption of normal diet.

There was no significant change in the urinary excretion of citrovorum factor but there was a significant fall in the urinary excretion of the folates (Strep. faecalis and L. casei) in the first 24 hours in the four patients studied (Cases 1, 2B, 4 and 5). Furthermore, the urinary folate excre-

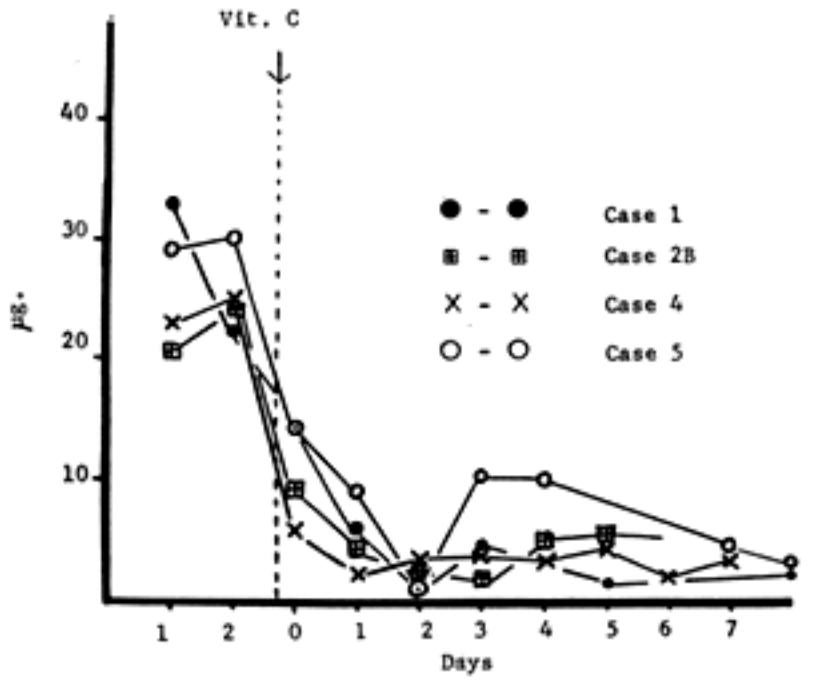

FIG. 2. Daily urinary excretion of folate (Strep. faecalis). In this and Figures 3, 4 and 5 vitamin C was administered at the point indicated by dotted line.

tion remained low for as long as eighteen days (Fig. 2 and 3). Despite this fall in urinary excretion, the serum and blood L. casei activity remained virtually unchanged in three patients (Fig. 4). Somewhat parodoxically there was a temporary rise in blood Strep. faecalis activity in three patients (Fig. 5).

Only one patient (Case 3, with jejunal diverticulosis) did not achieve full haematologic remission with ascorbic acid therapy alone. Despite an initial injection of 5 mg. citrovorum factor which reduced FIGLU excretion, and ascorbic acid therapy which produced a haematologic response, the serum L. casei activity fell

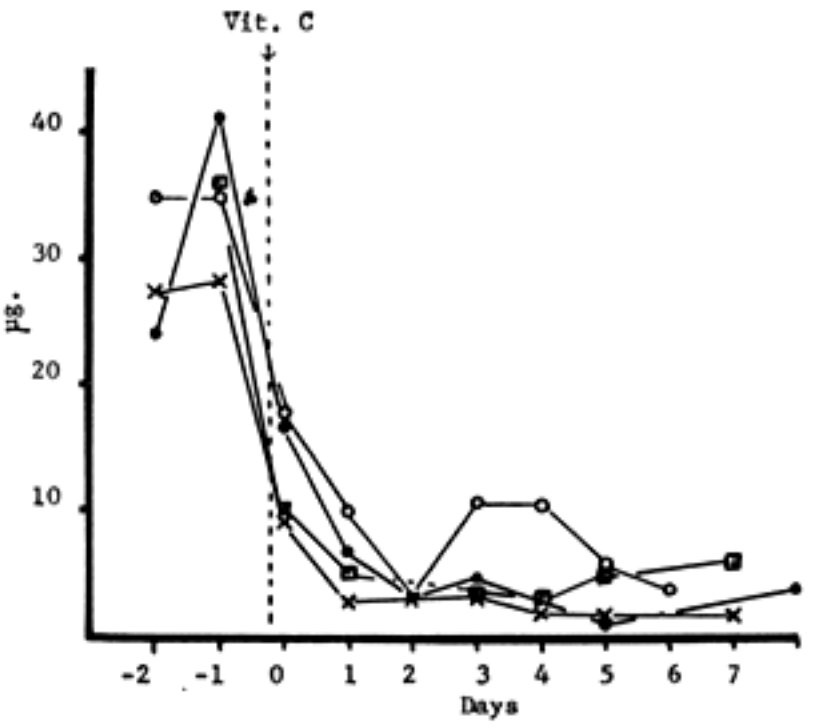

FIG. 3. Daily urinary excretion of folate (L. casei). Case symbols as in Figure 1.

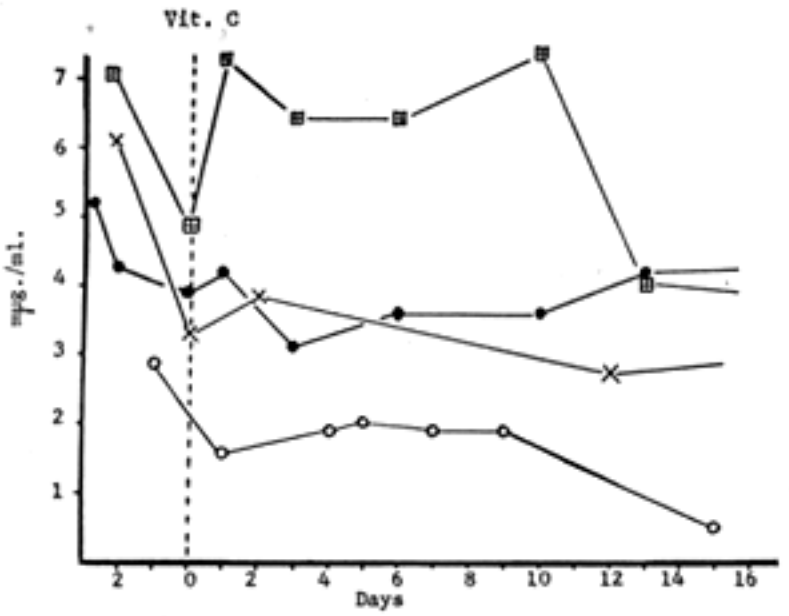

FIG. 4. Serum levels of folates (L. casei) following administration of sodium ascorbate. Case symbols as in Figure 1.

to extremely low levels. Haematologic improvement ceased after thirty-eight days and FIGLU reappeared in the urine. Complete remission was achieved only with folic acid therapy, at which time a folic acid excretion test was demonstrated to be abnormal (61 per cent: normal 70 to 120 per cent) [*27*].

In all patients seromucoid levels returned to normal with ascorbic acid therapy.

COMMENTS

The administration of ascorbic acid to four of the five patients recorded here led to complete haematologic remission; the fifth patient, with jejunal diverticulosis, needed folic acid to complete recovery. These findings are similar to those noted by many workers. Considering the character of the patients and their circumstances, more than one factor might be expected to play its part in the production of the anaemia of scurvy. However, some of these play a minor part. Thus there appears to be a reluctance to ascribe any significant role to the haemorrhage and subsequent blood destruction in the tissues and yet these are the most common manifestations. Falling or static blood counts are usually associated with evidence of further bleeding into the skin and subcutaneous tissues; bed rest alone will sometimes reduce this and result in haematologic improvement [*16,28,29*] On the other hand, we were unable to demonstrate any significant blood loss into the gastrointestinal tract using routine biochemical methods and neither could Goldberg [*6*] with the more sensitive chromium-labeled red cells technic.

It might be expected that iron deficiency plays some part since ascorbic acid aids the incorporation of iron into the haemoglobin molecule [*13*]. Only one of the seven patients with scurvy previously reported by us [*16*] required iron therapy and he had had a partial gastrectomy twenty years previously. Only one of Goldberg's six patients required iron therapy for complete remission. None of the five patients described in this paper needed supplementary iron and all had a normal serum iron level and total iron-binding capacity on attaining full haematologic normality. Cases of scurvy with iron deficiency

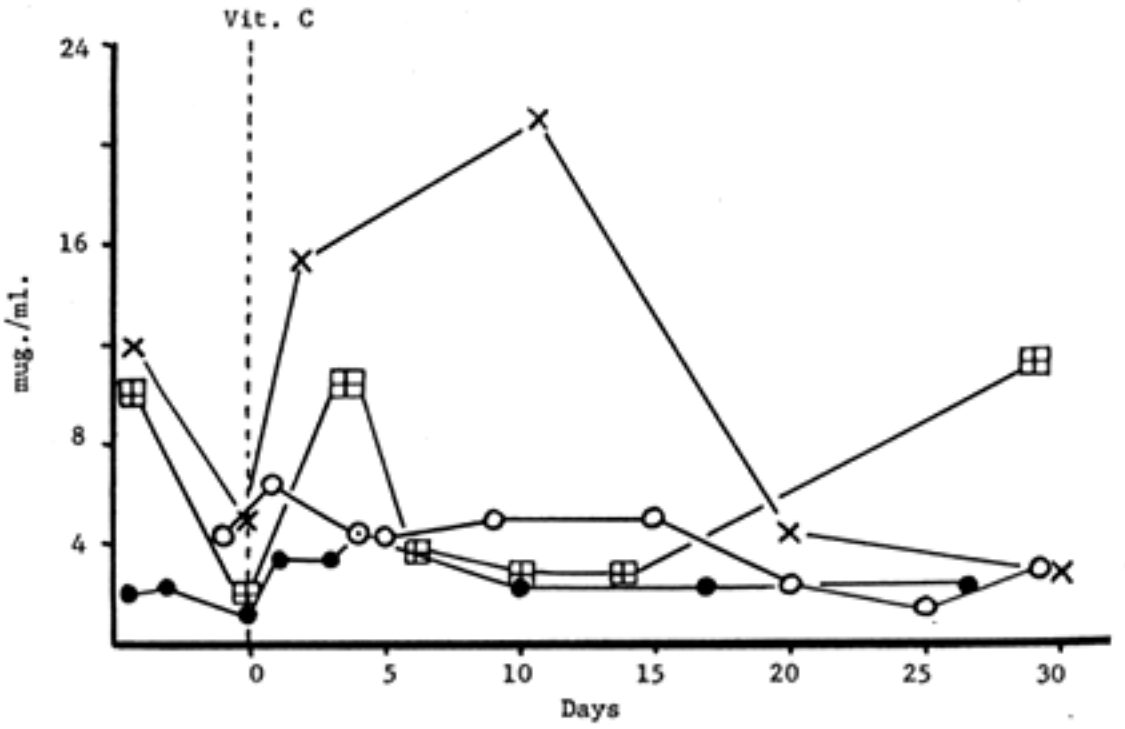

FIG. 5. Blood levels of folates (Strep. faecalis) following administration of sodium ascorbate. Case symbols as in Figure 1.

have of course been recorded, and this has been variously attributed to alimentary blood loss, malabsorption or a reduced dietary intake [*12*]. Many scorbutogenic diets have a low iron content but in light of the observations of Crandon, Lund and Dill [*30*] the onset of scurvy should long precede the development of iron deficiency.

Increased reticulocyte counts coinciding with falling or static blood values and elevation of serum bilirubin levels in three of the patients in this report could be explained by intra- or extravascular haemolysis. Reduced red cell survival as estimated by the Ashby technic [*8*] or with radioactive chromium-labeled red cells [*6,9*] could be explained in either way. Goldberg [*6*] also demonstrated that scorbutic cells survived significantly longer when transfused into normal subjects than they did in the scorbutic patient. He believed that an intravascular haemolytic process is the important factor in the production of anaemia and that it is readily reversible by the administration of ascorbic acid. However, the simultaneous presence in the serum of haptoglobin and methaemalbumin can best be explained by extravascular haemolysis (Table 1). Methaemalbumin is readily formed when haematin is added to normal serum *in vitro* but on adding haemoglobin, methaemalbumin appears only when the binding capacity of haptoglobin is exceeded [*31,32*]. In intravascular haemolytic states, methaemal bumin is found only when haptoglobin has disappeared from the serum as a complex with haptoglobin [*33*]. Haptoglobin was detectable in three of four patients in the presence of methaemalbumin, suggesting that heme rather than haemoglobin gains access to the plasma. This resembles the findings in haemorrhagic pancreatitis and may be interpreted to signify that the heme in scurvy is similarly derived from extravascular tissue haemorrhage with subsequent haemolysis. In Case 1, on the other hand, the total absence of haptoglobins may have been the result of intravascular haemolysis. Nevertheless, it is doubtful whether haemolysis plays any significant part in the production of the anaemia since the serum of the most severely anaemic patient in the series contained normal haptoglobins and no methaemalbumin.

Investigations into the bleeding diathesis of the patients with scurvy have revealed a number of minor abnormalities but no consistent pattern. Platelet counts were normal in all our patients. Cetingil, Ulutin and Karaca [*34*] claimed to have demonstrated a platelet defect in the scorbutic patient but neither Hart, Ploem, Panders and Verloop [*35*] nor we have been able to find any evidence of this, since the thromboplastin generation test using both donor and patients platelets showed no difference. Quick's one-stage prothrombin assay was normal in two of the five patients studied, and showed a mild reduction in the other three. In none of the patients was the two-stage prothrombin test performed but since the thromboplastin generation tests using the patient's absorbed plasma were normal, these three results were due to either a prothrombin or a factor VII deficiency. Bleeding time was prolonged slightly in only one patient and rather anomalously this was the only patient who had a negative tourniquet test.

Low serum levels of vitamin B_{12} of course occur in scurvy but are invariably associated with alimentary disorders in which defects of absorption of vitamin B_{12} occur. In pernicious anaemia, however, vitamin B_{12} appears to have a sparing effect upon ascorbic acid, [*36*] whereas Kahn and Brodsky [*37*] found that normal plasma levels of ascorbic acid could not be restored in a scorbutic patient despite therapy without replenishing the stores of vitamin B_{12}. The finding of normal urinary excretion of methylmalonic acid in three patients discounts any significant deficiency of vitamin B_{12}. However, restoration of normal marrow function or increased erythropoiesis following ascorbic acid therapy may have been the cause of the fall in serum levels of vitamin B_{12} in our patients and of the appearance of methylmalonic acid in the urine of the patient described by Kahn and Brodsky [*37*].

The cytology of the bone marrow has been described as normoblastic [*2–4,38*], macronormoblastic [*6*] and megaloblastic [*3,6,12,15,38–42*]; and although hypercellularity [*2,12,16*] is the common finding, hypocellularity has been reported [*43–45*]. This variation in marrow cytology in scurvy makes it difficult to interpret the role of vitamin C in erythropoiesis, but it is perhaps worthy of comment that its administration in our patients did not result in a rapid decrease in serum iron such as occurs in anaemias due to deficiency of vitamin B_{12} or folic acid when treated with the specific haematinic.

The interrelationship between ascorbic acid and folic acid remains to be considered and it

would seem that this provides the key to the occurrence of anaemia in scurvy, as has been suggested by Brown [*15*]. Approximately 10 per cent of patients with scurvy have no anaemia but this fact need not invalidate the argument that vitamin C may have a direct effect upon erythropoiesis. Red cell turnover may increase many times before anaemia becomes manifest. The increased rate of plasma clearance of radioactive iron in scurvy [*6*] could be taken as evidence of increased marrow function, had not a similar increase been noted in the disordered erythropoiesis of pernicious anaemia [*46*]. It has been claimed that megaloblastic anaemia in scurvy is solely due to folic acid deficiency [*7,47*] and it is possible that the apparent conversion of a megaloblastic to a normoblastic marrow by ascorbic acid alone, comparable to those patients described by Bronte-Stewart [*12*] and by Brown [*15*], has in some instances been due to the lack of rigid dietary control excluding folic acid during the period of observation. As Herbert and Zalusky [*48*] have demonstrated, such small amounts of folic acid that are contained in a normal diet are quite sufficient to produce haematologic remission in patients with folic acid deficiency. In the cases reported here, this possibility has been ruled out since the patients were kept on diets which had no therapeutic effect in patients with severe folic acid deficiency so that the primary factor in their remission must be assumed to be ascorbic acid therapy.

In scurvy, the urinary excretion of folates following 5 mg. test doses of folic acid is significantly diminished following ascorbic acid therapy [*16*]. This observation was paralleled by the rapid fall in urinary folate noted in the patients here following the administration of ascorbic acid. Herbert and Zalusky [*48*] demonstrated that ascorbic acid therapy may precipitate clinically overt folate deficiency, similar to that noted in Case 3. Such observations suggest that increased utilisation of folic acid results from specific therapy for scurvy. Absence of ascorbic acid leads to some disturbance of folate metabolism. For example, there is a normal or increased excretion of folates in the urine whereas the excretion of FIGLU was markedly increased in two patients who had normal serum levels of 5-methyltetrahydrofolic acid and decreased in two other patients following ascorbic acid therapy.

The nature of this disturbance is still speculative. Ascorbic acid is alleged to enhance the conversion of folic acid to a more physiologically active form, folinic acid [*14,49*], but there was no response to folinic acid in the patients recorded here so that this pathway is not disturbed. Vilter [*50*] has suggested a role for ascorbic acid which makes the sequence of erythropoietic change more understandable. Both ascorbic acid and vitamin B_{12} have a protective role in the formation of folic acid coenzymes. Since vitamin B_{12} is not deficient, this probably plays no part in the anaemia of scurvy. Ascorbic acid is involved in the protection of folic acid reductase, thus maintaining a higher concentration of folic acid coenzymes. In ascorbic acid deficiency, therefore, a profound disturbance of folate metabolism will result. This may account for the increased excretion of the pteroylglutamates that we have observed. Also a megaloblastic anaemia will result, the severity of which will depend on the degree of depletion of folates and the disturbance in their metabolism. This would explain why some patients respond completely to ascorbic acid therapy and others require supplementary folic acid.

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