SUBSCORBUTIC STATES

with emphasis on

SUBSCORBUTIC ANAEMIA

by

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Being a Thesis Presented for the Degree of

Doctor of Medicine

of The University of Glasgow

in the year One Thousand Nine Hundred and Forty Three.

" Let us cherish a sober mind, and take for granted that in our best performances there are latent many errors which in their own time will come to light...."

--- W.E.Gladstone, 1887.

Introduction.

This work was carried out in general practice, in a coalfield on the North-East coast of England, in the fourth year of war. It was an assay of the vitamin C nutritional level of a cross section of the population of this area, so far as could be judged from the cases seen in the course of practice, and an investigation of the part , if any, played by lack of vitamin C in the causation of the nutritional deficiency anaemia so frequently encountered here. (Personal observation).

It is generally considered that iron nutrional deficiency anaemia in adult males in Britain is a rarity (Davidson 1943), but it has been my experience in this area, over a number of years, to find a definite degree of anaemia present in a considerable number of male patients, where no source of bleeding could be found after a careful search. As the daily iron requirement is so small, somewhere in the region of 15 mgm. daily for women and children, and half this amount for men (Whitby and Britton 1942), it is extremely unlikely that iron is the deficient factor in all of these cases. Indeed, it would be surprising if lack of iron in the diet was the cause in any of the male cases.

The enquiry was prompted by two reasons. In the first place, one had the impression that there were a certain number of cases of apparently simple iron nutritional deficiency anaemia which did not respond either as rapidly or as fully to massive iron therapy as was to be expected, and the impression that the number of such refractory cases was rapidly increasing. In the second place, as both lay and medical press seemed intent on making the population "vitamin conscious" it appeared to be reasonable to enquire whether there was really either widespread or marked vitamin C subnutrition, if so whether a definite effect on the blood picture resulted, and lastly whether administration of the vitamin in synthetic form brought about any marked improvement in the blood picture in either normally nouished or subscorbutic cases.

Three questions were asked, and an attempt to answer them was the aim of this work. The questions were:-

(a) Is there any marked vitamin C subnutrition among the people of this area?

(b) Is vitamin C subnutrition accompanied by a constant and typical alteration in the blood picture?

(c) Has ascorbic acid therapy any effect on these nutritional anaemias when used alone or in combination with other haematinics?

The cases investigated were those usually seen in the course of general practice. Cases were selected as opportunity arose and time for investigation allowed. Only adults were included in the series because of the relative complexity of the tests employed. Harris, Passmore and Pagel (1937) state that the vitamin C content of the tissues, especially the suprarenal but also the liver, is diminished in fever. Wright (1938) states that in increased metabolism with or without pyrexia, in achlorhydria, colitis, pneumonia, tuberculosis, rheumatic fever, whooping cough and osteomyelitis, there was vitamin C deficiency whether the intake was adequate, as judged by the usual standards or not. Abbasy, Harris, Ray and Marrack (1935) state that there is a sharp drop in the vitamin C excretion rate in feverish colds which lasts for about one week.

Such cases as those noted above which lead to a diminished excretion rate were excluded, as were cases of haemolytic, haemorrhagic and aplastic anaemias.

The time chosen for the survey was late winter, spring and early summer, this being the period of the year when the intake of vitamin C is at its lowest.

When a case was presented, it was investigated on the following lines.

(1). A careful dietary history was taken and a routine clinical examination made.

(2). An estimation of the state of vitamin C nutrition of the subject was made, as shown by the amount of vitamin C excreted in the urine in 24 hours. Confirmation of the state of the reserves was obtained by test-dosing with ascorbic acid, either at the beginning of the investigation, or at the end of a control period. This estimation was performed in 100 cases.

(3). The blood picture of these cases was investigated, the routine followed in each case being the red cell count, reticulocyte count, haemaglobin estimation, colour index, non-differential white cell count, blood sedimentation rate reading and examination of the stained blood film. The blood sedimentation rate was measured in every case, but the complete blood examination was performed in only 87 of the 100 cases, the remaining 13 being unable or unwilling to allow complete investigation.

(4). From the foregoing data, the case was classified in 87 instances, and placed in one of the four following groups.

GROUP I. Cases showing adequate vitamin C nutrition and a blood picture within normal limits.

GROUP II. Cases showing unsatisfactory vitamin C nutrition and a blood picture within normal limits.

GROUP III. Cases showing unsatisfactory vitamin C nutririon and some degree of anaemia.

GROUP IV. Cases with adequate vitamin C nutrition and some degree of anaemia.

An analysis of the findings in each group was then made.

The third part of the survey, viz. observation of the effect of controlled therapy on the blood picture, was more difficult to perform. A few cases one did not feel justified in subjecting to prolonged investigation; some defaulted in one way or another; and a few had to be omitted because they developed some infection during the course of treatment. In all, 65 cases completed the course of treatment with ascorbic acid. A group of 22 cases treated by massive doses of iron is included for purposes of comparison.

The work may be arranged and presented under the following headings.

(1). A discussion of the sources, daily requirements and excretion rates of vitamin C in health and in disease.

(2). The accepted normal values for the relevant features of the blood picture.

(3). A discussion of the methods used in the investigation.
- (a). Selection of patients.
- (b). Details of the method employed for assessing the vitamin C nutrition of the subject.
- (c). The methods adopted for the blood examination.
- (d). Details of the methods of classification and of controlled therapy

(4). The incidence and extent of subnutrition found by other workers.

(5). The incidence and extent of subnutrition found in this series.

(6). The effect of vitamin C subnutrition on the blood picture, observed by others.

(7). Personal observations on the blood picture found in vitamin C subnutrition.

(8). The effects of vitamin C therapy in anaemia observed by others, with a note on dosage and

and hypervitaminosis C.

(9). Personal observations on the effect of administration of ascorbic acid in subscorbutic and non-scorbutic anaemias.

(10). General summary and Conclusions.

A list of references is given at the end of each chapter.

References.

Davidson L.S.P. Verbal communication, Glasgow, March, 1943.

Harris, Passmore and Pagel, 1937, Lancet,ii.,183.

Wright I.S., 1938, Ann. Intern. Med.,12,156.

Abbasy, Harris, Ray and Marrack, 1935, Lancet,ii.,1399.

Whitby L.E.H. and Britton C.J.C., Dis. of Blood,1942, 169.

I. A discussion of the sources, daily requirements and excretion rates of vitamin C in health and in disease.

Vitamin C may be called the anti-scorbutic vitamin. Administration of the vitamin in sufficient dosage prevents and can cure scurvy. Scurvy is a deficiency disorder which has been long known among men who are cut off from sources of fresh food, especially fruit and vegetables, for long periods. Captain Cook in 1776 showed that it could be prevented or cured in sailors by the administration of malt or fresh vegetables.

Hess (1918) points out that dried vegetables and fruits possess very little anti-scorbutic action, and states that orange peel has high anti-scorbutic power. According to Hutchison and Mottram (1936) the water-soluble vitamin C is most plentiful in oranges, lemons, grape fruit, fruits, tomatoes, fresh vegetables, all the green salad foods, especially the cresses, and potato. Potato is probably the main source of the vitamin for working class families. They state that vitamin C is very unstable, succumbing easily to oxidation and to temperature, and that one hour's boiling will destroy practically all the vitamin C in a vegetable; that vegetables dried in air lose all their anti-scorbutic property, but that, if dried below 40° C in vacuo they retain it; and that, in some vegetables (potatoes and tomatoes) the vitamin appears to be more resistant than in others (e.g. cabbage) and therefore stabilising substances have been postulated. Beaumont and Dodds (1941) state that vitamin C is present in cow's milk in only small quantities (0.02 mgm. per ml.) and that this content is lowered still further by pasteurisation.

According to Evans (1941) the vitamin will stand exposure to boiling point for some time in an acid medium, but it is destroyed by oxidation almost at once on heating in an alkaline medium. He states that the practice of adding alkali to preserve the colour of the vegetables when they are cooked is thoroughly bad. He gives the vitamin C content of various foods as follows:-

Food.	mgm. vitamin C per 100 gm. or 100 c.c.
Egg yolk	0 - 1.3.
Milk (cow's)	0.6 - 2.8
Bacon	Trace.
Beef (lean)	1.8.
Liver	25 - 75.
Fish	1.7.
Fish roe	4 - 14.

Food.	mgm. vitamin C per 100 gm. or 100 c.c.
Apples	0.1 - 20.
Lemon Juice	25 - 70.
Nuts	15 - 30.
Tomatoes	12 - 40.
Cabbage etc.	20 - 124.
Carrots	1.8 - 31.
Peas	4.8 - 40.
Potatoes	10 - 36.
Spinach	6 - 124.

Sugiura (1937), working in the city of Mukden, Manchukuo, found that the milk on sale had a low ascorbic acid content; he also found that the content varied a good deal. The vitamin C content was reduced 44% by sterilisation and the content was less in summer than in winter.

Schiaparelli and Buogo (1937) give the following food values:-

Food.	mgm. vitamin C per 100 gm.
Cow's milk	3.7.
Powdered cow's milk	2.2.
Human milk	2.2 - 4.1.
Lemons	54.5.

Food.	mgm. vitamin C per 100 gm.
Oranges	34.0 - 73.0.
Bananas	2.0.
Honey	1.1.

These workers state that the giving of orange juice to a woman increased the cevitamic acid of her milk from 2 to 4 mgm. per 100 grams.

Perry and Zilva (1932) attribute a high vitamin C content to the mango.

Svirbely and Szent-Gyorgyi (1932) attribute a very high content to paprika.

Alston (1942) investigated preserved fruit preparations. He found that the vitamin remained comparatively stable in blackcurrant puree and rose-hip syrup if these were kept in tins or bottles which were airtight and contained the minimum residual air.

Function of vitamin C.

Parsons and Smallwood (1935) consider that vitamin C functions in the body by forming an oxidation-reduction system, taking up oxygen in the tissues and subsequently, by its power of reversible oxidation functioning as an oxygen carrier and thereby playing an important part in the process of tissue respiration and metabolism. They consider it possible that vitamin C, like thyroxin, is responsible for cell metabolism and that its action on the cells of the bone marrow extends throughout the whole range of maturation from endothelial cell to adult erythrocyte and is not restricted to the maturation of the mormoblast as has been suggested by Witts (1932).

Let us next consider the various views held on the daily intake of the vitamin necessary to preserve health.

Heinemann (1938) points out that there are widely different requirements of vitamin C both for complete saturation and for protection against scurvy. For this latter purpose, 0.4 mgm. ascorbic acid per kilo of body weight, or even less, appears to be sufficient, but at least 0.8 mgm. per kilo is used daily by healthy saturated subjects. Active tuberculous patients require abnormally high amounts, although it is observed that high requirements are not specific for this disease. Patients with peptic ulcer show a low concentration of ascorbic acid in the blood. This is due chiefly to the dietary restiction which fails to provide a sufficient supply of vitamin C. Heinemann gives his findings in 12 cases of peptic ulcer--- these range from 1.7 mgm. ascorbic acid per litre whole blood to 3.1 mgm. per litre. None of these cases showed evidence of scurvy.

Wright (1938) states that the curative and maintenance dosage varies between 30 and 50 mgm. ascorbic acid daily by mouth. He states that the dosage varies greatly, however, and that he has had patients in whom 1,000 mgm. daily by mouth failed to cure the scorbutic condition. Intravenous administration, however, produced a rapid cure.

Abbasy, Harris, Ray and Marrack (1935) are of the opinion that an adult needs about 25 mgm. daily to prevent the slightest objectively determined prescorbutic ill-effects.

Dilling (1940) gives the daily requirement as about 500 international units, one international unit representing the activity of 0.05mgm. ascorbic acid.

Schultzer (1937) showed that, at least in a small group of patients, the results in deficiency states were as rapid using a dose of 40 mgm. intravenously as when 600 mgm. were given orally.

It would appear, therefore, that the daily requirement of vitamin C is not inconsiderable, the absolute minimum being between 15 and 25 mgm. daily. But this may not be the optimum figure. The optimal intake has been defined by Orr as the level above which no further improvement in health takes place. This amount has to be derived entirely from the food ingested. It will be noted from the lists given above that potatoes have a very high anti-scorbutic value, as have citrous fruits, the cabbage family and liver. Being acquainted with the dietic habits of the people of this area, it would not be surprising if the daily intake of a good proportion fell far short of this agreed minimum. The daily intake of the average working class woman, as judged from her dietary history, is far below the man of her class, a great difference being found in the relative consumption of potatoes. Vegetables do not figure prominently in the working class diet in this area at any time and they are even less conspicuous in a wartime winter. Fruit has been practically unobtainable. It is obvious, then, that the source of vitamin C has been definitely reduced in the past three years. This loss has not been replaced to any large extent by administration of synthetic vitamin C to adults, although children have had consideration in this respect.

It has been stated by Harris (1942) that vitamin C is lost in measurable amount in the sweat. Coal face work is one of the most strenuous of occupations and a great loss of sweat occurs in the course of a single 'shift'. It may be that the daily requirement in this class of worker is greater than the accepted minimum. Since the outbreak of war, canteens have been set up at each of the pit-heads in the district and are freely used by the miners. At these canteens a feature is a generous supply of vegetables, and, as a direct result of this, the vitamin C intake of these workers is probably greater than it would be if all their meals were taken at home.

Let us now turn to the question of the rate of excretion of ascorbic acid in the urine and the subject of "test-dosing".

Ascorbic acid is excreted in measurable amount in the urine, except in cases of gross undernutrition. The quantity excreted varies with the age of the patient, his intake both immediate and remote, and the requirements of his body.

According to Harris and Ray (1935) a young child should excrete between one and two mgm. ascorbic acid per day, a normal adult on a middle or higher working class diet between 15 and 30 mgm. per day.

Youmans, Corlette, Akeroyd and Frank (1936) suggest 20 mgm. per day as the lower limit of normal daily excretion.

Abbasy, Harris, Ray and Marrack (1935) suggest 2 mgm. per stone of body weight as about the normal rate, and Harris regards a daily excretion below 13 mgm. per day as being suggestive of vitamin C deficiency. It has also been demonstrated by Harris and Ray (1935) that individuals accustomed to getting too little vitamin C excrete less than those on a well balanced diet, and they state that this lower output is parallel with a diminished intake. They concluded that (i) undernourished cases excreted less than well nourished cases, (ii) that after cure they excreted the same amount as well nourished cases and (iii) that in adults a low urinary output and a low response to test doses go parallel with a history of vitamin C underfeeding.

It was also found by Harris and Ray (1935) that the output in the urine varies with the dietary intake in a peculiar manner. They found that normal well nourished humans behaved as if they had an appreciable reserve store, for, when the vitamin is temporarily witheld from the diet they continue to excrete it for a time at an almost steady rate, and administration of large doses shows a sudden peak in the rate of excretion. This means that the urinary output depends both on the immediate vitamin intake, and on the past nutritional history and state of saturation. Abbasy, Hill and Harris (1936) state that under ordinary circumstances the responses to test-doses are simply graded according to the resting level of excretion. They found that controls with a normal rate of excretion gave a peak response, usually on the first day, but certainly on the second day. They conclude that diminished excretion probably means that the intake is insufficient or that the reserves have been used up, in pyrexia, infection, or toxaemia.

The subject of test-dosing may now be discussed. This diagnostic procedure was introduced by Harris and Ray in 1935. The effect of a test-dose of 700 mgm. of ascorbic acid per 10 stones of body weight depends on the state of nutrition of the subject with regard to vitamin C. In normal individuals there is a sharp increase in the excretion of ascorbic acid, usually on the first day following the test-dose, but certainly on the second. A patient is regarded as unsaturated if the excretion does not show a rapid increase above the resting level. The degree of unsaturation is assessed by repeating the test-dose daily and counting the number of days required, until a peak response is obtained. The greater the number of days required, the lower the vitamin C reserves of the subject. Subjects who fail to reach a level of excretion of 50 mgm. per stone of body weight on the second day are regarded by Harris as being below standard.

Youmans, Corlette, Akeroyd and Frank (1936) suggest 30% of a test-dose of 600 mgm. ascorbic acid as the lower limit of urinary excretion in a well nourished subject.

According to Abbasy, Harris, Ray and Marrack (1935), the advantage of the test-dose is that it magnifies the relatively small differences seen on the scale of the resting level. Abbasy, Hill and Harris (1936) state that under ordinary circumstances the responses to test-doses are simply graded according to the resting level of excretion. The test-dose is therefore simply a method of magnifying the result obtained from testing the resting vitamin C excretion level. While Harris (1942) again stresses the importance of test-dosing before diagnosing vitamin C subnutrition, he admits in the same paper that an estimation of the resting level is a good general guide, especially when the tests can be based on 24 hour specimens.

References.

Youmans J.B., Corlette M.B., Akeroyd J.H., Frank H., 1936, Amer. J. Med. Sci., 191, 319.

Heinemann M., 1938. Journal Clin. Invest., 17., 671.

Wright I.S. 1938. Ann. Intern. Med., 12., 516.

Abbasy, Harris, Ray and Marrack. 1935. Lancet, ii., 1399.

Dilling W.J. 1940. British Encyclopaedia of Medical Practice, Surveys and Abstracts, 130.

Schultzer P., 1937. Bioch. J., xxxi., 1934, 1938.

References.

Harris L.J. 1942. Lancet, i., 645.

Harris and Ray. 1935. Lancet, i., 71, 462.

Abbasy, Hill and Harris. 1936. Lancet, 1413.

Szent-Gyorgyi. 1928. Bioch. J., 22, 1387.

Beaumont and Dodds. 1941. Recent Adv. In Medicine, London, 20.

Schiaparelli P. and Buogo G. 1937. Amer. J. Dis. Child., 52, 1451.

Sugiura M. 1937. Amer. J. Dis. Child., 52, 1453.

Hutchison and Mottram. 1936. Food and the Principles of Dietetics, London, 25.

Evans L. 1942. Starling's Principles of Human Phys., 861.

Parsons and Smallwood. 1935. Arch. Dis. Child., x, 335.

Perry E.O.V. and Zilva S.S., E.M.B., 1932. Prelim. Report on the vitamin content of the Mango.

Svirbely J.L. and Szent-Gyorgyi A. 1932. Nature, London, cxxix, 576, 690.

Alston J.M., 1942, Br. Med. J., i., 559.

Hess A.F., 1918, J.Amer. Med. Assoc., 71, 942.

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CHAPTER II. THE ACCEPTED NORMAL VALUES FOR THE RELEVANT FEATURES OF THE BLOOD PICTURE.

Let us consider first the red cell count. Originally it was taught that a figure of 5 million red cells per c.mm. blood in men and 4½ million red cells per c.mm. in women, was the normal reading. These figures were apparently based on an insufficient number of investigations. More recent enquiries have shown that there is a wide range in the normal figures. Whitby and Britton (1942) state that for ordinary clinical purposes it may be assumed that the normal adult red cell count varies between 4,200,000 and 6,400,000 per c.mm.; that 5½ millions is the average for the male and 4,800,000 for the female; and that a fair average, irrespective of sex, is 5,000,000 per c.mm. The same authors state that variations in a red cell count up to plus or minus 5% are of no significance, because such variations may occur in the same individual at different times of the day, and are within the

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bounds of reasonable experimental error. They consider that lowering of the red cell count below 4½ millions per c.mm. in men, or below 4 millions per c.mm. in women, indicates a definite anaemia which requires investigation.

The red cell count, may of course, be raised. It may be high in polycythaemia, it may be raised by concentration in such conditions as excessive sweating, polyuria, fever, vomiting and diarrhoea, or after severe burns. The count may be lowered by dilution in dropsical conditions.

No case which would lead to such mechanical alteration in the red cell count was included.

The figure of 4½ million red cells per c.mm. for men, and 4 million per c.mm. for women, were accepted as the lower limits of normality in this series.

There are several well known methods of estimating the haemoglobin level. Details of the method used will be given in the appropriate section of the paper. As with the red cell count, there is considerable difference of opinion as to what constitutes a normal haemoglobin reading. There is, as in the

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case of the red cell count, a wide normal range, according to Dreyer, Bazett and Pierce, (1920). The reading not only varies from one country to another, but from person to person and in the same person from one part of the day to another. Dreyer, Bazett and Pierce (1920) state that this diurnal variation is minimal between the hours of five and seven p.m. and suggest that haemoglobin readings should be made at this time of the day. We consider that unreliability has been ascribed to a particular method, in many cases when the fault actually lay in the technique employed and in the failure to follow the advice of Dreyer, Bazett and Pierce.

According to Price-Jones, Vaughan and Goddard (1935), there is no significant difference between the haemoglobin content of capillary and venous blood.

Whitby and Britton (1942) give the following figures:

i. Adult males (average) - 15.8 grams haemoglobin per 100 c.c. blood.

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ii. Adult females (average) - 13.7 grams haemoglobin per 100 c.c. blood.

iii. Average irrespective of sex - 14.5 grams haemoglobin per 100 c.c. blood.

iv. Adult male (lower limit of normal range) - 14 grams haemoglobin per 100 c.c. blood.

v. Adult female (lower limit of normal range) - 12 grams haemoglobin per 100 c.c. blood.

The figures of 14 grams haemoglobin per 100 c.c. blood for men, and 12 grams haemoglobin per 100 c.c. blood for women, were accepted as the lower limits of normality.

The colour index is simply an expression of the amount of haemoglobin per red cell compared with the normal. The normal amount is unity, which denotes a normal sized, saturated corpuscle. A reduction of the colour index indicates a fall in haemoglobin per red cell. A high colour index must indicate an enlargement of the cell. It does not indicate supersaturation. The normal colour index range is from 0.9 to 1.1. This range allows for experimental error.

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We now come to a consideration of the stained blood film. From a well stained film a good deal of useful information may be gleaned. Let us now briefly consider a few salient features of a normal blood film.

The cells should be between 6.7 and 7.7 u. (Price-Jones, Vaughan and Goddard (1935). Anisocytosis should not be a prominent feature - the coefficient of variation by Price-Jones method is from 5.3 to 7.3 per cent, according to Whitby and Britton (1942). The cells should stain uniformly and well. Nucleated red cells should not be apparent in a normal blood film unless regeneration is proceeding at a rapid pace, in which case the megaloblasts and erythroblasts are not haemoglobinised. Reticulocytosis, stippling and polychromasia are usually signs of active regeneration and are not seen to any great extent in a normal film.

Price-Jones, Vaughan and Goddard (1935) state that the blood of a healthy adult usually contains from 0 to 2 per cent of reticulocytes. This is the value they found over a series of 81 cases, counting 1,000 red cells in each case. These reticulocytes are

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young red cells, and if the treatment given in any anaemia gives a reticulocyte response followed by an increase in the red cell count, it may be assumed that such treatment is rational. The reticulocyte response to be expected during effective treatment varies with the type of anaemia and with the therapeutic agent employed (minot and Heath,1932). In effective iron therapy the reticulocyte response is governed both by the initial red cell count and the haemoglobin level, but more by the latter than the former. Thus, if the haemoglobin level is low, there is a considerable reticulocyte response, even though the initial red cell count is quite high, whereas, in the response of a macrocytic anaemia to liver treatment the reticulocyte response is negligible if the initial red cell count is high. The degree of reticulocyte response will thus depend both on the type of the anaemia and on the treatment employed.

The normal physiological range of the white cell count is from 4,000 to 10,000 per c.mm., according to Garrey and Bryan (1935), who also state that

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though some people may have a level which is relativ- ely low, none show a greater physiological range than a difference of 6,000 per c.mm. between their upper and lower normal limits. Medlar (1929) states that, as a rule, no significance can be attached to varia- tions of less than 50% in the total count, and that variations up to 100% frequently occur in average healthy individuals, without any clinical disease being manifest.

Exercise, bathing, food intake, digestion, exposure to sun all cause a rise in the leucocyte count. There is also the condition known as "afternoon tide" which comprises the appreciable rise in the leucocyte count which occurs in all persons in the afternoon. (Whitby and Britton,1942).

The white cell count was introduced in this survey as a check on the clinical condition of the patient. As has been stated earlier in the paper, febrile and infective conditions lead to a diminished excretion of vitamin C in the urine. A leucocytosis is not uncommonly found in infective conditions where

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pyrexia is not noted, and thus may be of definite value in excluding cases unsuitable for this investigation.

Lastly, let us consider briefly the blood sedimentation rate.

If an anti-coagulant is added to a specimen of blood, the mixture set up in a vertical tube and allowed to stand,the corpuscles settle and leave a clear supernatant plasma. The distance sedimented by the corpuscles depends on the time allowed, on the red cell content, and on certain physiological and pathological conditions. In a given time it is relatively constant for normal human beings and is remarkably constant for any given individual in health. (Harvey and Hamilton,1936). Harvey and Hamilton emphasise that while it is not a specific test for disease, it is a valuable index of health and returning health. The rate of settling is known as the sedimentation rate.

The sedimentation rate is increased with a fall in the red cell count and this is most marked in severe acute anaemias. According to Gregg (1937), the increase

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in rate in chronic anaemias is less marked (unless of course, there is also a toxic or infective factor at work). A correction for anaemia may be applied in terms of the haemoglobin content.

The rate is slightly increased at the menses and to a much greater extent in normal pregnancy, especially after the fourth month, and returns to normal within one month of parturition (Whitby and Britton,1942)

The main factor in the production of an increased blood sedimentation rate, as well as the most important practical factor, is the extensive body cell destruction which occurs in infections and toxic conditions. The rate is increased in all conditions where there is tissue breakdown, or where foreign protein enters the blood, except for localised infections of mild grade, such as apical dental infection (Whitby and Britton,1942) The rate is therefore increased in most infections and toxaemias, after injection of vaccines and other foreign proteins, in fractures, operations and in cancer.
An increased rate occurs in active tuberculosis and rheumatoid arthritis, but it is not diagnostic of these conditions. The test is as non-specific as fever or leucocytosis, but is more sensitive and may indicate disease in the absence of either of these signs.

The test was made in all cases as a check on the clinical examination. As has been stated above, the test is more delicate than either fever or leucocytosis, and an increased rate is frequently present when there are no other obvious signs of tissue destruction. Since infections and toxaemias lead to a diminished urinary output of vitamin C (v.s.), even in the presence of adequate intake, it seemed wise to use the test as an indicator of the possible presence of such interfering factors. Anaemia, per se, causes an increased rate, and in Paul Wood's case, cited below, the rate was very greatly increased in what apparently was a true scorbutic anaemia. It would not have been prudent, therefore, to exclude a case simply because the sedimentation rate was increased, but any cases found to have a greatly increased rate were reviewed in the light of this finding before being considered suitable material. The sedimentation rate was again checked at the end of treatment so that one could exclude intercurrent infection and interference with vitamin C metabolism during the period of treatment.

Westergren (1926) gives the following figures, which hold only when his method is used:-

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Men	-	Normal	1-3 mm. in 1st hour.
Men	-	Borderline	4-7 mm. in 1st hour
Women	-	Normal	(1-) 4-7 mm. in 1st hour.
Women	-	Borderline	8-11 mm in 1st hour
Children	-	The same standard can be applied as in women.	

References.

Whitby and Britton, Disorders of the Blood, 1942, London, 40, 41, 42, 108.

Dreyer G., Bazett H.C., and Pierce H.F., 1920, Lancet, ii, 588.

Price-Jones, C., Vaughan J.M. and Goddard H.M., 1935, J. Path. Bact., 40, 503.

Garrey W.E. and Bryan W.R., 1935, Physiol. Rev., 15, 597.

Medlar E.M., 1929, Amer. Journal. Med.Sci., 177, 72.

Harvey W.F. and Hamilton T.D., 1936, Edinburgh Med.Journal, 43, 29.

Gregg R.O., 1937, J. Lab.Clin.Med., 22, 786.

Gibson H., 1938, Proc.Royal Soc.Med., 31, 309.

Westergren A., 1926, Amer. Rev. Tuberc., xiv., 94.

Minot and Heath, 1932, Amer. J. Med. Sci., 183, 110.

CHAPTER III. A DISCUSSION OF THE METHODS USED IN THE INVESTIGATION.

(a) The selection of suitable subjects.

It has been stated in the introduction that the investigation was confined to adults, male and female. It is difficult to conduct prolonged investigation on children in general practice without having a considerable number of defaulters.

The patients included in the series were, for the most part, representative of that type of case frequently seen in general practice, the case in which no gross organic disease can be discovered, apart, perhaps, from a variable degree of anaemia which has no obvious cause. Their disabilities are often of minor degree, but are of considerable importance when viewed from either the personal or the economic aspect. These people never feel really fit, they are unable to carry out their duties satisfactorily for any prolonged period, and their relapse rate is high when they are not under treatment or supervision.

Most of the cases were drawn from working class or poor class homes. Incomes in the area

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were, however, high compared with pre-war days and actual financial poverty was uncommon. Any dietary restriction was due to a failure of the usual food-stuffs to make their appearance on the market, and to a marked disinclination on the part of some people to make full use of the food available.

Visitors were not included in the series because of the brevity of their stay and the fact that their diet had been derived from a different part of the country.

Cases were investigated either on their first appearance, or after they had recovered from a minor illness. Any case suffering from a condition leading to a diminished excretion of vitamin C - e.g. achlorhydria, active tuberculosis, rheumatoid arthritis, rheumatic fever, osteomyelitis, common cold, pneumonia, acute colitis, or any other toxic or infective state, was excluded, as we have already stated in the introduction. It is recognised that disorders due to vitamin deficiencies are brought forth, or intensified by adverse conditions. e.g. infectious diseases, fatigue or various excesses may precipitate a deficiency disorder in inadequately nourished subjects.

One had to be convinced after reviewing the evidence of the clinical examination, white cell count and blood sedimentation rate reading that no condition likely to interfere with vitamin C absorption, metabolism or excretion was present. An estimation of the acidity of the gastric juice was not made in all cases - consent to pass a Ryle's tube could not always be obtained. But there are usually sufficient clinical grounds for suspecting achlorhydria, such as anorexia, epigastric pain frequently more marked after a meat meal, eructations, flatulence, constipation or diarrhoea. Glossitis, which is of frequent occurrence, is usually painless and may only be noted if looked for. Any case clinically suggestive of achlorhydria had the gastic juice examined and found to have free hydrochloric acid before being admitted to the series.

One of the objects of this investigation was to discover whether mild degrees of vitamin C subnutrition were associated with anaemia; it was therefore essential that the group of cases selected should be, as it were, hand-picked, and that no case suffering from an anaemia due to a different and recognisable cause should be included. Naturally, one would not have been

/justified

justified in assessing the vitamin C nutritional status of a case suffering from, for example, pernicious anaemia, associating the blood picture with this nutritional status and then attributing the blood changes to any alteration in the nutritional state which there might happen to be. It was therefore decided at the outset that any case which showed the clinical characteristics of an aplastic, acute haemolytic or ordinary haemorrhagic anaemia should be excluded, as should cases of such dyshaemopoietic type as pernicious anaemia, non-tropical sprue, megalocytic anaemia of pregnancy, gastric carcinoma, myxoedema, thyrotoxicosis, etc.

The anaemia of scurvy, as is explained in Chapter V1., has been variously attributed to bone marrow aplasia, haemolysis, haemorrhage and dyshaemopoiesis. No cases of gross aplastic or acute haemolytic anaemia were encountered during the investigation and so no question was raised as to the suitability of these types of case. Any case found to be suffering from an haemorrhagic or post-haemorrhagic anaemia of either acute or chronic types was carefully assessed and in only one instance was it considered

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that the bleeding might conceivably be due to lack of vitamin C. This was in case No. 74 (Group III,3). In this particular case,epistaxis, haemoptysis, haematemesis, melaena, haematuria and menorrhagia were in turn associated with a low vitamin C nutritional state. Detailed features of the case are given in the appropriate section of this paper. Any other cases of haemorrhagic anaemia which were encountered had an obvious cause such as menorrhagia due to uterine subinvolution, bleeding piles, and , in one instance, haematuria of unknown origin; these cases were relatively easy of diagnosis and exclusion. Dyshaemopoietic anaemias of the kind listed above, were scheduled for exclusion from the series. Actually the only three cases of this type which were encountered and excluded were 2 cases of Addisonian anaemia and 1 case of megalocytic anaemia of pregnancy.

Such, then, was the composition of the cases selected. They were, generally speaking, people suffering from no gross organic disease, no condition known to interfere with vitamin C absorption, metabolism or excretion, and possessing a blood picture either within normal limits or showing the characters of a simple anaemia.

(b) <u>The method employed for assessing the vitamin C Nutrition of the subject</u>.

As has already been stated, an estimation of the amount of vitamin C excreted in twenty-four hours in the urine is a fairly reliable guide to the nutritional state of the subject, so far as that vitamin is concerned. Such an estimation of the resting excretion level was made in all of the cases at the beginning of the investigation. Test-dosing was performed at the outset in some cases, at the end of a control period in the others. The reason for this is explained below.

Vitamin C, first isolated from oranges and from the suprarenal cortex in 1928 by Szent-Gyorgyi as hexuronic acid, is a powerful reducing agent and is rapidly oxidisable in the air. The chief difficulty in performing a quantitive estimation of the vitamin is due to this instability. A preservative must therefore be added to the urine immediately it is voided, unless titration is proceeded with immediately, Glacial acetic acid has been used to this end by Harris and Ray (1935). Even when treated thus, the loss of vitamin C is fairly rapid. According to Harrison (1942), some oxidation

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may occur in as short a period as four hours when this method is used, but this oxidation can be reversed by treatment with sulphuretted hydrogen up to a period of about eighteen hours.

It was found to be more practicable to use the method of Sendroy and Miller (1939) in this series. The method is as follows:-

To a stoppered winchester of 750 c.c. capacity and of dark glass add 75 mls. of 5 Normal Sulphuric acid (approximately 135 mls. conc. sulphuric acid per litre), 0.75 mls. of 0.1 M 8 Hydroxy-quinoline solution (1.45 grammes per 100 c.c. of 95% ethyl alcohol) and 1.5 c.c. toluene. The urine to be tested is either passed directly into the winchester, or added to it immediately after passing. These workers claim this to be a very satisfactory preservation method. They claim that when stored in the cold, the loss of titratable ascorbic acid is limited to 2%-5% over 24-48 hours.

At a temperature fluctuating about 20o C and preserved by this method, the rate of loss is as follows (Rose 1942) :-

/ 0 days.................. 4.42

0 days	4.42
1 day	4.42
2 days	4.39
3 days	4.20
4 days	4.20
5 days	3.82
6 days	3.76

This rate of loss is much slower than when the urine is preserved by the glacial acetic acid method. The efficiency of Sendroy and Miller's method was checked in 20 cases before being adopted as the preservation method in this series, and it would appear that the method is thoroughly reliable.

The 24 hour specimen was collected in each case at the outset of the investigation. Each patient was given a dark stoppered winchester of 1,500 c.c. capacity containing double the quantity of the preservative suggested by Sendroy and Miller as being sufficient for a bottle of 750 c.c. capacity; and a printed slip was handed to him, giving full instructions for the collection of the specimen. The points stressed in the instructions were:

(1) That all the urine passed over a period of 24 hours should be collected.

(2) That the urine should be passed into the bottle direct or the total passing added and the stopper replaced within one minute of being voided.

(3) That the bottle should then be shaken and stood in a cool place until required again.

(4) That the bottle should be returned as soon as possible after completion of the collection.

According to Sendroy and Miller, excess of preservative does not interfere with the test.

The vitamin C content of the winchester was then estimated by a modification of the method of Harris and Ray (1935), using Tillman's reduction indicator, 2:6 dichloro-phenol-indophenol. Standardised tablets of the indicator, containing at issue the titration equivalent of one milligram of ascorbic acid, were used. To prepare a solution of the dye, a tablet was crushed and completely dissolved in boiling distilled water, the volume then being made up to 50 c.c.

The volume of urine in the winchester i.e. the quantity passed in twenty-four hours, was measured, and the portion required for titration filtered. It was found that by filtration of the urine prior to titration a better end point was obtained.

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Into a glass beaker was measured 5 c.c. dye solution and 1-2 c.c. glacial acetic acid. The filtered urine was run into the beaker at such a speed that titration was complete in from ½-2 minutes (ascorbic acid does not reduce the dye instantaneously; it requires some thirty seconds, according to Harris. And if the titration is not complete within two minutes substances other than ascorbic acid cause reduction). The titration is complete when the pink colour of the acidified dye is just discharged. The volume of urine required to bring about the reduction was then read and noted.

Occasionally there is some difficulty in determining the exact end-point. This is especially so if the ascorbic acid content of the urine is so low that one cannot be sure whether the dye has been reduced or so diluted that the pink colour cannot be detected. This, however, only occurs with very low concentrations. A more definite end-point is obtained if the urine is filtered prior to titration and a comparator used. A solution for comparison can be obtained by adding to a second beaker 5 c.c. water and 1-2 c.c. glacial acetic acid, and then running in filtered urine from a second burette at the same rate and at the same time as titration is proceeding.

The solid dichlorindophenol remains practically unaltered under normal conditions for at least two months. The solution of the dye, however, deteriorates rapidly, and has to be discarded in about one week. In this series it was discarded every three days. Each tablet of the solid dye was stated to be the titration equivalent of 1 mgm. ascorbic acid, but its potency was checked against a known quantity of ascorbic acid each time a fresh batch of solution was made, as will be explained in the pages which follow.

Let us consider, for the sake of calculation, that each tablet was the titration equivalent of 1 mgm. ascorbic acid. One tablet was dissolved in 50 c.c. water. Five c.c. dye solution was therefore the equivalent of 0.1 mgm. ascorbic acid. The amount of urine required to reduce 5 c.c. dye solution therefore contained 0.1 mgm. ascorbic acid.

Let x equal the number of c.c. urine required.

Then x c.c. urine contains 0.1 mgm. ascorbic acid.

Then 100 c.c. urine contains $\frac{100}{x} \times \frac{1}{10} = \frac{10}{x}$

/Therefore

Therefore $\frac{10}{\text{number of c.c. urine used}}$ equals the number of milligrams of ascorbic acid per 100 c.c. urine. Since the volume of urine passed in twenty-four hours has been measured, we can calculate the number of milligrams of ascorbic acid excreted daily by the individual thus:

Let Y equal the number of mgm. ascorbic acid per 100 c.c. urine.

Let Z equal the quantity of urine excreted in twenty-four hours in c.c.

Then $Y \times \frac{Z}{100}$ equals the total excretion of ascorbic acid in mgms. per day.

As has been stated, the solid dye does not keep its full equivalent indefinitely, therefore each fresh batch of dye solution prepared was tested for potency. The method of checking was as follows (Harrison (1942), with personal modification and explanatory notes).

Dissolve 25 mgm. pure ascorbic acid in water to 50 c.c. and titrate against 0.01 N iodine solution. Measure 5 c.c. of the ascorbic acid solution (i.e. 2.5 mgm. ascorbic acid) into a boiling tube, and

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run in the 0.01 N iodine solution from a micro-burette, using starch as indicator. This should require 2.84 c.c. N iodine solution.

One may explain this conclusion of Harrison as follows:

$C_6H_8O_6$(the molecular formula of ascorbic acid) $\equiv I_2$

The molecular weight of ascorbic acid is 176 (12 x 6 plus 1 x 8 plus 16 x 6)

The molecular weight of iodine is 127 x 2 , i.e. 254. But N solution iodine contains 127 grams iodine per litre. Therefore 0.01 N iodine contains 1.27 grams per litre. Therefore 200 c.c. 0.01 N iodine solution contains $\frac{1.27}{5}$ grams i.e. 254 mgm. iodine. Therefore 176 mgm. ascorbic acid require 200 c.c. of 0.01 N iodine. Therefore 2.5 mgm. ascorbic acid require $\frac{200}{176}$ x 2.5, i.e. 2.84 c.c. of 0.01 N iodine solution.

The potency of the ascorbic acid solution having thus been checked, it was used to standardise the dye solution as follows:

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To 12.5 c.c. dichlorophenol-indophenol solution i.e., the titration equivalent of 0.25 mgms. ascorbic acid, add 4 c.c. glacial acetic acid, and run in the 0.05 ascorbic acid solution from a microburette until the colour is discharged.

1 c.c. ascorbic acid solution contains 0.5 mgm. ascorbic acid. Therefore 0.5 c.c. adcorbic acid solution contains 0.25 mgm. ascorbic acid.

But 12.5 c.c. dichlorophenol-indophenol solution is the titration equivalent of 0.25 mgm. ascorbic acid.

Therefore it should need 0.5 c.c. ascorbic acid solution to discharge the colour of the dye.

The exact ascorbic acid equivalent of the dye is then calculated and noted.

An estimation of the resting level of excretion was made in all cases. Those cases scheduled for vitamin C therapy were also test-dosed at the outset (see page 10). This had the dual effect of confirming or refuting the evidence of the

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resting excretion rate and of replenishing the reserves rapidly in the cases where these were diminished. Those cases serving as controls, and from whom ascorbic acid was being deliberately withheld, were only test-dosed when their control period had passed.

References.

Harris and Ray, Lancet,1935,i.,71.

Harrison, Chemical Methods in Clinical Medicine, 1943,298.

Rose,C.F.M., 1942, The Monthly Bulletin,Path & Bact. Lab. Assist. Assoc., 8, 4.

The methods adopted for blood examination.

So far as possible, the hour chosen for the blood examination was the same in every case, namely between the hours of five and seven p.m. (G.M.T.). As we have already stated, the diurnal variation in the haemoglobin estimation is minimal between these hours. In women, the time around the menstrual period, and the duration of pregnancy and the puerperium were avoided, because at these times the sedimentation rate is liable to be physiologically increased.

The blood for the red cell count, white cell count, haemoglobin estimation, reticulocyte count and the stained film was obtained from the nail bed of the thumb. The puncture was made by a blood "gun" to give uniformity of puncture and so avoid the ever present tendency to apply pressure in inadequately punctured cases. The blood was allowed to well up freely and the first drop was in all cases discarded.

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The red cell count was performed in the usual way, a standardised pipette being employed. The usual precautions regarding accuracy of measurement, thorough mixing of blood and diluting fluid and the discarding of a few drops of the diluted blood prior to placing in the counting chamber were observed. A Thoma counting chamber was employed, and the number of red cells in 320 small squares was counted in every case. The counting chamber was charged, 160 squares counted, the chamber emptied and cleaned and then re-charged. The counting process was then repeated, 160 squares again being counted. A reasonably large number of cells was thus counted, so leading to greater accuracy.

The haemoglobin estimation was made by the acid haematin method. Decinormal hydrochloric acid was placed in the graduated tube up to the mark '10', and 20 c.mm. blood thoroughly mixed with this. The period allowed for conversion of oxyhaemoglobin to acid haematin varies from worker to worker. The period advised by the Standing Committee on Laboratory Methods, University of Glasgow, is

/ "Exactly"

"exactly" one minute; by Whitby and Britton, thirty to forty minutes; and by Scott Thompson, Glazebrook and Millar, four hours. In this series of estimations a period of thirty minutes was allowed to elapse before the distilled water was added.

The tube used for the estimations was graduated in both percentage and in grammes of haemoglobin per 100 c.c. of blood. As there are great variations in the method of graduating these tubes, the equivalent values for the tube employed will now be given:

100%	equals	16	grammes	haemoglobin	per	100	c.c.	blood.
97%	"	15.6%	"	"	"	"	"	"
86%	"	13.7	"	"	"	"	"	"
91%	"	14.5	"	"	"	"	"	"
88%	"	14	"	"	"	"	"	"
76%	"	12	"	"	"	"	"	"

According to Whitby and Britton (1942) 15.6 grammes haemoglobin per 100 c.c. blood is the average figure in adult males, 13.7 grammes being the average figure in females; and neglecting sex differences, the average is 14.5 grammes per 100 c.c. blood,

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The white cell count was made in the usual way, using 1/20 dilution. The white cell content of 1/10 c.mm. was counted three times and an average struck.

The blood films were stained in the usual manner by Leishman's stain and examined under low power, high power and oil immersion lenses.

Reticulocytes were counted after staining with brilliant cresyl blue and counterstaining with Leishman's stain.

The blood sedimentation rate was estimated by the method of Westergren (1926) his tecnique being followed in every detail. Blood was usually obtained from the median basilic vein without using prolonged compression. A special syringe with a stop device which gave an accurate mixture of 2 volumes of 3.8% sodium citrate with 8 volumes of blood was employed. Westergren tubes, graduated from zero at the upper end to 200 mm. at the lower end were employed for the actual sedimentation.

/ Readings

Readings of the distances sedimented by the red cells in one and two hours were made.

References.

Standing Committee on Laboratory Methods, University of Glasgow, 1927,21.

Whitby L.E.H. and Britton C.J.C., Disorders of the Blood, London,1942, pp. 512,40.

Scott Thompson, Glazebrook and Millar, Journal of Hygiene,1942,42,2.

Westergren A., American Review of Tuberculosis, N.Y., 1926, 14, 94.

(d) Details of the method of classification.

After a routine clinical examination, blood examination and estimation of the vitamin C nutritional status of the individual we were able to classify the case on two criteria, viz.

i. Whether anaemia was present or not, and

ii. Whether there was vitamin C subnutrition or not.

Anaemia was considered to be present if the red cell count was below 4½ millions per c.mm. in men, or below 4 millions per c.mm. in women, or if the haemoglobin content was below 14.0 grammes per 100 c.c. blood in men, or below 12.0 grammes per 100 c.c. in women. (vide pp. 22.)

The state of the vitamin C reserves was estimated by (a) finding the resting excretion rate in all cases at the outset and (b) test-dosing all cases; some were test-dosed at the outset, the remainder after a control period, as is explained later in the chapter. In those cases where test-dosing was performed at the outset, the positive or negative result obtained was accepted as a definite proof of the nutritional state of the case. But in those cases

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where test-dosing was postponed, subnutrition was considered to be a possibility if the resting excretion rate was below 15 mgm. per day. Thus any case with a daily excretion of less than 15 mgm. per day was temporarily placed in the undernourished group, and, if necessary, re-classified on the evidence of the subsequent test-dose.

Four possible combinations of the above two criteria were therefore possible, thus -

i. No anaemia, vitamin C nutrition satisfactory.
ii. No Anaemia, vitamin C nutrition unsatisfactory.
iii. Anaemia, vitamin C nutrition unsatisfactory.
iv. Anaemia, vitamin C nutrition satisfactory.

This method of classification was adopted and the features of each group are tabulated below:

Group	State of vit. C reserves.	R.B.C. in millions	H.globin in gms./ 100 c.c.
1.	Satisfactory	Above $4\frac{1}{2}$ in men, above 4 in women	Above 14 in men, Above 12 in women.
11.	Unsatisfactory	"	" " "
111.	Unsatisfactory	Below $4\frac{1}{2}$ in men, below 4 in women	Below 14 in men Below 12 in women.
IV	Satisfactory	"	" " "

It will be seen that any given case will fall into one or other of these groups.

An analysis of the features of each group was made to discover whether there were any features peculiar to Group 111. This will be discussed in Chapter V11.

It was found necessary to subgroup the cases for the purpose of administering controlled therapy. One of the questions to be answered was - has ascorbic acid therapy any important place in the treatment of nutritional deficiency anaemia, either non-scorbutic or sub-scorbutic. To answer this and other pertinent questions, each group was subdivided as follows:

(a) To patients in this subgroup was administered
    Ferri et Ammon Cit. gr. 40 t.d.s. and
    Acid Hydrochlor. Dil. dr.i t.d.s.

until no further response could be obtained as indicated by a rise in the red cell count, reticulocyte count, or haemoglobin content, or until it was obvious that no response was likely to be obtained.

/ (b)

(b) To patients in this subgroup was administered Ferri et Ammon Cit. gr 40 t.d.s., Acid Hydrochlor. Dil dr. i t.d.s. and Ascorbic acid mgm. 50 t.d.s.

(c) This subgroup received ascorbic acid mgm. 50 t.d.s. only.

(d) The patients placed in this subgroup were patients who had already been saturated with ascorbic acid, and whose response to a test-dose was satisfactory. They were transfers from subgroup C. To them was administered Ferri et Ammon Cit. gr. 40 t.d.s. and Acid Hydrochlor. Dil. i. t.d.s.

(e) The patients in this subgroup had, so far as possible, been saturated with iron. To them was administered ascorbic acid mgm. 50 t.d.s.

When a case was placed in a subgroup, the treatment appropriate to that subgroup was continued so long as the blood picture continued to improve. Even though no response was obtained, treatment was administered for a minimum period

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of 4 weeks before transfer to another subgroup was considered. All patients who received ascorbic acid therapy were test-dosed in addition and found to be saturated before that line of treatment was discontinued.

This method of subgrouping for the purpose of administering controlled therapy may appear at first sight to be cumbersome, but when we consider the information obtainable from it, it would seem to be justifiable. One of the questions to be answered was - has ascorbic acid therapy any place of importance in the treatment of nutritional deficiency anaemia, either subscorbutic or non-scorbutic ? By adopting the method described above, one was in a position to observe the effects of

i. Ascorbic acid alone,

ii. Ascorbic acid given before, with or after iron therapy and

iii. Iron alone,

and so attempt to define the place, if any of of ascorbic acid in the rational treatment of

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these asaemias.

The results will be discussed later in the paper.

It will be noted that those cases undergoing iron therapy also received drachm doses of Acid Hydrochlor. dil. According to Mettier and Minot (1931) iron is more readily absorbed from an acid than alkaline medium, and so every possible facility for rapid iron absorption was given to those cases serving as controls to the cases undergoing ascorbic acid therapy.

Finally, let us return to the criteria on which classification was based. We have accepted the lower limits of normality as

i. 4½ million red cells per c.mm. blood in men and
   4 million red cells per c.mm. blood in women.

ii. 14 grams haemoglobin per 100 c.c. blood in men,
   12 grams haemoglobin per 100 c.c. blood in women.

iii. A daily urinary vitamin C excretion of 15 mgm.

Let us consider the red cell count and haemoglobin level first. These figures have been accepted as the lower limits of normality, but it

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is agreed that persons having such readings (or readings even slightly higher than these), are frequently encountered, who are nevertheless suffering from a minor degree of anaemia. So that, while it may be a normal reading, it is not necessarily an optimum one. It is a common experience to find a woman with, for example, a red cell count of 4,200,000 and a haemoglobin level of 12.2 grams per 100 c.c. blood, who is none the less suffering from a mild degree of anaemia, and whose red cell count and haemoglobin level can be materially improved by appropriate therapy.

How, then, are we to judge whether a reading around the lower limits of normality is indicative of a mild degree of anaemia ? The only practical method in such cases is that of submitting the patient to therapeutic trial and observing the result. If the picture improves, and the improvement can be maintained by appropriate treatment, then the patient has had some degree of anaemia. This appears to to be a point of some practical importance.

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It has been found that these borderline cases are of frequent occurrence in practice, and if a rigid lower limit is laid down to cover each and every case, a goodly number of cases will fall above these rigid limits, even though they be suffering from a minor degree of anaemia. It has also been my experience to find that, both objectively and subjectively, the improvement resulting from adequate and appropriate treatment far outpaces the relatively small improvement recorded in the blood picture.

So with the vitamin C excretion rate. A daily excretion below 15 mgm. has been accepted as a possible indicator of subnutrition. At the same time, one has no desire to be dogmatic about this, or any other figure. Other things being equal, the resting excretion rate, as judged by an analysis of a 24 hour specimen, is a reasonably reliable guide to the state of vitamin C nutrition of the subject. But cases above, as well as below this level were included in the series, and it must be understood that the figure is a purely arbitrary one adopted for classification purposes, and confirmed or refuted by the subsequent evidence

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of the test-dose.

Without setting up some such standards for the blood readings and vitamin C excretion, classification would not have been possible, the investigation would have been unwieldy, and an analysis of the findings would have been much more difficult.

Such were the reasons for the necessarily somewhat artificial classification on the two specified criteria.

Reference.

Mettier S.R. and Minot G.R. 1931. Amer. J. Med.Sci., 181,25.

CHAPTER IV

THE INCIDENCE AND EXTENT OF SUBNUTRITION FOUND BY OTHER WORKERS.

In the 16th and 17th centuries, scurvy as an entity was recognised for the first time. It has been suggested however, that Hippocrates and Galen recognised the condition, and that Pliny meant scurvy when he described how the soldiers of Germanicus, after a two years campaign on the Rhine, suffered from loosening of the teeth and weakness of the limbs. (They apparently recovered on eating a plant known as Herba Britannica.)

In 1534, Euricius Cordus wrote on scurvy, and a few years later, Johannes Agricola devoted some of his writing to the same subject. It had long been recognised that scurvy was a disease affecting soldiers, sailors and those cut off from fresh foods for long periods. The disease was apparently very prevalent in the 17th and 18th centuries, a result of the absence of fresh food in winter time. In 1675, Gideon Harvey, physician to the King, wrote a treatise on the subject, scurvy apparently being prevalent in London at that time. Although the potato, which incidentally has a very high antiscorbutic value, was introduced into this country by Sir Walter Raleigh in 1585, its cultivation on a large scale was not started until the end of the 18th century.

In 1778 de Mertans recognised that the antiscorbutic property of vegetables was diminished by cooking. The recognition of scuryy as an entity and a knowledge of the ease of its prevention, led to a reduction in the incidence of the disease. But in the latter half of the 19th century, dried patent foods for children made their appearance on the market, and the incidence of infantile scurvy again rose steeply.

In 1883 the nature of scurvy was finally settled by Barlow, but the real cause was still unknown. In 1912 Holst and Frolich showed that animals could be rendered scorbutic by placing them on a certain type of diet, and Sir Gowland Hopkins suggested the name "vitamin C" for the missing factor. In 1928 Szent-Gyorgyi isolated a crystalline substance from the adrenal cortex of the ox, from orange, and from cabbage; this substance was hexuronic acid. It was found to have strong antiscorbutic activity, and in 1932 the name was changed to ascorbic acid. This is accepted by most people as being identical with vitamin C, the antiscorbutic factor.

A great deal of work has been done by Harris on vitamin C nutrition. He states that outbreaks of scurvy occurred in all the belligerent civilian populations in the 1914-1918 war, not excluding Great Britain and the United States of America. In 1940 he published the results of a survey on the vitamin C level of 35 boys at an elementary school. He found that 14 were below standard, and of these 5 were classified as showing a relatively severe deficiency. At a home for waifs and strays, where the diet was very good and included one and one third pints of milk daily, cooked green vegetables at least three times weekly and an orange each day for several weeks before the investigation, none of the 29 boys examined were below standard. He found intermediate results in boys having intermediate diets.

In 1942 Harris published the results of his findings on the vitamin C levels of schoolchildren and students in wartime, using the modified saturation method. 700 mgm. ascorbic acid per 10 stones of body weight were given daily, and the number of days counted until the body's reserves were approaching saturation, as indicated by the rise to a plateau in the curve of urinary excretion. The smaller the subject's past intake, the longer the delay before the saturation was attained. He carried out the test in springtime when the body's reserves are at their lowest. He found that (a) poor class children had a lower rate of excretion than those from a well conducted residential institution and (b) both groups were lower than when examined before the war. He therefore concluded that the decrease was due to a diminished supply of foods rich in vitamin C. He found that controls who had been given graded supplements were higher in their reserves and that the middle classes were less affected than the poor classes.

Orr, in 1936, suggested that more than half of the population of Great Britain received less than the reputed optimum allowance of vitamin C.

Earle (1942) found no evidence of avitaminosis in apparently healthy Trinidadians.

Rafsky and Newman (1941) investigated the retention and excretion of ascorbic acid in 25 "normal" subjects between 66 and 83 years, 14 men and 11 women living at home, ambulatory, and free from active disease. In 10 of these cases, oranges, tomatoes and lemons were part of their dietetic intake. They found they could only saturate, and keep saturated, two out of the twenty-five patients. In the others, high retention values were observed.

Croft and Snorf (1939) investigated the blood ascorbic acid level in 100 unselected patients. The patients were nearly all of an economic and social status that would presuppose a sufficiency of any desired diet. They made the figure of 0.40 mgm. ascorbic acid per 100 c.c. plasma, the arbitrary lower limit of normality, and considered figures below this indicative of vitamin C deficiency. In 38 patients, the concentration was found to be below normal. Although the levels were as low in some cases as in scurvy, no cases of scurvy were seen. They found oral sepsis, dental caries, and spongy gums much more common in the deficient group, these conditions being present in 64% of the "deficient" cases. 13% of this group were edentulous, and only 24% had gums and teeth in good condition. This deficient group, on their history or symptoms, showed a high percentage (84%) of disease or dysfunction of the gastro-intestinal tract of a degree that had led to a diet lacking in vitamin C for from one month to three years. 40% had active peptic ulcers. Only four instances of active infections were recorded. Croft and Snorf concluded that gastro-intestinal dysfunction or disease was the greatest aetiological factor, being present in 84% of the deficient group. They found no mucous or epidermal petechiae. Of the non-deficient group, they considered the dietary history satisfactory in 71% of cases; the remainder had been on doubtful or deficient diets. Only 10% had caries, spongy gums or gingival infections, 6% were edentulous and 84% of this group had teeth and gums in good condition.

Davidson (1928) cites three cases of vitamin C subnutrition in three cases suffering from digestive tract upset. In the first case, a woman of 25 years who had had a cholecystectomy, transduodenal feeding had had to be adopted. Eight weeks after the cholecystectomy, she started to show signs of vitamin C deficiency, such as areas of necrosis on the incisors and canines just above the gum margins. Within one week of adding citrous fruits, the process was completely arrested. In the second case, again a female aged 22 years, and suffering from peptic ulcer, there was restriction of diet. After a period of 22 days on alternating starvation and restricted diet or transduodenal feeding, bleeding spongy gums were noted. The juice of three oranges made neutral to litmus with sodium bicarbonate was administered, and in ten days, the bleeding had disappeared. In the third case, a male aged 36 years, there was also digestive upset. He had been on a desultory Sippy diet for three years. He was found to be suffering from vitamin C subnutrition - he was undernourished, the gums were bleeding, the eyes were prominent, and skin dry, and there were purpuric spots and ecchymoses on the inner aspect of the thighs. Improvement set in after four days of vitamin C rich diet and the improvement was continuous. Davidson emphasises the importance of accessory food factors in special diets as a prophylactic against the development of a deficiency disease, and the ensuing possibly detrimental effects on the lesion under treatment.

Wright (1938) working in America, stated that pre-clinical scurvy was a very common disease among all economic classes. In his series of over 200 cases of definite scurvy, he saw 5 cases in doctors or their families, and eight cases in nurses. More than 50% of his cases could easily have afforded the preventive citrous fruits; many were wealthy, and one owned a large orange grove. The causes of the disease, apart from poverty, were distaste of citrus fruits and other foods containing large amounts of vitamin C; allergic and gastro-intestinal sensitivity to such foods; diets prescribed by physicians for the treatment of ulcers, colitis, etc.,; faddist diets; winter diets; and inability to use vitamin C when taken by mouth.

There are many references in the literature to either single or small groups of cases suffering from scurvy. Its incidence is widespread, cases being reported from America by Hirschfeld (1929), from East Africa by Esler (1929), from Scandinavia by Öknell, from Germany by Meulengracht (1929), from Australia by Frecker (1927), from Britain by Norgate (1923), by McLelland (1921), by Dunlop and Scarborough (1935), to cite but a few.

Youmans, Corlette, Akeroyd and Frank examined 15 subjects whose diets were suspected of being inadequate in vitamin C.12 excreted less than 20 mgm. daily. Of 16 normal subjects whose diets were supposed to be adequate, 8 also excreted less than 20 mgm. per day. In the latter group subsequent inquiry suggested a limited intake, for one or another reason, of vitamin C among those with the low daily excretion. Of the 31 subjects, 21 excreted only a small part of a large test-dose of the vitamin in the 24 hours when it was administered, suggesting a small reserve store in the body. In general, the tests of saturation corresponded to the tests of daily excretion and the probable dietary intake of the vitamin.

Bourne (1938) reported the occurence of the latent scurvy in the dietetic treatment of peptic ulcer. (Bourne G., 1938, B.M.J., i., 560.).

To summarise, we may state that, even in peace-time, the diet of a large part of the population of Great Britain was below the minimum necessary for health, at least so far as vitamin C was concerned; that the position is now worse than it was before the onset of the present war; that the position is shown much more acutely in poor and working class homes, in Britain, than in the middle and upper classes; and that a comparable situation arose in the last war. It would appear that elderly people find difficulty in absorbing vitamin C, even when this is present in adequate amount in the diet. It would also appear that while the subscorbutic state in Britain can generally be attributed to economic stress, this is not the case in America. Isolated cases of scurvy are periodically reported from many countries, and the pre-scorbutic state must be of frequent occurrence and wide distribution.

As we have noted above, Croft and Snorf drew conclusions from an investigation of the blood ascorbic acid level in unselected patients. They apparently did not exclude patients with conditions which, per se, lead to an increased vitamin C metabolism, and according to Golden and Garfinkel (1942) an investigation of the blood ascorbic acid level is not the method of choice because of the complexity of the technique and the uncertainty of the preservation methods.

R E F E R E N C E S.

Holst A. and Frolich T., Zeitschr. Hyg.und.Infect., 1912, 72, 1.

Szent-Gyorgyi A., 1928, Bioch J., xxii., 1387.

Harris L. J. Br. Encyc. Med. Pract., xii., 582.

Harris L. J. Lancet 1940, ii., 259.

Harris L. J. Lancet 1942, i., 642.

Orr J. B. Food, Health and Income, 1936.

Rafsky H. A. and Newman B., Amer. J.Med.Sci.,1941. 201, 749.

Wright I.S. 1938, Ann.Intern.Med.,12,523.

R E F E R E N C E S. (contd).

Croft J. D. and Snorf L. D. 1939, Amer. J.Med Sci., 198, 403.

Golden Walter R.C. and Garfinkel Leo, 1942, Journ.Biol. Chem., 447.

Davidson P.B.,Journ. Amer. Med. Ass., 1928, 1014.

Hirschfeld I. 1929, J.Amer. Dent. Ass., 16, 796.

Esler A.R.,1929, Kenya and East African Med. J. 5, 350.

Oknell H.,Acta. Med. Scandinav.,1928, 68, 176.

Meulengracht, Ugesk. f. laeger, 1927, 89, 165.

Frecker E.W., M.J. Australia, 1927, i, 274.

Norgate R.H., Lancet, 1923, ii, 511.

McLelland R.S., Lancet, 1921, ii., 608.

Dunlop D.M. and Scarborough H., Ed.M. Journal,1935,XL11, 476.

Youmans J.B. Corlette M.B., Akeroyd J.H., and Frank H. Amer. J. Med. Sci., 1936, 191., 319.

Earle K.V., 1942, Br.Med. J. i, 255.

CHAPTER V.

THE INCIDENCE AND EXTENT OF VITAMIN C SUBNUTRITION FOUND IN THIS SERIES.

The vitamin C nutritional status of 100 cases was assessed. The amount of vitamin C excreted in the urine in 24 hours was noted. Test-dosing was subsequently instituted and the effect of the test-dose on the excretion of the vitamin charted. The methods of estimating the urinary vitamin C and of administering a test-dose and reading the result have already been given in detail on pages 17 & 18 .

The type of case investigated has been described in Part III.

On the pages which follow are tabulated the results, with details of each case investigated. Most of the headings used on the table are self-explanatory, but we may explain what is to be understood by (a) dietetic habits and (b) state of vitamin C reserves.

(a) A dietary history was obtained in each case, the patient being allowed to describe his various meals. After this spontaneous description, particular enquiry was made regarding the quantity consumed of fresh fruit, green vegetable both raw and cooked, root vegetable, potato, fruit juices, canned fruit and vegetable and sources of synthetic vitamin C. From this history, whose reliability naturally varied from case to case, some idea was usually obtained of the dietetic habits of the individual. It will be noted that "dietetic habits" are classified as being either "satisfactory" or "unsatisfactory." The term "satisfactory" is used, to indicate that the person probably partook of sufficient vitamin C to meet his requirements and to spare. The term "unsatisfactory" means that sources of vitamin C did not figure prominently in the diet concerned.

(b) As has already been pointed out, the body, in healthy well fed subjects, behaves as if it had appreciable reserve stores of vitamin C. A body with a positive vitamin C balance usually excrètes from 15-30 mgm. per day in the urine, and administration of a large dose of the vitamin leads to a sudden rise in the rate of excretion. One is justified in drawing a conclusion on the state of these reserves after a resting excretion rate has been measured and the test-dose result assessed. In the following table, the state of the vitamin C reserves is noted as being "satisfactory" where administration of a test-dose leads to a sudden peak in the rate of excretion (a resting excretion rate of more than 15 mgm. per day is accepted as tentative evidence of good nutrition pending the performance of test-dosing.). The reserves are stated to be "unsatisfactory" where administration of a test-dose does not give a sudden peak in the rate of excretion, or the resting excretion rate is below 15 mgm. per day. There remain, of course, the possibilities of a low excretion rate combined with a satisfactory response to the test-dose, and a high resting excretion rate combined with an unsatisfactory response. In either instance, one would accept the evidence of the test-dose. It might then be asked, "Why assess the resting excretion rate at all?" It was performed in this series to see whether the results given by that method agreed in every case with the results given by the test-dose, and so decide whether one could rely simply on a resting excretion rate reading in deciding whether or not a subject was undernourished. In addition, as will be explained in full later in the paper, ascorbic acid was deliberately witheld, in the first instance, from a number of the cases. Thus, test-dosing of every case at the outset was not possible, but in those cases in which it was deferred, it was performed at the end of a control period, before they had received any ascorbic acid therapy. Thus, in those cases where test-dosing was deferred, the resting excretion rate was used as a rough indicator of the state of the reserves. One thus had an idea of the type of case with which one was dealing, the case could be temporarily classified, treatment instituted, and the case, if necessary, re-classified on the evidence of the subsequent test-dose.

TABLE 1.

Investigation of vitamin C nutritional level of 100 cases.

Case No.	Age.	Sex.	M.S.W.	Occupation.	Dietetic habits.	Social status.	Disease or dysfunction of gastro-int. tract.	Oral Condition.	Daily vit. C excrtn.	Test dose.	State of vit. C reserves.
1.	15.	F.	S.	Shop assistant.	Unsatis-factory.	Working.	No.	Satisfactory.	14 mgm.	Neg.	Unsatisfactory.
2.	45.	M.	M.	Surveyor.	Satisfactory.	Middle.	No.	Satisfactory.	21 mgm.	Pos.	Satisfactory.
3.	28.	M.	S.	Miner.	Satisfactory.	Working.	Habit Yes. dyspepsia.	Satisfactory.	30 mgm.	Pos.	Satisfactory.
4.	48.	F.	M.	Domestic.	Satisfactory.	Working.	No.	Edentulous Palate worn.	12 mgm.	Neg.	Unsatisfactory.
5.	43.	M.	M.	Miner.	Satisfactory.	Working.	No.	Carious teeth.	21 mgm.	Pos.	Satisfactory.
6.	35.	M.	M.	Miner.	Unsatis-factory.	Working.	Habit Yes. dyspepsia.	Carious teeth.	17 mgm.	Pos.	Satisfactory.
7.	43.	M.	M.	Miner.	Unsatis-factory.	Working.	No.	Edentulous.	18.7 mgm.	Pos.	Satisfactory.
8.	27.	F.	M.	Domestic.	Unsatis-factory.	Middle.	No.	Satisfactory.	13 mgm.	Neg.	Unsatisfactory.
9.	53.	M.	W.	Miner.	Satisfactory.	Working.	No.	Carious teeth.	18 mgm.	Pos.	Satisfactory.
10.	19.	F.	S.	Factory worker.	Unsatis-factory.	Working.	No.	Satisfactory.	12 mgm.	Neg.	Unsatisfactory.
11.	50.	F.	S.	Nurse.	Unsatis-factory.	Middle.	No.	Edentulous Palate worn.	12 mgm.	Neg.	Unsatisfactory.
12.	29.	M.	S.	Labourer.	Satisfactory.	Working.	Healed Yes. peptic ulcer.	Satisfactory.	24 mgm.	Pos.	Satisfactory.
13.	19.	F.	S.	Domestic.	Unsatis-factory.	Working.	No.	Satisfactory.	14 mgm.	Neg.	Unsatisfactory.
14.	40.	F.	M.	Domestic.	Unsatis-factory.	Working.	No.	Edentulous Palate worn.	9 mgm.	Neg.	Unsatisfactory.
15.	50.	M.	M.	Miner.	Satisfactory.	Working.	No.	Edentulous Palate worn.	15.5 mgm.	Pos.	Satisfactory.
16.	49.	M.	M.	Miner.	Satisfactory.	Working.	No.	Edentulous Palate worn.	18.3 mgm.	Pos.	Satisfactory.
17.	65.	M.	M.	Pilot.	Satisfactory.	Working.	No.	Edentulous Palate worn.	19.2 mgm.	Pos.	Satisfactory.
18.	46.	F.	M.	Domestic.	Satisfactory.	Working.	Habit Yes. dyspepsia.	Edentulous Palate worn.	22 mgm.	Pos.	Satisfactory.
19.	27.	F.	M.	Domestic.	Satisfactory.	Middle.	No.	Satisfactory.	20 mgm.	Pos.	Satisfactory.
20.	70.	F.	S.	Domestic.	Satisfactory.	Middle.	No.	Edentulous Palate worn.	18 mgm.	Pos.	Satisfactory.

TABLE 1. (contd).

Case No.	Age.	Sex.	M.S.W.	Occupation.	Dietetic habits.	Social status.	Disease or dysfunction of gastro-int. tract.	Oral Condition.	Daily vit. C excrtn.	Test-dose.	State of vit. C reserves.
21.	47.	F.	W.	Domestic.	Unsatis-factory.	Working.	No.	Edentulous Palate worn.	12 mgm.	Neg.	Unsatisfactory.
22.	48.	F.	M.	Domestic.	Satisfactory.	Middle.	No.	Edentulous Palate worn.	17 mgm.	Pos.	Satisfactory.
23.	51.	M.	M.	Dockyard worker.	Unsatis-factory.	Working.	Yes. Mucous colitis.	Edentulous Palate worn.	14.5 mgm.	Pos.	Satisfactory.
24.	25.	F.	M.	Domestic.	Unsatis-factory.	Middle.	No.	Satisfactory.	11.5 mgm.	Neg.	Unsatisfactory.
25.	60.	F.	M.	Domestic.	Satisfactory.	Working.	No.	Edentulous Palate worn.	21.5 mgm.	Pos.	Satisfactory.
26.	24.	F.	S.	Factory worker.	Satisfactory.	Working.	No.	Satisfactory.	16.5 mgm.	Pos.	Satisfactory.
27.	35.	F.	M.	Domestic.	Unsatis-factory.	Working.	No.	Satisfactory.	17 mgm.	Pos.	Satisfactory.
28.	45.	M.	M.	Miner.	Satisfactory.	Working.	No.	Edentulous Palate worn.	24 mgm.	Pos.	Satisfactory.
29.	37.	F.	M.	Domestic.	Unsatis-factory.	Working.	No.	Edentulous Palate worn.	10 mgm.	Neg.	Unsatisfactory.
30.	26.	F.	M.	Domestic.	Unsatis-factory.	Middle.	No.	Satisfactory.	13.5 mgm.	Neg.	Unsatisfactory.
31.	71.	F.	W.	Domestic.	Unsatis-factory.	Working.	No.	Edentulous Palate worn.	13 mgm.	Neg.	Unsatisfactory.
32.	42.	M.	M.	Miner.	Satisfactory.	Working.	No.	Edentulous Palate worn.	17 mgm.	Pos.	Satisfactory.
33.	41.	F.	M.	Domestic.	Unsatis-factory.	Working.	No.	Edentulous Palate worn.	12 mgm.	Neg.	Unsatisfactory.
34.	50.	F.	M.	Domestic.	Satisfactory.	Working.	No.	Edentulous.	20 mgm.	Pos.	Satisfactory.
35.	57.	F.	M.	Domestic.	Satisfactory.	Working.	No.	Edentulous Palate worn.	22 mgm.	Pos.	Satisfactory.
36.	39.	F.	M.	Domestic.	Satisfactory.	Working.	No.	Edentulous Palate worn.	20 mgm.	Pos.	Satisfactory.
37.	18.	F.	S.	Nurse.	Unsatis-factory.	Working.	No.	Satisfactory.	15.6 mgm.	Neg.	Unsatisfactory.
38.	70.	F.	S.	Retired School teacher.	Unsatis-factory.	Middle.	No.	Edentulous Palate.	12.5 mgm.	Neg.	Unsatisfactory.
39.	40.	F.	S.	School teacher.	Unsatis-factory.	Middle.	No.	Satisfactory.	13 mgm.	Neg.	Unsatisfactory.
40.	33.	M.	M.	Sea Captain.	Satisfactory.	Middle.	No.	Satisfactory.	26 mgm.	Pos.	Satisfactory.

TABLE 1. (contd).

Case No.	Age.	Sex.	M.S.W.	Occupation.	Dietetic habits.	Social status.	Disease or dysfunction of gastro-int. tract.	Oral Condition.	Daily vit. C excrtn.	Test-dose.	State of vit. C reserves.
41.	50.	M.	M.	Grocer.	Satisfactory.	Middle.	No.	Edentulous: Palate.	17.5 mgm.	Pos.	Satisfactory.
42.	39.	F.	M.	Domestic.	Satisfactory.	Working.	No.	Edentulous: Palate.	18 mgm.	Pos.	Satisfactory.
43.	46.	F.	M.	Domestic.	Satisfactory.	Working.	No.	Edentulous.	20 mgm.	Pos.	Satisfactory.
44.	52.	M.	M.	Miner.	Satisfactory.	Working.	Yes. Flatulent dyspepsia.	Edentulous: Palate.	25 mgm.	Pos.	Satisfactory.
45.	40.	F.	M.	Domestic.	Satisfactory.	Working.	No.	Edentulous: Palate.	12.5 mgm.	Neg.	Unsatisfactory.
46.	50.	M.	M.	Miner.	Satisfactory.	Working.	No.	Edentulous: Palate.	20 mgm.	Pos.	Satisfactory.
47.	36.	M.	M.	Seagoing Engineer.	Unsatis-factory.	Middle.	No.	Satisfactory.	16.8 mgm.	Pos.	Satisfactory.
48.	53.	F.	W.	Domestic.	Unsatis-factory.	Working.	No.	Edentulous.	7.5 mgm.	Neg.	Unsatisfactory.
49.	32.	F.	M.	Domestic.	Unsatis-factory.	Middle.	No.	Edentulous: Palate.	14 mgm.	Pos.	Satisfactory.
50.	20.	F.	S.	Domestic.	Unsatis-factory.	Working.	No.	Edentulous.	9 mgm.	Neg.	Unsatisfactory.
51.	41.	M.	M.	Miner.	Satisfactory.	Working.	No.	Edentulous: Palate.	16 mgm.	Pos.	Satisfactory.
52.	33.	M.	M.	Clerk.	Satisfactory.	Working.	No.	Edentulous: Palate.	20 mgm.	Pos.	Satisfactory.
53.	26.	F.	M.	Domestic.	Unsatis-factory.	Working.	No.	Edentulous: Palate.	15.7 mgm.	Pos.	Satisfactory.
54.	25.	F.	M.	Domestic.	Unsatis-factory.	Middle.	No.	Satisfactory.	14 mgm.	Neg.	Unsatisfactory.
55.	16.	M.	S.	Dock Worker.	Satisfactory.	Working.	No.	Satisfactory.	22 mgm.	Pos.	Satisfactory.
56.	47.	M.	M.	Dock Worker.	Unsatis-factory.	Working.	No.	Edentulous: Palate.	13.8 mgm.	Neg.	Unsatisfactory.
57.	39.	F.	M.	Domestic.	Unsatis-factory.	Working.	No.	Edentulous.	3 mgm.	Neg.	Unsatisfactory.
58.	50.	M.	M.	Grocer.	Satisfactory.	Working.	No.	Edentulous: Palate.	19.6 mgm.	Pos.	Satisfactory.
59.	40.	F.	M.	Domestic.	Satisfactory.	Working.	No.	Edentulous.	23 mgm.	Pos.	Satisfactory.
60.	24.	M.	M.	Miner.	Satisfactory.	Working.	No.	Carious teeth.	15 mgm.	Pos.	Satisfactory.

TABLE 1 (Contd).

Case No.	Age.	Sex.	M.S.W.	Occupation.	Dietetic habits.	Social status.	Disease or dysfunction of gastro-int. tract.	Oral condition.	Daily vit. C excrt.	Test dose.	State of vit. C reserves.
81.	55.	F.	M.	Domestic.	Unsatis-factory.	Working.	No.	Edentulous: Palate worn.	9.2 mgm.	Neg.	Unsatisfactory.
82.	20.	F.	M.	Factory Worker.	Satisfactory.	Working.	No.	Satisfactory.	11 mgm.	Neg.	Unsatisfactory.
83.	34.	F.	M.	Domestic.	Unsatis-factory.	Working.	No.	Edentulous: Palate worn.	4.1 mgm.	Neg.	Unsatisfactory.
84.	48.	F.	M.	Domestic.	Satisfactory.	Middle.	No.	Edentulous: Palate.	13.2 mgm.	Neg.	Unsatisfactory.
85.	51.	M.	M.	Ambulance driver.	Unsatis-factory.	Working.	Yes. Ulcer diathesis.	Edentulous: Palate.	17 mgm.	Pos.	Satisfactory.
86.	35.	F.	M.	Domestic.	Unsatis-factory.	Working.	No.	Carious teeth: spongy gums.	11.5 mgm.	Neg.	Unsatisfactory.
87.	25.	F.	M.	Bakeress.	Unsatis-factory.	Working.	No.	Satisfactory.	20 mgm.	Pos.	Satisfactory.
88.	20.	F.	S.	Bank Clerk.	Satisfactory.	Working.	No.	Satisfactory.	16 mgm.	Pos.	Satisfactory.
89.	20.	M.	S.	Bricklayer.	Satisfactory.	Working.	No.	Satisfactory.	25 mgm.	Pos.	Satisfactory.
90.	30.	F.	M.	Domestic.	Satisfactory.	Working.	No.	Satisfactory.	23 mgm.	Pos.	Satisfactory.
91.	54.	F.	M.	Domestic.	Satisfactory.	Middle.	No.	Edentulous: Palate.	17 mgm.	Pos.	Satisfactory.
92.	64.	M.	M.	Miner.	Satisfactory.	Working.	No.	Edentulous: Palate.	20 mgm.	Pos.	Satisfactory.
93.	55.	M.	M.	Miner.	Satisfactory.	Working.	No.	Edentulous: Palate.	19 mgm.	Pos.	Satisfactory.
94.	60.	M.	M.	Miner.	Unsatis-factory.	Working.	No.	Edentulous.	10 mgm.	Pos.	Satisfactory.
95.	34.	M.	M.	Miner.	Unsatis-factory.	Working.	Yes. Ulcer diathesis.	Edentulous: Palate worn.	15.5 mgm.	Neg.	Unsatisfactory.
96.	33.	M.	M.	Joiner.	Satisfactory.	Working.	No.	Satisfactory.	14.5 mgm.	Pos.	Satisfactory.
97.	61.	M.	M.	Miner.	Satisfactory.	Working.	No.	Edentulous: Palate worn.	13.3 mgm.	Pos.	Satisfactory.
98.	64.	M.	M.	Miner.	Satisfactory.	Working.	No.	Edentulous: Palate worn.	19 mgm.	Pos.	Satisfactory.
99.	37.	M.	M.	Miner.	Unsatis-factory.	Working.	Yes. Ulcer diathesis.	Edentulous.	20 mgm.	Pos.	Satisfactory.
100.	47.	M.	M.	Engineer.	Satisfactory.	Working.	No.	Edentulous: Palate.	24.5 mgm.	Pos.	Satisfactory.

Let us now attempt an analysis of these findings.

One hundred cases were examined, 62 being females and 38 males. We shall deal separately with the sexes. 62 of these cases were found to have satisfactory vitamin C reserves and 38 to have unsatisfactory reserves.

A. Males. 38 cases.

Of the 38 males examined, 35 showed a satisfactory vitamin C nutritional state. Only 3 were found to have an unsatisfactory balance, i.e. slightly less than 8% of the males were undernourished. This "unsatisfactory" group is tabled below for ease of reference.

"Unsatisfactory" group. - male.

Case No.	Age.	Diet.	Occupn.	Social status.	Gastro-int. tract.	Oral condition.
56.	47.	Unsatis.	Docker.	Working.	Nil.	Dentures.
73.	60.	Satis.	Grocer.	Working.	Nil.	Dentures.
95.	34.	Unsatis.	Miner.	Working.	Healed peptic ulcer.	Dentures.

These cases do not appear to have any features in common apart from the facts that they were all three married, that they were of working class and that they were edentulous but wore satisfactory dentures. In two of the cases, the dietetic history given at the outset appeared to be unsatisfactory from the point of view of the vitamin C content; in case number 56 the diet was restricted because of the patient's fear of his blood pressure rising, in case number 95 because of the ulcer diathesis. The diet in both of these cases consisted largely of bread and butter or margarine, milk, milk puddings, breakfast cereals and biscuits, with an occasional addition of boiled or steamed fish. They ate very little potato, no green vegetable, no fruit, and very little meat. In case number 73, the history given by the patient suggested a satisfactory intake. He was, however, suffering from a slight degree of arteriosclerosis, and this may have interfered to some extent with his absorption of the vitamin.

.92% of the males showed a satisfactory vitamin C balance, so far as could be judged from the examination of a 24 hour specimen of urine and subsequent observation of the result of test-dosing.

B. Females. 62 cases.

In all, 62 females were examined. Of this number, 27 were found to have satisfactory vitamin C reserves and 35 to have unsatisfactory reserves.

This was an impressive finding. It meant that over 56% of the females examined in this series of cases, were suffering from some degree of vitamin C subnutrition. The dietary histories usually agreed with this finding, the potato, vegetable, and fruit intake being very low in most of the undernourished cases. In a few cases, however, the dietary history and subsequent findings did not agree. Some patients would not admit to partaking of an unbalanced diet - one was impressed by the studied inclusion of a green vegetable daily in the dietetic history, if not actually in the diet, of some of the more prosperous members of the community.

At what ages was subnutrition found to be most prevalent?

Females - 62 cases.

Age group. (years).	Number with unsatis-factory reserves.	Number with satisfactory reserves.
0 - 9.	0	0
10 - 19.	4	0
20 - 29.	10	7
30 - 39.	9	8
40 - 49.	8	4
50 - 59.	3	6
60 - 69.	0	1
70 - 79.	1	1
TOTAL.	35	27

It will be seen that the majority of the cases were between 20 and 49 years of age. It is also noticeable that in each age group under 49 years, there is a preponderance of undernourished over wellnourished cases. This is not in agreement with the common conception of adult scurvy as a disease of bachelor males over middle age.

It is also noteworthy that four of the cases occurred in girls under 19 years who had completely abstained from green vegetables over a period of several years.

Let us next examine the cases from the marital status.

	"Unsatisfactory" group.	"Satisfactory" group.
Married.	22	22
Single.	9	5
Widowed.	4	0
TOTAL.	35	27

It will be seen that there was an equal number of "satisfactory" and "unsatisfactory" married women, while there were nearly twice as many spinsters undernourished as there were nourished. None of the few widows examined were well nourished.

It is of some importance to examine the question as it affects married women, with a little more care.

Married - 44 cases.

"Unsatisfactory" group. (22 cases).

Husband at home. 12.

Husband away from home. 10.

"Satisfactory" group. (22 cases).

Husband at home. 20.

Husband away from home. 2.

We find that in the "unsatisfactory" group of 22 cases, the husband was living away from home in 10 instances, either in the forces or in the factories; while in the "satisfactory" group of 22 cases, the husband was living away from home in only 2 instances. Subnutrition was thus found to be more common among women whose husbands were living away from home. Here we may have a clue to one of the causes of vitamin C subnutrition in women. The women who were now living alone, and had only themselves and perhaps a young child for whom to cater, did not always maintain their previous standards of living. Not being engaged on heavy work herself, having no husband with a healthy appetite for whom to cook, perhaps living on a greatly reduced income, she did not always give as much time or thought to the selection and preparation of dishes as she ought to have done. Another not unimportant reason for the greater incidence of vitamin C subnutrition among these women who are living alone, may be that gardens and allotments tend to fall into disuse when the menfolk are absent.

Similar reasons may be advanced to account for the greater incidence of subnutrition among widows.

When an occupational analysis of the cases was made, housewives were found to figure prominently among the undernourished. 49 housewives were examined.

27 were found to be undernourished, 22 to be satisfactorily nourished.

This incidence is so high that the prescorbutic state might almost be regarded as an occupational disorder in this area. In industry, precautions are taken against employees contracting industrial disease. The women who are running our homes should be protected from this, among the many other risks to which they are peculiarly exposed, and the importance of taking regular, well balanced meals should be impressed upon them. It is not uncommon to find a prescorbutic mother virtually administering daily test-doses of vitamin C to a perfectly well nourished child; it might also be impressed on the mothers that proper management of the child's diet obviates, to a large degree, the need for the administration of vitamins in an artificial form.

Two hospital nurses and two school teachers were included in the series. In all four cases, they were found to be undernourished.

Five factory workers were examined. Most of their meals were derived from the factory canteen. In four of the cases, the reserves were satisfactory, in one case, unsatisfactory. This "unsatisfactory" case had voluntarily abstained, over a long period, from sources of vitamin C which were available at the works canteen.

As has already been stated, Harris and Ray, (1935) demonstrated that individuals accustomed to getting too little vitamin C, excrete less than those on a well balanced diet, and that this lower output is parallel with a diminished intake. They also concluded that, in adults, a low urinary output and a low response to test-doses, go parallel with a history of vitamin C underfeeding. Did these statements hold in the present series of cases?

In 83 of the present 100 cases, the dietetic history was a reliable guide to the nutritional state, the history agreeing with the results found on test-dosing.

In 5 of the cases, a satisfactory history was given, but an unsatisfactory state found. Four of the cases were females, the fifth, a male who has already been discussed. The four women were cases number 4, 45, 82 and 84.

Case No.	Age.	M.S.W.	Occupn.	Dietary.	Gast-int. upset.	Daily vit. C excren.	Test dose.
4.	48.	M.	House.	Satis.	No.	12 mgm.	Neg.
45.	40.	M.	House.	Satis.	No.	12.5 mgm.	Neg.
82.	20.	M.	Factory.	Satis.	No.	11 mgm.	Neg.
84.	48.	M.	House.	Satis.	No.	13.2 mgm.	Neg.

It will be noted that the resting excretion rate in each case was not far short of the figure suggested by Harris as the minimum in well nourished subjects, viz. 13 mgm. per day. In none of the four cases was there found any gastro-intestinal, metabolic or renal upset likely to interfere with the absorption, metabolism or excretion of vitamin C. One must therefore conclude that in these four cases, the history was unreliable.

Twelve cases who gave an unsatisfactory dietetic history were subsequently found not to be undernourished. The only explanation to be offered is that either the history was inaccurate, or they had built up sufficient reserves in the previous summer to allow of a reasonable resting excretion, and a quick overflow into the urine on administration of a test-dose.

We must therefore conclude that the history obtained from the patient was a true guide to his nutritional status in only 83% of the cases.

Abbasy, Hill and Harris (1936) state that, under ordinary circumstances, the responses to test-doses are simply graded according to the resting level of excretion. Accepting Harris' figure of 13 mgm. as the daily excretion level below which subnutrition is a probability, one would expect to find that all cases excreting more than 13 mgm. per day would give a positive response to the test-dose, while all cases excreting less than 13 mgm. per day would give a negative response. This was the finding in 88 of the patients investigated. In the remaining 12, the impression given by the initial resting excretion rate estimation, was not quite in accordance with the result of the test-dose. The test-dose was repeated on the 2nd day if the result on the 1st day was negative. A negative result after a second test-dose was accepted as conclusive evidence of subnutrition. The findings were as follows :-

(a) 26 cases had an excretion of below 13 mgm. per day. Test-dosing in all of these cases gave negative results. This means that the resting excretion rate and test-dose result were, in all these cases, parallel.

(b) 4 cases had an excretion of 13 mgm. per day. Test-dosing in each of these four cases gave negative results. This means that the resting excretion rate and test-dose result were, in all four cases, contrary.

(c) 70 cases had an excretion rate of more than 13 mgm. per day. Test-dosing in 62 of these cases gave positive results; in 8 cases, negative results were given. The resting excretion rate and test-dose result were therefore parallel in 62 cases, contrary in 8 cases. Details of these 8 cases in whom were found a resting excretion rate of over 13 mgm. and a negative result to test-dosing, are given below.

Case No.	Age.	Sex.	Daily vit. C excretion.	Test-dose result.
1.	15.	F.	14 mgm.	Negative.
13.	19.	F.	14 mgm.	Negative.
30.	26.	F.	13.5 mgm.	Negative.
37.	18.	F.	15.6 mgm.	Negative.
54.	25.	F.	14 mgm.	Negative.
56.	47.	M.	13.8 mgm.	Negative.
84.	48.	F.	13.2 mgm.	Negative.
95.	34.	M.	15.5 mgm.	Negative.

It will be noted that the highest excretion rate encountered which gave a negative result to test-dosing, was 15.6 mgm. per day, and that six of the remaining eight cases shown above were between 13 and 14 mgm. per day.

How are we to account for this apparent paradox? A number of cases just above the 13 mgm. per day line gave a negative result with test-dosing, while others with a similar rate of excretion gave a positive result. We may advance three possible explanations, viz :-

(a) The specimen of preserved urine alleged to have been a 24 hours. specimen, may have been the volume passed over a longer period. As we have stated in a previous section, precise instructions were given to each patient of the method to be adopted for collecting the specimen. A few patients may have included both the urine passed at the beginning as well as at the end of the 24 hour period. This naturally would have led to a figure higher than the true 24 hour reading.

(b) As no attempt was made to influence the diet of the patient while under test, he may have partaken of a sufficiently large amount of vitamin C on that particular day, to give a rise in the resting vitamin C excretion level, even though he was not saturated with the vitamin.

(c) A test-dose of 700 mgm. ascorbic acid per 10 stones of body weight was administered to each patient, and if necessary, repeated on the second day. There were several possible sources of error here, viz :-

i. Reduced potency of the ascorbic acid tablets.

ii. The full dose may, or may not, have been swallowed by the patient.

iii. Instructions were given to each patient to the effect that the tablets must not be exposed to light, or dissolved in water until immediately before use. These instructions may, or may not, have been followed.

iv. There may have been difficulty or delay in absorption of the ascorbic acid from the gut, or in its excretion in the urine.

According to Abbasy, Harris, Ray and Marrack, (1935) the standard test-dose should give a response - i.e. a sudden rise in the excretion of ascorbic acid - at the latest on the second day in normally nourished persons. They consider that normal healthy adults with an intake of 45 mgm. ascorbic acid per day, will excrete about 30 mgm; those with an intake of 15 mgm. will excrete about 15 mgm. per day. They also consider that where the intake is about 15 mgm. per day, the response to a single test-dose is in the region of 40-50 mgm. This is a sufficiently large response to be easily recognisable on titration, and one cannot therefore conceive an erroneous interpretation of the test-dose result. Youmans, Corlette, Akeroyd and Frank (1936) suggest an excretion of 30% of a test-dose (600 mgm. adults) as the lower limit of normality.

Lastly, some small experimental error in titration must be anticipated, even with experience and a careful technique. Such an error may have accounted for the paradox, and merely serves to emphasise the importance of the test-dose before arriving at a final conclusion. It is, of course, much more difficult to arrive at an exact end-point where the ascorbic acid content of the urine is low than where the content is high.

The resting excretion rate indicated the true state of vitamin C reserves in only 88% of the cases.

The highest resting excretion rates encountered in the series were :-

(a) Males. - 33 mgm. ascorbic acid per day.

(b) Female. - 23 mgm. per day.

The lowest resting excretion rates were :-

(a) Male. - 6 mgm. per day.

(b) Female. - 3 mgm. per day.

The average daily excretion irrespective of sex was 16.09 mgm. per day.

The average daily excretion in males was 19.3 mgm. per day, and in females, 14.4 mgm. per day.

It is very obvious from these figures and from the details already given, that the nutritional state of the men was much better than that of the women.. Of the 100 cases, 82 were of working class and 18 were of middle class.

Of the 82 working class patients, 53 were found to have satisfactory vitamin C reserves, 29 to have unsatisfactory reserves.

Of the 18 middle class patients, 9 were found to have satisfactory reserves, 9 to have unsatisfactory reserves. In this small series of cases, there was a higher percentage of subnutrition among the middle class patients than among the working class. This agrees with Wright's finding (1938) that pre-clinical scurvy is a common condition in all economic classes.

As we have stated in the previous chapter, some workers have found a definite relationship between a deranged alimentary tract and vitamin C subnutrition. Croft and Snorf (1939) found oral sepsis, dental caries and spongy gums much more common in the deficient group, and found many cases with disease or dysfunction of the gastro-intestinal tract in this group. Davidson (1928) has cited cases of scurvy in gastro-intestinal tract disease. Barlow (1883) states that the intestinal condition in some of his cases was healthy, in others, not so. He found vomiting to be very exceptional, anorexia to meat vegetable and potato, common. A few had diarrhoea. Two of his cases had hysterical objections to meat and vegetable respectively, one of them even crying and vomiting when a plate of meat was placed in front of her. Another of his cases even screamed if vegetable was placed near her.

In the present series, 11 patients gave a history or presented symptoms suggestive of gastro-intestinal disease or dysfunction - the type of gastro-intestinal upset is indicated in each case in Table 1 - but in only 2 of these cases were unsatisfactory vitamin C reserves found. The two cases with unsatisfactory reserves were cases number 65 and number 95.

Case number 65, as will be seen by referring to the Table, was a married woman aged 40. She was apparently in good health when she was first examined - there was no suggestion of gastro-intestinal upset. She was found to have a resting excretion of 12 mgm. per day, and test-dosing gave negative results. The patient did not return for further investigation or treatment, and she was next heard of three weeks later. An urgent call was sent at 11.p.m., and on arrival at the patient's house, she was found to have had a haematemesis of about 5 ounces. This was apparently due to an acute gastric ulcer. The usual treatment for this condition was adopted, and, in addition, ascorbic acid was administered by mouth. After a period of ten days, the reserves were found to be satisfactory. The patient made a rapid and uninterrupted recovery, and there has been no recurrence of either symptoms or signs.

Case number 95 was a miner aged 34 years, who had a healed peptic ulcer. He had been living on a diet which was deficient in vitamin C for some time. His resting excretion rate was 15.5 mgm. per day, but his response to test-dosing was unsatisfactory, and he was therefore classified as having unsatisfactory reserves. On subsequent interrogation, he confirmed the fact that his specimen was a 24 hour one, and that he had swallowed the whole of the test-dose on each occasion. One might therefore suggest that there was delay in the absorption of ascorbic acid from the gut in this case, and that that was the reason for the negative result to the test-dose.

In this series of 100 cases, there were found 11 cases who were suffering, or had suffered, from gastro-intestinal disease or disorder. These 11 cases were composed of the following, viz :-

i. There were 5 cases of what one might term "habit dyspepsia." This term was used to cover the dyspeptic states due to such causes as ill-balanced, ill-cooked or irregular meals, inadequate mastication, air-swallowing, etc. No organic disease was found in any of these cases, nor was achlorhydria present.

ii. There were 4 cases who had previously suffered from proven peptic ulcer, which had now healed, so far as could be ascertained on clinical grounds.

iii. There was one case, a female, who developed an acute peptic ulcer 3 weeks after her vitamin C nutritional state had been investigated.

iv. There was one case suffering from long-standing mucous colitis which was, at the time of investigation, in a quiescent phase.

Of these 11 cases, only two were found to be suffering from vitamin C subnutrition. They were case number 65, who later developed the acute peptic ulcer, and case number 95, the case who had a healed peptic ulcer; in both cases, there had been an inadequate diet over a long period of time.

We must therefore conclude that gastro-intestinal disease or dysfunction was not closely associated with the cases of vitamin C subnutrition found in this area. Of the 38 cases found to be undernourished, only two had gastro-intestinal disease or disorder, past or present, while of the 62 well-nourished cases, 9 were suffering, or had suffered, from such disease or disorder.

Barlow T. (1883) states that in a typical early case in an infant, the gums are frequently natural apart from a small erosion; but in 15 out of 31 cases which he collected, there was some morbid oral condition. In four of the cases, there was sponginess of the gums with a tendency to bleed, and a putrid odour. Nine cases showed varying degrees of mouth affection from slight swelling round newly cut teeth to general swelling of both gums, and in one case, swelling of the lower lip also.

Hanke (1933) states that both gingivitis and dental caries may be affected by a lack of vitamin C. They claim that both these forms of dental disease can be lessened by giving massive doses of orange and lemon juice.

Pitts (1935) states that in scurvy, affecting either adults or children, the disease affects the supporting structures of the teeth and leads to gingivitis and progressive loosening, changes which are, in many respects, comparable to pyorrhoea. He suggests that vitamin C in conjunction with vitamins A and D, may be necessary for a normal development and function of the teeth, and that any deficiency of this vitamin may be reflected in a lower resistance of the teeth to disease.

Croft and Snorf (1939) found oral sepsis, spongy gums and dental caries much more common in cases suffering from vitamin C subnutrition than in well-nourished cases. Let us examine the position as we found it here.

It is rather difficult to assess the relationship between the dental and the nutritional state in an area such as this. Conservative dental work is not appreciated, and one finds that the bulk of patients over 25 to 30 years of age, are either edentulous or have gross dental caries, with or without pyorrhoea alveolaris. Of the 100 cases assessed :-

i. 27 had an apparently healthy mouth. The teeth were good, there was no obvious pyorrhoea alveolaris, nor was there any sponginess of the gums. In a few of these cases, odd teeth were missing, usually replaced by a partial denture.

ii. 8 cases were found to be suffering from caries and/or pyorrhoea. Case number 86 was found to be suffering from dental caries and marked sponginess of the gums which bled readily; she had no pyorrhoea.

iii. 13 cases were completely edentulous, and did not wear dentures.

iv. 52 cases were edentulous, but wore satisfactory dentures.

As we have stated, 38 of the 100 cases examined suffered from vitamin C subnutrition. Of these 38 undernourished cases :-

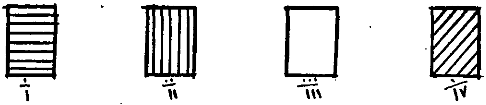
i. 12 had a satisfactory dental condition.

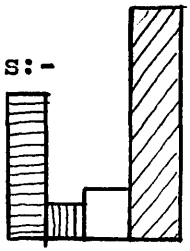
ii. 3 had an unsatisfactory dental condition, i.e. were suffering from caries, pyorrhoea or spongy gums.

iii. 4 were completely edentulous.

iv. 19 were completely edentulous, but wore satisfactory dentures.

This finding may be represented diagramatically thus:-





"Undernourished" group

Of the 62 cases found to be well-nourished with vitamin C :-

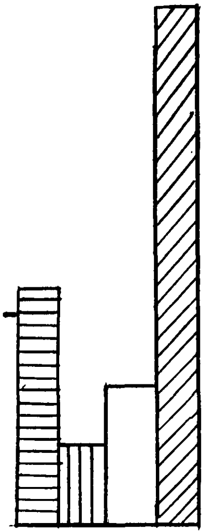
i. 15 had a satisfactory dental condition.

ii. 5 had an unsatisfactory dental condition.

iii. 9 were completely edentulous.

iv. 33 were completely edentulous, but wore satisfactory dentures.

This finding may be represented diagramatically thus:-



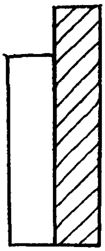
"Well-nourished" group

We see that the form of the graph is essentially the same in each group.

Stated in a different way :-

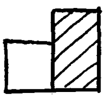
i. Of the 27 cases with a healthy mouth, 12 were undernourished, and 15 well-nourished.

i.



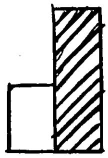
ii. Of the 8 cases with an unhealthy mouth, 3 were undernourished, and 5 were well-nourished.

ii.



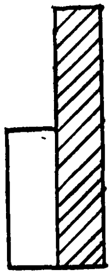
iii. Of the 13 cases who were completely edentulous, 4 were undernourished and 9 were well-nourished.

iii



iv. Of the 52 cases who were edentulous but wore a satisfactory palate, 19 were undernourished, and 33 were well-nourished.

iv



Again we see that the form of the graph is essentially the same in each group.

These findings may be tabulated as follows :-

No. of cases examined. 100.	No. of cases with sub-nutrition. 38.	No. of well-nourished cases. 62.
Dental condition good. (27 cases).	12 (31.5% of 38).	15 (24% of 62)
Unsatis. dental condition. (8 cases).	3 (8%).	5 (8%).
Edentulous, no dentures. (13 cases).	4 (10.5%).	9 (14.5%).
Edentulous with dentures. (52 cases).	19(50%).	33 (53.5%).
TOTAL.	38(100%).	62(100%).

When the percentage figures are compared, it will be seen that the dental condition of the well-nourished group was almost parallel with that of the ill-nourished. There is no suggestion that oral sepsis or dental caries were more common in the ill-nourished group. One case showing definite sponginess of the gums did, however, fall into the deficient group. This was case no. 86, a female, aged 35 years, married, her husband serving abroad. She had six young children, all living at home. Her diet was greatly restricted, consisting mainly of bread and margarine, tea, milk puddings, a little jam, occasionally potato and very rarely any fresh fruit or vegetable. Her excretion rate was 11.5 mgm. ascorbic acid per day, and she gave a negative response on test-dosing. She bruised particularly easily, and had done so for some years. Unfortunately, ascorbic acid therapy was interrupted as a result of enemy action, and exact figures of the progress of the case were not obtained.

We may note that between 60% and 70% of both the well and the ill-nourished groups, were completely edentulous. We may also note that the ratio of well-nourished to ill-nourished cases, was lowest in that group who had a satisfactory dental condition. These findings do not agree with those of Croft and Snorf; the reason for the difference in the findings may be that, <u>in the present group of cases,</u> where a satisfactory dental condition was found in a working class family, it was usually in one of its younger female members, the very type in whom the intake of vitamin C is often restricted. Twelve cases with a satisfactory dental condition and inadequate reserves are shown in Table 1. Of these 12 cases, 11 were females below the age of 30 years, and the remaining one was a female aged 40 years. No male with a satisfactory dental condition and an unsatisfactory nutritional state was found, although the reverse held good, viz. cases were encountered where the teeth were carious, but the nutrition good. Of the eight cases suffering from caries, five had satisfactory vitamin C reserves. In the remaining three cases, the reserves were unsatisfactory. The five well-nourished cases who were suffering from caries were composed of 4 males and 1 female. The three ill-nourished cases who were suffering from caries were females. All that one is justified in saying is that, of the four females who had carious teeth, three were suffering from vitamin C subnutrition; and that of the four males who had carious teeth, none were suffering from subnutrition.

Let us now give a short resume of the more important conclusions which we have so far reached.

(1) In a series of 100 cases, 62% were found to be satisfactorily nourished, and 38% appeared to be suffering from vitamin C subnutrition.

(2) The prescorbutic state was found in both middle and working class patients.

(3) Only three male cases were found to be undernourished (i.e. less than 8% of the males), and the subnutrition in these males was due to restricted diet in two of the cases, and was probably due to arteriosclerotic malabsorption in the third case.

(4) Of the 62 females examined, thirty-five (i.e. 56% of the females) were found to be undernourished. The majority of the cases were between 20 and 50 years of age, and in each age group under the age of 49 there was a preponderance of undernourished cases. Spinsters and widows were found more often to be undernourished than well-nourished. Subnutrition was found to be especially common among housewives, especially among those whose husbands were living away from home.

(5) The dietetic history was a reliable guide to the nutritional state of the subject in only 83% of the cases.

(6) The impression of the nutritional status of the

subject gained by estimating the resting excretion rate of vitamin C was correct in 88% of the cases; in the other 12% the resting excretion level was on or near the borderline figure of 13 mgm. per day, and in these borderline cases, one is not justified in diagnosing subnutrition without test-dosing and watching the result. If, however, the resting excretion is well above or below the 13 mgm. per day line, it would appear justifiable to rely solely on this resting rate.

(7) The average rate of excretion of vitamin C in the urine appeared to be higher in males than in females (adult).

(8) There was no evidence of a higher incidence of gastro-intestinal disease or disorder among the undernourished cases than among the well-nourished.

(9) There was no evidence of a higher incidence of dental caries or pyorrhoea alveolaris in the undernourished group; this group, however, contained the only case found to have spongy gums.

REFERENCES.

Barlow T. 1935. Arch. Dis. Child., x, 232.

Hanke M.T. 1933. Diet and Dental Health, Chicago.

Pitts A.T. 1935. Arch. Dis. Child., x, 295.

Chapter VI.

The blood changes noted by other workers in cases of vitamin C subnutrition.

Most authorities are agreed that anaemia, if not a necessary feature, is at least a common occurrence in scurvy. While this thesis is not concerned with fully developed scurvy, it is concerned with minor degrees of vitamin C subnutrition, and we must examine and assess the changes found in the fully developed disease before we can expect to appreciate any minor deviations from normal which we may find in the lesser degrees of subnutrition. Let us now examine the various views held on the subject. We propose to give in detail the picture drawn by some of the workers, since only in this way can we understand the pathology of the condition and correlate the findings of the various workers.

Barlow (1883) stated that the anaemia in some of his cases of infantile scurvy was profound and that, besides the pallor, there was sometimes noted a peculiar sallow, muddy tint in the complexion.

In 1917 Leitner described the occurrence of scorbutic anaemia. Hess (1920) gave details of the features of the blood in scurvy. He considered the anaemia to be of the secondary type, the haemoglobin being reduced to a much lower level than the red cells, and he found the colour index to be as low on occasion as 0.5. Microscopically he noted poikilocytosis, anisocytosis and central pallor of the red cells, which were sometimes enlarged. Occasionally he noted the presence of exceptionally large red cells resembling the dropsical cells of chlorosis; and on occasion there were seen nucleated red cells, myeloblasts and megaloblasts.

Weill and Mouriquand (1922) described the occurrence of anaemia due to lack of vitamin C, before symptoms of frank scurvy appeared. This anaemia resisted iron but responded to lemon juice.

Liotta (1923) described scorbutic anaemia, and suggested that the anaemia may be due to increased blood destruction as well as to haemorrhage and malnutrition.

Meyer and McCormick (1928) showed that definite cytological changes occurred in the blood of guinea-pigs in experimental scurvy, and that these changes became evident after 10 days on a scurvy producing diet. The changes consisted in a decrease in the number of the red cells, the haemoglobin and the colour index; an apparent decrease in the blood fragility; a relative decrease in the number of lymphocytes; an absolute increase in the polymorphs and an increase in the number of reticulocytes, nucleated red cells and leucocytes. They concluded that the eosinophile, basophile, monocytic and transitional cell counts were not characteristic of scurvy. In the microphotographs which they show, red cell vacuolation, poikilocytosis and anisocytosis are marked, reticulocytes are conspicuous and nucleated red blood cells are seen.

Davidson (1928) reported the occurrence of anaemia in patients taking the diet given for the treatment of gastric ulcer.

Mettier, Minot and Townsend (1930) state that scurvy in adults is apt to develop following a prolonged period of nutritional instability, and thus manifest a chronic condition. They consider that chronicity favours the production of anaemia. In a large group of cases of adult scurvy, they would expect to find about 35% with a red cell count between 2 and 3 millions per c. mm., a similar percentage with between 3 and 4 millions and the remainder showing slight or no anaemia with red cell counts above 4 millions per c. mm.. They find that the haemoglobin is usually reduced, so that a colour index about 1 is the rule; and while achromia of the red cells is not a feature, slight central pallor is not infrequently seen. They consider that if haemorrhage has been an important factor in the production of the anaemia, a greater degree of achromia and a lower colour index are to be expected. The platelets are usually normal or slightly increased and the leucocytes in uncomplicated cases are usually between 4,000 and 6,000 per c. mm.. They find that a slight lymphocytosis frequently occurs in which are found about 3% atypical lymphoid cells. Examination of the stained film shows anisocytosis to be more pronounced than poikilocytosis and occasionally a sufficient number of non-achromic cells slightly larger than normal are encountered to suggest the possibility of pernicious anaemia. About 1% of the red cells are usually polychromatic, and nucleated erythrocytes may appear in the peripheral blood.

The same workers describe the bone marrow in one of their cases of vitamin C subnutrition. The tissue which they obtained by sternal puncture showed a moderate cellular hyperplasia and contained a few isolated fat cells. There were scattered, small, varying sized groups of nucleated red blood cells. Many myelocytes were present, among which were several eosinophiles. Adult polymorphs and megakaryocytes occurred in moderate numbers. There was no evidence of marrow fibrosis. They also describe the marrow obtained by sternal puncture during treatment with a vitamin C rich diet and at the peak of a reticulocyte response; this specimen showed more nucleated red blood cells and more mitotic figures among the precursors of those cells.

Mettier, Minot and Townsend therefore conclude that when vitamin C has been chronically deficient, its replenishment in the body in some way promotes the development of nucleated erythrocytes.

Rohmer and Bindschedler (1932) recorded the results of an examination of the blood in 15 cases of infantile scurvy. They found anaemia in only 7 instances and concluded that anaemia could not be regarded as an essential feature of scurvy.

Baar, quoted by the above workers, found anaemia in only one third of his cases.

Rohmer and Bindschedler (1932) investigated 22 anaemic infants. In 6 of these the anaemia was unaffected by iron but when vitamin C was given in addition a prompt cure resulted. They therefore suggest that vitamin C may have some effect on the metabolism of iron.

Mettier and Chew (1932) placed guinea-pigs on a scurvy-producing diet. After from 12 to 15 days on the deficient diet, the red cell count began to drop slowly; a few days before death the fall became much more rapid.

The total fall in each pig was between 1 and $2\frac{1}{2}$ millions. The fall in the haemoglobin content of the blood did not take place simultaneously with the red cell alteration. The time interval between the feeding of the diet and the haemoglobin decrease was usually 5 to 10 days longer than that necessary to produce a change in the total number of red blood cells. There was a drop in the haemoglobin concentration ranging from about 2.5 to approximately 7.1 gm. per 100 c.c. below the normal. Thus, in all animals showing signs of scurvy, there developed a distinct anaemia. After the fall in the red cell count and in the haemoglobin concentration , there was considerable alteration in the character of the red cells, viz. polychromatic and stippled erythrocytes appeared in the peripheral blood and there was slight poikilocytosis and anisocytosis. Beginning 3 or 4 days before the death of the animal from scurvy, there appeared moderately increasing numbers of reticulocytes in the peripheral circulation, and decreasing numbers of mature red cells. There was a terminal rise in the reticulocytes from about 0.2% to as high as 9%. It therefore appeared that with failure of the bone marrow to maintain a normal number of erythrocytes in the peripheral circulation, there was a release of cells of a younger stage of maturation, and that this replacement was never sufficiently great to prevent an advancing degree of anaemia in the scorbutic animal. Their conclusion was that a state of retarded erythrocyte maturation was induced when sources of vitamin C were witheld from the diet, and that, while undernutrition and blood loss may partly account for the anaemia of scurvy, it is not the whole reason. They considered that there were no definite grounds for stating that increased blood destruction occurs. They considered that delayed erythropoiesis was the fundamental lesion and the real cause of the anaemia of vitamin C subnutrition.

Witts (1932) believes that in vitamin C subnutrition a microcytic anaemia results.

Shipley (1933) stated that some degree of anaemia was present in all the cases of scurvy seen at Johns Hopkins Hospital. He reported one case with a red cell count of 2,480,000/c.mm. and a haemoglobin level of 29%.

Parsons (1933) reported the occurrence of anaemia in children which responded to vitamin C after treatment with other haematinics had failed. He stated, however, that all authorities did not regard anaemia as an essential feature of scurvy.

Still (1934) stated that the blood in scurvy shows no characteristic change in its cell count. A simple secondary anaemia, with diminution especially of haemoglobin has been found in some cases; but this is not always so.

Still has found the number of red cells rather above the normal in some pronounced cases of scurvy.

Bondurant (1934) found an orthochromic, normocytic anaemia in scurvy.

Dunlop and Scarborough (1935) described the blood picture in 2 cases of scorbutic anaemia. In their first case, the red cell count was greatly reduced, as was the haemoglobin level. There was a definite reticulocytosis in the untreated case. In their second case, neither the red cell count nor the haemoglobin level were greatly reduced and there was only a slight reticulocytosis. They give the following figures.

<u>1st. case.</u>

Red cell count ---------------- 2,040,000/c.mm.

Haemoglobin --------------------48%.

Reticulocytes ----------------- 14.6%

W.B.C.------------------------- 5,800/c.mm.

The blood film showed a marked anisocytosis, slight poikilocytosis, a number of microcytes and megalocytes, but no nucleated red cells. There was a slight relative lymphocytosis. No abnormality of either coagulation or bleeding times was noted.

<u>2nd. case.</u>

Red cell count ---------------- 4,320,000/c.mm.

Haemoglobin --------------- 70%.

Reticulocytes ------------- 3%.

W.B.C. -------------------- 6.200/c.mm.

No such definite alteration in the stained film as was encountered in case I was noted.

Frohlich (1935) mentions that anaemia may occur in the slighter cases of infantile scurvy.

Wood (1935) recorded a case of gross scurvy with gross anaemia, in a female aged 53 years. She had longstanding dietary restriction. In this case, who was grossly deficient in vitamin C, the figures were as follows

Red cell count --------------- 3,200,000/c.mm.

Haemoglobin(Haldane) --------- 58%.

Colour index ----------------- 0.9.

Blood sedimentation rate
(Westergren) 1st. hour ------- 54 mm.

He describes the anaemia as being normocytic and normochromic.

Rohmer and Bezssonoff (1935) state that in prescorbutic dystrophy there occurs an anaemia in some cases which reacts only to a combination of iron and vitamin C.

Parsons and Smallwood (1935) state that a macrocytic anaemia due to vitamin C subnutrition is more common in adults than in children. They attribute the anaemia of scurvy to deficient oxygenation of the marrow. Ascorbic acid, in their opinion, takes up oxygen and releases it to the tissues. In the case of the marrow this relative anoxaemia leads to delay in maturation of the red cells right through the range, from endothelium to mature cell, and to degeneration and aplasia of the marrow. They also suggest that the type of the anaemia depends on the degree of the deficiency, being normocytic and normochromic where the deficit is slight and only the later stages of maturation are affected, and being macrocytic when the deficit is more severe and the earlier stages of maturation impaired.

The same workers cite a case of scurvy in an infant in whom the red cell count was $5\frac{1}{2}$ millions/c.mm. and the haemoglobin 98%. In a series of 6 cases they found anaemia in only 4, and the anaemia reached a severe degree in only 1 case. In this 1 case the red cell count was 2,310,000/c.mm. the haemoglobin 35% and the colour index 0.76. The bleeding, clotting and clot retraction times were normal. There was no fall in the platelet count; the differential white cell count showed no constant changes. They found that a moderate degree of anisocytosis occurred but that the cells were of normal average diameter.

Parsons and Smallwood (1935) also state that proof of the existence of anaemia in latent scurvy had, up to the time of their writing, been lacking; it did seem feasible to them, however, that if frank scurvy was due to grossly defective vitamin C intake, then less severe defects should produce a state of affairs predisposing to the onset of acute manifestations. They state that it is quite conceivable that a child who has nutritional anaemia may develop scurvy and the anaemia of scurvy, and that therefore, for its complete cure, both iron and vitamin C would be required. They add, however, that those occasional cases of nutritional anaemia which, in their experience, have proved resistant to iron have never shown any haematological improvement as the result of intensive vitamin C therapy.

Barron and Barron (1936) produced a cobalt polycythaemia in rabbits -- they found a subsequent rise in both the red cell count and haemoglobin level. When ascorbic acid and cobalt sulphate were injected simultaneously no polycythaemia occurred. When ascorbic acid was injected after the production of the polycythaemia a decrease in the haemoglobin and red cell levels resulted; this decrease was, however, only temporary. They therefore concluded that ascorbic acid is probably one of the regulators of the red cell level in the circulating blood.

Nisenson and Cohen (1937) reported a case of scurvy with a marked megalocytic anaemia, a colour index of 1.16, and a reticulocyte count of 2.5%.

Cardelle (1937) reported a case of prescorbutic anaemia in a girl of 10 months who had not received any fruit juice. She had been breast fed for 1½ months and from then onwards she had cow's milk. Persistent anorexia, painful joints and stationary weight were the most characteristic symptoms. The haemoglobin was 45% and there was considerable diminution in the red cell count. The x-ray findings were those of early scurvy. Rapid improvement in all the conditions, including that of the blood, was brought about by antiscorbutic treatment.

Parsons (1938) states that the effect of vitamin C upon the maturation of the red cells parallels that of thyroxin, and that while an anaemia is characteristic of vitamin C deficiency, it is not universal. He is of the opinion that anaemia is more common in adults than in children who are undernourished. He found that between 70% and 80% of those undernourished adult patients who had their blood examined showed some degree of anaemia. He stated that he had no doubt that severe scurvy might occur without any anaemia developing in some instances.

Parsons also describes the various types of anaemia which he has seen due to vitamin C deficiency. He has seen anaemia both of the orthochromic normocytic type and of the macrocytic type, both forms being cured by the administration of ascorbic acid without any other alteration in the diet and without giving any drug; and he suggests that such results furnish conclusive evidence that vitamin C is essential for the normal maturation of the red cell. In a series of 14 children suffering from scurvy he has seen 3 cases of orthochromic normocytic anaemia, 2 cases of normocytic slightly hypochromic anaemia, and 2 cases of macrocytic anaemia, all of which were cured by administration of ascorbic acid. He also states that the character of the anaemias in adults are similar to those in children and points out that in those cases where haemorrhages are extensive a post-haemorrhagic blood picture may be superimposed and the anaemia may then become hypochromic and, in extreme cases truly microcytic. Parsons is of the opinion that the action of vitamin C on the cells of the bone marrow extends throughout the whole range of the maturation of the red cell viz. from the reticulo-endothelial cell to the adult erythrocyte, and is not restricted to a small portion of its maturation. He believes that the anaemia of scurvy results from a slowing down of the whole process of erythropoiesis and that this slowing may become so great as to produce degeneration and aplasia of the red bone marrow. As a result of this, an anaemia, usually orthochromic and normocytic is produced; on occasion there is disproportionate slowing of red cell development at the stage of maturation of the megaloblast to the normoblast and a macrocytic anaemia then occurs.

Jennings and Glazebrook (1938) described the blood picture in 2 cases of adult scurvy. In the first case, which showed a severe degree of anaemia, the findings were such that an Addisonian anaemia was at first suspected. The red cell count dropped to the low level of 1,900,000/c.mm, the haemoglobin was 40% and the colour index 1.05. The white cell count was reduced to 3,000/c.mm.. A most striking feature was the constant reticulocytosis of from 3% to 4%. Examination of the stained blood film demonstrated a definite megalocytosis, anisocytosis and polychromasia. They found the bone marrow to be hyperplastic and noted a shift back towards the megalocytic type; in this case the gums were healthy and the patient edentulous.

In the second case which they describe, the degree of anaemia was not so severe. The red cell count was 3,420,000 per c.mm., the haemoglobin 67%, the colour index 0.98, the white cell count 6,000/c.mm. and the reticulocytes only 0.8%. Again the marrow was found to be hyperplastic and showed a shift back. The anaemia was of the orthochromic and normocytic type. In this case the gums were spongy and the teeth carious.

Croft and Snorf (1939) found no correlation between anaemia and a low blood ascorbic acid concentration. Six patients with low blood ascorbic acid levels and anaemia were given 75 -- 100 mgm. ascorbic acid daily by mouth but no reticulocytosis resulted. They suggest that the dosage may have been insufficient to give such a response. Taking the normal plasma ascorbic acid level as being between 0.6 and 1.12 mgm. per cent, and levels under 0.2 mgm per cent as indicating serious deficiency, they found very low blood ascorbic acid levels in several cases which had no clinical manifestations.

Scott Thompson, Glazebrook and Millar (1942) investigated the relationship between vitamin C and anaemia. They investigated adolescents admitted to an institution from working class homes where the dietary was probably less than optimum. The institution diet contained only negligable amounts of vitamin C and it was found that large amounts of the vitamin were required to produce saturation. A number of their cases were given 50 -- 200 mgm. ascorbic acid daily for over 2 months, the others receiving no such addition; at the end of this time titrations were made on the haemoglobin levels of both sets of boys. The technique which they used for these titrations was scrupulously accurate -- they tried to standardise the puncture, used specially accurate methods of measuring the blood and the hydrochloric acid, and 4 hours after adding the blood to the acid they made the titration by a photoelectric method. They found that the average haemoglobin reading in the control group of 133 boys on the institution diet was 89.5% and that the average of the 60 boys who had had large amounts of ascorbic acid for 2 months was 90.4%. These workers had undertaken this investigation to discover whether mild degrees of vitamin C deficiency were associated with mild degrees of anaemia, and from their findings they concluded that there probably was no such association.

Possible causes for the occurrence of anaemia in vitamin C subnutrition have already been mentioned. Mettier Minot and Townsend (1930) suggest the following 3 etiological factors.

(1). Undernutrition and intercurrent infection. These are considered to be merely contributory factors.

(2). Loss of blood, especially from the intestinal tract, which need not be of severe degree.

(3). Insufficient red cell production. The observations of these workers suggest to them that the inadequate function of the bone marrow may be directly dependent on a chronic lack of vitamin C, although in any given case some other factor or factors may intensify the process.

Mettier, Minot and Townsend also suggest that in the anaemia of scurvy and in the anaemias responding to iron therapy, the effective substance hastens the maturation of the normoblasts, which permits a reticulocyte response. This response, as will be described later in the paper, tends to occur more rapidly than in pernicious anaemia; the increased speed could be accounted for by the fact that the normoblast is a less primitive cell than the megaloblast, so that the former could mature sooner than the latter.

Barlow (1883) stated that the anatomical basis of the limb affections is the sub-periosteal haemorrhage and that this haemorrhage probably accounts for some of the anaemia.

Parsons and Smallwood (1935) consider that the anaemia of scurvy results from a slowing down of the whole process of erythropoiesis, which may be so marked as to result in marrow degeneration and aplasia, the resulting anaemia being therefore usually orthochromic and normocytic; in chronic cases associated with large haemorrhages into the tissues and from the mucous membranes a post-haemorrhagic blood picture becomes superimposed and the anaemia may then become hypochromic; and it appears to these workers that in extreme cases it might even become truly microcytic. On this hypothesis, they would expect a megalocytic anaemia to occur occasionally, the result of a disproportionate slowing up of red cell development at the stage of maturation of megaloblast to normoblast. They do not entirely agree with Witts' theory that the stage of arrest is always at the level of maturation of normoblast to erythrocyte; they believe that the anaemia of vitamin C deficiency is not a microcytic anaemia in the sense that an iron deficiency anaemia is, and they see no reason why an arrest at the normoblastic level should lead to a microcytosis.

The bone marrow in the scorbutic state is described by several workers.

Harris (1927) gives details of his post-mortem findings in a case of scurvy. He describes the manifestations of scurvy on the bone marrow as consisting of areas of haemorrhage, areas of excessive development of fibrous tissue and areas of gelatinous marrow devoid of blood-forming cells. He concludes that the anaemia in scurvy is not only due to the succession of haemorrhages, but is due to the formation of gelatinous marrow with failure of differentiation of the marrow into erythroblastic and leucoblastic areas. In his specimen, some healing of the scorbutic process was present at the bone ends in contiguity with the epiphyseal line, and in this situation a few normal marrow cells were seen which he regarded as a clear indication that the process of healing was not dependent on extension of normal tissue but on a special differentiation in situ as a result of blood borne substances.

Mettier and Chew (1932) on studying the bone marrows of guinea-pigs which had died of scurvy, found the development of the cells at a standstill. The medullary cavities were filled with a greyish-red mass, soft in consistence. Under the microscope, the cellularity was greatly increased over the normal. The fatty marrow tissue was almost entirely replaced by cells of the erythropoietic series . There were great numbers of nucleated red blood cells, mainly of the normoblastic variety. There was little evidence of active cellular maturation.

In the bone marrows of guinea-pigs which had been killed during a reticulocyte crisis there was found a striking difference from the findings in the untreated case. Adult erythrocytes appeared in the marrow in much larger numbers. There were an increased number of cells showing mitotic figures. These findings suggest a specific effect of vitamin C on the bone marrow.

Parsons and Smallwood (1935) consider that the conclusions drawn by Mettier, Minot and Townsend from 2 samples of bone marrow obtained by sternal puncture are unjustified, They state that it is a well known fact that hyperplasia is frequently followed by degeneration and aplasia, especially in the bone marrow, and they consider that Mettier, Minot and Townsends' findings may quite possibly represent a stage prior to gelatinous degeneration. They consider that the picture of increased numbers of normoblasts actively dividing is an indication of greater production, rather than maturation of these marrow elements. They suggest that the earthy tint of the skin may be an indication of injury to the bone marrow.

Naegeli (1923), MacCallum (1928), Shipley (1933), and Holt and McIntosh (1933) all describe a disappearance of blood-forming tissues from the bone-marrow and their replacement by fibrous tissue.

Witts (1932) suggested that, in the absence of vitamin C, the marrow becomes hyperplastic and normoblastic, and a microcytic anaemia results.

Jennings and Glazebrook (1938) found the marrow in one case to be hyperplastic and to show a shift back towards the megalocytic type.

From a review of the literature on the subject the following general conclusions may be drawn.

(1). That while anaemia is a common feature in scurvy, it is by no means a constant one.

(2). That anaemia is also a common feature in subscorbutic states, and that it is of similar type and incidence as the fully fledged scorbutic anaemia.

(3). That the anaemia is of more frequent occurrence in adults than in children.

(4). That the anaemia of vitamin C subnutrition is usually orthochromic and normocytic, both the red cell count and haemoglobin level falling, but that it may become megalocytic if the subnutrition is severe, or micricytic if severe haemorrhages occur. The anaemia may be of mild or very severe degree.

(5). That a reticulocytosis, which varies directly as the severity of the untreated case, is a feature.

(6). That there are no significant changes in bleeding time, coagulation time, clot retraction time, white cell count or platelet count.

(7). That in the stained film anisocytosis is commonly seen, poikilocytosis may occur and red cell vacuolation may be a feature. Nucleated red cells may appear in the peripheral circulation.

(8). That the cause of the anaemia is probably a variable degree of bone-marrow damage, leading to insufficient production of red cells, and that subnutrition and haemorrhage may play some part.

(9). That the marrow changes are, essentially, a gradual disappearance of blood forming tissue and its replacement by fibrous tissue.

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References.

Leitner P., Wien. Klin. Woch., 1917, xxxi, 978.

Hess A.F., Scurvy Past and Present, Philadelphia and London, 1920, p. 209.

Weill L., and Mouriquand C., Congres franc. de Med., 1922.

Liotta D., Arch. di Farmacol. Sperimentale, 1923, pp 36, 76.

Meyer A.W., and McCormick L.M., Stanford Univ. Pub., Univ. Series, Med. Sc., 1928,ii,No.2, p. 199.

Davidson P.B., Journ. Amer. Med. Assoc., 1928, 40, 1014.

Mettier S.R., Minot G.R. and Townsend W.C., Journ. Amer. Med. Assoc., 1930, 95, 1089.

Mettier S.R. and Chew W.B., 1932, Journ. Exper. Med., lv,971.

Shipley P.G., Dis. Inf. and Child., Parsons and Barling, London, 1933, 325.

Parsons L.G., 1933, Br. Med. Journ., ii., 631.

Bondurant W.W., 1934, Texas State Journ. Med., 29., 565.

Dunlop D.M. and Scarborough H., 1935, Ed. Med. J., 42.,476.

Wood P., 1935, Lancet, ii., 1405.

Parsons L.G. and Smallwood W.C., 1935, Arch. Dis. Child., 10, 327.

Barron A.G. and Barron E.S.G., 1936, Pr oc. Soc. Exp. Biol., N.Y., 35, 407.

Nisenson A. and Cohen A.G., 1937, Amer. J. Med. Sci., 194., 63

Cardelle G., 1937, A mer. J. Dis. Child., 52.,1452.

Parsons L.G., 1938, Lancet, i.,65.

Jennings G.H., and Glazebrook A.J., 1938, Br. Med. J., 784.

Croft and Snorf, 1939, Amer. J. Med. Sci., 198., 403.

Scott Thompson, Glazebrook A.J. and Millar, 1942, Journ. of Hygiene, 42., 2.

Witts L.J., 1932, Lancet, i., 495.

Barlow T., reprint Arch. Dis. Child., 1935, x., 229.

Rohmer P. and Bindschedler J.S., 1932, Acta. Paed., Uppsala, XIII, 399.

Still G.F., 1934, Dis. of Children, London, 162.

Frolich T., 1935, Arch. Dis. Child., X., 309.

Rohmer P. and Bezssonoff N., 1935., Arch. Dis. Child., X,319.

Harris H.A., 1927, Quart. J. Med., Oxford, XXI, 499.

MacCallum W.G., 1928, Textbook of Pathology, Philadelphia, 927

Naegeli O., 1923, Blutkrankheit und Blutdiagnostik,Berlin,364.

Holt L.E. and McIntosh R., 1933, Dis. Inf. and Child., New York, 10th. edn., 223.

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Chapter VII.

The alterations noted in the blood picture in the present series of cases.

The second major issue was "Is vitamin C subnutrition, short of frank scurvy, accompanied by a constant and typical alteration in the blood picture" ?. In an attempt to answer this question, the vitamin C nutritional state and the blood picture in each of 87 cases were correlated. To this end, a system of "Grouping" was adopted; this has already been discussed in detail in Chapter III, but we may here re-iterate the main features.

Group I cases were those showing a positive vitamin C balance and no anaemia.

Group II cases were those showing a negative vitamin C balance and no anaemia.

Group III cases were those showing a negative vitamin C balance and anaemia.

Group IV cases were those showing a positive vitamin C balance and anaemia.

We have already noted that any given case will fall into one or other of these Groups. In the following 6 pages these 87 cases are set out in their appropriate groups with details of each case.

Group I.

Features of group (a) excretion rate satisfactory and
(b) no anaemia.

Case No.	Age.	Sex.	M.S.W.	Daily vit. C excretion.	Test dose.	Red cell count.	Ret'cyte count.	H.globin. % :grams.	C.I.	B.S.R. 1 : 2.	Examination of stained blood film.
1.	45	M.	M.	21 mgm.	Pos.	5,500,000	2%	92%:14.9	0.9	4 : 8	No gross abnormality seen.
2.	40	M.	M.	17 mgm.	Pos.	4,850,000	1%	95%:15.2	1.0	9 : 20	No gross abnormality seen.
3.	43	M.	M.	18.7 mgm.	Pos.	4,970,000	1%	90%:14.3	0.9	4 : 9	No gross abnormality seen.
4.	29	M.	S.	24 mgm.	Pos.	4,650,000	1%	90%:14.3	1.0	10 : 18	No gross abnormality seen.
5.	33	M.	M.	26 mgm.	Pos.	4,940,000	1%	90%:14.3	0.9	2.5 : 6	No gross abnormality seen.
6.	52	M.	M.	25 mgm.	Pos.	4,520,000	1%	93%:15.0	1.0	8 : 18	No gross abnormality seen.
7.	24	F.	S.	16.5 mgm.	Pos.	4,210,000	0.5%	85%:13.7	1.0	6 : 12	No gross abnormality seen.
8.	41	M.	M.	16 mgm.	Pos.	4,600,000	0.5%	101%:16.2	1.1	3 : 6	No gross abnormality seen.
9.	32	F.	S.	15.5 mgm.	Pos.	4,150,000	1%	88%:14.2	1.1	4 : 10	No gross abnormality seen.
10.	64	M.	M.	20 mgm.	Pos.	4,665,000	0.4%	92%:14.9	1.0	2 : 4	No gross abnormality seen.
11.	55	M.	M.	19 mgm.	Pos.	5,200,000	1%	94%:15	0.9	6 : 10	No gross abnormality seen.
12.	60	M.	M.	16 mgm.	Pos.	4,610,000	0	94%:15	1.0	5 : 10	Anisocytosis +.
13.	33	M.	M.	14.5 mgm.	Pos.	4,900,000	0.2%	96%:15.4	1.0	5 : 9	No gross abnormality seen.
14.	64	M.	M.	19 mgm.	Pos.	4,800,000	0.2%	88%:14.2	0.9	8 : 12	Anisocytosis +.
15.	61	M.	M.	13.3 mgm.	Pos.	4,700,000	0.1%	96%:15.4	1.0	5 : 8	Anisocytosis + : Poikilocytosis +.
16.	37	M.	M.	20 mgm.	Pos.	4,840,000	0.2%	92%:14.9	0.95	3 : 6	No gross abnormality seen.
17.	47	M.	M.	24.5 mgm.	Pos.	4,600,000	1%	100%:16	1.1	3 : 6	No gross abnormality seen.

Blood sedimentation rate in mms. at the end of one and two hours.

GROUP II.

Features of group (a) excretion rate unsatisfactory and
(b) no anaemia.

Case No.	Age.	Sex.	M.S.W.	Daily vit. C excretion.	Test dose.	Red cell count.	Ret'cyte count.	H,globin. % :grams.	C.I.	B.S.R. 1 : 2	Examination of stained blood film.
1.	20	F.	M.	11 mgm.	-ve	4,580,000	0.5%	85%:13.7	0.9	7 : 16	No gross abnormality.
2.	37	F.	M.	12 mgm.	-ve	4,570,000	1%	82%:13.1	0.9	7 : 13	Central pallor.
3.	53	F.	W.	7.5 mgm.	-ve	4,500,000	1%	81%:13	0.9	12 : 20	Anisocytosis + +.
4.	55	F.	M.	9,2 mgm.	-ve	4,000,000	1%	83%:13.3	1.0	7 : 13	No gross abnormality.
5.	48	F.	M.	13.2 mgm.	-ve	4,025,000	1%	77%:12.3	0.95	8 : 17	Anisocytosis + + : Poikilocytosis +.
6.	21	F.	S.	12 mgm.	-ve	4,735,000	1.5%	76%:12.2	0.8	6.5 : 12	Central pallor.
7.	32	F.	S.	13 mgm.	-ve	4,300,000	1%	82%:13.1	1.0	9 : 16	No gross abnormality.
8.	19	F.	S.	14 mgm.	-ve	4,400,000	0.2%	78%:12.4	0.9	6 : 12	No gross abnormality.
9.	29	F.	M.	12.5 mgm.	-ve	4,370,000	1%	78%:12.4	0.9	5 : 12	Anisocytosis + +.

GROUP III.

Features of group (a) excretion rate unsatisfactory and
(b) anaemia.

Case No.	Age.	Sex.	M.S.W.	Daily vit. C excretion.	Test dose.	Red cell count.	Ret'cyte count.	H.globin. %: grams:	C.I.	B.S.R. 1 : 2.	Examination of stained blood film.
1.	25	F.	M.	11.5 mgm.	-ve	5,150,000	1%	74%:11.9	0.7	9 : 18	Anisocytosis + : Central pallor.
2.	45	F.	M.	12 mgm.	-ve	3,260,000	1%	56%:9	0.9	14 : 24	Anisocytosis +.
3.	30	F.	W.	6.5 mgm.	-ve	3,600,000	0.5%	47%:7.5	0.7	11 : 20	Anisocytosis + + + : Poikilocytosis + + + Central pallor : Few megalocytes.
4.	27	F.	M.	13 mgm.	-ve	3,810,000	1%	72%:11.5	1.0	7 : 15	No gross abnormality.
5.	41	F.	M.	12 mgm.	-ve	3,710,000	2%	31%:5	0.4	8 : 18	Anisocytosis + : Ring staining.
6.	50	F.	S.	12 mgm.	-ve	4,000,000	2%	75%:12	1.1	10 : 20	Anisocytosis + + + : Poikilocytosis +. Few megalocytes.
7.	35	F.	M.	12 mgm.	-ve	3,775,000	1%	85%:13.7	1.1	9 : 18	No gross abnormality.
8.	40	F.	S.	13 mgm.	-ve	3,800,000	1%	70%:11.1	0.9	4 : 8	No gross abnormality.
9.	28	F.	M.	8 mgm.	-ve	3,665,000	1%	70%:11.1	1.0	5 : 9	Few megalocytes, poikilocytosis ++, Anisocytosis +++.
10.	15	F.	S.	14 mgm.	-ve	4,500,000	1%	50%:7.9	0.55	40:70	Anisocytosis +, ring staining.
11.	37	F.	M.	10 mgm.	-ve	3,900,000	1%	40%:6.4	0.5	14:30	Anisocytosis +, poikilocytosis +, ring staining.
12.	19	F.	S.	12 mgm.	-ve	3,560,000	0.5%	74%:11.9	1.0	5 :12	No gross abnormality.
13.	47	F.	W.	14 mgm.	-ve	4,000,000	0.5%	74%:11.9	1.0	9 :19	No gross abnormality.
14.	26	F.	M.	13.5 mgm.	-ve	4,500,000	1%	74%:11.9	0.8	9 :17	No gross abnormality.
15.	32	F.	M.	14 mgm.	-ve	4,215,000	1%	68%:10.9	0.8	10:19	Anisocytosis +, poikilocytosis +, few megalocytes.
16.	20	F.	S.	9 mgm.	-ve	4,120,000	1%	74%:11.9	0.9	3 : 7	No gross abnormality.
17.	25	F.	M.	14 mgm.	-ve	3,125,000	1%	62%;10	1	2 : 5	Anisocytosis +, poikilocytosis +.
18.	39	F.	M.	3 mgm.	-ve	3,150,000	0.5%	70%:11.1	1	10:25	Anisocytosis +, poikilocytosis +.
19.	31	F.	M.	10 mgm.	-ve	3,875,000	0.5%	65%:10.5	0.8	3 : 7	Anisocytosis +, few megalocytes.

GROUP III continued.

Features of group (a) excretion rate unsatisfactory and
(b) anaemia.

Case No.	Age.	Sex.	M.S.W.	Daily vit. C excretion.	Test dose.	Red cell count.	Ret'cyte count.	H.globin. % :grams	C.I.	B.S.R. 1 : 2.	Examination of stained blood film.
20.	60	M.	M.	6 mgm.	-ve	4,700,000	1%	85%:13.7	0.9	2 : 5	No gross abnormality.
21.	35	F.	M.	11.5 mgm.	-ve	4,410,000	1%	70%:11.1	0.9	8 : 20	Central pallor : Anisocytosis + Poikilocytosis +.
22.	40	F.	M.	12.5 mgm.	-ve	4,280,000	0.5%	74%:11.8	0.85	5 : 15	No gross abnormality.
23.	34	F.	M.	4.1 mgm.	-ve	4,430,000	0.5%	67%:10.7	0.8	6 : 15	No gross abnormality.
24.	40	F.	M.	9 mgm.	-ve	3,600,000	0.5%	51%:8.1	0.9	6 : 14	Anisocytosis + +.
25.	34	M.	M.	15.5 mgm.	-ve	4,550,000	0.2%	77%:12.3	0.85	6 : 11	Anisocytosis + : Poikilocytosis + Central Pallor : Few megalocytes.

GROUP IV.

Features of Group-------- (a) Excretion rate satisfactory and
(b) Anaemia.

Case No.	Age	Sex	M.S.W.	Daily vit C excretion.	Test dose.	Red cell count.	Ret'cyte count.	H.globin. % :grams.	C.I.	B.S.R. 1 : 2.	Examination of stained blood film.
1.	50	M.	M.	20 mgm.	Pos.	5,110,000.	1%.	80 :12.8.	0.8.	5 : 9	No gross abnormality.
2.	28	F.	M.	17.5 mgm.	Pos.	4,400,000	1%.	76 :12.2	0.9.	52:86.	Central pallor; anisocytosis + ; poikilocytosis +.
3.	46	F.	M.	20 mgm.	Pos.	4,790,000.	0.5%.	87 :14.	0.9.	4: 8.	No gross abnormality.
4.	28	F.	S.	16.5 mgm.	Pos.	3,540,000.	1%.	70 :11.2.	1.	6:14.	No gross abnormality.
5.	36	M.	M.	16.8 mgm.	Pos.	4,260,000.	1%.	78 :12.5.	0.9.	7:16.	No gross abnormality.
6.	43	M.	M.	21 mgm.	Pos.	5,270,000.	0.5%.	85 :13.6.	0.8.	6:12.	Anisocytosis +.
7.	49	M.	M.	18.3 mgm.	Pos.	5,000,000.	1%.	77 :12.3.	0.8.	23:43.	No gross abnormality.
8.	61	M.	M.	19 mgm.	Pos.	5,140,000.	0.5%.	82 :13.	0.8.	11:20.	Central pallor.
9.	50	F.	M.	20.5 mgm.	Pos.	3,910,000.	0.6%.	75 :12.1.	1.	8:19.	Few megalocytes, Central pallor, anisocytosis ++,
10.	60	F.	M.	21.5 mgm.	Pos.	3,900,000.	1%.	84 :13.5.	1.	8:19.	No gross abnormality.
11.	46	F.	M.	22 mgm.	Pos.	4,225,000.	1%.	58 :9.4.	0.7.	7:15.	Central pallor, anisocytosis ++.
12.	39	F.	M.	20 mgm.	Pos.	3,910,000	1%.	67 :10.6.	0.85	5:15.	No gross abnormality.
13.	39	F.	M.	18 mgm.	Pos.	3,390,000.	0.5%.	48 :7.6.	0.7.	10:23.	Central pallor, anisocytosis +.
14.	38	F.	M.	15.5 mgm.	Pos.	4,260,000	0.6%.	73 :11.9.	0.8.	4: 8.	Central pallor.
15.	33	M.	M.	20 mgm.	Pos.	3,900,000	0.5%.	81 :13.2.	1.	6:12.	No gross abnormality.
16.	20	M.	S.	25 mgm.	Pos.	5,520,000.	0.2%.	77:12.5.	0.7.	3: 6.	Poikilocytosis +, Central pallor, anisocytosis ++.
17.	50	M.	M.	15.5 mgm.	Pos.	4,320,000.	0.4%.	78:12.6.	0.9.	7:14.	Anisocytosis +, poikilocytosis +.
18.	70	F.	S.	18 mgm.	Pos.	3,420,000.	0.5%.	70:11.	1.	18:50.	Anisocytosis +.
19.	48	F.	M.	17 mgm.	Pos.	4,220,000.	0.5%.	75:11.9.	0.9.	8:15.	No gross abnormality.
20.	27	F.	M.	20 mgm.	Pos.	3,510,000.	0.4%.	65:10.5.	0.9.	7:17.	Anisocytosis +.

GROUP IV. --- continued.

Features of Group ----------- (a) Excretion rate satisfactory and
(b) Anaemia.

Case No.	Age	Sex	M.S.W.	Daily vit.C excretion.	Test dose.	Red cell count.	Retic. count.	H.globin. %:grams.	C.I.	B.S.R. 1 : 2.	Examination of stained blood film.
21.	50.	F.	M.	20 mgm.	Pos.	3,630,000.	0.6%.	70:11.2.	1.	15:39.	No gross abnormality.
22.	16.	M.	S.	22 mgm.	Pos.	3,900,000.	1%.	68:10.9.	0.8.	10:18.	Central pallor, anisocytosis +, poikilocytosis +.
23.	50.	M.	M.	19.6 mgm.	Pos.	4,220,000.	1%.	84:13.4.	1.	6:12.	No gross abnormality.
24.	55.	F.	M.	21 mgm.	Pos.	3,900,000.	0.5%.	73:11.6.	0.9.	7:16.	Anisocytosis +, poikilocytosis +.
25.	38.	F.	M.	17 mgm.	Pos.	4,600,000.	1.5%.	73:11.6.	0.8.	9:20.	No gross abnormality.
26.	57.	F.	M.	22 mgm.	Pos.	4,720,000.	1%.	74:11.9.	0.8.	9:20.	Anisocytosis ++, poikilocytosis ++.
27.	26.	F.	M.	15.7 mgm.	Pos.	3,420,000.	1%.	68:11.	1.	6:20.	No gross abnormality.
28.	35.	F.	M.	17 mgm.	Pos.	4,200,000.	0.6%.	67:10.8.	0.8.	9:18.	Central pallor, anisocytosis +, poikilocytosis +.
29.	51.	M.	M.	17 mgm.	Pos.	4,660,000.	1%.	84:13.5.	0.9.	3:6.	No gross abnormality.
30.	42.	M.	M.	17 mgm.	Pos.	4,265,000.	1%.	79:12.6.	0.9.	10:20.	No gross abnormality.
31.	18.	F.	S.	16.5 mgm.	Pos.	3,410,000	0.5%.	74:11.9.	1.	8 :18.	Anisocytosis +.
32.	25.	F.	M.	20 mgm.	Pos.	3,645,000.	1%.	79:12.6.	1.	11:25.	Anisocytosis +, poikilocytosis +.
33.	20.	F.	S.	16 mgm.	Pos.	3,370,000.	1%.	74:11.9.	1.1.	7:18.	Anisocytosis +.
34.	30.	F.	M.	23.mgm.	Pos.	3,800,000.	0.5%.	80:12.8.	1.1.	10:20.	Anisocytosis +.
35.	54.	F.	M.	17 mgm.	Pos.	3,700,000.	0.2%.	80:12.8.	1.1.	8:16.	No gross abnormality.
36.	51.	M.	M.	14.5 mgm.	Pos.	3,860,000.	1%.	80:12.8.	1.	3:10.	Anisocytosis +, poikilocytosis ++.

The group distribution of the cases is as follows.

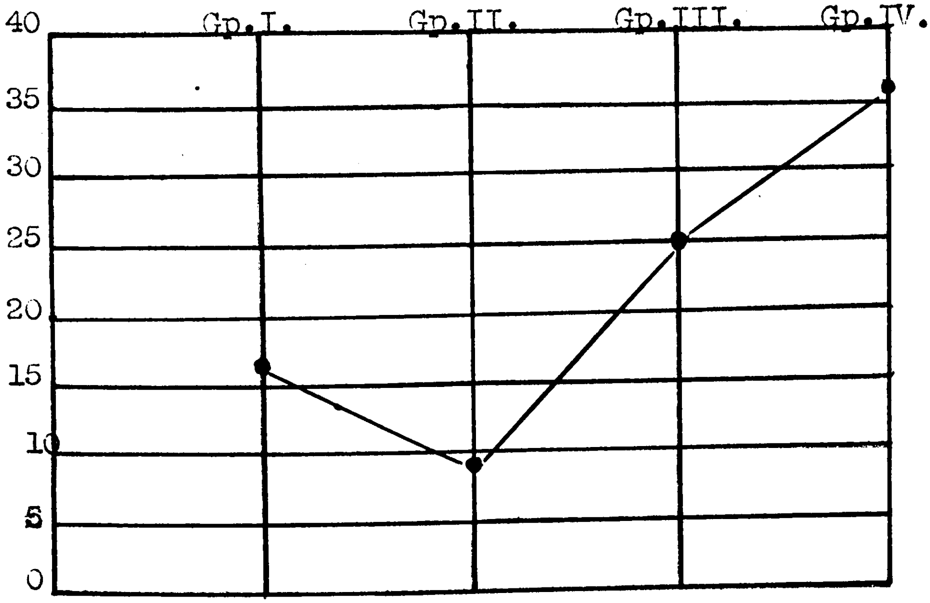
<u>Group I.</u>---------------- 17 cases.

<u>Group II.</u> -------------- 9 cases.

<u>Group III.</u>-------------- 25 cases.

<u>Group IV.</u>--------------- 36 cases.

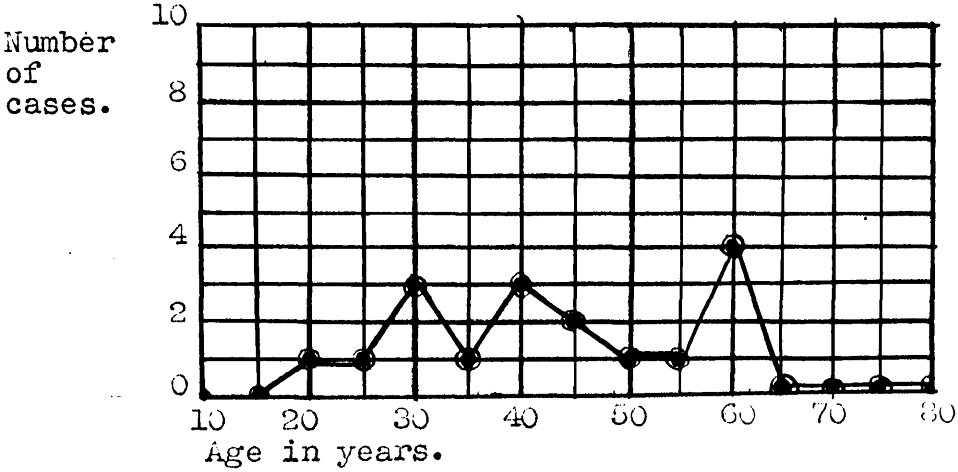
This distribution may be graphically represented thus.



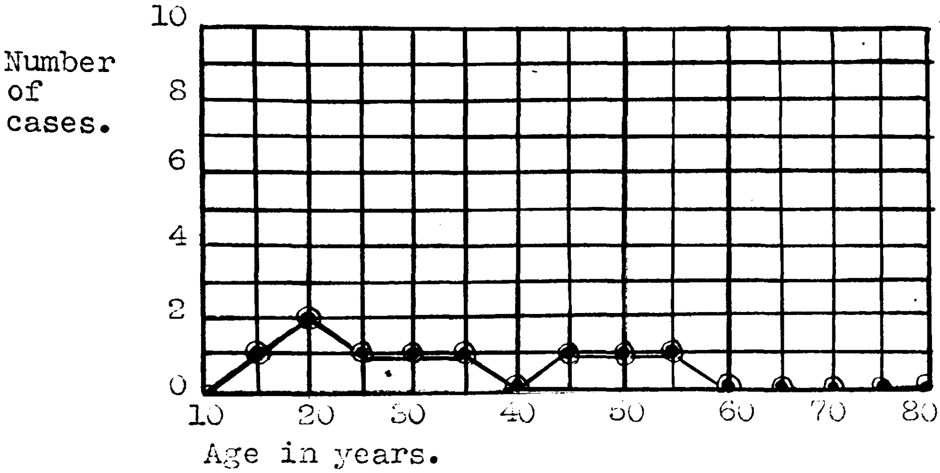
It is noteworthy that of the 87 cases examined, 61 had some degree of anaemia, according to our accepted standards; and that, of these 61 anaemic subjects, 25 or 41% were suffering from vitamin C subnutrition (Group III) and 36 or 59% were well nourished (Group IV). Of the remaining 26 cases whose blood picture was within normal limits 9 or 35% were ill-nourished (Group II) and 17 or 55% were well-nourished(Group I).

Below are set out 5 graphs, the first four showing the age incidence of the cases in the corresponding Group, the fifth graph showing the age incidence of the whole series of cases.

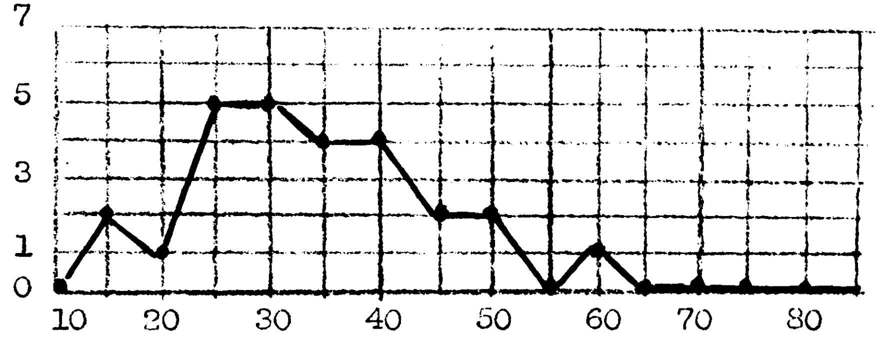
<u>Graph I.</u>-- showing the age incidence of the 17 cases in Group I.



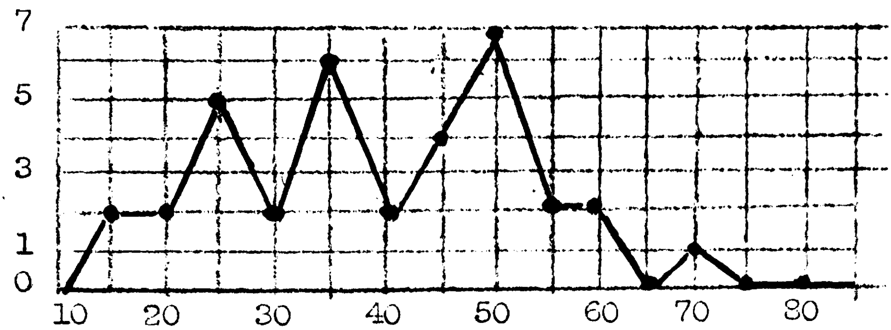
<u>Graph II.</u>-- showing the age incidence of the 9 cases in Group II.



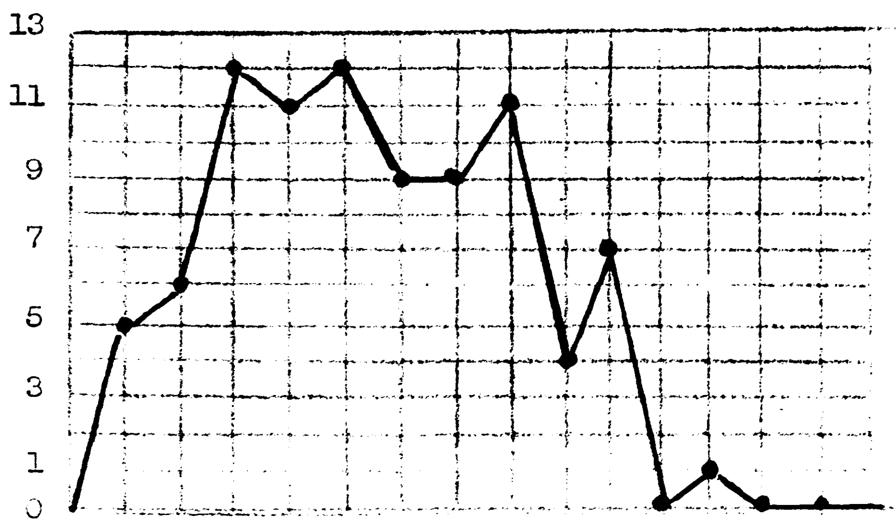
Graph III.--- showing the age incidence of the 26 cases in Group III.



Graph IV.--- showing the age incidence of the 35 cases in Group IV.



Graph V.--- showing the age incidence of the whole series of 87 cases.



Graphs I, III and IV have a definite resemblance to the composite graph V, but graph II differs in that it does not ascend in the centre. Only 3 of the 9 patients in Group II are over the age of 35 years; the other 6 are definitely young people. These young patients may find it easier to exist on a lowered vitamin C intake and still maintain a blood picture within normal limits, while the older patients tend to drop into the anaemic Group III.

Remarks. Apart from this slight difference in Group II, the age incidence of the various groups parallels the age incidence of the whole series.

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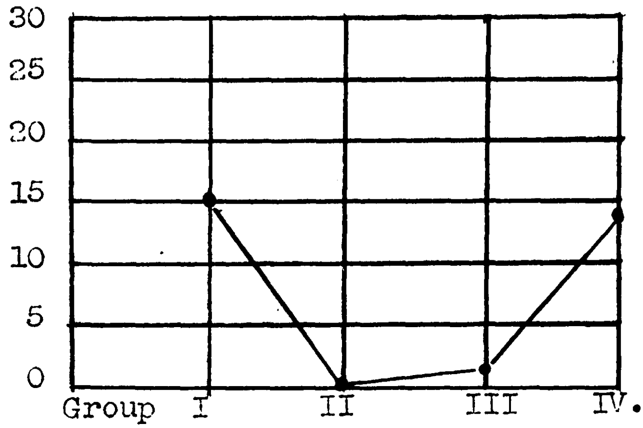
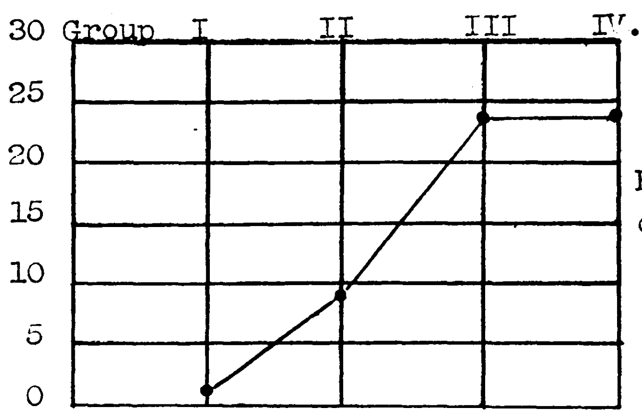
Let us now resolve the Group distribution of the cases into its male and female components.

Group.	Males. Total 30 cases	Females. Total 57 cases.
I. 17 cases.	15.	2.
II. 9 cases.	0.	9.
III. 25 cases	2.	23.
IV. 36 cases	13.	23.

This may be graphically represented.



Male distribution.



Female distribution.

We now note that there is a marked difference between the male and female "group" distribution; for, while the males are well represented in Group I and are practically absent from Groups II and III, the females are practically absent from Group I and are well represented in Groups II and III. There is no marked difference in the representation in Group IV.

We note that, of 15 anaemic males, only 2 suffered from subnutrition; of 46 anaemic females, 23 suffered from subnutrition.

Let us now examine the features of each Group closely, dealing separately with the sexes. In each Group the average red cell count, haemoglobin level, colour index and reticulocyte count was calculated for the male and female components separately. Thus, in Group I there were 15 males and 2 females. Let us deal first with the males.

Total red cell count of the 15 males --------- 72,345,000/15cmm

Average red cell count is therefore $\frac{72,345,000}{15}$ i.e.4,856,000

Total Hb. percentage of the 15 males ----- 1,403.

Average Hb. percentage is therefore $\frac{1,403}{15}$ i.e. 93.5%

and the average colour index is calculated in the usual way from the average red cell count and the average haemoglobin level.

Below are shown these average Group readings in both sexes.

A. Males ---- 30 cases.

Group Number.	Average R.B.C.	Average Hb. - %.	Average C.I.	Average Retic. count.
I. - 15 cases.	4,856,000	93.5%	0.96.	0.7%
II.- 0 cases.	----------	-----	----	----
III. 2 cases.	4,625,000.	81%.	0.875.	0.6%.
IV.- 13 cases.	4,571,000,	79.5%.	0.87.	0.8%

We note that, of the 30 males, 28 had a sufficiency of vitamin C (viz. Groups I and IV). This left only 2 undernourished cases, both with some degree of anaemia. The number of ill-nourished cases was therefore too small to allow of comparison and conclusion between the average blood findings in the well- and the ill-nourished male groups.

In the case of the females there is a better distribution of cases for the purposes of contrast and of comparison. Group I is comparable only with Group II i.e. a group whose members have a sufficiency of vitamin C and a blood picture within normal limits is being compared with a group whose members are short of vitamin C but whose blood pictures fall within normal limits. Similarly Group IV. (well-nourished but anaemic) is comparable with Group III (undernourished and anaemic). Let us now consider the several features of the blood picture in the females.

B. Females. ------ 57 cases.

Group Number.	Average R.B.C.	Average Hb.- %.	Average C.I.	Average retic. count.
I.---2 cases.	4,180,000.	86.5%.	1.03.	1.05%.
II.- 9 cases.	4,386,000.	80.2%.	0.91.	0.9%.
III.23 cases.	3,932,000.	65%.	0.83.	0.9%.
IV.-23 cases.	3,907,000.	72.2%.	0.93.	0.7%.

A. The red cell count. There is no significant difference between the average counts of Group I and Group II cases. The red cell count is slightly higher in the undernourished Group, but the number of cases in Group I is insufficient from which to draw conclusions. It has already been mentioned that Still (1934) found cases with advanced scurvy and a blood count above normal; all one can say in the present case is that we have 2 cases whose vitamin C nutrition did not appear to be satisfactory but whose red cell count was within normal limits.

In Group IV (well-nourished) and in Group III (ill-nourished) the average cell counts are almost identical; there are 23 cases, which is a reasonably large number, in each Group. We are therefore justified in saying that mild degrees of vitamin C subnutrition, taken over a series of cases, does not appear to be associated with gross alteration in the red cell count.

B. The average haemoglobin levels.

When we compare the average haemoglobin levels we find that

i. the average level is 6.3% higher in Group I (well-nourished) than in Group II (ill-nourished). The number of cases is, however, too small to have any significance.

ii. the average level is 7.2% higher in Group IV (well-nourished) than in Group III(ill-nourished). This difference, while not large, is significant when taken over a fair number of cases.

C. The average colour index.

When we compare the average colour indices, we find

i. that the index is 0.12 higher in Group I (well-nourished) cases than in Group II (ill-nourished) cases.

ii. that the index is 0.1 higher in Group IV (well-nourished) cases than in Group III (ill-nourished) cases.

D. The reticulocyte count. There is no significant deviation from normal in the reticulocyte count of any Group, nor is there any significant difference between the reticulocyte counts in the various Groups.

To sum up we may say

i. that there is no significant difference in the red cell counts of comparable well- and ill-nourished Groups.

ii. that the average haemoglobin level is significantly higher in the well-nourished Group.

iii. that the colour index, the index of corpuscular saturation, is higher in the well-nourished Group.

iv. that there is no significant deviation from normal in the reticulocyte count in any of the Groups.

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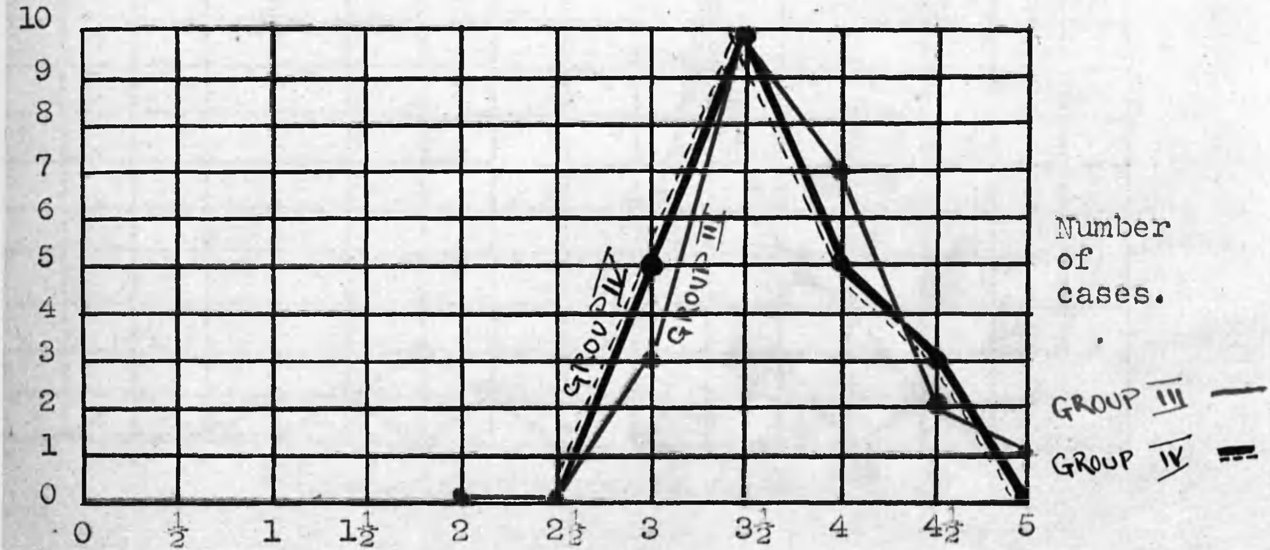
So far we have dealt with Group averages generally. Let us now examine some of the Groups a little more closely. Below are drawn 4 graphs showing the distribution of the red cell counts, haemoglobin levels, colour indices and reticulocyte counts of the members of Groups III and IV females. No graphs of the male cases are shown for the simple reason that there are no males in Group II (male) and so there is no contrast for Group I; and there are only 2 cases in Group III (males), again an insufficient number for comparative purposes. Similarly the graphs of Groups I and II females are omitted as there are only 2 cases in Group I females. But in Groups III and IV females we have suitable comparative material in sufficient numbers from which to draw conclusions.

In the case of the red cell count graph, each reading is plotted to the nearest half million below the actual count;

in the haemoglobin level graph, to the nearest 5% below the actual level; and in the colour index graph, to the nearest 0.1 below the calculated figure. Thus, if the red cell count is 3,500,000 or 3,950,000/c.mm., it is plotted in either case as $3\frac{1}{2}$ millions; if the haemoglobin level is 75% or 79% it is plotted in either case as 75%; and if the colour index is between 0.9 and 0.99 it is plotted as 0.9.

The graphs are shown on the four following pages.

Graph I. -- Red cell count graphic distribution, of Groups III and IV. ---- Females.



Red cell count in millions per c. mm. blood.

Analysis of Graph I.

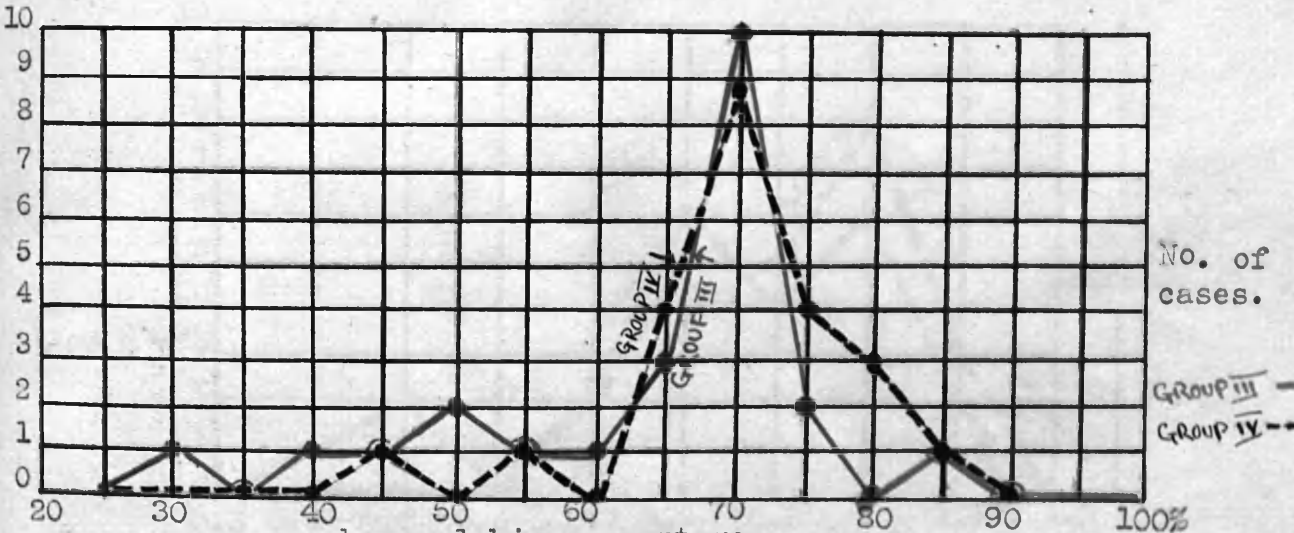
i. The curves of both groups are very similar in general appearance.

ii. The peak of both curves is at 3½ millions, 10 cases having this count in each Group. i.e. there is no "shifting" of the peak of one curve relative to the peak of the other.

iii. The divergences in the curves are of small degree and more or less cancel each other.

We may therefore state that there are no significant differences between the red cell count curve of the ill-nourished Group III and that of the well-nourished Group IV (females).

Graph II. -- haemoglobin levels, graphic distribution of Groups III and IV. ---- Females.



Analysis of Graph II.

i. The peak of both curves is at the 70% mark, ten cases in Group III and nine cases in Group IV having this level. i.e. there is no relative "shifting" of the peaks.

ii. In Group III there are 7 cases below 65%; in Group IV there are only 2 cases below 65%.

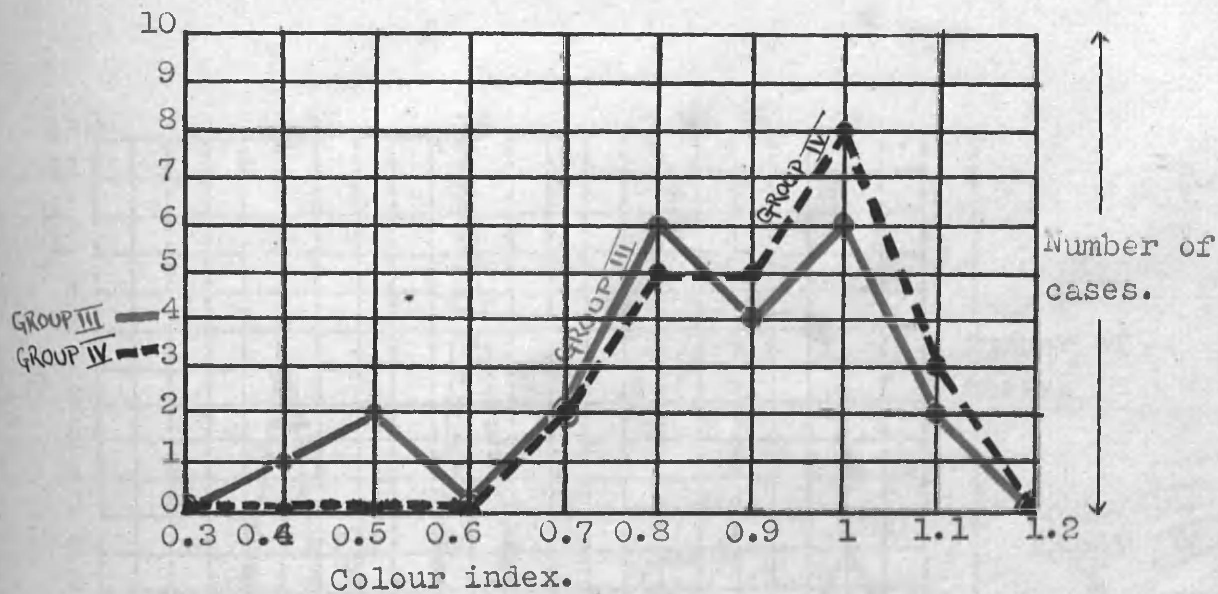
iii. In Group III there is a sudden return to the baseline, only 2 cases having levels of more than 70%; while in Group IV the return to the baseline is more gradual, 8 cases having levels of more than 70%.

iv. Although there is no relative "shift" of the peak, there is a relative "shift to the right" of the body of the Group IV graph.

We may therefore state that more cases are found to have a reasonably high Hb. level in the well-nourished than in the undernourished Group.

Graph III.---- Colour index, graphic distribution.

Groups III and IV. ---- Females.



Analysis of Graph III.

i. There are obvious differences in the general appearance of the two curves.

ii. The peaks of the two curves do not coincide, that of Group III being splintered into 2 components at 0.8 and 1.0, while the single peak of Group IV. is at 1.0. i.e. there is a "shift to the right" of the Group IV curve.

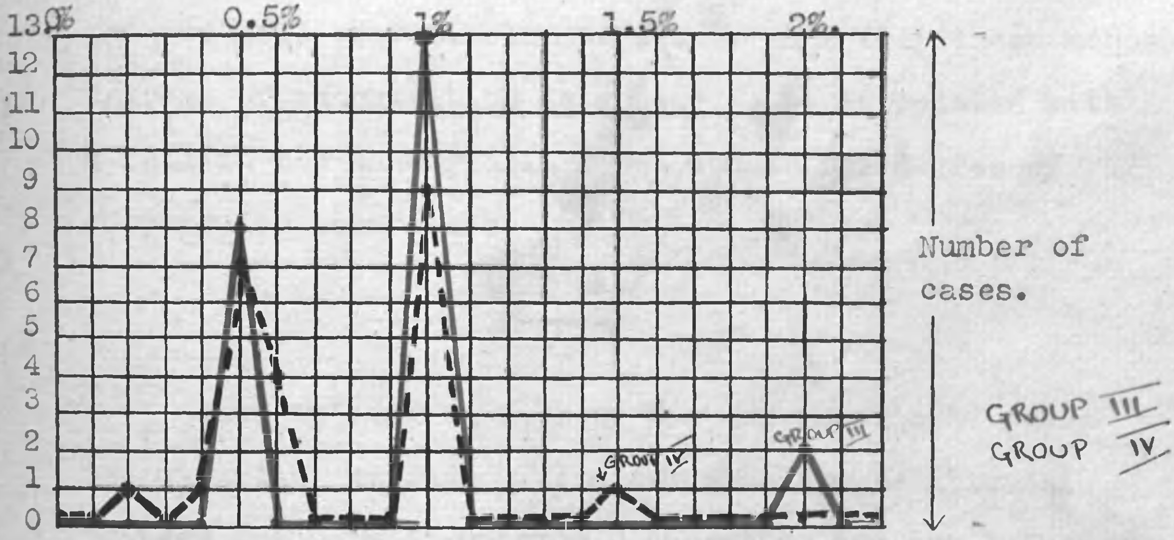
iii. In group III there are 3 cases with a colour index below 0.7, while in Group IV there are no cases with a colour index below 0.7.

iv. Both Groups fall fairly rapidly from their respective peaks to the baseline.

We may therefore remark that the undernourished Group III has a greater number of unsaturated cases than the well-nourished Group IV.

Graph IV. ---- Reticulocyte count, graphic distribution.

Groups III and IV. ---- Females.



Analysis of Graph IV.

i. There are slight differences in the two curves, which are, however, essentially similar in general form.

ii. The peak of each curve is at 1%, a secondary peak being noted in each curve at 0.5%.

iii. The remaining small oscillations appear to be without significance.

iv. No case in either Group was found to have a reticulocyte count over 2%. We may therefore state that there was no abnormality of the reticulocyte count in any case, in either Group III or Group IV, nor was there any significant difference between the Groups as regards reticulocyte distribution.

From an examination of these 4 graphs we may state that minor degrees of vitamin C subnutrition do not appear to be associated with significant alterations in either the red cell or reticulocyte counts; but that these minor degrees of subnutrition do appear to be associated with a fall in the haemoglobin level and in the degree of corpuscular saturation.

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When we come to examine the undernourished cases individually, we do not find any absolute relationship between the rate of vitamin C excretion and the haemoglobin level. For example, in Group III, case number 5 was found to be excreting 12 mgm. per day while her haemoglobin level was only 31%; yet case number 23, with a haemoglobin level of 67% was excreting only 4.1 mgm. per day; and case number 18, with a haemoglobin level of 70% was found to be excreting only 3 mgm. per day. But only 1 case out of the 9 in Group III who had a haemoglobin level of over 70% was found to be excreting less than 11.5 mgm. per day viz. case number 16, and even this excretion rate was comparatively high at 9 mgm. per day. So also in Group II, in which all the haemoglobin levels are within normal limits, the excretion rates do not sink to such low levels as are to be found in the undernourished and anaemic Group III.

We may state that no case with a grossly diminished vitamin C excretion was noted as having a satisfactory haemoglobin level.

There does not appear to be any constant relationship between the vitamin C nutritional state and the blood sedimentation rate of the subject. The sedimentation rate of each case is shown in the case sheets at the beginning of this chapter; in some instances the rate of fall was rather rapid and in these the case was carefully reviewed and considered to be suitable before being admitted to the series.

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We now come to the most equivocal part of the blood examination, viz. the examination of the stained blood film and the report thereon. Unless one performs, for example, a Price-Jones curve on each case, one is merely reporting one's impression of a variable number of microscopic fields. But we consider that, with training and experience, and using a microscope to which one has grown accustomed, one can judge, with a reasonable degree of accuracy, the size of the cells under review; this clinical impression of cell size was checked and substantiated on many occasions by actual measurement of an enlarged image of the cells. It will be seen that the presence of megalocytes is noted in some cases; the enlarged size of these cells was in all cases confirmed by direct measurement of the image.

Anisocytosis and poikilocytosis present very little difficulty or discussion. But their degree varies from one case to another. Thus the difference in cell size may be very marked and obvious at a glance, or it may be relatively slight; therefore anisocytosis is noted as being slight, moderate or marked. So also with poikilocytosis; the abnormally shaped cells may be scanty, quite noticeable or present in abundance; the poikilocytosis is then noted as being slight, moderate or marked.

Any case showing noticeable alteration in staining reaction is noted e.g. polychromasia; and any case showing poverty of staining power is noted as showing either "central pallor" or, in extreme cases, "ring staining".

Any nucleated red cells encountered are noted. Having made these explanations, let us proceed with the analysis of our findings.

Group I.

Features. ---- vitamin C nutrition good, no anaemia.

Number of cases. ---- 17.

In 14 of the cases, no gross abnormality is noted; in the remaining 3 cases, some slight abnormality is seen. These

3 cases are ---

i. Case No. 12 ---- slight degree of anisocytosis.

ii. Case No. 14 ----- slight degree of anisocytosis.

iii. Case No. 15 ---- slight degree of anisocytosis and of poikilocytosis.

The outstanding feature of the blood films in Group I is their essential normality; in the 3 exceptions listed above, the degree of anisocytosis is so slight that no significance can be attached to it.

Group II.

Features. ------ vitamin C subnutrition, no anaemia.

Number of cases. ---- 9.

In 4 cases (i.e. in 44%), no abnormality of the blood film is found. In the remaining 5 cases (56%), some abnormality is noted, thus,

i. Case No. 2 ---- central pallor.

ii. Case No. 6 ---- central pallor.

iii. Case No. 3 ---- moderate degree of anisocytosis.

iv. Case No. 9 ---- moderate degree of anisocytosis.

v. Case No. 5 ---- moderate degree of anisocytosis and slight degree of poikilocytosis.

This group of cases has a red cell count and a haemoglobin level within the accepted limits of normality. But central pallor and anisocytosis are frequently found in a blood where some degree of anaemia exists. We may therefore suggest that in 6 of the 9 cases in this Group there actually is a slight degree of anaemia, associated with the vitamin C subnutrition.? In the remaining 3 cases, subnutrition appears to be associated with a normal red cell count and a normal haemoglobin level.

Group III.

Features. ---- vitamin C subnutrition, anaemia.

Number of cases. ---- 25.

In 10 of the cases (40%), no gross abnormality is noted on examination of the stained film.

In the remaining 15 cases (60%), some abnormality is found, thus,

i. Anisocytosis. This is present in all 15 cases in the following degree,

Slight ---- 11 cases.

Moderate -- 1 case.

Marked ---- 3 cases.

ii. Central pallor is present in 7 of the 15 cases, amounting to ring staining in 3 of these.

iii. Poikilocytosis is noted in 9 cases in the following degree,

Slight ---- 7 cases.

Moderate ---1 case.

Marked -----1 case.

The 2 cases showing moderate and marked poikilocytosis have a markedly diminished excretion rate, viz. 8 mgm. per day and 6.5 mgm. per day respectively.

iv. Megalocytes are noted in 6 cases.

v. No nucleated red cells are seen.

Group IV.

Features.---- vitamin C nutrition good, anaemia.

Number of cases. ------ 36.

In 16 of the cases (44%), no gross abnormality is seen on examination of the stained film.

In the remaining 20 cases (i.e. in 56% of the cases) some abnormality is noted, thus,

i. Anisocytosis. This is present in 18 instances in the following degree,

Slight ---- 14 cases.

Moderate -- 4 cases.

Marked ---- 0 cases.

ii. Central pallor is present in 9 of the 20 cases.

iii. Poikilocytosis is noted in 9 cases in the following degree,

Slight ---- 7 cases.

Moderate -- 2 cases.

Marked ---- 0 cases.

The 2 cases showing moderate degrees of poikilocytosis are cases No. 26 and 36 in Group IV, with an excretion rate of 22 mgm. and 14.5 mgm. per day respectively.

<u>iv. Megalocytes</u> are noted in only 1 case.

<u>v. No nucleated red cells are seen.</u>

Approximately only 40% of the cases in Groups II,III and IV show a normal blood film. In the remaining 60% in each Group some abnormality is clinically detectable, the degree of abnormality varying from case to case and from Group to Group. The best way in which to appreciate the Group differences is to show the findings in tabular form thus,

	Gp.I.	Gp.II.	Gp.III.	Gp.IV.
Aniso-cytosis.	Infrequent; slight degree.	Frequent; moderate degree.	<u>Very frequent;</u> may be of marked degree.	<u>Very frequent;</u> never of marked degree.
Poikilo-cytosis.	Infrequent; slight degree.	Infrequent; slight degree.	<u>Very frequent;</u> usually slight but may be moderate or marked.	<u>Very frequent;</u> may be slight or moderate but never marked.
Central pallor.	None noted.	Frequent.	Frequent.	Frequent.
Megalo-cytosis.	None noted.	None noted.	Frequent.	Infrequent.
Nucleated red cells	None noted.	None noted.	None noted.	None noted.

The differences between Groups II, III and IV are, for the most part, differences in degree. As will be seen by referring to the above table, there are very few differences between Group III and Group IV, but the features peculiar to Group III are

i. The occasional striking degree of the anisocytosis.
ii. The occasional striking degree of the poikilocytosis.
iii. The relative frequency of cases showing megalocytes in the stained blood film.

These striking differences occur in only a few of the cases, however, and are not an essential feature of the Group.

In the other undernourished Group, viz. Group II, no such striking changes are noted. It may be that the marked alterations in size and shape of the erythrocytes are only found as a result of vitamin C subnutrition when subnutrition and anaemia occur together.

Let us briefly summarise these findings.

(1). Of the 87 cases examined, 61 have some degree of anaemia.
(2). Subnutrition is infrequently found in the anaemic men (2 cases), but is of frequent occurrence in the anaemic women (32 cases).
(3). No case possessing a grossly diminished vitamin C excretion is noted as having a satisfactory haemoglobin level. Young patients may find it easier to exist on a lowered vitamin C intake and still maintain a blood picture within normal

limits.

(4). The red cell and reticulocyte counts are approximately the same in the well- and the ill-nourished groups. Minor degrees of subnutrition do not appear to be directly associated with significant alterations in the red cell or reticulocyte counts.

(5). The haemoglobin level and the colour index are, on the average, lower in the undernourished group than in the well-nourished group. It would therefore appear that minor degrees of vitamin C subnutrition are associated with a fall in the haemoglobin level and in the degree of corpuscular saturation.

(6). The prescorbutic state is not constantly associated with a typical abnormality of the stained blood film. The film may show no obvious abnormality. Or, if it does so, the abnormality is likely to take the form of a varying degree of anisocytosis and poikilocytosis; in some cases megalocytes may be seen, with or without central pallor. It must be realised, however, that none of these changes are pathognomonic of prescorbutic anaemia; nor are they, in toto or in part, constant in the prescorbutic state.

CHAPTER VIII.

THE EFFECTS OF VITAMIN C THERAPY IN ANAEMIA, OBSERVED BY OTHER WORKERS, WITH A NOTE ON DOSAGE AND OVERDOSAGE.

Mettier, Minot and Townsend (1930) noted 5 cases, in each of whom a definite and rapid increase of the reticulocytes occurred in response to a diet rich in vitamin C. The reticulocytes in all instances began to increase on the 3rd. and 4th. days and reached the peak of their rise on from the 5th. to the 8th. day. This reticulocyte response was coincident with an increase of the red blood cells which usually continued rapidly to a normal level.

The same workers also treated 2 elderly male patients with typical scurvy as follows:-

(1). For 6 days they were fed on a diet deficient in vitamin C. No change in the reticulocyte count was noted during this time.

(2). For the next 6 days in one patient, and for the next 7 days in the other, iron in adequate doses was added to the diet. No change in the reticulocyte count was noted within 8 days of starting iron administration.

(3). Orange juice, 575c.c. daily, was now added to the diet. Within 3 days, the reticulocytes began to increase from a stationary level of 2.5% in the 1st. case and reached 9.7% and 9.9% on the 4th. and 5th. days respectively. In the 2nd. case the picture was very similar. The reticulocytes rose from a resting level of 1% to 3% on the 3rd. day, and 4% - 5% on the 6th., 7th., and 8th. days; this rise was followed by a return to a low level on the 12th. day.

In the 1st. case, the haemoglobin dropped from 73% to 55% and the red cells fell from 3, 800,000/c.mm. to 2,500,000/c.mm. during the 12 days when the patient took food deficient in vitamin C. But 24 days after starting to take orange juice the red cells had risen to 4,000,000 per c.mm. and the haemoglobin to 90%. In the 2nd. case, the anaemia increased very little during the 13 days when vitamin C was witheld from the diet. After 41 days of anti-scorbutic treatment the haemoglobin rose from 55% to 83% and the red cells from 2,100,000/c.mm. to 3,800,000/c.mm. The slight reticulocyte response and sluggish improvement in this case is attributed to his poor general condition.

Mettier, Minot and Townsend therefore concluded that food rich in vitamin C causes a reticulocyte response and rapid blood regeneration in scurvy, and that iron does not cause such a response.

Mettier and Chew (1932) found that administration of orange juice to scorbutic guinea-pigs gave a reticulocyte crisis which started on the 3rd. day, increased rapidly and reached the peak of production on the 5th.-7th. day. Thereafter the reticulocyte count progressively fell and the red cell count progressively rose. They noted a simultaneous rise in the haemoglobin level. They found that the duration and degree of the reticulocyte response varied from animal to animal. They did not find that the reticulocyte response varied inversely with the initial red cell count as Minot and his co-workers had found in the liver treatment of pernicious anaemia.

Rohmer and Bindschedler (1932) found 6 cases of infantile anaemia which did not respond to iron alone but responded to a combination of iron and vitamin C.

Parsons (1933) states that one of his cases who had a red cell count of under 2½ millions per c.mm. and a haemoglobin level of 33%, responded rapidly to the administration of orange juice-- in 17 days the red cells had risen to over 5 millions per c.mm. and the haemoglobin to 70%. In the same paper, Parsons reminds us that copper should not be given with vitamin C because the vitamin is oxidised in the presence of copper.

Parsons and Smallwood (1935) found that, on administering orange juice to a case of scurvy, there was a reticulocyte crisis which reached its maximum on the 8th. day. There was also a rapid rise in the red cell count and in the haemoglobin level, the red cell count being doubled in 15 days.

Dunlop and Scarborough (1935) noted a reticulocyte response in the anaemia of scurvy when ascorbic acid was added to the diet. Two adult male patients were given 60 mgm. pure ascorbic acid daily, corresponding to 3 oz. orange juice. This treatment was continued for 17 days in one case and for 19 days in the other. In addition to recovery from scurvy, the anaemia was specifically affected by the ascorbic acid. Dunlop and Scarborough state that <u>anaemia may be the only symptom in persons on a deficient diet when other evidences of frank scurvy are missing</u>. They found improvement in both the haemoglobin level and the red cell count in both of their cases. In the first case the red cell count rose from 2,040,000 to 5,600,000 per c.mm. and the haemoglobin from 48% to

100% over a period of 3 months; in the 2nd. case the red cell count rose from 4, 320,000 to 6, 120,000 per c.mm. and the haemoglobin from 70% to 95% in a similar period. Ascorbic acid was administered in the 1st. case for a continuous period of 17 days and in the 2nd. case for a continuous period of 19 days. In both cases a reticulocytosis continued during the period of treatment with ascorbic acid. Dunlop and Scarborough consider that the increase in the reticulocyte and red cell counts could not be due to an improvement in nutrition, since the diet remained at its original level and the patient did not gain weight.

Jennings and Glazebrook (1938) report 2 cases responding to the oral administration of ascorbic acid 600 mgm. daily by mouth. In both of the cases orange juice was given as a supplement before saturation was obtained; so we do not know whether the whole response would have been obtained with ascorbic acid alone. From a scrutiny of their results, it would appear that there was a definite rise in the reticulocyte count in both cases.

Scott Thompson, Glazebrook and Miller (1942) administered ascorbic acid in doses of from 50 to 200 mgm. daily to boys on a deficient institution diet for a period of over 2 months. They kept a control group who did not receive the ascorbic acid. At the end of this time they estimated the haemoglobin levels of both sets of boys and found no significant differences. From this experiment they concluded that minor degrees of vitamin C subnutrition are probably not associated with mild degrees of anaemia. Full particulars of their experiment are given in Chapter VI.

Barron and Barron (1936) concluded that ascorbic acid is probably one of the regulators of the red cell level in the circulating blood.

Croft and Snorf (1939) gave 6 patients with low blood ascorbic acid levels and anaemia 75 - 100 mgm. ascorbic acid daily by mouth, but no reticulocyte response occurred. They considered that there was no relationship between a low blood ascorbic acid concentration and anaemia.

Stephenson, Penton and Korenchevsky (1941) treated senile dements with ascorbic acid but found no significant alteration in the red cell counts or in the haemoglobin levels.

Elmby and Warburg (1937) investigated the adequacy of ascorbic acid as an anti-scorbutic agent. Following

Szent-Gyorgyi's work, the synthetic ascorbic acid and the natural vitamin C had come to be regarded as being identical in every respect. Elmby and Warburg, however, were impressed by the fact that some patients presenting typical prescorbutic and scorbutic symptoms and signs did not respond as well to administration of synthetic ascorbic acid ~~as well~~ as was to be expected. This raised the question as to whether there was some factor missing from the synthetic preparation which was present in the natural vitamin C. They investigated 29 cases who had a haemorragic diathesis and a low blood ascorbic acid concentration. They found that oral administration of ascorbic acid cured the condition and raised the blood ascorbic acid level to normal in all but 3 of the 29 cases, and that even intravenous administration of ascorbic acid to these 3 refractory cases did not bring about cure. As soon as these 3 cases were given large doses of lemon juice by mouth, however, there was a rapid improvement in the clinical condition and a rapid return of the blood ascorbic acid to a normal level. Elmby and Warburg postulate the existence of a co-vitamin which is present in natural sources of vitamin C or is manufactured in a normal bowel and suggest that the presence of this co-vitamin is necessary for the absorption and retention of vitamin C.

Let us briefly summarise the findings of these workers.

(1). Anaemia may be the only sign in persons on a deficient diet when other signs of frank scurvy are missing.

(2). In scorbutic anaemia a reticulocytosis occurs in response to natural vitamin C, starting about the 3rd. day, reaching the peak at about the end of the 1st. week and returning to a normal level in about 12 days.

(3). There is a rapid regeneration of red cells and a rapid rise in the haemoglobin level in response to orange juice in scorbutic anaemia.

(4). A reticulocytosis followed by a rapid regeneration of red cells and a rise in the haemoglobin level in response to ascorbic acid was noted by Dunlop and Scarborough.

(5). The body is apparently able to store ascorbic acid which exerts its effect for a considerable period.

(6). Croft and Snorf found no relationship between a low blood ascorbic acid level and anaemia. They passed judgement on a small series of 6 cases and assumed that a reticulocyte response is the only indication of an improvement in the blood picture in response to treatment. As we have stated in Chapter VI, an estimation of the blood ascorbic acid level is not always a reliable guide to the nutritional state of the subject.

(7). Scott Thompson, Glazebrook and Miller concluded that there was no relation between minor degrees of vitamin C subnutrition and mild degrees of anaemia. One may criticise this conclusion. A blood examination was not made at the start of the investigation in either group of boys, and so the original haemoglobin levels were not known; it is only stated that the boys were not obviously anaemic. In the second place, the cases were not assessed individually, and if any case should have shown improvement it would not have been noted. It is not stated which percentage scale they were using, and so we do not know whether the children had even the slightest degree of anaemia. If they were using a Sahli scale, 90% would have corresponded to a haemoglobin level of 15.6 grams per cent, a level which has been accepted as the average finding in healthy men. According to Whitby and Britton (1942), the haemoglobin level during the period of active growth is 12.5 - 13.8 grams per cent, or 90% - 100% on the Haldane scale. Thus, even if the Haldane scale were being used, it would appear that the children were not suffering from anaemia at all.

(8). Elmby and Warburg have cast doubts on the identity of ascorbic acid and natural vitamin C. It would appear, however, that ascorbic acid is, in most cases, an efficient substitute.

The dosage of ascorbic acid or of vitamin C employed for therapeutic purposes varies from worker to worker. Schultzer (1937) suggested that 40 mgm. ascorbic acid intravenously was as effective as 600 mgm. by mouth. Dunlop and Scarborough (1935) gave 60 mgm. pure ascorbic acid daily by mouth to 2 adult male patients who were suffering from scurvy and scorbutic anaemia, in both cases with excellent results. Jennings and Glazebrook (1938) cited 2 cases responding to the oral administration of ascorbic acid, 600 mgm. daily by mouth. Scott Thompsom, Glazebrook and Miller (1942) gave 50 - 200 mgm. daily by mouth for a period of 2 months. Croft and Snorf (1939) gave 75 - 100 mgm. ascorbic acid daily by mouth without effect. Elmby and Warburg (1937) had to employ the juice of 10 lemons every day in 3 refractory cases before they responded fully. Mettier, Minot and Townsend (1930) administered 575 c.c. orange juice daily.

We have discussed the question of the daily requirement of vitamin C at some length in Chapter I; we may conclude that approximately 25 mgm. daily is sufficient to prevent scurvy and to maintain a reasonable semblance of health. Dunlop and Scarborough, employing 60 mgm.

daily, obtained satisfactory results in the treatment of gross scurvy. Other workers, using larger doses have not obtained uniformly satisfactory results. It would appear that either ascorbic acid will be effective in moderate doses e.g. 60 mgm. per day, or it will not give satisfactory results at all; natural vitamin C must then be given to these refractory cases.

In this series of cases a dosage of 50 mgm. ascorbic acid t.d.s. was employed. It was felt that, by employing this slightly heavier dosage, no harm could be done and there would be a greater working margin.

Various factors tend to interfere with the therapeutic effect of ascorbic acid, viz.,

(1). The incontrovertible fact that a synthetic product is being employed.

(2). In the presence of intestinal disease, there may be some difficulty in absorption.

(3). There may be an inability to retain the substance.

(4). Various toxaemias may interfere with its effect on the bone marrow.

There does not appear to be much danger in administering large doses of ascorbic acid. Wright (1938) states that he has given 10,000 mgm. ascorbic acid intravenously in a single dose to one man, and has given 1,000 mgm. daily for many months intravenously without any ill-effects, the excess beyond saturation being excreted.

Rafsky and Newman (1941) state single doses of 1,000 mgm. ascorbic acid cause diarrhoea in some cases.

Rietschel (1939) states that children and adults who were given large doses of vitamin C showed an increase of thrombocytes, became restless and had diarrhoea. The author took on each of 5 consecutive days 500 mgm. ascorbic acid and found an increase of the thrombocytes in his blood; he also suffered from sleeplessness and diarrhoea. The symptoms disappeared immediately he discontinued the ascorbic acid. He concludes that there is no danger of hypervitaminosis when one is on an ordinary diet, but that there may be such a danger in hospitals where vitamin C is used for therapeutic purposes. He considers the danger to be small, since vitamin C is easily oxidised, but he considers that hypervitaminosis is likely to appear when vitamin C is given in doses of from 200 to 500 mgm. daily over long periods.

Discussion.

In a review of the literature, we are struck by the fact that most of the work in connection with the haematinic effect of ascorbic acid has been performed on the anaemia of fully developed scurvy. Apart from the inadequately controlled investigation of Scott Thompson, Glazebrook and Miller, no serious attempt to investigate the possible relationship between minor degrees of vitamin C subnutrition and anaemia appears to have been made. Frank scurvy is the expression of extreme vitamin C subnutrition and this subnutrition may be of any degree of severity. The various opinions on the effect of vitamin C on the blood picture mostly deal with the anaemia of developed scurvy; but we are entitled to assume that minor degrees of subnutrition are associated with less definite alterations in the blood picture and possibly with less definite responses to specific treatment. So that, while the specific response of scorbutic anaemia to treatment may manifest itself by a reticulocyte crisis, we cannot expect the response in the anaemia of the minor degrees of subnutrition to be so dramatic. While the reticulocyte response may not be so clear cut, we can expect the general form of the response to specific treatment to be parallel with, but on a lower plane than, the response of gross scorbutic anaemia to effective treatment.

References.

Mettier, S.R., Minot, G.R. and Townsend, W.C., 1930. J. Amer. Med. Assoc., 95., 1089.

Mettier, S.R. and Chew, W.B., 1932. J. Exp. Med., IV., 971.

Parsons, L.G., 1933. B.M.J., ii., 631.

Parsons, L.G. and Smallwood, W.C., 1935. Arch. Dis. Child., 10., 327.

Dunlop, D.M. and Scarborough, H., 1935. Ed.M.J., 42.,476.

Jennings, G.H. and Glazebrook, A.J., 1938. B.M.J., ii.,784.

Scott Thompson, Glazebrook, A.J. and Miller, 1942. J. of Hygiene., 42., 2.

Barron, A.G. and Barron, E.S.G., 1936. Proc. Soc. Exp. Biol., N. Y., 35., 407.

References. (cont.)

Croft, J.D., and Snorf, L.D., 1939. Amer.J. Med. Sci., 198, 403.

Stephenson, W., Penton, N. and Korenchevsky, V., 1941. B.M.J., ii., 839.

Elmby and Warburg, E., 1937. Lancet, ii., 1363.

Schultzer, P., 1937. Bioch. J., 1934, 1938.

Rafsky and Newman, H.W., 1941. Amer. J. Med. Sci., 201, 749.

Wright, I.S., 1938. Ann. Interm. Med., 12, 525.

Rietschel, H., 1939. Klin. Wschr., 18, 923.

Rohmer, P. and Bindschedler, J.S., 1932. Acta. Paed., Uppsala, XIII., 399.

Chapter IX.

PERSONAL OBSERVATIONS ON THE EFFECT OF ADMINISTRATION OF ASCORBIC ACID IN SUBSCORBUTIC AND NON-SCORBUTIC ANAEMIAS.

Keefer and Yang (1929) made the following statement-- "In determining the value of any treatment of anaemia in man, the increase in the haemoglobin and red blood cells must be followed before and after therapy and the results compared. In some cases, an increase in the reticulocytes is an indication of the beneficial effects of therapy. However, when the increase in the haemoglobin and red blood cells occurs without an increase in the reticulated cells, the rate of haemoglobin increase must serve as a criterion".

On Keefer and Yang's thesis, improvement as the result of specific treatment may take the form of a reticulocytosis, increased red cell count and raised haemoglobin level; or the improvement may simply appear as an increase in the red cell count and haemoglobin level.

It is, however, possible that only the haemoglobin level may show a rise in response to specific treatment, neither reticulocytosis nor marked rise in the red cell count being noted.

On these 3 criteria of improvement, viz. reticulocytosis, increased red cell count and increased haemoglobin level, the results of therapy were assessed in the present series. In this series of cases, a reticulocyte response to treatment was presumed if the reticulocyte count rose above 2% and was followed by a rise in the red cell count. As we have stated in Chapter II, the normal reticulocyte limits are 0 -- 2%, and any case with a count above 2% must be considered abnormal; while the abnormality may simply be a physiological increase of blood production e.g. following haemorrhage or excessive blood destruction, it is nevertheless an abnormality. We have already seen in Chapter VIII that, if a reticulocyte response to ascorbic acid occurs, it starts on the 3rd. day and reaches its peak at the end of 1 week. In the present series, a reticulocyte count was performed on 2 occasions, on the first occasion before treatment had started, and on the second occasion 7 days after starting treatment.

While examination of the bone marrow is frequently the best guide to the progress of a case under treatment, it is not a procedure which can be widely adopted in general practice and for this reason was not performed in this series of cases.

On the following pages are given details of the observed effects of ascorbic acid administration on the red cell count, reticulocyte count, and haemoglobin level in each of 64 cases.

The results of massive iron therapy in 22 cases, and the results of massive iron therapy supplementary to ascorbic acid treatment in another 13 cases are also tabulated. These results may be presented under the following headings.

(A). Details of the results of massive iron therapy on the blood picture in 22 cases.

(B). The results of ascorbic acid therapy on the blood picture in 16 cases previously treated to the limit of their improvement with iron.

(C). The results of combined ascorbic acid and iron therapy in 5 cases.

(D). The results of ascorbic acid therapy in 43 cases.

(E). The results of massive iron therapy in 13 cases previously treated to the limit of improvement with ascorbic acid.

The case numbers occupy the 1st. column in the various charts which follow. In the 2nd. column is shewn the Group to which the case originally belonged, followed by the number of the case in that Group. e.g. IV (14) indicates that she was case number 14, Group IV; we can then identify the case and find all relevant particulars by referring to Chapter VII, and finding case number 14 in the Group IV chart.

The expressions "significant" and "insignificant" are frequently used in the text in this chapter. A rise or fall in either the red cell count or the haemoglobin level is considered to be "significant" if the alteration is greater than 5%. It is considered to be "insignificant" and possibly due to experimental error if the alteration is less than 5% in single cases. Experimental error and physiological variation can account for small alterations of no significance in single cases, but, if the level of a whole series of cases shifts, even if the shift is less than 5%, we have a change in level of very definite significance.

Let us now proceed to an examination of our findings.

CHART A

DETAILS OF THE OBSERVED RESULTS OF MASSIVE IRON THERAPY ON THE BLOOD PICTURE IN 22 CASES

Ferri et Ammon cit gr.XL t.d.s. and Acid Hydrochlor. Dil. dr. i t.d.s. administered in each case.

				BLOOD PICTURE BEFORE TREATMENT.						BLOOD PICTURE AFTER TREATMENT					RESULT.		
Case No.	Group	Age	Sex	Red cell count	Ret. Ct.	Haemoglobin % (grams)	C.I.	B.S.R. 1 : 2	Dur'n of treatment	Ret. ct.	Red cell count	Haemoglobin % : grams	C.I.	B.S.R. 1 : 2	Red cell count	Ret. ct.	H.globin %
1.	III(1)	25	F	5,150,000	1%	74%(11.8)	0.7	9 : 16	5 weeks	0.8%	5,000,000	86% (13.6)	1	9 : 18	-150,000	-	+ 12%
2.	III(2)	45	F.	3,260,000	1%	56%(8.9)	0.9	14 : 24	8 weeks	2%	4,200,000	81% (13)	1	14 : 24	+940,000	-	+ 25%
3.	III(16)	20	F.	4,120,000	1%	74%(11.8)	0.9	3 : 7	4 weeks	0.8%	4,200,000	76% (12.1)	0.9	3 : 7	+ 80,000	-	+ 2%
4.	III(4)	27	F.	3,810,000	1%	72%(11.5)	1	7 13	8 weeks	1%	4,250,000	86% (13.6)	1	9 : 16	+440,000	-	+ 14%
5.	III(5)	41	F.	3,710,000	2%	31%(5)	0.4	8 : 18	12 weeks	3%	4,000,000	72%(11.5)	0.9	6 : 14	+290,000	Cris.	+ 41%
6.	III(6)	50	F.	4,000,000	2%	75%(11.9)	1.1	8 : 18	4 weeks	1%	4,000,000	78%(12.4)	1	10 : 20	-	-	+ 3%
7.	III(7)	35	F.	3,775,000	1%	85%(13.6)	1.1	8 : 16	4 weeks	0.4%	4,090,000	88%(14.1)	1.1	9 : 18	+315,000	-	+ 3%
8.	III(8)	40	F.	3,800,000	1%	70%(11.1)	0.9	4 : 8	4 weeks	1%	4,480,000	79%(12.6)	0.9	4 : 8	+680,000	-	+ 9%
9.	IV(1)	50	M.	5,110,000	1%	80%(12.7)	0.8	5 : 9	4 weeks	0.6%	5,110,000	87%(13.9)	0.9	5 : 9	-	-	+ 7%
10.	IV(4)	28	F.	3,540,000	1%	70%(11.1)	1	6 : 14	6 weeks	0.5%	4,300,000	82%(13.1)	1	6 : 14	+760,000	-	+ 12%
11.	IV(5)	36	M.	4,260,000	1%	78%(12.5)	0.9	7 : 16	5 weeks	1%	4,300,000	88%(14.1)	1	7 : 16	+ 40,000	-	+ 10%
12.	IV(6)	43	M.	5,270,000	0.5%	85%(13.5)	0.8.	6 : 12	5 weeks	1%	5,300,000	90%(14.3)	0.9	6 : 12	+ 30,000	-	+ 5%
13.	IV(7)	49	M.	5,000,000	1%	77%(12.3)	0.8	23 : 43	5 weeks	0.5%	4,630,000	87%(14.)	0.9	20 : 40	-370,000	-	+ 10%
14.	IV(8)	61	M.	5,140,000	0.5%	83%(13.2)	0.8	11 : 20	4 weeks	1%	5,090,000	84%(13.2)	0.8	11 : 20	- 50,000	-	+ 1%
15.	IV(9)	50	F.	3,910,000	0.6%	75%(12.1)	1	8 : 19	6 weeks	1.4%	4,900,000	98%(15.7)	1	4 : 10	+990,000	-	+ 23%
16.	IV(10)	60	F.	3,900,000	1%	84%(13.4)	1	8 : 19	4 weeks	1%	4,320,000	91%(14.5)	1	8 : 19	+420,000	-	+ 7%
17.	IV(11)	46	F.	4,225,000	1%	58%(9.4)	0.7	7 : 15	11 weeks	0.2%	4,340,000	80%(12.7)	0.9	4 : 7	+115,000	-	+ 22%
18.	IV(12)	39	F.	3,910,000	1%	67%(10.6)	0.85	5 : 15	6 weeks	0.2%	4,000,000	78%(12.5)	1	4 : 10	+ 90,000	-	+ 11%
19.	IV(13)	39	F.	3,390,000	0.5%	48%(7.6)	0.7	10 : 23	8 weeks	1%	4,500,000	88%(14.1)	1	6 : 11	1110,000	-	+ 40%
20.	IV(14)	38	F.	4,260,000	0.6%	74%(11.9)	0.8	4 : 8	6 weeks	1%	4,260,000	81%(13.)	1	4 : 8	-	-	+ 7%
21.	IV(15)	33	M	3,900,000	0.5%	81%(13.2)	1	14 : 34	4 weeks	0.6%	4,000,000	87%(14.)	1.1	6 : 12	+100,000	-	+ 6%
22.	IV (16	20	M.	5,520,000	0.2%	77%(12.5)	0.7	3 : 6	4 weeks	0	5,400,000	78%(12.4)	0.7	3 : 6	-120,000	-	+ 1%

The above Chart A shows the effect of massive iron and dilute hydrochloric acid therapy on the blood picture in 22 cases.

8 of the cases belong to Group III (undernourished Vit. C).

14 of the cases belong to Group IV (well-nourished Vit. C).

Below are tabulated and contrasted the responses of the 2 Groups to iron.

	No. of cases.	Final average red cell count.	Final average H.globin	Final extremes of H.globin.
Gp.III females	8	4,278,000.	80.75%	72% -- 88%.
Gp.III males	0	----------	------	-----------
Gp.IV females	7	4,374,000.	85.5%.	78% -- 98%.
Gp.IV males	7	4,883,000.	86%.	78% -- 90%.

Unfortunately, Group IV males have no Group III males with whom to contrast.

There is very slight difference in the average red cell counts of Group III and Group IV. But there is a marked difference between the haemoglobin levels of the Groups; the well-nourished members of this series, as a group, reacted more fully to iron therapy than did the ill-nourished members. At the end of treatment they had reached a haemoglobin level approximately 5% higher than the level attained by the ill-nourished members. And not only had they reacted better as a group, but they had reacted better individually as will be seen from a scrutiny of the column showing the "final extremes of haemoglobin".

To summarise we may say that

(a). The final haemoglobin level of the well-nourished (Group IV) females is 4.75% higher than the final haemoglobin level of the undernourished (Group III) females.

(b). The final figures after treatment are

<u>Females --- 15 cases.</u>

Final average red cell count ---------- 4,322,000/c.mm.

Final average haemoglobin ------------- 83%.

<u>Males.</u>

Final average red cell count ---------- 4,883,000/c.mm.

Final average haemoglobin ------------- 86%.

We may conclude that the response to iron was greater in those cases who had adequate reserves of vitamin C in the present series.

CHART B.

DETAILS OF THE OBSERVED RESULTS OF ASCORBIC ACID THERAPY ON THE BLOOD PICTURE IN 16 CASES PREVIOUSLY TREATED TO THE LIMIT OF THEIR IMPROVEMENT BY MASSIVE DOSES OF IRON. Ascorbic Acid mgm. 50 t.d.s. administered by mouth in each case.

				BLOOD PICTURE BEFORE TREATMENT						BLOOD PICTURE AFTER TREATMENT					RESULT.		
Case No.	Origin. Group	Age	sex	Red cell count	Ret' ct.	H.globin % :grams	C.I.	B.S.R. 1 : 2	Dur'n of treat't.	Ret. ct.	Red cell count	H.globin % : grams	C.I.	B.S.R. 1 : 2	Red cell count	Ret. ct.	H.globin %
1.	111(8)	40	F.	4,480,000	1%	79%(12.6)	0.9	4 : 8	4 weeks	0.5%	4,500,000	87%(13.9)	1	3 : 7	-	-	+ 8%
2.	111(1)	25	F.	4,350,000	0.8%	86%(13.7)	1	9 : 18	4 weeks	0.2%	4,590,000	91%(14.5)	1	7 : 17	+ 240,000	-	+ 5%
3.	111(6)	50	F.	4,000,000	1%	78%(12.4)	0.9	10 : 20	4 weeks	2%	4,130,000	87%(13.9)	1	8 : 15	+ 130,000	-	+ 9%
4.	111(4)	27	F.	4,250,000	1%	86%(13.7)	1	9 : 16	4 weeks	0.5%	4,500,000	86%(13.7)	1	6 : 14	+ 250,000	-	-
5.	111(7)	30	F.	4,090,000	0.4%	88%(14)	1.1	9 : 18	4 weeks	0	4,000,000	88%(14)	1.1	5 : 12	- 90,000	-	-
6.	1V(8)	61	M.	5,090,000	1%	84%(13.4)	0.8	11 : 20	4 weeks	0.5%	4,900,000	91%(14.5)	0.9	9 : 14	- 190,000	-	+ 7%
7.	1V(1)	50	M.	5,110,000	0.6%	87%(13.9)	0.9	5 : 9	4 weeks	2%	5,500,000	88%(14)	0.8	5 : 9	+ 390,000	-	+ 1%
8.	1V(6)	43	M.	5,300,000	1%	90%(14.3)	0.9	6 : 12	4 weeks	0.5%	5,300,000	101%(16.1)	1	5 : 10	-	-	+ 11%
9.	1V(7)	49	M.	4,630,000	0.5%	87%(13.9)	0.9	20 : 40	4 weeks	0.5%	4,560,000	92%(14.6)	1	23 : 43	- 70,000	-	+ 5%
10.	1V(5)	36	M.	4,300,000	1%	88%(14)	1	7 : 16	4 weeks	0.2%	4,300,000	90%(14.3)	1	4 : 7	-	-	+ 2%
11.	1V(4)	28	F.	4,300,000	0.5%	82%(13.1)	1	6 : 14	4 weeks	0.5%	4,200,000	75%(11.9)	0.9	6 : 14	- 100,000	-	- 7%
12.	1V(10)	60	F.	4,320,000	1%	91%(14.5)	1	8 : 19	4 weeks	0.2%	4,420,000	89%(14.2)	1	8 : 16	+ 100,000	-	- 2%
13.	1V(14)	38	F.	4,260,000	1%	81%(12.9)	1	4 : 8	4 weeks	1%	4,200,000	86%(13.7)	1	4 : 8	- 60,000	-	+ 5%
14.	1V(15)	33	M.	4,000,000	0.6%	87%(13.9)	1.1	6 : 12	4 weeks	3%	4,920,000	104%(16.6)	1	6 : 11	+ 920,000	Crisis	+ 17%
15.	1V(12)	39	F.	4,000,000	0.2%	78%(12.4)	1	4 : 10	4 weeks	1%	4,230,000	85%(13.6)	1	4 : 9	+ 230,000	-	+ 7%
16.	1V(16)	20	M.	5,400,000	0.	78%(12.4)	0.7	3 : 6	4 weeks	1%	5,200,000	84%(13.4)	0.8	3 : 6	- 200,000	-	+ 6%

This chart is discussed on the following pages.

(B). Ascorbic acid was administered to 16 cases who had already been saturated with iron. (A case was considered to be iron saturated when continued administration of Ferri et Ammon Cit., gr. xl t.d.s. and Acid Hydrochlor. dil. dr.i t.d.s. failed to provoke any further rise in the haemoglobin level; administration of the iron and the acid was continued in all cases for a minimum period of 4 weeks, even though no response was obtained). Thus, the supplementary effect, if any, of ascorbic acid on the blood picture of cases of nutritional anaemia who had been efficiently treated with iron could be measured and recorded.

The ascorbic acid was administered as Tab. Ascorbic Acid (B.D.H.) mgm. 50. The dosage employed was mgm. 50 t.d.s. by mouth, the tablets not being dissolved until immediately before swallowing. This treatment was continued for 4 weeks, or for a longer period if the "limit of improvement" had not been attained in that time.

In all, 16 cases were examined in this manner. Five of these were females and had <u>originally</u> belonged to Group III and so were suffering from vitamin C subnutrition. The remaining 11 had <u>originally</u> belonged to Group IV and were well-nourished.

Let us consider the reactions of the ill- and well-nourished Groups separately.

(1). The response of the undernourished members to ascorbic acid.

Number of cases.------ 5 (Gp. III).

Sex. ----------------- females.

i. The red cell count.

3 cases show an insignificant rise in the count.

1 case shows no alteration.

1 case shows a fall (insignificant).

In no case is a reticulocyte crisis seen.

Average red cell count before ascorbic acid ----- 4,234,000/c.mm

Average red cell increase ---------------------- 110,000/c.mm.

Average red cell count after ascorbic acid ------ 4,344,000/c.mm

There is therefore no significant alteration in the red cell count in these 5 cases.

ii. The haemoglobin level.

3 cases show significant increases.

2 cases show no alteration.

The increases are as follows --

Case 1 ---- 8%.

Case 3 ---- 9%.

Case 2 ---- 5%.

It is interesting to note that those cases with the lowest haemoglobin levels give the greatest responses, viz.

Case No. 3 --- Hb. 78% --- increase 9% --- total 87%.

Case No. 1 --- Hb. 79% --- increase 8% --- total 87%.

Case No. 2*--- Hb. 86% --- increase 5% --- total 91%.

Case No. 4 --- Hb. 86% --- increase nil -- total 86%.

Case No. 5 --- Hb. 88% --- increase nil -- total 88%.

It is also of interest to note that the final haemoglobin levels of these 5 female cases fall within the narrow boundaries 86% -- 91% i.e. between 13.7 and 14.5 grams Hb. per 100 c.c. blood; and that the final average haemoglobin is 88% (approx. 14.1 grams/100 c.c. blood), a figure which is slightly higher than the average female haemoglobin level of 13.7 grams/100 c.c. and considerably higher than the minimum level of 12 grams/100 c.c. suggested by Whitby and Britton (1942).

Average haemoglobin level before ascorbic acid ---- 83.4%.

Extremes of haemoglobin before ascorbic acid ------ 78% -- 88%.

Average increase in haemoglobin ------------------- 4.4%.

Maximum increase in haemoglobin ------------------- 9%.

Extremes of haemoglobin after ascorbic acid ------- 86% -- 91%.

Final average haemoglobin ------------------------- 87.8%.

<u>(2). The response of the well-nourished members to ascorbic acid</u>

<u>Number of cases.</u> ----- 11.

<u>Sex.</u> ----------------- 7 males.
4 females.

We shall consider the sexes separately.

(a) Males. ---- 7 cases.

i. Red cell count.

2 cases show a significant increase.

2 cases show no alteration.

3 cases show an insignificant fall.

The falls in the count are not sufficiently large to be significant, the greatest fall being 200,000 (approx. 4%) and the average fall 150,000 (approx. 3%).

The increases in the count are, however, sufficiently large to possess some significance, viz.

Increase in case No. 7 -------- 390,000/c.mm. (approx. 8%).

Increase in case No. 14 ------- 920,000/c.mm. (approx. 20%).

No reticulocyte crisis was actually noted in case No 7 and so we do not know whether the ascorbic acid was really the cause of the increase. In case No. 14, however, a moderate but definite rise in the reticulocyte count was noted at the end of 7 days' treatment (3% reticulocytes), followed by a rapid rise in both the red cell count and the haemoglobin level.

Average red cell count before ascorbic acid --- 4,833,000/c.mm.

Average red cell increase ---------------------- 121,000/c.mm.

Average red cell count after ascorbic acid ---- 4,954,000/c.mm.

ii. The haemoglobin level.

5 cases show a significant increase.

2 cases show an insignificant increase.

The increases in the haemoglobin level range between 1% and 17%.

Case Number.	Original Hb. level.	% rise or fall.	Final Hb. reading.
7.	87%	plus 1%	88%.
10.	88%	plus 2%	90%.
9.	87%	plus 5%	92%.
16.	78%	plus 6%	84%.
6.	84%	plus 7%	91%.
8.	90%	plus 11%	101%.
14.	87%	plus 17%	104%.

Summarised, these findings are as follows --

Average haemoglobin level before ascorbic acid --- 86%.

Extremes of haemoglobin before ascorbic acid ----- 78% -- 90%.

Average increase in haemoglobin ------------------ 7%.

Maximum increase in haemoglobin ------------------ 17%.

Extremes of haemoglobin after ascorbic acid ------ 84% -- 104%.

Final average haemoglobin ------------------------ 93%.

<u>(b). Females.</u>

<u>Number of cases.</u> ---- 4.

<u>i. Red cell count.</u>

2 cases show an insignificant increase.

2 cases show an insignificant fall.

In both cases the increases are of moderate degree, viz.

230,000/c.mm. (approx. 5%) and 100,000/c.mm. (approx. 2%). The increases are not accompanied by an increase in the reticulocyte counts and no significance is attached to the higher readings.

In the other 2 cases the falls are slight, viz. 100,000/c.mm. (approx. 2%), and 60,000/c.mm. (approx. 1%), and are without significance.

We may therefore state that there are no significant alterations in the red cell counts in these 4 female cases.

Average red cell count before ascorbic acid --- 4,220,000/c.mm.
Average red cell increase ------------------------ 42,000/c.mm.
Average red cell count after ascorbic acid -----4,262,000/c.mm.

ii. The haemoglobin level.

2 cases show a significant increase in the haemoglobin level.
1 case shows an insignificant fall in the level.
1 case shows a significant fall in the level.

The significant fall is one of 7% in case number 11. This fall is accompanied by a marked fall in the red cell count. No dogmatic explanation of this paradox is offered, but the suggestion may be advanced that the withdrawal of the large "head" of iron led to the fall; no history of bleeding had been given, and the fall seems to be too large to be due to experimental error.

The cases showing the increases are

Case

Case Number.	Original Hb. level.	% rise or fall.	Final Hb. reading.
13.	81%.	plus 5%.	86%.
15.	78%.	plus 7%.	85%.

Summarised, the findings are as follows ---

Average haemoglobin before ascorbic acid ----- 83%.

Extremes of haemoglobin before ascorbic acid - 78% -- 91%.

Average increase in haemoglobin -------------- 0.75%.

Maximum increase in haemoglobin -------------- 7%.

Extremes of haemoglobin after ascorbic acid -- 75% -- 89%.

Final average haemoglobin ------------------- 83.75%.

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Let us shortly recapitulate our findings on these 16 cases who had first been saturated with iron, then treated with ascorbic acid.

(1). The 4 apparently well-nourished women showed no dramatic response to treatment with ascorbic acid, either by a marked increase in the red cell count or by an increase in the haemoglobin level. There was an average increase in the red cell count of 42,000/c.mm. (approx. 1%); no case with an increased reticulocyte count was noted, nor was any case found with an increase of more than 5% in the red cell count. One case only showed a significant rise of 7% in the haemoglobin level. The average increase in the haemoglobin level was only 0.75%.

(2). The five apparently ill-nourished women gave a definite response to ascorbic acid. There was no single significant rise in the red cell count, there was no reticulocytosis, and the average increase in the red cell count over the 5 cases was insignificant(2%). In 3 of the 5 cases there was a marked rise in the haemoglobin level, viz, 5%, 8%, and 9%, and the average rise in the haemoglobin level over the 5 cases was 4.4%.

(3). In the 7 apparently well-nourished men there was an average red cell increase of 121,000/c.mm. (approx. 2.5%). In 1 case there was a reticulocytosis of 3% followed by a 20% increase in the red cell count. There was a rise in the haemoglobin level in all 7 cases, the rise ranging from 1% to 17%, the average rise over the 7 cases being 7%.

(4). The average red cell increase over the 16 cases is 98,000/c.mm. blood (approx. 2%).

(5). The average haemoglobin increase over the 16 cases is 5.2%.

(6). The final average red cell count in the 9 women is 4,290,000/c.mm. and the final average haemoglobin in the same women is 86%.

(7). The final average red cell count in the 7 men is 4,954,000 per c.mm. and their final average haemoglobin is 93%.

We may summarise and conclude by stating that supplementary administration of ascorbic acid to these 16 cases who had previously been treated to the limit of their improvement with iron caused a haemoglobin rise of 5% or more in 10 of the 16 cases and an average increase in the haemoglobin level over the

16 cases of 5.2%. The effect of ascorbic acid on the red cell count was not so marked, but in 1 case there was a reticulocyte rise followed by a rapid and significant increase in the red cell count; curiously enough, this striking result was given by a male who had apparently been well-endowed with the vitamin prior to treatment. The average increase in the red cell count over the 16 cases was only 2%, an insignificant figure.

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Chart C.

Details of the observed results of combined ascorbic and massive iron therapy on the blood picture in 5 cases.

Ferri et Ammon Cit. gr. xl t.d.s., Acid Hydrochlor. Dil. dr. i t.d.s., and Ascorbic acid mgm. 50 t.d.s. administered to each case.

				BLOOD PICTURE BEFORE TREATMENT.						BLOOD PICTURE AFTER TREATMENT.					RESULT.		
Case No.	Group	Age	Sex	Red cell count.	Retic ct.	H.globin. % :grams.	C.I.	B.S.R. 1 : 2 .	Durn. of treatment	Retic ct.	Red cell count.	H.globin. % :grams.	C.I.	B.S.R. 1 : 2 .	Red cell count	Ret. ct.	H.globin.
1.	II(2)	48.	F.	4,570,000	0.5%	82: 13.1.	0.9.	7 : 13.	4 weeks	0.5%	4,700,000	92; 14.7.	1.	6 : 12.	+130,000	----	+. 10%.
2.	III(9)	28.	F.	3,665,000	1%.	70: 11.2.	1.	5 : 9.	4 weeks	0.6%	3,910,000	78: 12.5.	1.	5 : 9.	+245,000	----	+ 8%.
3.	III(11)	37.	F.	3,900,000	1%.	36: 5.7.	0.5.	14 : 30.	9 weeks	5%.	4,600,000	88: 14.	1.	4 : 8.	+700,000	Cris.	+ 52%.
4.	IV(17)	50.	M.	4,320,000	0.4%.	78:12.5.	0.9.	7 : 14.	4 weeks	0.8%.	4,200,000	86: 13.7.	1.	6 : 12.	-120,000	----	+ 8%.+
5.	IV(19)	48.	F.	4,220,000	0.5%.	74:11.8.	0.9.	8 : 15.	4 weeks	0.7%.	4,280,000	90: 14.3.	1.1	6 : 12.	+ 60,000	----	+ 16%.

This chart is discussed on the following pages.

Five cases were treated simultaneously with iron, acid hydrochlor. dil. and ascorbic acid. The responses of these cases to this treatment are shown in the above table. 3 of the cases are undernourished females, the 4th. a well-nourished female and the 5th. a well-nourished male. We shall consider the undernourished females first.

Undernourished females.

Number of cases ---- 3

i. Red cell count.

There is a rise in the red cell count in all 3 cases, of moderate degree in 2 of the cases and of marked degree in the 3rd. case. A reticulocytosis of 5% is noted at the end of the 1st. week in case No. 3.

Average red cell count before treatment ---- 4,045,000/c.mm.

Average increase in red cell count --------- 358,000/c.mm.

Final average red cell count --------------- 4,403,000/c.mm.

ii. The haemoglobin level.

There is a rise in the level in the 3 cases , of significant degree in each case.

Average haemoglobin before ascorbic acid ----- 63%.

Extremes of haemoglobin before treatment------ 36% -- 82%.

Average increase in haemoglobin--------------- 23%.

Highest increase in haemoglobin -------------- 52%.

Extremes of haemoglobin after treatment ------ 78% -- 92%.

Final average haemoglobin --------------------- 86%.

The final haemoglobin levels of the other 2 cases are 86% in the case of the well-nourished male, and 90% in the case of the well-nourished female.

Final average red cell count (4 women) ---- 4,372,000/c.mm.

Final average haemoglobin ----------------- 87%.

Final average red cell count (male) ------- 4,200,000/c.mm.

Final red cell count (male)---------------- 86%.

The combination of ascorbic acid with iron therapy does not appear to accelerate markedly the speed of the haemoglobin rise. The final average haemoglobin level of the 3 cases corresponds closely with the final average figure of the females treated with ascorbic acid after saturation with iron.

DETAILS OF THE OBSERVED RESULTS OF ASCORBIC ACID THERAPY ON THE BLOOD PICTURE IN 43 CASES.

Ascorbic Acid mgm. 50 t.d.s. administered by mouth to each case.

				BLOOD PICTURE BEFORE TREATMENT						BLOOD PICTURE AFTER TREATMENT.					RESULT		
Case No.	Group	Age	Sex	Red cell count	Ret. ct.	H'Globin % : grams	C.I.	B.S.R. 1 : 2	Dur'n of treatment	Ret. ct.	Red cell count	H.globin % :grams	C.I.	B.S.R. 1 : 2	Red cell count	Ret. ct.	H.globin % :grams.
1.	1(1)	45	M.	5,500,000	2%	92%(14.6)	0.9	4 : 8	4 weeks	2.2%	5,400,000	93%(14.8)	0.9	4 : 8	-100,000	-	+ 1%
2.	1(2)	40	M.	4,850,000	1%	95%(15.2)	1	9 : 20	4 weeks	1.4%	4,700,000	93%(14.8)	1	6 : 12	-150,000	-	- 2%
3.	1(3)	43	M.	4,970,000	1%	90%(14.3)	0.9	4 : 9	4 weeks	1.6%	4,800,000	90%(14.3)	0.9	4 : 9	-170,000	-	-
4.	1(4)	29	M.	4,650,000	1%	90%(14.3)	1	10 : 18	4 weeks	1%	4,700,000	90%(14.3)	1	10 : 18	+ 50,000	-	-
5.	1(5)	33	M.	4,940,000	1%	90%(14.3)	0.9	2.5 : 6	4 weeks	1%	5,000,000	90%(14.3)	0.9	12 : 5	+ 60,000	-	-
6.	1(6)	52	M.	4,520,000	1%	93%(14.8)	1	8 : 18	4 weeks	1%	4,700,000	94%(15)	1	8 : 18	+180,000	-	+ 1%
7.	1(7)	24	F.	4,210,000	0.5%	85%(13.5)	1	6 : 12	4 weeks	1%	4,400,000	85%(13.5)	1	6 : 15	+190,000	-	-
8.	1(8)	41	M.	4,600,000	0.5%	101%(16.2)	1.1	3 : 6	4 weeks	4%	5,000,000	103%(16.4)	1	3 : 5	+400,000	+	+ 2%
9.	11(3)	53	F.	4,500,000	0.4%	81%(13)	0.9	12 : 20	4 weeks	0.5%	4,500,000	82%(13.1)	0.9	6 : 12	-	-	+ 1%
10.	11(4)	55	F.	4,090,000	0.5%	83%(13.2)	1	9 : 13	4 weeks	1%	4,395,000	83%(13.2)	1	8 : 14	+305,000	-	-
11.	11(7)	32	F.	4,300,000	1%	82%(13)	1	9 : 16	4 weeks	0.4%	4,250,000	75%(11.9)	0.9	12 : 22	- 50,000	-	- 7%
12.	11(9)	29	F.	4,370,000	0.6%	78%(12.4)	0.9	5 : 12	6 weeks	0.2%	4,410,000	87%(14)	1	4 : 11	+ 40,000	-	+ 9%
13.	111(12)	19	F.	3,560,000	1%	74%(11.9)	1	5 : 12	4 weeks	1%	3,910,000	79%(12.6)	1	6 : 13	+350,000	-	+ 5%
14.	111(13)	47	F.	4,000,000	0.5%	74%(11.9)	1	9 : 19	4 weeks	0.4%	3,900,000	65%(10.4)	0.8	10 : 21	-100,000	-	- 9%
15.	1V(36)	51	M.	3,860,000	0.5%	80%(13)	1	3 : 10	4 weeks	1%	4,580,000	93%(15)	1	2 : 3	+720,000	-	+ 13%
16.	111(14)	26	F.	4,500,000	1%	74%(11.9)	0.8	9 : 17	4 weeks	1.5%	4,790,000	80%(12.9)	0.85	6 : 12	+290,000	-	+ 6%
17.	111(15)	32	F.	4,215,000	0.8%	68%(10.8)	0.8	10 : 19	6 weeks	0.4%	4,000,000	82%(13.1)	1	8 : 14	-215,000	-	+ 14%
18.	111(16)	20	F.	4,120,000	0.5%	74%(11.9)	0.9	3 : 7	4 weeks	1%	3,940,000	76%(12)	1	3 : 7	-180,000	-	+ 2%
19	111(17)	25	F.	3,125,000	1%	62%(10)	1	2 : 5	5 weeks	2.6%	3,930,000	81%(13)	1	2 : 5	+805,000	Cris	+ 19%
20.	111(18)	39	F.	3,150,000	1%	70%(11.2)	1	10 : 25	6 weeks	3.5%	4,000,000	82%(13.1)	1	8 : 14	+850,000	Cris	+ 12%
21	111(2)	45	F	3,260,000	0.5%	56%(8.9)	0.9	14 : 24	4 weeks	1%	3,280,000	58%(9.5)	0.9	14:24	+ 20,000	-	+2%

Chart D cotinued.

Case No.	Group	Age	Sex	BLOOD PICTURE BEFORE TREATMENT. Red cell count.	Retic ct.	H.globin. % :grams.	C.I.	B.S.R. 1 : 2.	Duration of treatment.	BLOOD PICTURE AFTER TREATMENT. Ret. ct.	Red cell count.	H.globin % :grams.	C.I.	B.S.R. 1 : 2	RESULT. Red cell count.	Ret. ct.	Haemo-globin.
22.	III(19)	31	F.	3,875,000	1.2%	65:10.5.	0.8.	3 : 7.	4 weeks.	1.5%	4,100,000	82:13.2.	1.	2 : 5.	+225,000	---	+17%.
23.	III(20)	60	M.	4,700,000	1%.	85:13.5.	0.9.	2 : 5.	4 weeks.	0.8%	4,500,000	93:14.9.	1.	2 : 5.	-200,000	---	+8%.
24.	III(23)	34	F.	4,430,000	0.8%	67:10.6.	0.8.	6 :15.	6 weeks.	1%.	4,500,000	80:12.7.	0.9	5 :12.	+ 70,000	---	+13%.
25.	III(24)	40	F.	3,600,000	1%	51:8.1.	0.7.	6 :14.	5 weeks.	0.6%.	3,500,000	50:7.9.	0.7.	6 :14.	-100,000	---	-1%.
26.	III(11)	37	F.	3,900,000	1%	40:6.3.	0.5.	14:30.	4 weeks.	0.6%.	3,700,000	38:6.	0.5.	14:30.	-200,000	---	-2%.
27.	IV(20)	27	F.	3,510,000	0.4%	65:10.5.	0.9.	7:17.	4 weeks.	3%	4,300,000	79:12.6	0.9.	7:17.	+790,000	cris	+14%.
28.	IV(22)	16	M.	3,900,000	1%.	68:10.8.	0.8.	10:18.	4 weeks.	1%.	4,000,000	68:10.8	0.8.	10:18.	+100,00	----	----
29.	IV(23)	50	M.	4,220,000	1%.	84:13.4.	1.	6:12.	4 weeks.	1%.	4,250,000	88:14.1	1.	6:12.	+ 30,000	----	+4%.
30.	IV(24)	55	F.	3,900,000	0.5%.	73:11.6.	0.9.	7:16.	4 weeks.	0.6%	3,800,000	70:11.2	0.9.	6:15.	-100,000	----	-3%.
31.	IV(26)	57	F.	4,720,000	1%.	75:11.9.	0.8.	9:20.	4 weeks.	1%.	4,700,000	86:13.8	0.9.	8:16.	- 20,000	----	+11%.
32.	IV(27)	26	F.	3,420,000	1%.	68:11.	1.	10:20.	4 weeks.	0.8%.	3,840,000	70:11.2	0.9.	8:14.	+420,000	----	+2%.
33.	IV(28)	35	F.	4,200,000	0.6%	67:10.8.	0.8.	9:18.	6 weeks.	0.4%	4,500,000	80:12.7	0.9.	8:14.	+300,000	----	+13%.
34.	IV(30)	42	M.	4,265,000	1%.	79:12.6.	0.9.	10:20.	4 weeks.	0.8%	4,250,000	86:13.7	1.	6:10.	- 15,000	----	+7%.
35.	IV(31)	18	F.	3,410,000	0.5%	74:11.9.	1.	8:18.	6 weeks.	3%.	3,900,000	86:13.8	1.1.	7:14.	+490,000	cris	+12%.
36.	IV(32)	25	F.	3,645,000	1%.	79:12.6.	1.	11:25.	4 weeks.	0.8%.	3,500,000	79:12.6	1.	11:25.	-145,000	----	----
37.	IV(33)	20	F.	3,370,000	1%.	74:11.9.	1.1	7:18.	4 weeks.	0.5%	3,600,000	74:11.9	1.	7:13.	+230,000	----	----
38.	IV(34)	30	F.	3,800,000	0.5%	80:12.9.	1.1	10:20.	4 weeks.	0.	4,000,000	79:12.6	1.	8:18.	+200,000	----	-1%.
39.	IV(35)	54	F.	3,700,000	0.2%	80:12.9.	1.1	8:16.	4 weeks.	0.5%	3,800,000	80:12.7	1.	8:15.	+100,000	----	----
40.	IV(29)	51	M.	4,660,000	1%.	84:13.4.	0.9.	3:6.	4 weeks.	1.5%	4,600,000	98:15.6	1.	3:6.	- 60,000	----	+14%.
41.	I(9)	32	F.	4,150,000	1%.	88:14.2.	1.1.	4:10.	4 weeks.	2%.	4,200,000	92:14.6	1.1.	4:10	+ 50,000	----	+4%.
42.	II(8)	19	F.	4,400,000	0.2%	78:12.4.	0.9.	6:12.	6 weeks.	0.4%	4,300,000	88:14.1	1.	5:10.	-100,000	----	+10%.
43.	III(25)	34	M.	4,550,000	0.2%	77:12.3.	0.85	6:11.	4 weeks.	0.4%	4,500,000	93:14.8	1.	5:10	-50,000	----	+16%.

This chart is discussed on the following pages.

(D). The effect of ascorbic acid on the blood picture in 43 cases.

We shall deal separately with the sexes.

(1). Females. ---- 29 cases.

(a) Group I ----- 2 cases.

i. The red cell count is insignificantly raised in both cases.

Average red cell count before treatment ---- 4,180,000/c.mm.

Average red cell increase ------------------- 120,000/c.mm.

Average red cell count after treatment ----- 4,300,000/c.mm.

ii. The haemoglobin level. In 1 case there is no alteration, in the other there is an increase of 4%.

Average haemoglobin before treatment ------- 86.5%.

Extremes of haemoglobin before treatment --- 85% -- 88%.

Average haemoglobin increase --------------- 2%.

Maximum haemoglobin increase --------------- 4%.

Extremes of haemoglobin after treatment ---- 85% -- 92%.

Average haemoglobin after ascorbic acid ---- 88.5%.

(b) Group II.---- 5 cases.

i. The red cell count shows no alteration in 1 case, an insignificant fall in 2 cases, an insignificant increase in 1 case and a significant increase of 6% in 1 case.

Average red cell count before ascorbic acid --- 4,332,000/c.mm.

Average red cell increase ---------------------- 39,000/c.mm.

Average red cell count after asc. acid -------- 4,371,000/c.mm.

ii. The haemoglobin level shows no alteration in 1 case, a significant fall in 1 case, an insignificant increase in 1 case and significant increases in 2 cases (10% & 9%).

Average haemoglobin before ascorbic acid --- 80.4%.

Extremes of Hb. before ascorbic acid ------- 78% -- 83%.

Average Hb. increase ---------------------- 2.6%.

Maximum Hb. increase ---------------------- 10%.

Extremes of Hb. after ascorbic acid -------- 75% -- 88%.

Average Hb. after ascorbic acid ------------ 83%.

(c). Group III ---- 12 cases.

i. The red cell count shows a significant increase in 4 cases, an insignificant increase in 3 cases and an insignificant fall in 5 cases. The greatest increase is one of 850,000 per c.mm., preceded by a reticulocytosis of 3.5%.

Average red cell count before ascorbic acid -- 3,811,000/c.mm.

Average red cell increase --------------------- 151,000/c.mm.

Average red cell count after ascorbic acid --- 3,962,000/c.mm.

ii. The haemoglobin level shows an insignificant increase in 2 cases, a significant increase in 7 cases, an insignificant fall in 2 cases and a significant fall in 1 case. The greatest increase is one of 19% in case No. 19, and the greatest fall is one of 9% in case No. 14.

Average Hb. before asc. acid --------------- 64.6%.

Extremes of Hb. before asc. acid ----------- 40% -- 74%.

Average Hb. increase ---------------------- 6.5%.

Maximum Hb. increase ---------------------- 19%.

Extremes of Hb. after asc. acid ------------ 30% -- 82%.

Average Hb. after ascorbic acid ----------- 71.1%

(d) Group IV.---- 10 cases.

i. The red cell count is significantly raised in 4 cases, insignificantly raised in 3 cases, and insignificantly diminished in the remaining 3 cases.

The maximum increase is noted in case No. 27, there being a reticulocytosis of 3% followed by a rise in the red cell count of 790,000/c.mm. blood.

Average red cell count before ascorbic acid --- 3,767,000/c.mm.
Average red cell increase ---------------------- 227,000/c.mm.
Average red cell count after ascorbic acid ---- 3,994,000/c.mm.

ii. The haemoglobin level is significantly increased in 4 cases, insignificantly increased in 1 case, insignificantly diminished in 2 cases and unaltered in the 3 remaining cases.

The maximum increase noted is one of 14% in case No. 27.

Average Hb. level before ascorbic acid ---- 73.5%.
Extremes of Hb. before ascorbic acid ------ 65% -- 80%.
Average Hb. increase ---------------------- 4.8%.
Maximum Hb. increase ---------------------- 14%.
Extremes of Hb. after ascorbic acid ------- 70% -- 86%.
Average Hb. after ascorbic acid ----------- 78.3%.

(2) Males.---- 14 cases.

(a). Group I. ---- 7 cases.

i. The red cell count is insignificantly diminished in 3 cases, insignificantly raised in 3 cases, and significantly raised in 1 case, viz. case No. 8 which shows a reticulocytosis of 4%.

Average red cell count before ascorbic acid --- 4,861,000/c.mm.
Average red cell increase ---------------------- 39,000/c.mm.
Average red cell count after ascorbic acid ------4,900,000/c.mm.

<u>ii. The haemoglobin level</u> is unaltered in 3 cases, insignificantly increased in 3 cases and insignificantly diminished in 1 case.

Average Hb. before ascorbic acid ---------- 93%.
Extremes of Hb. before ascorbic acid ------ 90% -- 101%.
Average Hb. increase --------------------- 0.3%.
Maximum Hb. increase --------------------- 2%.
Extremes of Hb. after ascorbic acid ------- 90% -- 103%.
Average Hb. after ascorbic acid ----------- 93%.

(b). Group III --- 2 cases.
<u>i. The red cell count.</u> There is an insignificant fall in both cases.
Average red cell count before asc. acid --- 4,625,000/c.mm.
Average red cell <u>decrease</u> ------------------ 125,000/c.mm.
Average red cell count after ascorbic acid -4,500,000/c.mm.

<u>ii. The haemoglobin level</u>
Average Hb. level before ascorbic acid ---- 81%.
Extremes of Hb. levels before asc. acid --- 77% -- 85%.

Average Hb. increase ------------- 12%.

Maximum Hb. increase ------------- 16%.

Extremes of Hb. after ascorbic acid ---- 93% -- 93%.

Average Hb. after ascorbic acid -------- 93%.

(c). Group IV. --- 5 cases.

<u>i. The red cell count</u> is significantly increased in 1 case (No. 15), insignificantly increased in 2 cases and insignificantly diminished in the remaining 2 cases. The greatest increase is recorded in case No. 15, the red cell count being <u>raised by 720,000/c.mm.</u>, no actual reticulocytosis being recorded at the end of 7 days.

Average red cell count before ascorbic acid -- 4,181,000/c.mm.

Average red cell increase --------------------- 155,000/c.mm.

Average red cell count after ascorbic acid --- 4,336,000/c.mm.

<u>ii. The haemoglobin level</u> is significantly increased in 3 cases, insignificantly increased in 1 case and unchanged in the remaining case.

Average Hb. before ascorbic acid ----------- 79%.

Extremes of Hb. before asc. acid ----------- 68% -- 84%.

Maximum Hb. increase ---------------------- 14%.

Average Hb. increase ---------------------- 8.6%.

Extremes of Hb. after ascorbic acid -------- 68% -- 98%.

Average Hb. after ascorbic acid ------------ 87.6%.

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Let us summarise our findings on these 43 cases treated by ascorbic acid .

<u>(1). Females.</u>

(a). In the 29 females examined there was a definite, though not high, reticulocytosis followed by a marked increase in the red cell count in 4 cases; 2 of the cases showing the reticulocytosis were vitamin C "undernourished", the other 2 being apparently "well-nourished". There was an <u>average</u> red cell increase in Group III (undernourished) cases amounting to 3% (approx). In Group IV the <u>average</u> increase amounted to 5%.

(b). In the same 29 cases the haemoglobin level was substantially increased in 13 instances. The individual increases were as follows ---

5%; 6%; 9%; 10%; 11%; 12%; 12%; 13%; 13%; 14%; 14%; 17%; 19%.

An increase was found in each Group excepting Group I, in which Group there was a significant rise in neither a single case nor in the group of cases. In the other Groups there was a noticeable average increase in the haemoglobin level

viz. Group II. --- increase 2.6%.
Group III. -- increase 6.5%.
Group IV. --- increase 4.8%.

(c). Final average red cell count -------- 4,067,000/c.mm.

Final average haemoglobin ----------- 77%.

(2). Males.

(a). In the 14 males examined there was a reticulocytosis of 4% in 1 case, which was followed by an increase in the red cell count amounting to 400,000/c.mm.. Over the 14 cases there was no significant alteration in the average red cell count.

(b). In the same 14 cases the haemoglobin level was substantially raised in 5 instances. The individual increases were as follows ---

7%; 8%; 13%; 14%; 16%.

There was no significant average increase in Groups I or II, but in Group III there was an average increase of 12% (this Group had only 2 cases, unfortunately), and in Group IV an average increase of 8.6%.

(c). Final average red cell count --------- 4,641,000/c.mm.

Final average haemoglobin ------------ 91%.

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CHART E.

DETAILS OF THE OBSERVED RESULTS OF MASSIVE IRON THERAPY ON THE BLOOD PICTURE IN 13 CASES PREVIOUSLY TREATED TO THE LIMIT OF THEIR IMPROVEMENT BY ASCORBIC ACID.

All cases test-dosed and found to be saturated before starting iron therapy. Ferri et Ammon. Cit. gr.xl t.d.s.administered to each case.

				BLOOD PICTURE BEFORE TREATMENT						BLOOD PICTURE AFTER TREATMENT					RESULT		
Case No.	Original Group	Age	Sex	Red cell count.	Retic count	H.globin % : grams	C.I.	B.S.R. 1 : 2.	Dur'n of treat't.	Ret ct.	Red cell count	H.globin. % : grams	C.I.	B.S.R. 1 : 2	R.B.C.	Ret.	H.G.
1.	II(3)	53	F.	4,500,000	0.5%	82%(13.1)	0.9	6 : 12	4 weeks	0.5%	4,500,000	83%(13.2)	0.9	6 : 12	-	-	+ 1%
2.	II(4)	55	F.	4,395,000	1%	83%(13.2)	1	8 : 20	4 weeks	0.8%	4,500,000	91%(14.5)	1	7 : 17	+ 105,000	-	+ 8%
3.	II(7)	32	F.	4,250,000	0.4%	75%(12)	0.9	12 : 22	4 weeks	0.5%	4,200,000	86%(13.7)	1	10 : 20	- 50,000	-	+ 11%
4.	III(19)	31	F.	4,100,000	1.5%	82%(13.1)	1	2 : 5	4 weeks	0.8%	4,200,000	83%(13.2)	1	2 : 5	+ 100,000	-	+ 1%
5.	III(13)	47	F.	3,900,000	0.4%	65%(10.3)	0.8	10 : 21	8 weeks	1%	4,300,000	84%(13.4)	1	8 : 17	+ 400,000	-	+ 19%
6.	III(18)	39	F.	4,000,000	1.5%	82%(13.1)	1	8 : 14	4 weeks	1%	4,000,000	85%(13.6)	1	8 : 14	-	-	+ 3%
7.	III(24)	40	F.	3,500,000	0.6%	50%(7.9)	0.7	6 : 14	8 weeks	1.8%	4,300,000	81%(12.9)	1	5 : 12	+ 800,000	-	+ 31%
8.	III(23)	34	F.	4,500,000	1%	80%(12.7)	0.9	5 : 12	4 weeks	0	4,500,000	80%(12.7)	0.9	5 : 12	-	-	-
9.	IV(32)	25	F.	3,500,000	0.8%	79%(12.6)	1	11 : 25	4 weeks	0.5%	3,720,000	76%(12.1)	1	11 : 25	+ 220,000	-	- 3%
10.	IV(27)	26	F.	3,840,000	0.8%	70%(11.2)	0.9	8 : 14	4 weeks	1.5%	4,250,000	76%(12.1)	0.9	7 : 13	+ 410,000	-	+ 6%
11.	IV(30)	42	M.	4,250,000	0.8%	86%(13.7)	1	6 : 10	4 weeks	1%	4,300,000	87%(13.9)	1	6 : 10	+ 50,000	-	+ 1%
12.	IV(33)	20	F.	3,600,000	0.5%	74%(11.8)	1	7 : 13	4 weeks	0.7%	3,560,000	74%(11.8)	1	7 : 13	- 40,000	-	-
13.	IV(28)	35	F.	4,500,000	0.4%	80%(12.7)	0.9	8 : 14	4 weeks	0	4,500,000	83%(13.2)	0.9	8 : 14	-	-	+ 3%

This chart is discussed on the following pages.

E. The effect of massive doses of iron, after saturation with ascorbic acid, on the blood picture in 13 cases.

We shall deal separately with the sexes.

(1). Females ---- 12 cases.

(a). Group II --- 3 cases.

i. The red cell count. 1 case shows an insignificant rise, 1 shows an insignificant fall and the 3rd. shows no alteration in the red cell count.

Average red cell count before iron ----- 4,382,000/c.mm.

Average red cell increase ----------------- 22,000/c.mm.

Average red cell count after iron ------ 4,404,000/c.mm.

ii. The haemoglobin level.

There is an insignificant increase in 1 case, a significant increase in the other 2 cases.

Average Hb. before iron -------------- 80%.

Extremes of Hb. before iron ----------- 75% -- 82%.

Average increase in Hb. -------------- 7%.

Maximum increase in Hb. (No. 3) ------- 11%.

Extremes of Hb. after iron ------------ 83% -- 91%.

Final average Hb. ---------------------- 87%.

(b). Group III females ----- 5 cases.

i. The red cell count. 2 cases show no alteration; 3 cases show an increase, the increase being insignificant in 1 case and significant in the other 2, viz. 400,000/c.mm. in

Case No. 5, and 800,000/c.mm. in case No. 7.

Average red cell count before iron ---- 4,000,000/c.mm.

Average increase ---------------------- 260,000/c.mm.

Average red cell count after iron ----- 4,260,000/c.mm.

ii. The haemoglobin level.

There is a significant increase in 2 cases, an insignificant increase in another 2 cases and no alteration in the 5th. case.

Average Hb. before iron ------------ 72%.

Extremes of Hb. before iron -------- 50% -- 82%.

Average increase in Hb. ------------ 11%.

Maximum increase in Hb. ------------ 31%.

Extremes of Hb. after iron. -------- 80% -- 85%.

Average Hb. after iron. ------------ 82.6%.

(c). Group IV. females ----- 4 cases.

i. The red cell count. 1 case shows no alteration in the count; the 2nd. shows an insignificant fall; the 3rd. shows an increase of 4% and the 4th. an increase of 8% (case No. 10 --- 410,000/c.mm.)

Average red cell count before iron ---------- 3,860,000/c.mm.

Average increase --------------------------- 160,000/c.mm.

Average red cell count after iron ----------- 4,020,000/c.mm.

ii. The haemoglobin level. 1 case shows an insignificant fall, 1 shows an insignificant rise, one shows no alteration and the 4th. shows a significant rise viz. case No. 10.

Average Hb. before iron ------------ 75.75%.

Extremes of Hb. before iron -------- 70% -- 80%.

Average increase in Hb. ------------ 1.5%.

Maximum increase in Hb. ------------ 6%.

Extremes of Hb. after iron --------- 74% -- 83%.

Average Hb. after iron ------------- 77.25%.

(2). Male ----- 1 case.

This case, No. 11, shows an insignificant increase in both the red cell count and the haemoglobin level.

Let us summarise our findings on these 13 cases treated with iron.

<u>(1). Females.</u>

(a). There was a significant increase in the red cell count in 3 cases, no reticulocyte response being noted on the 7th. day.

(b). There was a significant increase in the haemoglobin level in 5 of the 12 cases.

(c). Final average red cell count ----- 4,125,000/c.mm.

Final average haemoglobin -------- 82%.

<u>(2).Males.</u> ---- single case.

Final red cell count -------------4,300,000/c.mm.

Final Hb.------------------------- 87%.

We may conclude that ascorbic acid is not able to raise the haemoglobin to a normal level when given alone, even in those cases who are suffering from minor degrees of vitamin C subnutrition. These cases appear to require iron as well as ascorbic acid for complete cure. The cause of the anaemia in most of the cases is probably general subnutrition, and is not specifically due to hypovitaminosis C.

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<u>Summary of the more important findings of Chapter IX.</u>

(1). A higher final haemoglobin level was attained by vigorous iron therapy in women possessing apparently adequate vitamin C reserves than was attained in women having inadequate reserves. The final haemoglobin level was, on an average, 4.75% higher in the former than in the latter Group.

(2). Administration of ascorbic acid , mgm. 50 t.d.s by mouth, to 5 "undernourished" women who had reached their "limit of improvement" with iron led to a further rise in the haemoglobin level in 3 of the 5 cases. The individual increases in these 3 cases were 5%, 8%, and 9%, and the average haemoglobin increase over the 5 cases was 4.4%.

(3). Administration of ascorbic acid in the same dosage to

7 apparently well-nourished men, who had previously been treated to the limit of their improvement by iron, led to a reticulocytosis of 3% in 1 case followed by a rise of 920,000/c.mm. in the red cell count. There was an increase in the haemoglobin level in all 7 cases, the increases ranging from 1% to 17%, and averaging 7% over the 7 cases.

(4). Administration of ascorbic acid to 29 previously untreated females led to a reticulocytosis in 4 of the cases. Two of thes e cases were undernourished and two were apparently well-nourished. The reticulocytosis was in all 4 cases followed by a rise in the red cell count. There was a rise of 5% or over in the haemoglobin level in 13 of these 29 cases, the maximum recorded rise being 19%, and the average increase over the 29 cases being 5%.

(5). 14 males, previously untreated, were given ascorbic acid. There was a reticulocytosis of 4% in 1 apparently "well-nourished" case, followed by an increase of 400,000 per c.mm. in the red cell count. There was an increase of 5% or more in the haemoglobin level in 5 of the 14 cases, the maximum recorded rise being 16%, and the average rise in the haemoglobin level over the 14 cases being approximately 5%.

(6). 13 cases were saturated with ascorbic acid over a period of 4 weeks. After completion of this course, administration of iron in large doses brought about a rise in the red cell count in 3 cases and a significant rise in the haemoglobin level in 5 of the 13 cases.

(7). Improvement was effected, both by iron and by ascorbic acid, in the haemoglobin level of cases whose red cell and haemoglobin readings were considered to be within normal limits.

(8). There was no significant difference between (a) the final average red cell count and haemoglobin level in 15 females treated by iron alone and (b) the final figures in 25 cases treated with both iron and ascorbic acid, viz.,

Treatment	No. of cases.	Final average red cell ct.	Final average haemoglobin.
Iron.	15.	4,322,000.	83%.
Ascorbic after iron	9.	4,290,000.	86%.
Ascorbic and iron.	4.	4,372,000.	87%.
Ascorbic. alone.	29.	4,067,000.	77%.
Iron after ascorbic.	12.	4,125,000.	82%.

Treatment.	No. of cases.	Final average red cell count	Final average haemoglobin.
Iron.	15.	4,332,000/c.mm.	83%.
Ascorbic before, after or with iron.	25.	4,224,000/c.mm.	84.2%.

There are not a sufficient number of men from whom to draw conclusions.

Let us now draw a few conclusions from these findings.

(1). Ascorbic acid has a definite place in the treatment of nutritional anaemia at the present time, at least in this part of Great Britain.

(2). The best results from the massive iron treatment of nutritional anaemias are obtained in cases who possess adequate vitamin C reserves.

(3). Administration of ascorbic acid after completion of iron therapy frequently leads to a further increase in the haemoglobin level, often of striking degree. This further improvement not only occurs in vitamin C undernourished cases but in <u>apparently</u> well-nourished cases as well. We may conclude that these cases, while not strictly speaking "undernourished", do not possess the <u>optimum</u> reserve of vitamin C.

(4). The oral administration of ascorbic acid does not appear to have any effect on the red cell count or on the haemoglobin level of patients who have adequate vitamin C reserves and whose red cell count and haemoglobin levels are well within normal limits.

(5). Ascorbic acid should be administered as an adjuvant form of treatment to both "well-nourished" and "under-nourished" cases who suffer from nutritional anaemia. A haemoglobin level markedly higher than that attainable by iron alone is frequently reached by this means. In many of these cases, lack of iron is not the only cause of the anaemia; these patients frequently live on a diet which is grossly deficient in iron, vitamin C and perhaps extrinsic factor, and, depending on the substance which is most sorely missed depends the type of anaemia which developes. It is not surprising, therefore, that some cases suffering from what appears to be a simple iron nutritional anaemia should not respond as fully to massive iron therapy as one would expect, and that the response should be greater when the missing vitamin C is supplied. It is clear that iron by itself is not a complete cure for many of the cases of nutritional deficiency anaemia which we encounter. It therefore behoves us to impress on these patients the importance of a balanced diet in the prevention and treatment of anaemia; and if, for any reason, a restricted diet has to be maintained, it behoves us to supply vitamin C, either as the natural vitamin or as ascorbic acid.

(6). Ascorbic acid, in doses of 150 mgm. daily by mouth, can lead to an <u>increased reticulocyte count</u>, a consequent rise in the red cell count, and a rapid rise in the haemoglobin level in nutritional anaemics, even in those cases who <u>apparently</u> are not suffering from gross hypovitaminosis C. The reticulocytosis may be noted at the end of 7 days treatment. It may be present at an earlier or later phase of treatment, but this aspect of the subject was not closely studied. In a few cases there was a significant increase in the red cell count without a preceding increase in the reticulocyte count being noted; this may be accounted for by the fact that the reticulocyte count was checked only once after treatment had been started, viz. at the end of 7 days.

(7). When ascorbic acid is administered to anaemic subjects, no other form of treatment being employed, the haemoglobin level may be raised by as much as 19% in suitable cases. (We are not, of course, considering its effect on fully developed scorbutic anaemia).

(8). Ascorbic acid treatment is not a substitute for the iron treatment of nutritional anaemia; but it may enhance the effect of iron and lead to a higher final haemoglobin level than would have been attained by the use of iron alone.

(9). We note that the average red cell and haemoglobin figures are essentially the same in 2 groups of cases after they had been saturated with iron and with iron plus ascorbic acid respectively. A superficial analysis such as this might lead one to suppose ascorbic acid to be of no value in the treatment of the cases of nutritional anaemia here encountered. None of the cases treated by iron alone were, however, grossly undernourished, the lowest excretion being 11.5 mgm. per day; and even so there was a better response to iron from the well-nourished than from the undernourished cases. The incontrovertible fact remains that individual cases were found who showed marked and unmistakable responses to ascorbic acid.

(10). We should not accept a red cell count and haemoglobin level which lie within normal limits as being of necessity the <u>optimum</u> level for that particular case. A rise in one or other level may be obtainable by appropriate and adequate treatment.

(11). Although ascorbic acid appears to be a regulator both of the haemoglobin and red cell levels, its effect on the Haemoglobin level seems to be the more pronounced and the more constant, at least in the milder degrees of subnutrition with which we are here concerned.

(12). These experiments were carried out with ascorbic acid, a synthetic substitute for the naturally occurring vitamin C. If such results are obtained using the synthetic product, how much better might the results be from a better balanced diet and a more adequate supply of the vitamin in natural form.

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Reference.

Keefer C.S. and Yang C.S., 1929, Journ. Amer. Med. Assoc., 93., 575.

General Summary

and

Conclusions.

We embarked on this investigation in the 4th. year of the present war with the objects of discovering whether the subscorbutic state was prevalent in this part of North-East England; whether this state was in any way responsible for the refractory nature of some of the cases of nutritional anaemia here encountered; whether there was a blood picture which, by itself, was diagnostic or at least suggestive of vitamin C subnutrition; and whether oral administration of ascorbic acid was of value in the treatment of these nutritional anaemias.

Other workers have found evidence both of gross scurvy and of lesser degrees of subnutrition among the populations of Great Britain, America, Germany, France, Scandinavia, Africa and Australia, to mention but a few; and this subnutrition, in the experience of these workers, is not always a disease of the poorer classes. Orr, in 1936, however, found an inadequate intake of the vitamin in more than half of the population, and in his opinion this inadequate intake was directly related to inadequate income. Instances are cited of the occurrence of subnutrition in people whose diet is restricted from choice, from necessity, because of gastro-intestinal disease or disorder or because of some other restricting factor; and even of its occurrence in people whose economic status and general physical condition would presuppose both an adequate supply and an adequate intake of the vitamin. The incidence of scurvy appears to rise steeply in wartime --- Harris has found an increased incidence of the minor degrees of subnutrition in both middle- and poor-class children during the present conflict, and the incidence of frank scurvy during the last Great War was definitely increased. Until comparatively recently we have had no easy biochemical method of detecting subscorbutism and so the exact extent of minor degrees of subnutrition during the course of the 1914-1918 war is not known. But wherever one finds an increased incidence of sporadic cases of frank scurvy, one must expect a still greater increase in the incidence of subclinical cases; and vice versa, where we find a great increase in the number of subclinical cases, we have a greater number, or at least a potentially greater number, of fully developed cases. We now have a relatively easy method of detecting subscorbutism viz. estimation of the amount of vitamin C excreted in the urine in 24 hours and the response of this excretion level to a provocative- or test-dose of ascorbic acid.

In an attempt to estimate the vitamin C nutritional level of the people in this area, and indirectly to estimate their vitamin C intake, 100 adults were selected for investigation. Only cases whose nutritional state could be directly related to their dietary intake, both immediate and remote, were admitted to the series. We have explained how the vitamin C nutritional state of any subject depends on various factors, viz. (1) the intake of the vitamin, both immediate and remote, (2) the efficiency of absorption from the gut, (3) the state of health and the general metabolic level of the patient and (4) the ability of the kidney to retain vitamin C in the body. Therefore, any case who, on clinical or other grounds, was suspected to be suffering from any condition which might lead to inefficient absorption, excessive metabolism and destruction, or excessive renal excretion due to a low renal threshold was considered completely unsuitable for our present purposes and was discarded. In addition to the routine clinical examination the white cell count and the blood sedimentation rate of each case was assessed and so unsuitable cases who might happen to pass the clinical examination were weeded out.

The vitamin C nutritional state of these 100 cases was assessed by measuring the amount of vitamin C excreted in the urine in 24 hours (this should not be less, and is usually considerably more than 13 mgm. per day); and, by the subsequent test-dosing of each subject a more accurate estimate of the state of the vitamin C reserves was obtained. The evidence of the test-dose was in all cases accepted as the true index of the nutritional level of the subject. We have indicated how unstable is vitamin C and how precautions to ensure its adequate preservation must be taken unless the urine is examined immediately after voiding.

The expressions "well-nourished" and "ill-or under-nourished" frequently appear in the text. Unless stated otherwise these have to be taken as indicating the vitamin C nutritional state only and are in no way indicative of the general nutritional state of the subject.

We found that, in this series of 100 adults, 62% were adequately nourished, the remaining 38% being in what one might term the "subscorbutic" or "prescorbutic" state i.e. a borderline state of not quite adequate nutrition, unaccompanied by any gross clinical manifestations. This subscorbutic state was found in both middle and working-class patients; it was very infrequent in males and was then due to voluntary restriction of diet. The amount of vitamin C excreted in the urine was, on the average, higher in the male than in the female.

More than half (56%) of the females examined were found to be undernourished; the majority of these undernourished cases were spinsters, widows and married housewives, especially those whose husbands were living or serving away from home. In most of the cases the initial dietary history was a reliable guide to the nutritional state of the subject, a satisfactory history being associated with a satisfactory nutritional state, an unsatisfactory history being associated with an unsatisfactory state. But some of the less uneducated patients would not admit to existing on a diet which they well knew to be ill-balanced. We also found that an estimation of the vitamin C content of a 24 hour specimen of urine was a true guide to the nutritional state of the subject in the majority (88%) of the cases, but that, where the amount excreted in the urine in 24 hours was in the region of 13 mgm. per day, it was necessary to obtain the confirmatory evidence of the test-dose before deciding whether the case was adequately nourished. We would suggest that test-dosing is essential before giving an opinion on any case whose excretion rate is within plus or minus 3 mgm. of 13 mgm. per day; i.e. if a case excretes 16 mgm. vitamin C or over per day, without administration of a provocative dose, that case is well-nourished; if he excretes less than 10 mgm. per day, no toxic, infective or other interfering factor being present, then he would appear to be suffering from subnutrition; if, however, he excretes between 10 and 16 mgm. per day, administration of 1 or 2 test-doses will decide whether he is well- or ill-nourished.

Contrary to the findings of other workers, no evidence of a higher incidence of gastro-intestinal disease or disorder was found among the undernourished than among the well-nourished cases, nor did dental caries or pyorrhoea alveolaris appear to be unduly prevalent in the subscorbutic cases. The only case found to have definite sponginess of the gums was, however, inadequately nourished.

The degree of subnutrition in the present series did not appear to be very severe. The causes of the deficient intake varied from case to case; in one of the undernourished men the diet had been restricted because of his fear of raising his blood pressure; in another man the diet had been restricted because of his fear that a more liberal diet might cause a recurrence of his peptic ulcer. In women, ignorance or apathy were the main causes for the ingestion of an ill-balanced diet. Some of the women realised little or nothing of the nutritional value of fresh fruit and vegetables; a few definitely disliked garden produce in any form; the remainder simply did not take the trouble to procure an adequate diet, a large part of their intake consisting of bread and butter, margarine, or jam. The potato, though widely advertised as being a better source of nourishment than bread often went neglected. Poverty did not appear to be the cause of inadequate vitamin C intake in a single case. We may conclude that, although sources of vitamin C were at all times available, these sources were not always of the kind to which people had become accustomed; fruit was in short supply, and the lack of this commodity was not made good by an increased intake of vegetables, salads, potatoes etc. in every house. As a result of this diminished intake over a period of several years the vitamin C reserves became steadily less and a higher incidence of mild or moderate degrees of subnutrition made their appearance.

We have seen that a significant proportion of this series suffered from vitamin C subnutrition, a grim reminder that fully fledged scurvy is always in the background, and is not essentially a disease of polar explorers or of ship-bound mariners. We must remember that both the incidence and the degree of subnutrition may become greater as the war drags on. Any febrile condition, by increasing the daily requirement of the vitamin, is liable to aggravate a subscorbutic state and precipitate an attack of frank scurvy. Bearing in mind the prevalence of subnutrition, we must guard against this possible contingency by administering vitamin C,or the synthetic ascorbic acid, during the course of any feverish illness of some duration.

As regards the relationship between vitamin C nutrition and the blood picture, the findings and conclusions of many workers have been elaborated in Chapter VI. We do not propose to delay the reader by mere repetition of the contents of that chapter. Suffice it to say that the general conclusion of these various workers is that anaemia, while a common feature in scurvy, is by no means a constant one. Most of the workers give us the following description of the blood in scorbutic anaemia, viz.,--The anaemia may be of any degree of severity. While it is usually orthochromic and normocytic, it may be megalocytic if the degree of subnutrition is marked, or microcytic if haemorrhages are severe; reticulocytosis is a feature, being more marked in severe degrees of subnutrition. Anisocytosis, poikilocytosis and red cell vacuolation may be very noticeable, while megalocytes and nucleated red blood cells may occasionally appear in the peripheral circulation. The cause of the anaemia is generally considered to be a variable degree of bone marrow damage; subnutrition and haemorrhage, if severe , are considered to play but subsidiary parts.

Weill and Mouriquand (1922) described the occurrence of prescorbutic anaemia; Rohmer and Bindschedler (1932) found that 6 of 22 anaemic infants were refractory to iron but responded when vitamin C was also administered. The anaemia of the prescorbutic state appears to be of similar type to the fully developed scorbutic anaemia. Croft and Snorf (1939) and Scott Thompson, Glazebrook and Millar (1942) however, deny any connection between mild degrees of vitamin C subnutrition and anaemia.

Apart from the investigations of these few workers, no serious attempt seems to have been made to investigate the possible relationship between minor degrees of vitamin C subnutrition and anaemia, on anything approaching scientific lines. We have, of course, had numerous "impressions" of the value of vitamin C in the treatment of nutritional anaemia, and frequent claims made on totally inadequate data. Adequate attention has been paid to the gross manifestations of scurvy, but the minor degrees of subnutrition have not commanded the attention which they deserve. The interest raised by, and the amount of work done on , fully blown cases of scorbutic anaemia have, perhaps naturally, diverted the attention of most workers from subscorbutic anaemia, a less dramatic, but none the less important disability. Similarly, very little attention has been paid to the minor degrees of nutritional anaemia. How often do we accept a haemoglobin level of 90% (Sahli) in a man as being a normal reading when, with appropriate treatment, his level cound be raised to 100% or over. We may here re-iterate what has already been stated in this paper --- that the clinical improvement in these cases which is encompassed by appropriate treatment usually greatly outpaces the relatively small improvement noted in the haemoglobin level.

In the present survey 87 of the 100 cases whose vitamin C nutritional level had been assessed were subjected to a blood examination. In each of these 87 cases a red cell count, reticulocyte count, haemoglobin estimation and examination of the stained blood film were made. The white cell count and the blood sedimentation rate were also estimated in each case as a check on the clinical examination.

There was a careful selection of patients in an attempt to exclude anaemias other than those of nutritional origin.

Arbitrary lower limits of normality for the red cell count and haemoglobin level in both males and females were laid down, and any case whose red cell count, or haemoglobin level, or both, fell short of these lower limits was considered, for the purposes of this investigation, to be anaemic.

We thus obtained records of both the vitamin C nutritional level and the blood picture in each of 87 cases. Was there any relationship between the two.? Had vitamin C subnutrition any significant effect on the red cell count, haemoglobin level or reticulocyte count; or were there any typical alterations in the stained films of the subscorbutic cases ?. These were the questions raised; we have given details of the system of "grouping" employed in an attempt to answer these queries.

We found that, according to our standards, 61 of the 87 cases examined had some degree of anaemia. We also found that marked diminution of vitamin C excretion and a satisfactory haemoglobin level did not go together. It may be argued that <u>general undernutrition</u> may cause both a diminished iron and a diminished vitamin C intake, and that the iron diminution alone might cause the nutritional anaemia, the diminution in the vitamin C being merely incidental. This being the case, we should find no significant differences between the anaemias of the vitamin C well-nourished and the vitamin C undernourished groups; nor should there be a supplementary response to ascorbic acid. But we do find supplementary responses and we do find significant differences. The average haemoglobin level and colour index of the prescorbutic group of cases is significantly lower than the average haemoglobin level and colour index of the well-nourished group of cases. There does not appear to be any significant difference between the red cell count and the reticulocyte count of the 2 groups, however. From a careful analysis of the results it was concluded that anaemia is frequently, though not constantly, associated with the prescorbutic state in adults; that, while the anaemia may be orthochromic, it is more usually slightly hypochromic, the haemoglobin level falling proportionately more than the red cell count; that reticulocytosis is not a feature in the untreated case; that the stained film may show anisocytosis, or poikilocytosis, or megalocytosis, or central pallor, or any combination of these in any degree of severity; or, on the other hand, no gross abnormality of the stained film may be noted.

These findings are in general agreement with the reports of other workers on scorbutic anaemia. In only one particular is there a marked discrepancy, viz. over the question of the reticulocyte count. In no case in this present series was a reticulocytosis noted. This may be explained by the fact that the reticulocytosis of untreated scorbutic anaemia increases with increasing severity of the subnutrition; and none of the cases in the present series could be termed severely undernourished.

Some critics may object to the use of a Sahli haemoglobinometer. In our opinion the acid haematin method, in addition to being simple, is also reliable, provided exactly the same technique is employed on every occasion. An easy blood flow, rapid and accurate measurement of the blood and thorough mixing need no emphasis. But points on which emphasis might be laid are that, for comparative purposes, the period of day ought to be the same in each case, the technician ought to be the same one in each case, and the tube, comparator and pipette employed ought to be the same ones on every occasion. Inaccuracies have been ascribed to a method which should, in many cases, have been ascribed to the technician. Perhaps one of the greatest sources of inaccuracy is the variability of the time allowed for action of the acid on the blood before addition of the distilled water. If exactly the same time is allowed on every occasion the results are more constant. In this series exactly 30 minutes was allowed. Unfortunately, the Sahli haemoglobinometer is not yet checked for accuracy by the National Physical Laboratory, but any slight error in calibration of the tube affects every case to the same degree and does not affect conclusions in comparative work. The tube, pipette and comparator employed for making the haemoglobin estimations in this series of cases were checked for clinical accuracy in the Pathology department of the Sunderland Royal Infirmary; the number of grams of haemoglobin in a blood were estimated simultaneously by the Sahli haemoglobinometer and a Haldane haemoglobinometer which had previously been standardised by the National Physical Laboratory.

The last question which we had set out to answer was "Has ascorbic acid therapy any effect on these nutritional anaemias, when used alone or in combination with other haematinics" ?. In the course of the investigation ascorbic acid was administered to cases before, during and after massive iron therapy. It was witheld from some of the cases. We found that a higher final haemoglobin level was attained by vigorous iron therapy in those women who had adequate reserves than in those who had inadequate reserves, being on an average 4.75% higher in the former than in the latter group of cases. Ascorbic acid was found to exert a very definite effect on the haemoglobin level in both under-nourished and <u>apparently</u> well-nourished cases, the average increase being considerable and the increase in individual cases being at times very marked. The effect on the red cell count was not so striking, although a raised reticulocyte count followed by an increase in the red cell count was noted in a few cases during the course of ascorbic acid treatment. Let us now very briefly tabulate our more important findings and conclusions.

A. The vitamin C nutritional level of the 100 cases examined, with notes on incidence and other points of interest.

(1). 62% were found to be satisfactorily nourished, 38% to be undernourished.

(2). The subscorbutic state was found in both middle- and working-class patients.

(3). Only 3 males were found to be undernourished (i.e. less than 8% of the males), and the subnutrition in these cases was due to a voluntarily restricted diet in 2 of the cases and was probably due to arteriosclerotic malabsorption in the 3rd. case.

(4). Of the 62 females examined, 35 (i.e. 56%) were found to be undernourished.

(5). Spinsters and widows were found to be more often undernourished than well-nourished. Subnutrition was found to be especially common among housewives, especially among those whose husbands were living away from home.

(6). The dietetic history was a reliable guide to the nutritional state of the subject in only 83% of the cases.

(7). The impression of the nutritional state of the subject gained by estimating the resting excretion rate of vitamin C was correct in 88% of the cases. In the other 12% the resting excretion level was on or near the borderline figure of 13 mgm. per day.

(8). The average rate of excretion of vitamin C in the urine was higher in the males than in the females.

(9). There was no evidence of a higher incidence of gastro-intestinal disease or disorder among the undernourished cases than among the well-nourished cases.

(10). There was no evidence of a higher incidence of dental caries or pyorrhoea alveolaris in the undernourished group; this group, however, contained the only case found to have spongy gums.

B. The relationship between minor degrees of vitamin C subnutrition and the blood picture; and the incidence of nutritional anaemia in 87 consecutive cases.

(1). Of the 87 cases examined, 61 had some degree of anaemia according to our accepted standards.

(2). Vitamin C subnutrition was infrequently found in the anaemic men (2 cases), but was of frequent occurrence in the anaemic women (32 cases).

(3). No case possessing a grossly diminished vitamin C excretion rate was noted as having a haemoglobin level within normal limits.

(4). The average red cell and reticulocyte counts were approximately the same in the well-nourished and in the under-nourished groups.

(5). The average haemoglobin level and colour index were lower in the undernourished group than in the well-nourished group.

(6). The subscorbutic state was not constantly associated with a typical abnormality of the stained blood film. The film may show no obvious abnormality: or, if it does so, the abnormality is likely to take the form of a varying degree of anisocytosis and poikilocytosis; in some cases megalocytes may be seen, with or without central pallor

(7). Minor degrees of subnutrition were not constantly associated with anaemia. There was an obvious degree of anaemia in 74% of the undernourished cases.

C. The effect of oral administration of ascorbic acid on the blood picture.

(1). The final haemoglobin level attained after vigorous iron therapy was 4.75% higher in women with adequate vitamin C reserves than in women with inadequate reserves.

(2). Administration of ascorbic acid, mgm. 50 t.d.s. orally, to 5 "undernourished" women who had reached the limit of improvement under vigorous iron treatment, led to a rise in the haemoglobin level in 3 out of the 5 cases. The individual increases in these 3 cases were 5%, 8%, and 9%, and the average haemoglobin increase over the 5 cases was 4.4%.

(3). Administration of ascorbic acid in the same dosage to

7 <u>apparently</u> well-nourished men, who had previously been treated to the limit of their improvement with iron, led to a reticulocytosis of 3% in 1 case, followed by a rise in the red cell count; the red cell increase in this case was 920,000/c.mm. at the end of 4 weeks treatment. There was a rise in the haemoglobin level in all 7 cases, the increases ranging from 1% to 17%, and averaging 7% over the 7 cases.

(4). Administration of ascorbic acid to 29 previously untreated females led to a reticulocytosis in 4 of the cases. 2 of the cases were "undernourished" and 2 were apparently "well-nourished". The reticulocytosis was in all 4 cases followed by a rise in the red cell count. There was a rise of 5% or over in the haemoglobin level in 13 of these 29 cases, the maximum recorded rise being 19%, and the average increase over the 29 cases was 5%.

(5). 14 males, previously untreated, were given ascorbic acid. There was a reticulocytosis of 4% in 1 <u>apparently</u> "well-nourished" case, followed by a rise of 400,000/c.mm. in the red cell count. There was a rise of 5% or over in the haemoglobin level in 5 of the 14 cases, the maximum recorded rise being 16%, and the average rise in the haemoglobin level over the 14 cases was approximately 5%.

(6). 13 cases were saturated with ascorbic acid over a period of 4 weeks. After completion of this course, administration of iron in large doses brought about a rise in the haemoglobin level in 5 of the 13 cases, of significant

degree as well as a rise in the red cell count in 3 cases..
(7). Improvement was effected, both by iron and by ascorbic acid, in the haemoglobin level of cases whose red cell and haemoglobin readings were considered to be within normal limits.

We may conclude this paper by stating that
(1). In this part of North-East England, vitamin C subnutrition is widespread, especially among the married women whose husbands are living away from home, and among spinsters and widows. The degree of subnutrition does not appear to be severe. None of the cases need have fallen into this undernourished state, apathy being the cause in most cases, ignorance in a few.
(2). Nutritional anaemia, of varying degrees of severity, is extremely prevalent in this area.
(3) Anaemia is frequently, though not constantly, associated with the subscorbutic state in adults.
(4). No rigid law can be formulated to indicate which cases of nutritional anaemia either require or are likely to benefit from ascorbic acid.
(5). There is no blood picture diagnostic of the milder degrees of vitamin C subnutrition. Subscorbutic anaemia may be orthochromic, but is more usually slightly hypochromic, the haemoglobin level falling proportionately more than the red cell count.

(6). There is no reticulocytosis in the untreated case.

(7). The blood film in the subscorbutic state may show anisocytosis, or poikilocytosis, or megalocytosis, or central pallor, or any combination of these in any degree of severity.

(8). Ascorbic acid has a definite place in the treatment of nutritional anaemia at the present time, at least in this part of Great Britain.

(9). The subscorbutic state is responsible for the refractory nature of some of the cases of nutritional anaemia here encountered.

Pills and medicines are but poor substitutes for soundly balanced, properly cooked meals and we believe that more intensive dietetic education will, in time, lead to a virtual disappearance of the subscorbutic state from this area.

Finally, we wish to acknowledge our indebtedness to the many patients whose willing cooperation, often under difficult circumstances, made this investigation possible; to Mr. L. Haugh, of the Pathology Department, Sunderland Royal Infirmary, for technical advice on the various methods of preserving and estimating the urinary vitamin C; and especially to H.A.Cookson F.R.C.P.(E), F.R.C.S.(E), F.R.S.(E), Pathologist to Sunderland Royal Infirmary, who checked the accuracy of the tube, pipette and comparator employed in

making the haemoglobin estimations.