

THE ANAEMIA OF ADULT SCURVY<sup>1</sup>

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Is vitamin C necessary for erythropoiesis? No convincing answer to this question exists today, yet scurvy dates back to Biblical times, and it is now 20 years since pure synthetic ascorbic acid became available. Experimentally, anaemia has and has not been produced in the susceptible guinea-pig; clinically, anaemia has and has not been observed in manifest human scurvy. The latest reviews of this aspect of ascorbic-acid metabolism (Cartwright, 1947; Tötterman, 1949; Shafar, 1949) reflect this confusion. It is the purpose of the present report to show that anaemia is frequently seen in adult human scurvy, and that the anaemia, no matter how severe, responds promptly and completely to synthetic ascorbic acid.

*Patients Investigated and Methods*

Thirty-two adults, admitted to Groote Schuur Hospital from 1946 to 1950 with the diagnosis of scurvy, were studied. All had clinically obvious scurvy, usually of a severe degree. Coexisting deficiencies or other diseases were surprisingly rare. All were men ranging in age from 22 to 60 years, with the mean at 36½ years. All but one were Bantus. One (Case 13) died from coronary thrombosis soon after admission. Thirteen patients admitted consecutively (Cases 1 to 13) were subjected to an intensive analysis. The other patients were used merely to determine the incidence and severity of anaemia in adult scurvy, and were treated on admission with synthetic ascorbic acid added to the full mixed hospital diet. With respect to Cases 1 to 13 the following procedure was adopted. Great care was taken that no unprescribed ascorbic acid reached the patient. Each patient was given the diet on which he developed the disease. This was facilitated by the fact that all had consumed a similar diet, the staple diet of the Bantu in South Africa. It consisted of mealie-meal (maize) porridge without milk or sugar, 'stamped' mealies, bread without butter or jam, and black tea or coffee. Its vitamin-C content was zero. Protein 60 gm., fat 20 gm., carbohydrate 356 gm., iron 22 mg., aneurin 1.2 mg., riboflavin 0.65 mg., nicotinic acid 15 mg., and 1,850 calories constituted the average daily intake (Fox and Golberg, 1944). Variable preliminary control periods were instituted. Cases 1 and 4 received ascorbic acid within 24 hours of admission. Otherwise the shortest control period was two days (Cases 2 and 3). The other patients had control periods varying from four to 30 days. During this time Cases 6, 8,

<sup>1</sup> Received January 5, 1953.

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and 9 received parenteral vitamin B<sub>12</sub> in 30- $\mu$ g. doses daily for four days, oral folic acid in 15-mg. doses daily for five days, 400 to 500 mg. of intravenous iron divided over five days, and parenteral vitamin-B complex. Sufficient time was allowed between each of the above drugs to observe any haematological response. Unless otherwise indicated, the usual dose of ascorbic acid at the end of the control period was 1,000 mg. daily given intravenously, as saturation requirements were being tested at the same time by the method of Wright and Lilienfeld (1936). The ordinary full hospital diet was not allowed until the packed cell volume had passed 40 per cent. All patients remained in bed until pronounced cured except Case 5, who was ambulant as soon as ascorbic-acid therapy had started, and Case 12, who remained ambulant throughout. Full haematological investigations were performed according to the standard methods either daily or on alternate days. The bone-marrow from the iliac crest (Rubinstein, 1948) was studied in most cases initially and about two weeks after vitamin-C therapy had begun. In a few patients (Cases 6, 8, and 9), who were subjected to prolonged control periods, examinations were repeated weekly to assess the effect of vitamin B<sub>12</sub>, folic acid, and other substances, on the bone-marrow. The orthotolidine method (Budtz-Olsen, 1951) was used for the estimation of plasma-iron and total iron-binding capacity. Urinary and faecal urobilinogen excretion was measured by the petroleum-ether method of Watson (1936, 1937) and Schwartz, Sborov, and Watson (1944), and on certain occasions the simplified method of Maclagan (1946) was used to compute the daily faecal excretion from the four-day collection. The standard methods were used for fractional test meals with histamine stimulation, Price-Jones curves (Whitby and Britton, 1942), other haematological investigations (Wintrobe, 1946; Quick, Stanley-Brown, and Bancroft, 1935), serum-bilirubin (Malloy and Evelyn, 1937), and serum-protein pattern determinations (Wolfson, Cohn, Calvary, and Ichiba, 1948; Maclagan, 1944 *a, b*).

#### *Results*

*The incidence and severity of anaemia in adult scurvy.* The normal haematological standards for the adult Bantu man agree well with the normal figures acceptable in races of European origin (Bronte-Stewart and Hickley, 1953). The main haematological findings in all the patients, on their admission to hospital, are given in Table I. The Table shows that anaemia is common in adult scurvy. In over 80 per cent. of these consecutive patients the packed cell volume was below 40 per cent. It also shows that the anaemia in the majority was severe, the lowest recorded figure for the packed cell volume being 8 per cent. Most of the patients who were not anaemic showed the milder degrees of scurvy. One patient (Case 10) had been admitted for the second time. On his previous admission (Case 14) he was not anaemic, but the duration of his complaints was much shorter. In the few patients who were observed for prolonged control periods the anaemia grew worse. It was our impression that the degree of anaemia was proportional to the severity of the scurvy and to the duration of the lack of vitamin C.

*The morphology of the anaemia.* The anaemia in most cases was normocytic and normochromic, as shown by the mean cell-volumes and haemoglobin

TABLE I  
*Haematological State on Admission*

Only six of the 32 patients had a packed cell volume greater than 40 per cent.

Case number	Red cells (millions/c.mm.)	Haemoglobin (gm./100 ml.)	Packed cell volume (%)	White cells per c.mm.	Case number	Red cells (millions/c.mm.)	Haemoglobin (gm./100 ml.)	Packed cell volume (%)	White cells per c.mm.
1	1.5	4.6	14	3,400	17	2.1	5.2	17	4,280
2	3.9	14	37	7,040	18	3.6	11	31	7,200
3	2.0	6	20	8,600	19	3.9	12	39	3,600
4	2.2	7	23	6,500	20	2.8	8.5	26	3,920
5	2.7	8.2	22	12,200	21	1.7	5	14	2,200
6	1.8	5.4	15	3,900	22	1.5	5	17	8,360
7	6.3	18	59	7,150	23	2.8	7.5	24	4,550
8	2.7	7.3	23	7,200	24	2.2	7.5	25	8,960
9	2.8	7.3	23	8,150	25	0.8	4	8	2,750
10	2.8	8.6	26	7,600	26	1.0	4	12	2,900
11	4.3	13.6	42	6,700	27	1.8	5	15	4,250
12	2.9	8.8	27	6,000	28	3.5	9.4	28	3,950
13	1.5	4.6	15	6,200	29	1.6	4.7	14	6,700
14	5.3	16	51	8,880	30	2.8	8	26	5,600
15	4.7	14	42	5,900	31	4.3	15	41	6,650
16	5.3	14	45	6,150	32	4.0	12	38	4,500

TABLE II

*The Initial Haematological Indices, with the Mean Cell-Diameter Measurement and Standard Deviation, in relation to the Severity of the Anaemia*

A normocytic normochromic anaemia is the rule, with a tendency to macrocytosis and gross anisocytosis in the severer cases. The reticulocyte count on admission bears no constant relationship to the severity of the anaemia.

Case number	Packed cell volume (%)	Reticulocytes (%)	Mean cell-volume (c.μ)	Mean cell haemoglobin concentration (%)	Mean cell haemoglobin (μg.)	Mean cell-diameter (μ)	Diameter/thickness ratio
1	14	1.2	94.0	33.0	30.9	8.50 ± 0.87	4.7
2	37	2.0	94.8	37.9	35.9	..	..
3	20	1.0	101.2	30.0	30.3	..	..
4	23	8.0	106.0	30.2	31.8	7.97 ± 0.69	3.8
5	22	1.5	81.2	36.8	30.0	..	..
6	15	4.1	82.0	36.0	30.0	6.92 ± 0.69	3.2
8	23	7.4	84.0	32.4	27.0	7.18 ± 0.70	3.5
9	23	1.6	81.4	32.0	26.0	7.31 ± 0.52	3.8
10	26	3.3	94.2	33.0	31.0	7.59 ± 0.62	3.7
11	42	1.4	96.5	32.8	31.6	7.34 ± 0.48	3.2
12	27	1.6	93.1	32.6	30.0	7.36 ± 0.48	3.4
13	15	7.0	100.0	30.6	30.6	7.70 ± 0.89	3.6

indices in Table II. Among the severer cases of anaemia, macrocytic figures are seen in three (Cases 1, 4, and 13); in a fourth (Case 3) the mean cell-volume was increased, but no confirmation was made by a measurement of mean cell-diameter. In only one case, the most severe anaemia of the series (Case 1), was the bone-marrow megaloblastic. Poikilocytosis was rare, but the severer cases presented the more outstanding examples of anisocytosis, a feature which was also noticeable in their Price-Jones curves. In these severe cases marked variation was also seen in the haemoglobin-content of the red cells. Gross hypochromia was not a feature. The initial reticulocyte count was above normal in five cases (Table II); it had no constant relation to the severity of the anaemia. A constant feature, however, where the anaemia was severe enough, was a rise in the reticulocyte level following rest in bed, whether the count was normal initially or not. In three cases normoblasts were seen in the peripheral blood smear. Only one patient of the series (Case 5), admitted with epistaxis, bled externally. His initial white-cell count was slightly raised. In the other cases the white-cell counts were normal or low. In the patients who were studied for long control periods the white-cell count fell slowly until ascorbic acid was given. Normal numbers of platelets were seen in all the cases studied, and the bleeding, coagulation, and prothrombin times were normal.

*The bone-marrow in adult scurvy.* With the exception of Cases 2, 7, and 11, who were the least anaemic of the 13 patients in the series, the bone-marrow in all cases showed a similar picture of intense hypercellularity. The more severe the anaemia, the greater was this hypercellularity and the more primitive the predominant cell. The marrow was megaloblastic only in Case 1, although in many cases megaloblastoid forms were seen. The white-cell series appeared normal. When marrow examinations were repeated throughout the control periods there was no significant alteration, in spite of the use of other haematinics such as iron, folic acid, and vitamin B<sub>12</sub>. At the peak of the reticulocytosis which followed vitamin-C therapy the clumps or 'nests' of normoblasts which were seen prior to treatment were equally prevalent, but this similarity applied only to the degree of cellularity. Prior to treatment mitotic figures were rarely seen in these 'nests', and, when obvious, were in telophase, representing (Leitner, 1949) the 'lag' karyokinetic curve of decreased mitosis. The appearance was of numerous cells lying dormant. At the peak of the reticulocytosis, however, the marrow presented the picture of intense activity. Mitotic figures were frequently seen in the 'nests' with the prophase and metaphase predominating. Examinations performed two weeks after ascorbic-acid therapy had begun gave results indistinguishable from normal in all patients, whether iron, folic acid, or vitamin B<sub>12</sub> had or had not been given previously.

*Gastric juice.* Fractional test meals showed a histamine-fast achlorhydria in seven (Cases 1, 2, 3, 5, 6, 8, and 9) and hypochlorhydria in three (Cases 4, 11, and 13) of the 12 patients studied. Peptic activity was present in each case.

*Plasma-iron* was estimated in seven patients (Case 6, and Cases 8 to 13). In all it varied between 18 and 56  $\mu\text{g.}$  per 100 ml. The total iron-binding capacity was also very low. In Case 11 the levels of plasma-iron (28  $\mu\text{g.}$  per 100 ml.)

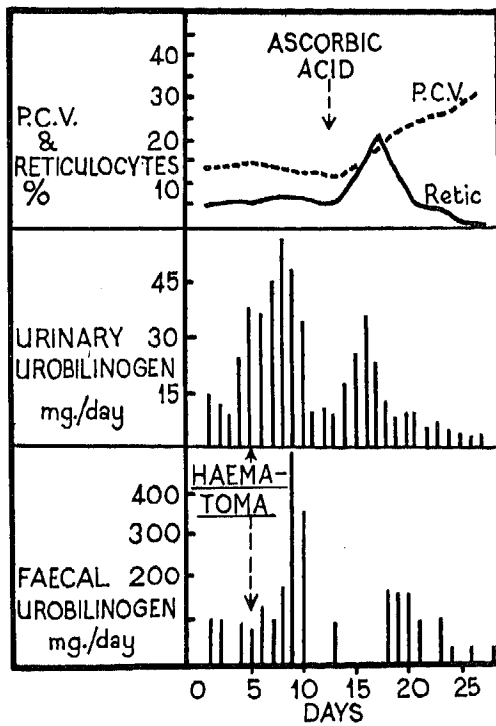


FIG. 1. Case 6. A capillary fragility test caused haematomata in the forearm. An increased urobilinogen excretion followed, but the reticulocytes and packed cell volume (P.C.V.) remained unchanged until ascorbic acid was given.

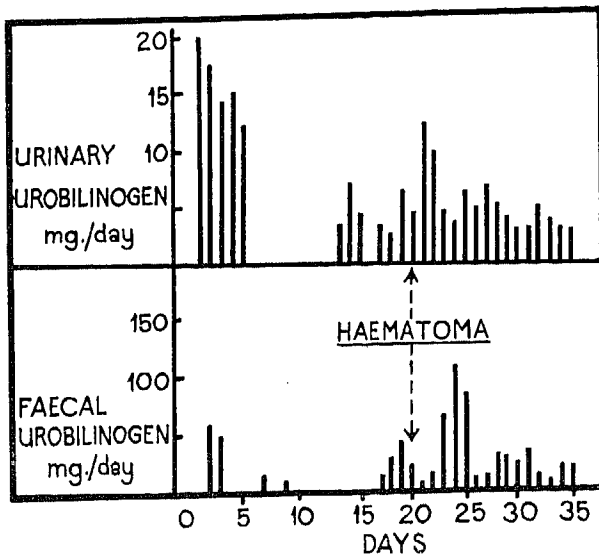


FIG. 2. Case 7. A case of scurvy without anaemia and without haematomata. The intramuscular injection of only 40 ml. of the patient's own blood resulted in an increased urobilinogen excretion.

and total iron-binding capacity ( $54 \mu\text{g.}$  per 100 ml.) were among the lowest found, yet the packed cell volume was 42 per cent. The outstanding feature common to these seven patients was the presence of one or more intramuscular haematomata. Unfortunately no anaemic patient who had scurvy without haematomata was available for study. In Case 8 the plasma-iron dropped from  $44 \mu\text{g.}$  to  $18 \mu\text{g.}$  per 100 ml. after the degree of haematoma-formation was

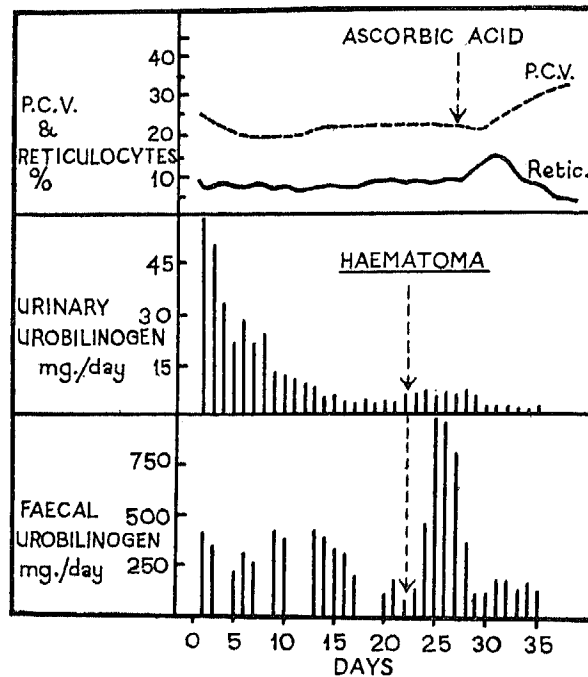


FIG. 3. Case 8. Deliberate haematoma-production, and the effect on the urobilinogen excretion and the anaemia. In this case no 'spill-over' into the urine occurred.

increased with a positive pressure cuff, yet no significant deterioration of the anaemic state followed. In two patients (Cases 10 and 12), to whom ascorbic acid was given soon after admission, the blood picture reached normal figures with the plasma-iron still low and the haematomata still palpable.

*Urobilinogen excretion.* With rest in bed there was a gradual fall in the urinary urobilinogen excretion, often to normal or nearly normal levels. This decrease was not seen in the faecal levels. Their fall lagged far behind that of the urinary levels, and in patients who received ascorbic acid soon after admission the faecal urobilinogen remained high for some time after treatment had begun. In the first patient studied (Case 6) it was noticed during the phase preceding treatment that the urinary urobilinogen began to increase, and a rise in the faecal urobilinogen soon followed, but no change in the reticulocytes, packed cell volume, or white-cell count took place (Fig. 1). The day before this increase, however, large haematomata had appeared while capillary fragility was being tested with the positive-pressure-cuff method of Wright and Lilien-

feld (1936). A non-anaemic scorbutic patient (Case 7), who had no deep haematomata, but much urobilinogenuria and other signs of grossly defective liver function, was subjected to the same manoeuvre of positive pressure with a blood-pressure cuff. As no haematomata resulted, 40 ml. of blood drawn from his vein was injected into his buttock. Even with this small amount of blood there was an immediate rise in the urinary urobilinogen level, followed later

TABLE III

*The Relation between the Degree of Anaemia, the Urobilinogen Excretion, and the Extent of Haematoma-Formation on Admission*

The normal range of faecal urobilinogen in 30 controls in the present series was from 16 mg. to 175 mg. per day; 83 per cent. of the estimations fell between 25 mg. and 100 mg. per day.

Case number	Packed cell volume (%)	Serum-bilirubin (mg./100 ml.)	Urobilinogen (mg./day)		Extent of haematoma
			Urinary	Faecal	
6	13	0	57.0	490	+++
7	53	0.5	19.6	57	Nil
8	22	0	59.9	390	++
9	23	0	21.2	328	++
10	26	0	15.8	303	++
11	42	1	20.8	310	++
12	27	0	13.5	125	+
13	15	0	63.0	510	++

by a rise in the faecal level, although the latter still remained within normal limits (Fig. 2). When the urobilinogen levels had settled, the small venesection was repeated but the blood was not injected, and no change occurred in the urobilinogen excretion. The fall in urinary urobilinogen with rest in bed, which was noted in all the patients, was allowed to reach normal or nearly normal levels in two other patients (Cases 8 and 9). Positive-pressure cuffs were then applied. In Case 8 the haematoma of the calf, which had decreased considerably in size, then became much larger and more painful. No significant change in the urinary urobilinogen excretion followed, but the faecal urobilinogen rapidly increased to more than 800 mg. per day. There was no change in the already elevated reticulocyte count, and the packed cell volume remained at 23 per cent. (Fig. 3). In Case 9 no haematomata resulted from the positive pressure. No change in urobilinogen excretion or in the haematological picture followed. Towards the end of the control period in this patient, the packed cell volume dropped fairly rapidly from 23 per cent. to 17.5 per cent., but the faecal urobilinogen excretion remained well within normal limits. The haematoma at this stage was barely palpable. From Table III, in which the urobilinogen level is compared with the blood state and the extent of haematoma-formation on admission, it will be seen that a high faecal urobilinogen level was found in a non-anaemic scorbutic patient with a large haematoma (Case 11). In another non-anaemic scorbutic patient who had no haematomata (Case 7) low levels were found. No anaemic scorbutic patient without haematomata was available

for study. Only one patient showed bilirubinaemia (Case 2). In this case both bile and urobilin were present in the urine.

*The effect of therapy.* Ten anaemic patients (Cases 1 to 6, 8 to 10, and 12) were available for study of the haematological progress.

*Rest in bed.* In all cases an improvement in the scorbutic state followed. No further bleeding was evident, and the haematomata slowly disappeared. Where

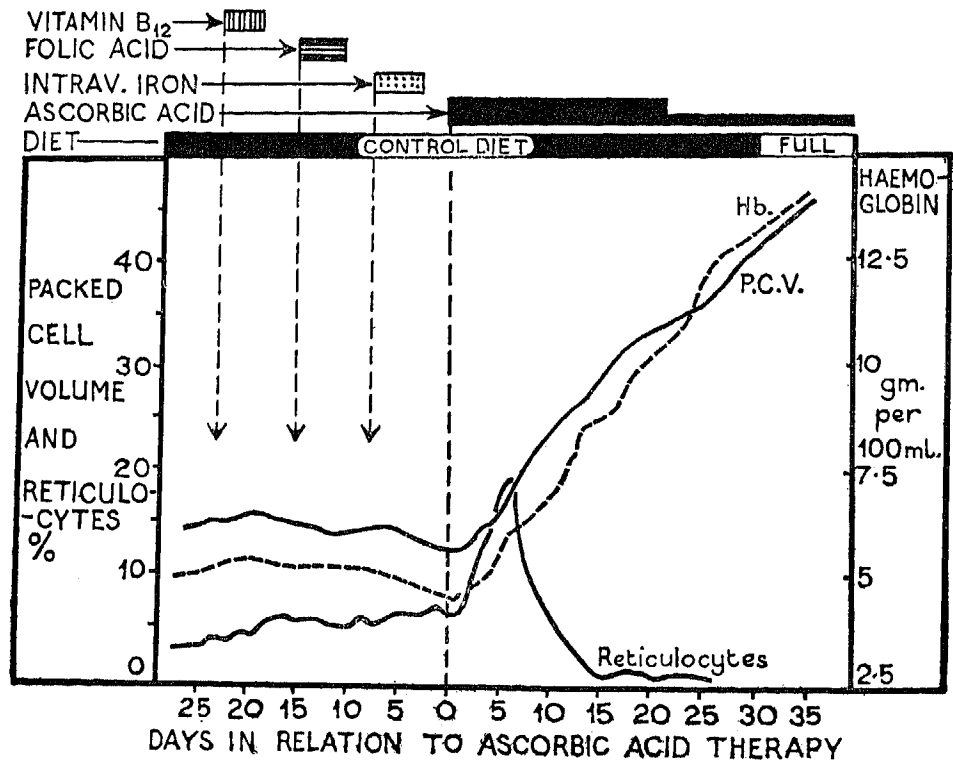


FIG. 4. Case 6. Vitamin B<sub>12</sub>, folic acid, and intravenous iron had no effect during the control period. There was an immediate response to synthetic ascorbic acid. The original diet was not changed until the packed cell volume (P.C.V.) reached 40 per cent.

the anaemia was severe the reticulocytes increased. In three patients (Cases 5, 6, and 9) a rise in the packed cell volume followed, but in two of these, in spite of a persistently raised reticulocyte count, the anaemia subsequently deteriorated. The third patient (Case 5) was the only one in whom obvious external bleeding could have contributed to the anaemia. His initial complaint was epistaxis. During the 10-day period prior to ascorbic acid therapy his packed cell volume rose by 4 per cent., but in the 10 following days it increased by 12.5 per cent. In this case the beneficial effect of ascorbic acid is graphically shown in Fig. 6.

*Haemopoietic and other agents.* In the three patients subjected to prolonged control periods (Cases 6, 8, and 9) neither vitamin B<sub>12</sub> nor folic acid had any effect on the peripheral blood, bone-marrow, or clinical features (Figs. 4 and 5). In the first five patients (Cases 1 to 5) no iron, additional to that present in the

diet on which the disease developed, was necessary to complete the haematological response that followed ascorbic-acid therapy. The other five anaemic patients (Cases 6, 8, 9, 10, and 12) received iron additional to that present in the diet. Iron was given intravenously to counteract any defective absorption that might be due to scurvy. In Cases 6, 9, 10, and 12 it was given during the control period prior to ascorbic-acid therapy. No effect was seen, either direct

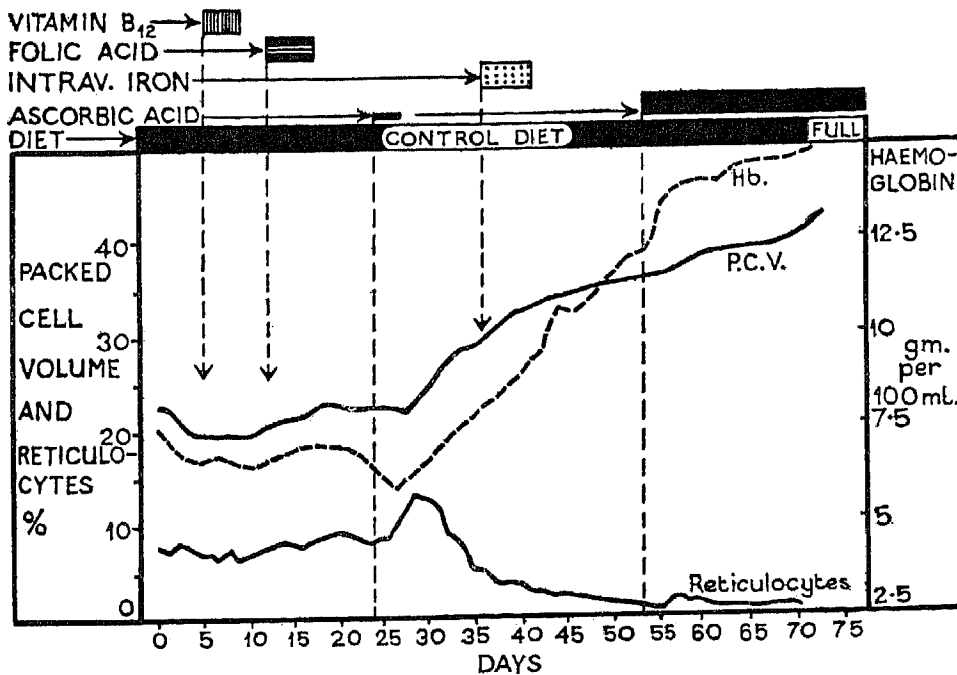


FIG. 5. Case 8. No double reticulocyte response occurred when iron was given after a small dose of ascorbic acid. Further ascorbic acid had to be given for full haematological remission.

or indirect, through hastening the subsequent response to ascorbic acid (Fig. 4). Parenteral iron was given to one patient (Case 8) after a small oral dose of ascorbic acid. No double reticulocyte response occurred; in fact, the rapid response to the oral ascorbic acid slowed down, and full regeneration did not occur until further ascorbic acid was given (Fig. 5). Other agents used were parenteral vitamin-B complex (six cases), penicillin (10,000 units daily) sucked in lozenge form (four cases), and parenteral streptomycin (1 gm. daily for 10 days) with oral succinylsulphathiazole (one case). No effect on the anaemia was seen. In one patient seen subsequently to the present series adrenocorticotrophic hormone, in one dose of 25 mg., led to epistaxis so alarming that blood transfusion and ascorbic acid had to be given immediately.

*Ascorbic acid.* The synthetic ascorbic acid used in the present investigation was found to have no effect on anaemia resulting from other causes such as infection, malignant disease, or iron deficiency, or on pernicious anaemia in relapse. In the present series of 32 cases of adult scurvy, no anaemic patient

failed to respond rapidly and completely to synthetic ascorbic acid. Except one patient, Case 25, who received blood transfusions, Cases 14 to 32 received no haemopoietic agents other than those that may be present in a full mixed

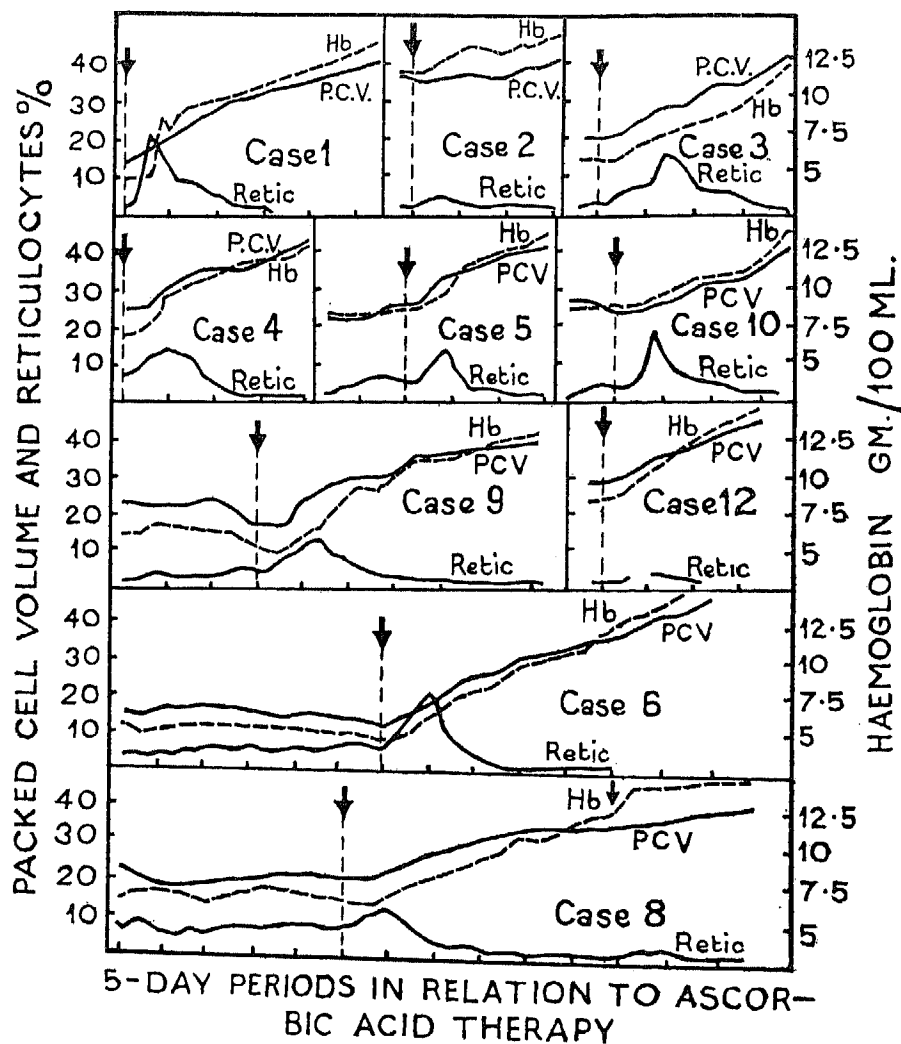


FIG. 6. No patient failed to respond promptly and completely when synthetic ascorbic acid was added (indicated by arrow) to the diet on which the disease developed.

hospital diet. It was possible, therefore, that other deficient factors were supplied in this diet. As regards Cases 1 to 12, whose original dietary environment remained unchanged, the results in the anaemic patients are illustrated graphically in Fig. 6. Where the anaemia was severe enough a prompt reticulocytosis, reaching its peak between the fourth and sixth day, followed ascorbic-acid therapy. In every case a complete and rapid haematological recovery was seen; no other factor such as adequate protein intake, iron, folic acid, or vitamin B<sub>12</sub>, was necessary before, during, or after ascorbic acid therapy, whether these

factors were deficient at the same time or not. The response appeared to be no less rapid in the two patients who became ambulant at the onset of ascorbic acid therapy (Cases 5 and 12). The fact must be emphasized again that this response occurred without any alteration of the basic environment. The mixed hospital diet was given, in Cases 1 to 12, only after the packed cell volume had reached normal figures.

#### *Discussion*

Those who feel that vitamin C is not an essential factor in erythropoiesis have brought forward many points which bear discussion. Recent investigations have shed light on many of these points. Aron (1939) and Sigal (1939) have shown that the inconstancy with which anaemia appears in the scorbutic guinea-pig is due to the size and age of the animal. Animals below 500 gm. in weight are liable to die from infection before the onset of anaemia, which is the last feature to develop in the scorbutic syndrome. Jennings and Glazebrook (1938) suggested that a similar trend in the development of the full scorbutic syndrome exists in man. The results obtained in the present series of patients agree with this suggestion. The presence or absence of anaemia in a given case of scurvy would then depend on its severity. This view would invalidate the approach of Croft and Snorf (1939), Liu, Chu, Yu, Hsu, and Cheng (1941), Lozner (1941), and Schulze and Morgan (1946), who tested the effect of ascorbic acid on an anaemia associated with a biochemically low ascorbic-acid status. In many of their subjects clinical evidence of scurvy was not present. Subsequently it was shown in experimental human scurvy (Medical Research Council, 1948) that the ascorbic-acid content of the white-cell-platelet layer, which provides the most reliable biochemical test, could be zero for as long as 40 days before follicular hyperkeratosis appeared. The absence of any ill health during the pre-clinical phase, and the fact that the minimum daily requirement of ascorbic acid lay in the region of 10 mg., were two further points established in this experiment.

The failure to produce anaemia in experimental human scurvy has been one of the strongest arguments used against the erythropoietic function of vitamin C in the organism. In no case, however, was the severe stage of scurvy reached. Considerations of wound healing (Crandon, Lund, and Dill, 1940) and certain unpleasant cardiovascular effects (Medical Research Council, 1948) terminated these experiments. Even the loss of six litres of blood during the control period (Crandon, Lund, and Dill, 1940), without the development of anaemia, is no argument against a specific influence of vitamin C on the erythron. From the evidence that has so far accumulated, both in the guinea-pig and man, it would appear that at this early stage of scurvy the erythron is functioning normally. When Ungley (1938) reported a spontaneous reticulocytosis and haematological remission in a scorbutic patient taking a diet low in vitamin-C content, a train of reports appeared disclaiming the specificity of vitamin C in the anaemia of adult scurvy. The absence of deterioration of the anaemia during prolonged control periods in bed, with a diet low in vitamin C, led to similar conclusions

by Ralli and Sherry (1941). It is noteworthy that prior to 1938 the specificity of vitamin C in this form of anaemia had been accepted. This was due to the confirmation of the beneficial effect of orange juice (Shattuck, 1928; Mettier, Minot, and Townsend, 1930; Nisenson and Cohen, 1937; Young, 1938) by the use of synthetic ascorbic acid (Vaughan, 1934; Dunlop and Scarborough, 1935; Jennings and Glazebrook, 1938). Apparently the control diets used by Ungley and by Ralli and Sherry were not completely devoid of vitamin C. In view of subsequent information as to the very small daily requirement of the vitamin, such a diet would not provide adequate control. The profound influence of the metabolic demand on the clinical features of scurvy was not widely known at that time. The resolution of the haemorrhagic manifestations of scurvy during rest in bed, and their reappearance when the patient was made ambulant, were illustrated clearly by Schultzer (1936, 1937), Vilter, Woolford, and Spies (1946), and Brown (1951). There is reason to believe that even in the severer stages of scurvy the tissues are never completely depleted of vitamin C (Pirani, 1952). Considering the small daily requirement, and the fact that rest in bed will release more vitamin C for erythropoiesis, it is reasonable to expect some haematological remission when the patient is kept in bed. The necessity of a preliminary period of rest in bed, before any other measures are tried, now becomes obvious.

Scurvy is a disease resulting from a dietary deficiency. Anaemia itself may result from deficient factors in the diet. The deficiency may depend on the increased demand for vitamin C caused by infection or other diseases, and such diseases by themselves may be potent causes of an associated anaemia. With such complex possibilities, controlled experiment is impossible if the environment in which the disease developed and flourished is altered. An adequate diet, and even a diet lacking in vitamin C, may allow the surreptitious treatment of a combined deficiency, whereas a severely restricted diet, if continued for prolonged control periods, may allow the development of new deficiencies to distort the haematological picture. A multiple deficiency or diseased state may explain the variable morphology that has been described in the anaemia of adult scurvy. This fact has further confused the issue. Unfortunately confirmation by measurements of the mean cell-diameter is rarely found. Reports on the bone-marrow, or serial bone-marrow studies, in scurvy are also rare. They are available in publications by Harris (1928), Mettier, Minot, and Townsend (1930), Mettier and Chew (1932), Wolbach (1937), Jennings and Glazebrook (1938), Israëls (1943), McMillan and Inglis (1944), Vilter, Woolford, and Spies (1946), May, Sundberg, Schaar, Lowe, and Salmon (1951), and Proehl and May (1952). Hypocellular and hypercellular, normoblastic and megaloblastic bone-marrow pictures have been described. Parsons and Hawksley (1933), Parsons and Smallwood (1935), and Parsons (1938) attempted to explain this variability by a slowing of erythropoiesis which may become disproportionate, while Jennings and Glazebrook (1938) suggested that the macrocytosis is related to the severity of the lack of vitamin C. The latter view receives support from the results seen in the present series, and from Proehl and May (1952), who have recently

shown that a megaloblastic anaemia becomes superimposed as the anaemia of scurvy progresses in monkeys. Undoubtedly age, the quantity of endogenous vitamin C, the severity of the anaemia, and possible complicating deficiencies and diseases are factors to be considered when interpreting these conflicting findings. In some cases the haemorrhagic tendency in scurvy may be responsible for the variable blood picture by producing a superimposed hypochromic anaemia. It has been suggested that the anaemia may be due entirely to this mechanism (Wintrobe, 1946; Proehl and May, 1952), so that the response to ascorbic acid would be due merely to cessation of the loss of blood. Against this view is the observation made by McMillan and Inglis (1944) and Vilter, Woolford, and Spies (1946), that no correlation exists between the extent of ecchymosis or of external loss of blood and the severity of the anaemia. In the patients of the present series who were studied over long control periods, the haematomata slowly disappeared, yet the anaemic state slowly deteriorated. Induction of further bleeding into the tissues with positive-pressure cuffs did not lead to any sudden deterioration of the anaemia. Neither in its morphology nor in its response to iron therapy, nor in the capacity of the plasma to bind iron, did this anaemia resemble that due to chronic loss of blood.

Differentiation, on morphological grounds, of the anaemia of adult human scurvy from the deficiency dyshaemopoietic anaemias can be exceedingly difficult. The appearance of the peripheral blood smear, the leucopenia, the histamine-fast achlorhydria, and the occasionally associated megaloblastic bone-marrow have led to an erroneous diagnosis of pernicious anaemia. Recent studies have focused interest on the relation between folic acid and ascorbic acid in the metabolism of certain aminoacids (Sealock, Perkinson, and Basinski, 1941; Sealock and Lepow, 1948; Woodruff and Darby, 1948; Woodruff, Cherrington, Stockell, and Darby, 1949; Luhby and Wheeler, 1949; Rogers and Gardner, 1949; Morris, Harpur, and Goldbloom, 1950; Vilter, Horrigan, Mueller, Jarrold, Vilter, Hawkins, and Seaman, 1950; May, Nelson, Salmon, Lowe, Lienke, and Sundberg, 1950). In the scorbutic monkey megaloblastic bone-marrow has reverted to normal after the use of folic and folinic acid (Proehl and May, 1952). It is significant, however, that the experimental diet in these monkeys was poor in folic acid, and that the control animals developed anaemia in spite of ascorbic-acid supplements. The latter anaemia, however, was much later in onset than that of the test animals. Thus the exact relationship of this experimental anaemia in scorbutic monkeys to the anaemia of adult human scurvy is uncertain. In the treatment of adult human scurvy, an ordinary hospital diet lacking vitamin C (Vilter, Woolford, and Spies, 1946), with liver supplement (Jennings and Glazebrook, 1938) and with vitamin-C-free liver (Mettier, Minot, and Townsend, 1930) failed to induce a response until vitamin C was given. In the present series neither vitamin B<sub>12</sub> nor folic acid was effective in the three patients studied; none of these three, however, had a megaloblastic bone-marrow.

The low plasma-iron levels and the low total iron-binding capacity in the present series seemed to bear a close relationship to the intramuscular

haematomata, and therein showed a state analogous to the experimental anaemia resulting from a sterile intramuscular turpentine abscess, produced in the dog by Cartwright and Wintrobe (1949). Only in this respect, however, were the two forms of anaemia similar. There was no correlation between the presence or severity of the anaemia and either the plasma-iron level or the extent of haematoma-formation. An increase of haematoma-formation by a positive-pressure cuff had no effect on the anaemic state, but a marked rise in the faecal excretion of urobilinogen followed. It appeared that extravascular haemolysis in the haematoma could account for most of the excess in faecal urobilinogen. The absence of bilirubinaemia in these patients is akin to the results obtained by Pass, Schwartz, and Watson (1945) after the intravenous injection of haematin. Proehl and May (1952) have since shown, in the scorbutic monkey, that the increase of faecal urobilinogen coincided with the onset of periorbital haemorrhages and bleeding elsewhere into the tissues. In the present series, therefore, the plasma-iron, total iron-binding capacity, and faecal urobilinogen excretion were of no assistance in determining the mechanism of the anaemia. They appeared to be related to the intramuscular haematoma, which in turn appeared to have no direct relation to the anaemia.

It now seems unlikely that an intravascular haemolytic mechanism can have caused these increases of faecal urobilinogen. Otherwise it would have been an attractive explanation of the inconstant reticulocytosis seen in the severer stages of untreated scurvy, as Vilter, Woolford, and Spies (1946) originally suggested. Vaughan (1934), on the other hand, felt that such reticulocytosis was due to stimulation of the bone-marrow by repeated haemorrhages. In the present series the inconstant reticulocyte level on admission, compared with the constantly elevated level soon after rest in bed was instituted in all cases in which the anaemia was sufficiently severe, might suggest that some endogenous source of vitamin C was stimulating the bone-marrow. There is evidence that such a source exists, since the scorbutic patient is never completely depleted of vitamin C (Pirani, 1952). Through decreasing the demand made by functions such as locomotion, more vitamin C may become available for more vital functions such as erythropoiesis. In the milder cases, as previous reports have shown, this source may be sufficient for full regeneration when the patients are no longer ambulant. Where the body stores are less adequate a state is produced analogous to the slight reticulocytosis, without subsequent haematological remission, found in pernicious anaemia when an inadequate dose of liver has been given (Minot and Castle, 1935). The very small amount of vitamin C necessary both for the cure (Barnes, 1947) and for the prevention (Medical Research Council, 1948) of scurvy fits well with this hypothesis. In the present series of patients, when an exogenous source of vitamin C became available, the picture did not change except in degree. The reticulocytosis became even more marked, and the bone-marrow, although its appearance was similar to that found before treatment, was the scene of intense activity.

From the present study it seems unnecessary to postulate that a combined deficiency is responsible for the anaemia that is found in uncomplicated adult

human scurvy. Anaemia develops and persists in scurvy in spite of adequate supplies of iron, vitamin B<sub>12</sub>, and folic acid. Of the 26 anaemic patients in the series none failed to respond to synthetic ascorbic acid. In 10 patients whose diet remained unchanged after admission, this response was prompt and complete without any other factor being necessary before, during, or after such treatment.

It is a great pleasure to acknowledge the assistance I have received from Dr. Linder, from Dr. Meiring, from the Department of Chemical Pathology, and from Dr. Budtz-Olsen, who in addition performed the plasma-iron estimations. Dr. Moll, Dr. Mirvish, Professor Brock, and Professor Forman gave me access to patients under their care. I am indebted to Dr. L. Mirvish and Dr. Merskey for helpful criticism, and to the Council of Scientific and Industrial Research, Nutrition Unit, for laboratory facilities.

## APPENDIX

### *Case Reports*

The dietary and environmental history was similar in each case. All were male Bantus from distant rural and somewhat primitive areas. They had migrated to the city for the sole purpose of earning money as rapidly as possible. The money was to be used to buy cattle, which is the index of wealth among the rural Bantu. The cattle, in most instances, provided the traditional 'labola' or marriage fee, so that the quality or number of the proposed wives determined for each man his length of stay in the city. Strict economy was therefore practised. This meant that the cheapest food with the greatest bulk was chosen, regardless of its lack of nourishing qualities. Usually its vitamin-C content was nil. At the same time his preference for over-cooking in an open iron pot over a wood fire would ensure the complete destruction of any remaining trace of vitamin C. So steadfastly did the men follow their purpose that it was not uncommon to hear that they had left an employer who provided a midday meal, because it was thought that more money could be earned from an employer who was not so considerate. Since they were unskilled, their work was in the class of heavy manual labour. Time, as we know it in months and years, means little to the rural Bantu. For this reason the patient's age and the duration of his complaints can only be approximate. All showed the classical features of the scorbutic syndrome: follicular hyperkeratosis, particularly noticeable on the anterior aspect of the thighs and ulnar border of the forearms; perifollicular haemorrhage, which is very difficult to recognize in the black skin; localized or diffuse gingival hyperplasia at the site of dental caries; and certain other features, which will be mentioned in each case. To avoid unnecessary repetition, only the haematological features which are not contained in the text (Tables I to III and Figs. 1 to 6) will be mentioned in the case reports.

*Case 1.* D. Z., of Nyasa origin, aged 33 years, was admitted with a chronic non-healing ulcer of one month's duration, which had followed continual trauma from a shoe. It was situated just in front of the left medial malleolus. There was a haematoma near the right cubital fossa, and a haematoma of the right thigh. There was a mild pyrexia. The erythrocyte sedimentation rate (Westergren

method, uncorrected) was 60 mm. after the first hour. The serum-bilirubin was 0.5 mg. per 100 ml., and the serum thymol and colloidal gold reactions were normal. Ascorbic acid was given on the first day. The ulcer was completely healed by the 19th day, and two weeks later no signs of scurvy were to be seen.

*Case 2.* S. N., of Tanganyika origin, aged 47 years, was admitted with a painful haematoma of the left calf of two weeks' duration. A firm spleen was palpable 2 cm. below the costal margin. The erythrocyte sedimentation rate was 8 mm. after the first hour. The serum-bilirubin was 3.2 mg. per 100 ml., and bile and urobilin were present in the urine. The serum-albumin was 4.0 gm. and the serum-globulin 3.9 gm. per 100 ml. The thymol turbidity was 4 units, the thymol flocculation 3, and the serum colloidal gold reaction 4. Ascorbic acid was added to the control diet on the third day; it rapidly cured the anaemia and the scorbutic features, but had no effect on the defective hepatic function and the splenomegaly. No malarial parasites were ever isolated. He had come from a hyperendemic malarial area before migrating to Cape Town three years previously.

*Case 3.* D. M., of Tanganyika origin, aged 40 years, was admitted with a painful haematoma of the right popliteal fossa and thigh of two weeks' duration. A firm spleen could be felt 2 cm. below the costal margin. The serum-bilirubin, thymol turbidity, and colloidal gold reaction were normal. The serum-albumin was 3.5 gm. and the serum-globulin 2.8 gm. per 100 ml. Urobilin was present in the urine. The erythrocyte sedimentation rate (uncorrected) was 120 mm. after the first hour. Ascorbic acid was given after a three-day control period, and there was a rapid clinical response. The splenomegaly remained unchanged. No malarial parasites were ever isolated. He, too, had come from a hyperendemic malarial area before migrating to Cape Town.

*Case 4.* J. J., of Xhosa origin, aged 49 years, was admitted with a painful haematoma of the right calf which had lasted one week. There was a serous effusion into the neighbouring knee-joint, and pitting oedema over the anterior aspect of the right leg. The serum-bilirubin and the colloidal gold and thymol reactions were normal. The serum-albumin was 3.8 gm. and the serum-globulin 2.2 gm. per 100 ml. The erythrocyte sedimentation rate (uncorrected) was 60 mm. after the first hour. Ascorbic acid was given on the first day and discontinued on the 20th day, by which time all clinical features had disappeared except the follicular hyperkeratosis, which was just detectable. Vitamin-B complex and full diet caused no further rise in the packed cell volume.

*Case 5.* J. C., of Xhosa origin, aged 25 years, was admitted with epistaxis of two days' duration. His nostril was plugged, and no further bleeding occurred. The serum-bilirubin was normal, but the serum colloidal gold and thymol reactions were grossly abnormal. The serum-albumin was 3.3 gm. and the serum-globulin 2.6 gm. per 100 ml. The erythrocyte sedimentation rate (uncorrected) was 40 mm. after the first hour. After 10 days ascorbic acid was given and the patient was allowed to get up. Three weeks later the serum-albumin had increased to 4.5 gm. per 100 ml., and acid (14 units) appeared in the stomach after histamine stimulation. The liver-function tests remained grossly abnormal. Vitamin-B complex and full diet caused no further improvement.

*Case 6.* E. C., of Tanganyika origin, aged 27 years, was admitted after having collapsed three days previously. Seven weeks before, haematomata had appeared in his left forearm after trauma; other haematomata had followed

in the left leg. He had gone to bed, and after two weeks the swellings had subsided. Giddiness and syncope followed the assumption of the erect position. On examination there was pigmentation of the skin overlying a thickening of the underlying muscles at the site of the former swellings. There were splinter haemorrhages of the finger nails, and a firm spleen was palpable 2.5 cm. below the costal margin. No malarial parasites were demonstrable, but he had originated from a hyperendemic malarial area. There was no bilirubinaemia, and the thymol and colloidal gold reactions were normal. The serum-albumin was 4.0 gm. and the serum-globulin 3.0 gm. per 100 ml. The capillary fragility test caused large haematomata in the forearm (page 313, Fig. 1). The erythrocyte sedimentation rate (Westergren, uncorrected) was 126 mm. after the first hour. The mild pyrexia noted on admission rose to 101° F. after the fresh haematomata had appeared. During the control period of approximately 30 days, several haematinic agents were tried without success. The packed cell volume dropped slowly from 15 per cent. to 12.5 per cent., while the reticulocyte percentage remained fairly constant in the region of 7. During this time, in spite of a constant fluid intake, the urinary output steadily lessened, and the liver-function tests showed no change. Penicillin lozenges had no effect on the gingival hyperplasia, but the pain and oral fetor were improved. The pyrexia, urinary output, leucopenia, and erythrocyte sedimentation rate soon reverted to normal after ascorbic-acid therapy. The serum-albumin rose to 4.6 gm., and the serum-globulin dropped to 2.7 gm., per 100 ml. The skin became shiny and less dry, but the follicular hyperkeratosis did not disappear until five weeks later. The pigmented areas gradually faded. The splenomegaly did not change.

*Case 7.* J. K., of Zulu origin, aged 50 years, was admitted because of progressive weakness and a painful mouth of three weeks' duration. Follicular hyperkeratosis, perifollicular haemorrhages, and gingival hyperplasia were the scorbutic signs, but there were well advanced mental, tongue, and skin signs of vitamin-B complex deficiency. The latter features disappeared promptly when vitamin-B complex was given. Full diet and vitamin C were given five weeks later because, being mildly scorbutic and without anaemia, he acted as a control in the studies of urobilinogen excretion (page 313, Fig. 2). The erythrocyte sedimentation rate remained between 3 mm. and 8 mm. per hour, and the serum-bilirubin, 0.5 mg. per 100 ml., was unchanged at the end of this period. The liver-function tests were grossly abnormal, and remained so. The serum-albumin dropped from 5.3 gm. to 4.6 gm., and the serum-globulin from 3.2 gm. to 2.7 gm., per 100 ml. The packed cell volume fell from 59 per cent. to 53 per cent., and it is possible that the initial figures were due to the slight dehydration noticed on admission. The fractional test meal disclosed no change, free acid being present before and after histamine.

*Case 8.* Q. S., of Xhosa origin, aged 35 years, was admitted with painful swellings of the left calf and right forearm. The swellings were haematomata. The erythrocyte sedimentation rate (uncorrected) was 60 mm. per hour. The icteric index was 14, but there was no bilirubinaemia, and the thymol and colloidal gold reactions were normal. The serum-albumin rose from 3.4 gm. to 4.1 gm., and the serum-globulin dropped from 2.5 gm. to 1.5 gm., per 100 ml., during the control period. Pyrexia did not disappear until ascorbic acid was given, and the increase in the haematoma after the application of a positive-pressure cuff caused the temperature to rise further. The effect of this manoeuvre on the urobilinogen excretion is shown in Fig. 3 (page 314), and the plasma-iron decreased at the same time from 44  $\mu$ g. to 18  $\mu$ g. per 100 ml. Nine days later, the fifth day after the first dose of ascorbic acid, it was 56  $\mu$ g., and

the packed cell volume was 27 per cent., 5 per cent. higher than before treatment. Twenty days after treatment had begun the packed cell volume was 36 per cent., the plasma-iron 86  $\mu\text{g.}$  per 100 ml., and the total iron-binding capacity 393  $\mu\text{g.}$  per 100 ml. Folic acid, vitamin B<sub>12</sub>, and vitamin-B complex had no effect during the control period of 26 days. No iron was given until the reticulocytosis following three daily oral doses of 100 mg. ascorbic acid had settled. Thirty days later more ascorbic acid had to be given, as the packed cell volume had remained stationary at 36 per cent. for the previous 10 days (page 317, Fig. 5). Full diet was given two weeks later. Seventeen days after the initial ascorbic acid the haematomata were barely palpable, but the colloidal gold reaction and thymol flocculation had become abnormal, and remained abnormal up to the time of discharge from hospital. The achlorhydria remained histamine-fast.

*Case 9.* F. G., of Nyasa origin, aged 24 years, was admitted with a painful haematoma of the right calf and an effusion of the right knee-joint of two weeks' duration. For four weeks he had difficulty in eating owing to painful, swollen gums, which did not bleed. Just before the onset of his illness he had had an episode of diarrhoea. The uncorrected erythrocyte sedimentation rate was 130 mm. after an hour. There was no bilirubinaemia, but the serum colloidal gold and thymol reactions were abnormal. The serum-albumin was 4.6 gm. and the globulin 2.4 gm. per 100 ml. The plasma-iron was 44  $\mu\text{g.}$  per 100 ml. On a few occasions a small amount of fresh blood was noticed in the stools. Bacteriological examination was negative, and streptomycin with sulphasuccidine had no effect. No blood was seen in the stools after ascorbic-acid therapy. At the end of the 17-day control period the haematomata had almost resolved, but the sulphasuccidine may have contributed towards the low normal levels of faecal urobilinogen at that time. Folic acid, vitamin B<sub>12</sub>, and intravenous iron had no effect. On the 10th day of the control period the packed cell volume was 1.5 per cent. higher, but a drop of 5 per cent. during the next seven days led to the administration of ascorbic acid. As in Cases 6 and 8, an increased urinary output followed, and the erythrocyte sedimentation rate fell rapidly to normal. The serum colloidal gold and thymol reactions remained abnormal even on a full diet, which was given five weeks after ascorbic-acid therapy had begun. Free gastric acid (6 units) reappeared, and reached 13 units after histamine. The mild pyrexia, as in the other severe cases, reverted to normal after ascorbic acid.

*Case 10.* E. M., of Pondoland, aged 59 years, was admitted with haematomata of the left calf and arm, and right thigh, of more than three weeks' duration. Two months previously he had noticed painful, swollen gums for the first time. Seven months previously he had been in hospital for 10 days with scorbutic changes in the skin and gums, but no haematomata were palpable. There was no anaemia (Case 14, page 311, Table I). The duration of his complaints at the time of that admission to hospital was only two weeks; during the 10 days in hospital he received 500 mg. ascorbic acid intravenously daily for four days, and 300 mg. by mouth three times daily for the remaining period. According to his dietary history no further vitamin C was consumed for the next seven months. The erythrocyte sedimentation rate (uncorrected) was 68 mm. after the first hour. The serum-albumin was 4.5 gm. and the serum-globulin 2.8 gm. per 100 ml. The serum colloidal gold and thymol reactions were slightly abnormal, and did not change after treatment. There were 7 units of free acid before, and 20 units after, histamine; these amounts increased to 62 units and 78 units three weeks after treatment. There was radiological evidence of healed

pulmonary tuberculosis in the right apical region. Repeated examinations of the sputum were negative. The serum-bilirubin was 0.5 mg. per 100 ml. The plasma-iron and total iron-binding capacity were low. Ascorbic acid was given on the sixth day. Eighteen days later, when the packed cell volume was 41 per cent., full diet was given. An apparent diuretic effect of ascorbic acid was again noticeable, and the mild pyrexia returned to normal. The unusual feature of this case was the failure of the erythrocyte sedimentation rate to return to normal. No cause for this could be found.

*Case 11.* T. P., of Nyasa origin, aged 27 years, was admitted with a painful haematoma of his right calf of three weeks' duration. There was a mild pyrexia. The serum-bilirubin was 1 mg. per 100 ml., the serum-albumin 2.8 gm., and the serum-globulin 3.7 gm. per 100 ml. After two weeks of ascorbic-acid therapy this ratio was still abnormal (albumin 3.8 gm., globulin 4.2 gm. per 100 ml.). The serum colloidal gold and thymol reactions remained normal throughout. The plasma-iron was 28 $\mu$ g. and the total iron-binding capacity 54 $\mu$ g. per 100 ml. There was no achlorhydria. There was slow regression of the haematoma after ascorbic-acid therapy, and the plasma-iron level rose slowly to normal figures. The packed cell volume was 46 per cent. on his discharge.

*Case 12.* G. M., of Xhosa origin, aged 55 years, when first seen had a haematoma of the right calf of one month's duration. He remained ambulant throughout the period of observation. There was no bilirubinaemia; the serum-albumin was 3.7 gm. and the serum-globulin 3.8 gm. per 100 ml. The serum colloidal gold and thymol reactions were normal. The plasma-iron was low. Unfortunately the reticulocytes were not counted between the second and sixth days of ascorbic-acid therapy, but the packed cell volume rose from 27 per cent. to 46 per cent. in only 18 days. The clinical features, in spite of ambulation, rapidly improved.

#### *Summary*

1. From a study of 32 cases it appears that anaemia has a high incidence in adult scurvy, and can be very severe.

2. In 10 consecutive cases of adult scurvy with anaemia, a prompt and complete haematological response followed the addition of pure ascorbic acid alone to the diet on which the disease developed. This response occurred without requiring an adequate aminoacid intake, iron, extrinsic factor, or other vitamin before, during, or after vitamin-C therapy, whether these factors were deficient in the diet or not. Folic acid, vitamin B<sub>12</sub>, and intravenous iron failed to have any influence on the bone-marrow or blood picture, but no patient failed to respond to synthetic ascorbic acid. To the other anaemic patients no known haemopoietic factor, other than that already present in the ordinary hospital diet with added ascorbic acid, was given. In each case a rapid and complete haematological recovery occurred.

3. Morphologically the anaemia is normochromic and normocytic, but occasionally macrocytic in the more severe cases. Variation of the size and haemoglobin-content of the red cells becomes more noticeable as the anaemia progresses. Variation in shape was less frequently seen. Reticulocytes may or may not be present in increased numbers in the severer cases. When rest in bed is instituted this increase is constant.

4. The bone-marrow, although often hypercellular in appearance, shows decreased mitosis. Occasionally megaloblasts are seen. This appearance, with the frequent leucopenia and a histamine-fast achlorhydria, may combine with the above features to simulate other deficiency dyshaemopoietic anaemias of the megaloblastic type. Pepsin activity is normal.

5. Increased excretion of pigment, suggestive of a haemolytic process, was shown to be due to extravascular haemolysis in the haematoma. No correlation existed between the state of anaemia and the fluctuations in pigment excretion.

6. The haematoma appeared also to be responsible for the low levels of plasma-iron and iron-binding capacity.

7. It is felt that the conflicting views as to the participation of ascorbic acid in erythropoiesis have been due to the following causes: (1) Ascorbic acid by itself has no proved effect in combating any condition other than scurvy. (2) Both anaemia and scurvy may result from dietary deficiencies, and to prove that one is the result of the other a very strict dietary control is necessary. The simplest, and yet most accurately controlled, diet is probably that on which the disease developed. (3) Lack of vitamin C, when it causes anaemia, is chronic and takes the form of clinically obvious scurvy; it therefore does not mean merely the chemical state of 'unsaturation'. (4) Influences exerted by haemorrhage, by other deficiencies, and by diminished metabolic requirements (such as occur with rest in bed) should be eliminated before the effect of pure synthetic ascorbic acid on erythropoiesis is assessed.

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