VITAMIN C NUTRITION.

John B. Macdonald, M. B., Ch.B.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

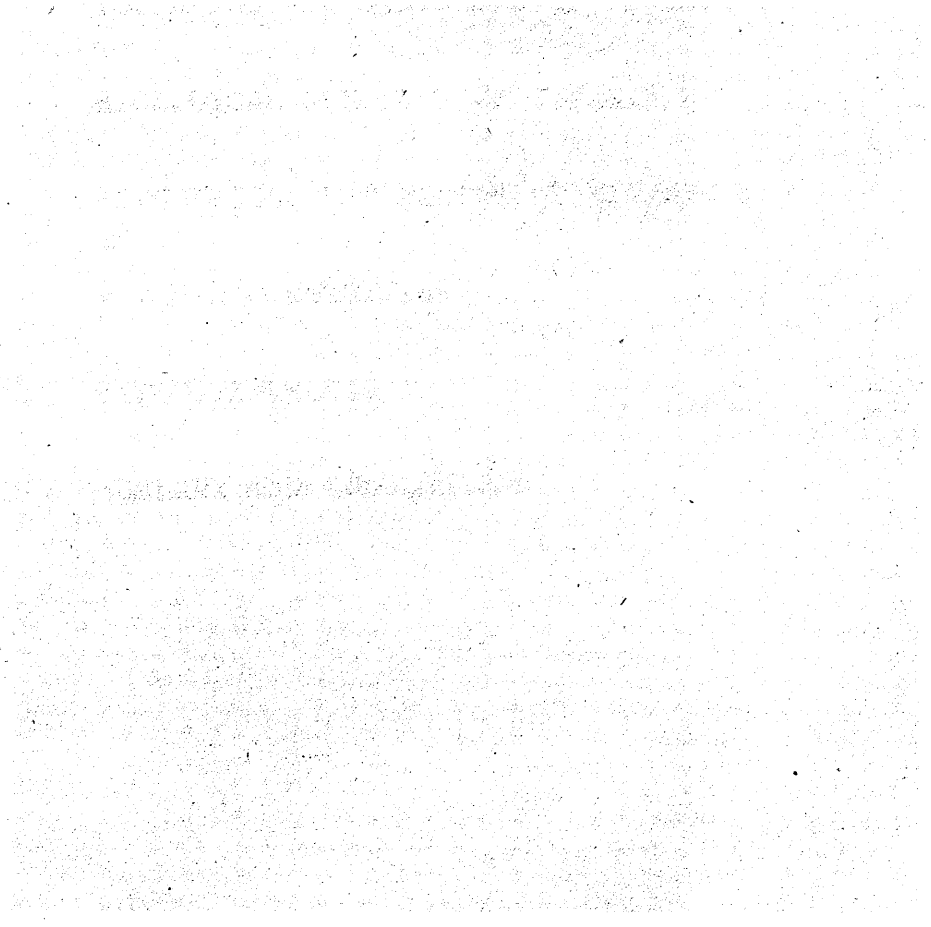
Introduction.

Throughout the time I have spent on this work I have been struck by one outstanding fact - namely that scurvy is still the only disease-entity which can be definitely ascribed to lack of vitamin C.

In contrast, the immense amount of research carried out since the isolation and synthesis of ascorbic acid, in a praiseworthy effort to associate its deficiency with some constant clinical symptom or syndrome, has led merely to the recognition of an altered bodily state denoted by the term hypovitaminosis C. The greatest benefit however must accrue if profession and layman alike are led to realise the prime importance of defective nutrition, especially in childhood, and to take a healthy interest in the physiological requirements of the human body.

The object of this paper is to give a survey of the stages/

stages of Vitamin C research, the results of some investigations carried out personally, and finally to summarise what, in my opinion, are the important factors related to the clinical use of Vitamin C.



Discussion will be under the following headings:-

I. SCURVY

II. THE NEGATIVE FACTOR

III. ISOLATION AND SYNTHESIS OF VITAMIN C

IV. BIOLOGICAL FUNCTION OF VITAMIN C

V. BIOCHEMICAL ESTIMATION OF VITAMIN C

VI. VITAMIN C NUTRITION

VII. HYPOVITAMINOSIS

VIII. SUMMARY AND CONCLUSIONS.

I. SCURVY.

Several centuries have elapsed since scurvy was first recognised as a disease which tended to occur especially in sea-faring men. Many theories were held as to its etiology.

In 1651, Glisson[1] described a disease occurring in infants and he recognised this as being identical in nature with cases of adult scurvy. Bachstrom[2] in 1734 pointed out, while reviewing the numerous theories as to the cause of scurvy, that abstinence from fresh vegetable food and greens was the only true etiological factor. Lind, working at about the same time, performed an experiment on board the "Salisbury" and arrived at the same conclusion. Ingerslev[3] and Cheadle[4] again emphasised the real cause of the disease but it was not generally accepted until Barlow[5] in 1883 published his classical description of the essential symptomatology and etiology of infantile scurvy.

About/

About this time, the first evidence backed up by experimental work, of the necessity of certain unknown substances in the diet for normal growth and indeed for the preservation of life itself came from the school of Bunge at Basle.

II. THE NEGATIVE FACTOR.

Disease and the materies morbi were firmly associated in the minds of the profession and the conception of a 'negative factor' as a cause of morbid processes proved most difficult to grasp. Nevertheless the work on these unknown substances continued. Holst (1907) and Frölich (1912) showed that scurvy could be readily induced in guinea-pigs by the withdrawal of greenstuff only from the ordinary diet and that it could be readily cured by reintroducing these into the diet. Furst (1912) made a valuable observation when he showed/

showed that the germinating cereal or pulse would prevent the onset of scurvy if given in an otherwise scorbutic diet, whereas the dry germ failed to do so. When the term vitamine, later changed to vitamin was introduced by Funk the anti-scorbutic vitamin was given the label C.

It had been shown therefore that there was in fresh fruits and vegetables some unknown substance (called vitamin C) which would if administered, prevent the onset of scurvy and further that this substance was not present in the cereal or pulse until germination was allowed.

The vitamin theory has since been firmly established.

It was natural that the next problem to engage the scientific worker was the isolation and identification of the vitamin.

III. ISOLATION AND SYNTHESIS OF VITAMIN C.

In/

In what way did this vitamin appear in the course of germination? Was there a precursor? Where was the vitamin formed and what was its nature? These were a few of the questions requiring an answer.

Although much work was done the next important step forward was in 1928 when Szent-Györgyi[6], in the course of studying the chemistry of the adrenal gland and the function of peroxidase systems, described a new carbohydrate derivative which he called hexuronic acid. He was able to isolate this substance also from cabbages and from orange juice. This opened up a new field of investigation and in 1932 Tillmans and Hirsch[7] found that the anti-scorbutic property of some natural products was related directly to the presence of a reducing substance which, they suggested, might be identical to the hexuronic acid of Szent-Györgyi[8]. King isolated crystalline vitamin C from orange juice and identified it with hexuronic acid/.

acid. In the same year hexuronic acid was chemically synthesised, given the formula $C_6H_8O_6$ and named l-ascorbic acid.[8] This substance was shown to have full anti-scorbutic activity and in fact to be identical with vitamin C.

Svirbely and Szent-Gyorgyi[9] described the chemical nature of this substance. They showed that only the laevo-rotatory form is active, the d-isomer being totally inactive. The first oxidation product of l-ascorbic acid is unstable and reversible and effective as an anti-scorbutic.

Ascorbic acid is a white or yellowish-white crystalline powder. Acid titration corresponds with the formula $C_6H_8O_6$ (Waugh).[10] It is decomposed when heated above 185°C. It oxidises on exposure to air or light. In an inert atmosphere it is quite stable to moderate heat even in alkaline solution. It has a high reducing power and oxidation of the acid is reversible. This is how it probably exerts its biologic activity/

activity (see later). It is oxidised and reduced alternately giving off and taking up two H atoms, thus acting as a hydrogen carrier.[11] McKinnis and King[12] showed that the active part of vitamin C diffuses rapidly through a collodion membrane. The acid was shown to retain its anti-scorbutic activity even when in the reversibly oxidised form.[6] This accounted for the fact that the juice of fruits on expression rapidly became oxidised, unless acid extracts were used, and failed to have any reducing power on dyes (as mentioned later) although anti-scorbutic activity was retained; it was also shown that after treatment with H_2S the reducing power of these juices was regained. This oxidation of ascorbic acid to its reversibly oxidised form occurs only under aerobic conditions and can be inhibited by the use of cyanide.[13,14,15.]

Ray[16] published in 1934 the results of his experiments,

in/

in which he set himself to find out how the vitamin was formed in the germination of the seed and what were the precursors. Adopting the methods of Brown and Morris[17] he used nutrient solutions containing different organic compounds and examined for the production of vitamin C in the embryo in each case at the end of seven days growth. He found that hexoses produced the best formation of ascorbic acid and concluded that ascorbic acid is formed in the plant embryo from hexoses present in the seeds.

Animals derive their vitamin C from plants directly or indirectly. It is known however that some animals (e.g. the guinea pig) are more susceptible to the development of scurvy than others and it has been postulated that this may depend on the ability of certain animals to store the vitamin and possibly to the ability to synthesise it. There has, however, been no report of this being the case in man. The human

organism/

organism relies entirely on a supply of vitamin C from the vegetable world.

IV. BIOLOGICAL FUNCTION OF VITAMIN C.

The biological rôle of ascorbic acid is still a subject for discussion and there appears to be much contradiction in the literature. Its most remarkable character is the high reducing power already mentioned and in virtue of this it would appear to play an important part in maintaining the oxidation-reduction equilibrium in cellular metabolism. The hexuronic acid oxidase in the adrenal cortex and in cabbage leaves described by Szent-Györgyi[6,18.] has been accepted as evidence of the fundamental importance of ascorbic acid in tissue respiration, the vitamin acting as a hydrogen transport agent between organic metabolites and indirectly molecular oxygen. In the active tissues of higher plants two oxidative enzymes/

enzymes specifically related to vitamin C are found.[18,19.] It is a fact that vitamin C is found in higher concentrations in actively metabolic tissues such as sprouting seeds, fresh fruits and tubers. It would appear to play an important rôle in these tissues. In plants the acid is found almost entirely in reduced form.

In animals vitamin C is found most abundantly in glandular tissues. The function of the vitamin as in plants is that of a hydrogen transport agent in cellular metabolism and it is also believed to regulate the colloidal condition of intercellular substances. Most of the gross effects of scurvy can be explained on this basis.[20,21.] Fish and Harris[22] found that in the absence of vitamin C formative cells such as odontoblasts and osteoblasts lost their function. The general conclusion to be drawn is that vitamin C has a vital part to play in

cellular/

cellular life and if absent or deficient will lead to changes in metabolism which may be present for a considerable time before producing even microscopic evidence of tissue damage. The importance of recognising this fact is obvious.

The readiness with which ascorbic acid is oxidised especially if catalysts be present, such as copper, which is present in all biological fluids[23] necessitates the use of cyanide as an inhibitor[13,14,15] even in acid solutions at pH2.[23.] Oxidation of the acid occurs readily at pH above 7.6[15] and hence the use of acid and cyanide to preserve vitamin C in biological fluids.

V. BIOCHEMICAL ESTIMATION OF VITAMIN C.

The identification of vitamin C and its remarkable reducing property led Tillmans et alii[24,7.] to introduce the use of reversible dyes of high oxidation potential, the indophenols, for the purpose of estimating the amount of vitamin C in biological fluids/

fluids.[25] Barron pointed out that the estimation was accurate provided the titration was performed rapidly in acid solution, as the rate of re-oxidation of these dyes is low. The dye used is 2.6 Dichlorophenol-indophenol.

Similarly the presence of the vitamin in plant and animal tissues can be demonstrated by the use of silver nitrate solution, a black precipitate of metallic silver being formed in tissues rich in the vitamin.[26] Levine[27] pointed out that ascorbic acid is the most powerful reducing agent amongst a number of organic compounds of biological significance which display reducing powers (e.g., thio-compounds, aldehydes).

In the determination of vitamin C in biological fluids, an acidified protein-free filtrate is titrated by running into it a freshly made aqueous solution of 2.6 dichlorophenol-indophenol from a graduated micro-burette. The reaction occurring/

occurring can be shown thus:-

Dichlorophenol-Indophenol Solution (Blue) → White leukobase → Excess of Dye solution (Pink) in acid solution.

The end point is reached when the pink colour, apparent in the fluid, persists. This indicates no further reduction of the dye or that the vitamin C present has been completely oxidised. The dye is reduced to a white leukobase by the ascorbic acid present in the filtrate. In the examination of biological fluids (blood, urine, etc.) for the presence of vitamin C the presence of other indophenol-reducing substances must be considered, so as to avoid erroneous results. Of these the principal ones are glutathione, ferro-salts, cysteine and ergothionine. Titration in an acid medium excludes errors with the first two-mentioned substances and [28,29.] cysteine and ergothionine can, if desired, be removed by precipitation with mercuric acetate followed by treatment with H_2S or can be removed with barium acetate. [30,31.] [32.] Johnson and Zilva [33]

state/

state that the presence of these substances is unable to change conditions so much as to compromise the results of the titration. Harris and Ray[34] allege that the error in titration can be avoided by acidifying the fluid to a pH of 2.5, ascorbic acid being the only substance titrated rapidly with this reaction. They add however that the specimen should be tested as soon after collection as possible and if this is not possible that it should be kept in dark bottles in an ice-chest with the addition of glacial acetic or sulphuric acid sufficient to give a pH of 2. Other workers[35] state that if titrations be carried out rapidly there is no interference by these other reducing agents. The important observation was made by Hopkins and Millikan[36] that ascorbic acid reduces indophenol three hundred and fifty times as fast as does material precipitated by mercuric acetate. (Cysteine, ergothionine).

A/

A further controversial point is that ascorbic acid may occur in biological fluids, especially blood, in both its reduced state and also as oxidised dehydro-ascorbic acid.[37] Musulin and King, however, deny this and state that such reports are in error because of secondary oxidation. Glutathione apparently plays an important part in maintaining the vitamin in a reduced state in vitro and in vivo. Blood, however, contains one or more enzymes as well as traces of copper[23] which catalyse the aerobic oxidation of ascorbic acid.[14,38] Cyanide, as already indicated, inhibits this action.[13,14,15]

In estimating the vitamin C content of urine, blood and other biological fluids, a protein-free filtrate is usually first obtained. To deproteinise, metaphosphoric acid appears to be in most favour. Trichloracetic acid was used by many workers but Fujita and Iwatake[39] studied the/

the relative stability of ascorbic acid in metaphosphoric and trichloracetic acid solutions. They showed that no loss occurred in metaphosphoric acid solution for five hours as compared with the greater loss in trichloracetic acid solution. Metaphosphoric acid exerts a protective effect against both atmospheric and trichloracetic acid oxidation and the rate of reaction with indophenol is not appreciably affected by the presence of the acid.[37.]

In my own investigations I have used the method described by Ingalls.[40.] Urine or oxalated blood is preserved with potassium cyanide and after deproteinisation with metaphosphoric acid the filtrate obtained by pipetting off the supernatant fluid after centrifugalisation is titrated against an aqueous solution of the indophenol dye. The estimations of blood were all carried out within 30 minutes after withdrawal of specimens. The 2.6 dichlorophenol-indophenol tablets used each contained the equivalent amount

of/

of the dye which is reduced by 1 milligram of ascorbic acid. This was verified by titration against pure synthetic ascorbic acid on several occasions during my investigations.

VI. VITAMIN C NUTRITION.

The diagnosis of frank scurvy, provided this disease is kept in mind, is readily made. In the adult the characteristic gingivitis and multiple haemorrhages associated very often with a secondary anaemia, and pains in the limbs should immediately lead one to a careful history of the dietary intake during the preceding months. In infantile scurvy the main features are general irritability especially when the child is handled, tenderness in the lower limbs, often with a swelling or swellings near the lower end of the thighs and on radio-

logical examination the typical subperiosteal haemorrhages in the femora and cessation of osteogenesis in the long bones. At all ages the result of the therapeutic test is also proof of the etiology of the disease.

Prior to the isolation of the vitamin, determination of vitamin C deficiency and of the protective value of various vegetable substances utilised the finding of Höjer[41,42] who, in 1924, showed that as scurvy develops in guinea-pigs, the odontoblasts in the teeth degenerate nearer and nearer to the root of the tooth and imperfect formation of dentine results, these histological findings being portrayed by examination of the incisor teeth.

Lund and others[43] using lemon-juice, dried hips and ascorbic acid determined the protective value of each of these substances against the development of scurvy in guinea-pigs fed on a vitamin C-free diet. They examined the incisor teeth and also estimated by titration the

amount/

amount of ascorbic acid present in each substance used, the comparison between the results showing good concordance.

On isolation of the vitamin Tillmans[7] observed that many foodstuffs reduced the indophenol dye, and later by an extraction process followed by titration in acid solution this was made the basis for a specific quantitative method of chemical estimation (Birch, Harris & Ray).[29,44] The best natural sources of the vitamin have since been fully determined.[45] Fixsen and Roscoe give full lists of ascorbic acid contents of foodstuffs. Here it will suffice to state that orange and lemon juice have been shown to contain from 40-70 mgm. ascorbic acid per 100 gm. Blackcurrants are particularly rich in vitamin; tomato-juice contains from 20-30 mgm. per 100 gm. Fresh meat also contains the vitamin in appreciable quantity. Drying processes entail a considerable loss of activity. In the pasteurisation of milk vitamin

C/

C is largely destroyed. It is of special interest to note that specimens of fresh cow's milk have only a quarter to a third of the antiscorbutic activity of human milk and after pasteurisation and standing, little activity remains.[34] Hence the necessity of orange-juice as a supplement to artificial feeding.

The treatment of scurvy is a diet containing large amounts of vitamin C, which can be given as orange-juice or in the form of the synthetic ascorbic acid now available.[34] Harris and Ray showed that in average human milk an infant receives daily about 30 to 50 mgm. of ascorbic acid and this forms a useful basis for calculating the normal requirements. In scurvy much larger quantities will be required to speed recovery.

The interesting fact was pointed out[46,33] that when the guinea-pig or the human being is on a vitamin C free diet, excretion/

excretion of ascorbic acid continues in the urine and the body tissues are markedly depleted before there is any external indication of scurvy. A wide zone of vitamin deficiency therefore exists between scurvy and optimum health. The biochemical methods now available for vitamin C estimation enable us to make quantitative studies in this zone of malnutrition. Direct titration of vitamin C may be performed in specimens of blood,[15,47,48,49] urine,[34] and cerebro-spinal fluid.[50.]

The conception of a sub-optimal state of vitamin C nutrition arose and this has been variously called sub-clinical scurvy, incipient or latent scurvy and hypo-vitaminosis C.[51,52.] Some paediatricians have maintained for many years that such a state existed in infants, the symptomatology being rather indefinite (fretfulness, sallowness) but improvement occurring on an increase of vitamin/

vitamin C foodstuffs in the diet. A similar state in adults has been described.[53.] Again, McCarrison[54] drew attention, in 1931, to the fact that vitamin under-nutrition of a chronic nature might occur without being severe enough to produce the classical symptoms or signs of scurvy.

How are we to assess the level of vitamin C nutrition and so determine the presence of a sub-optimal state?

The methods available may be grouped as -

(1) Biochemical

(2) Clinical

The biochemical methods depend upon the determination of the amount of ascorbic acid present in urine and blood by titration with indophenol dye. It has been shown that the results obtained by such methods compare closely with those obtained by biologic assay.[35,50.]

Johnson and Zilva[33] showed that under normal conditions

of/

of existence the urinary excretion of ascorbic acid varies and is conditioned by the amount consumed in the diet and also by the amount already present in the body. They also made another interesting observation, namely that when, upon the ingestion of a constant daily amount of ascorbic acid, the organism excretes a constant daily amount then that organism is, as they called it, 'saturated' as regards vitamin C.

The estimation of the quantity of ascorbic acid in the urine has been widely used as a method for the determination of the vitamin C nutrition of the body, the result depending, as it does, on the dietary intake both immediately prior to the collection of urine and also for some time previously.

Apart from finding the number of days, on a constant fixed dose of ascorbic acid, required to produce saturation, the state of vitamin C nutrition may be determined and has in/

in fact been applied clinically, by administering a test dose of ascorbic acid (such as 700 mgm. per 10 st. body weight) orally or intravenously and calculating by urinary analysis the percentage amount of the dose which is excreted in the urine within a fixed number of hours.[34,55,56.]

Wright, Lillienfeld, and MacLenathen[56] recommend the intravenous administration of the vitamin in the determination of the state of nutrition, as by this method defective absorption from the bowel is no longer an interfering factor. They gave 1000 mgm. of ascorbic acid intravenously and state that of this amount 400 mgm. should be excreted within the first five hours if vitamin C nutrition is satisfactory. This large dose was chosen because it was considered that in this way percentage of error in titration of the urine when excretion values were low, would be reduced. The

patients/

patients also could continue on normal diet without producing any important changes in the results. By blood analysis these observers showed that the amount of ascorbic acid in the blood rose rapidly to a height and then fell away rapidly during the first hour and a half and gradually came back to normal. They showed that the better the state of vitamin C nutrition (as determined by blood and urine values) the greater was the percentage of test dose excreted within the first five hours.

Faulkner and Taylor[57] observed the relationship of the level of blood serum ascorbic acid to urinary output in normal subjects and in patients with scurvy and chronic infections. Their report also dealt with the relationship of the concentration of ascorbic acid in the serum to the so-called saturation point and they produced/

produced evidence suggesting the existence of a renal threshold for vitamin C at a level of 1.4 mgm.%. They consider that the controlling factor for the excretion of ascorbic acid in the urine is the level of vitamin in the blood. This finding would appear to account satisfactorily for the various results obtained by the use of the test dose in persons with different levels of vitamin C nutrition.

The titration method for the estimation of ascorbic acid may be equally well applied to whole blood or to blood plasma.[35,58,50] Estimations are usually done on blood plasma and as Stephens and Hawley[59] have shown, the values of whole blood and of plasma ascorbic acid are very similar unless in cases of leukaemia. These workers determined the partition of ascorbic acid in the blood, using whole blood, plasma, packed red corpuscles and white cells. They found that the content in packed white cells

was/

was consistently higher than in any of the other fractions but since in subjects with normal white cell counts the leucocytes comprise less than 1% of the total blood volume, the high concentration of ascorbic acid in the leucocytes will have little effect on the values obtained for whole blood.

Quantitative estimations of vitamin C may be carried out on other biological fluids (cerebro-spinal fluid, milk, etc.) but these are not so generally in use.

As has already been pointed out it has been claimed that the quantity of ascorbic acid excreted in the urine is dependent on the vitamin content of the blood which in turn is a measure of the amount taken in the diet immediately prior to the estimation and also of the amount previously present in the body. The patient should therefore preferably be fasting and certainly must not have consumed any quantity of/

of vitamin C just shortly before the collection of urine or blood samples.

With regard to urinary analysis many modifications of technique have been and still are utilised. The total amount of ascorbic acid excreted in twenty-four hours is estimated. Some workers titrate a sample from a twenty-four hour collection, others collect a three or five hour specimen for analysis.

One of the simplified procedures is described by Harris and Abbasy.[60] They collected a three hour specimen of urine and consider this sufficiently accurate for routine surveys of vitamin C excretion. They argue that although the estimation of the actual total excretion of vitamin C in twenty-four hours is better than the mere measure of concentration, as the latter fluctuates considerably according to the volume of urine, nevertheless if an/

an adult patient is known to be excreting neither more nor less than the average bulk of urine and the concentration of ascorbic acid is found to be consistently above the limit of .02 - .03 mgm. per c.c. daily it may safely be assumed that his vitamin reserves are adequate. The same argument applies to the child, except that the level of .01 to .02 mgm. per c.c. may be substituted. The quantity of the adult's or child's daily excretion is taken to be normal if he excretes a 'normal' volume of urine for the 3-hour period. Other workers state the daily output of vitamin C in the healthy adult to be 15 - 28 mgm. This agrees with the figures of Harris and Ray,[34] who also gave 1 - 2 mgm. as the normal 24-hour excretion of vitamin C in infants. Readings below these would of course be considered as sub-normal, and indicative of deficient vitamin C nutrition.

'Saturation'/

'Saturation' tests have also been used both in adults[33] and in young children.[61,62] There is little doubt that they are of value as a guide to the vitamin C reserves of the body, the shorter the time taken for saturation the better the vitamin C nutrition and if used constantly by one worker, the comparative results obtained will obviously be of considerable value in assessing the state of nutrition. Again differences in technique exist and the assessment of the results obtained depends on this. As van Eekelen[63] has pointed out the rapidity of absorption of an oral test dose may be great if the tissues are poor in vitamin C whereas with an intravenous test dose, the overflow into the kidneys of a big dose even if the patient be 'unsaturated', may simulate the peak of saturation, and yet a similar oral dose given the following day shows no large excretion.

The standard test dose is 70 mgm. of ascorbic acid per stone/

stone of body weight. A response should occur on the first or certainly on the second day.[60] The amount required to be given before a satisfactory response is obtained, calculated by subtracting two standard test doses from the total amount of vitamin required, is a measure of the degree of hypovitaminosis.

The estimation of ascorbic acid in blood is another procedure largely employed in assessing the vitamin C nutrition.[35,58,50,64,65.] A fasting specimen is removed from a vein and the estimation made as already described. The procedure is simple and the analysis rapidly carried out. Over a large series of results the generally accepted minimum normal level of ascorbic acid in blood (plasma) varies between .65 mgm.% to .8 mgm.%.[50,64.]

The relative value of urinary and blood analysis has been investigated by numerous workers.[64,65.] Schroeder[65] showed

that/

that change in the blood content of ascorbic acid preceded demonstrable change in the urine and he considered that the blood reading was the better criterion. In urinary estimations fluctuation in the amount of urine passed must be taken into account and also other possible variations in renal function. Blood estimation gives a measure ab initio of the eventual existence of a hypo- or avitaminosis.

(2) In addition to biochemical tests, other methods mainly clinical in their application have been described when trying to determine the state of vitamin C nutrition.

The capillary resistance test was introduced originally by Göthlin[66] who, working on the known haemorrhagic diathesis of scurvy, demonstrated decreased capillary resistance to pressure in children in various schools in North Scandinavia. Many of these children were receiving a diet poor in vitamin C. The raising of the intracapillary pressure is carried out by/

by the application of a sphygmo-manometer band around the upper arm at a given pressure for a given time. A crop of petechiae distal to the site of the pressure due presumably to a diminished capillary permeability, occurs in scorbutic subjects.[67,68] This test has been widely used and Schultzer demonstrated that a progressive hypovitaminosis C manifested itself in a lowered capillary resistance test. It is, however, well known and has been pointed out, among others, by Schultzer, that this test lacks specificity. He reports cases where the capillary stasis test remained markedly positive even after prolonged injections of ascorbic acid.[69]

Another clinical test, the intradermal injection of indophenol dye and the direct observation of the time taken for its decolourisation was introduced first by Rotter.[70] Portnoy and Wilkinson[71] considered a slow decolourisation of value as an indication of lowered vitamin C nutrition but

more/

more recently Poncher and Stubenrauch[72] have severely criticised the test and found that their results are quite out of agreement with those obtained by the former workers. This test is based on the assumption that ascorbic acid being present in all body and intercellular fluid, will decolourise indophenol dye injected intradermally, quickly or slowly depending on the actual amount of vitamin present. As yet its value is not proven.

The clinical tests are therefore not specific but may be used in conjunction with the less fallible analytical procedures.

These then are the methods available at present for diagnosing the state of vitamin C nutrition.

Göthlin[73] made the observation that in order to restore to normal and to keep normal a diminished capillary resistance, arising as a result of shortage of vitamin C, the minimum/

minimum amount of ascorbic acid required daily for an adult is 25 mgm. This physiological minimum optimal dose has been administered to numerous patients[60] and it is found that those receiving this amount excrete at a level of not less than 13 mgm. daily and show good responses to the standard test doses (70 mgm. per stone body weight) generally on the first day but always on the second day of the test. Somewhat similar figures have already been stated to be the average normal daily output and the 'normal' response to the test dose. During the years of growth more will be required per unit of body weight. Eekelen and Wolff[74] showed that where the intake of fresh fruits is more adequate than usual the values in the excretion test are correspondingly higher and subnormal values are very much rarer.

The question arises as to the value of these tests as evidence of the nutritional state of the body as far as vitamin/

vitamin C is concerned. The fallacies which may arise due to faulty technique and lack of understanding in the assessment of the results have been pointed out but even allowing for skilful technique, which should be acquired by constant repetition, do the results obtained really represent a true estimate of vitamin nutrition? In his Withering lectures, Parsons[75], during his survey of nutrition, points out the importance of realising that the conception of nutrition should not be limited to food - 'alimentation'. An individual may be well fed and yet badly nourished. Cathcart has defined good nutrition as 'the state of well-being which characterises the individual who is physically and psychologically sound.' Not only must there be an adequately balanced and properly cooked supply of food; many other factors (environment, fresh air, cleanliness, sleep, worry, monotony) influence the state of nutrition. The presence of disease will/

will be a factor. Apart from diet then, absorption and ultimate utilisation of the food are of prime importance. Defectivity in any of these may produce deficiency symptoms. There is an individual variation in the amounts required of the various food products including of course the vitamins and, as Parsons[75] points out, some individuals require more of certain articles of diet than others. In the cure and prevention of recurrence of rickets some children require much more vitamin D than others. It is recognised that it is difficult to have any standards of nutrition and malnutrition. Each clinician has his own standard which may vary from year to year. We accept deficiency diseases such as scurvy as definite evidence of malnutrition and yet the scorbutic infant is not infrequently 'well-nourished' and developed. Again the 'overfed' infant who grows very rapidly often gets rickets. The usual standards of height, weight, vital capacity, etc. are/

are perhaps useful but not altogether satisfactory.

Apart from clear cut deficiency diseases have we any clinical evidence of malnutrition? It has already been stated that a wide range of sub-optimal nutrition exists between optimum health and the actual appearance of scorbutic symptoms in a subject fed on a vitamin C free diet. This sub-optimal state or hypovitaminosis occurs where there is defective ultimate utilisation of sufficient vitamin C - a similar suboptimal state no doubt exists with regard to all the other essential food substances. This is being more and more recognised and a study of the subnormal state leads one to enquire into the question of under what favourable circumstances a deficiency of the particular food substance will occur. It should always be remembered then that the optimal amount of vitamin or other foodstuff required for any particular patient may vary depending as it does on so many

46,33.

factors/

factors which may play a part in any individual case. To state a definite daily optimal dose of vitamin for adults or for children is then not to be considered as infallible. Every feature of every case should be carefully considered; some patients will require more than others.

VII. HYPOVITAMINOSIS.

Hypovitaminosis C is the suboptimal state of vitamin C nutrition as distinct from frank scurvy. There is no definite clinical symptom or syndrome which is pathognomonic of this state and it is therefore impossible to diagnose its presence on purely clinical grounds. Biochemical estimations of blood and urine if carried out carefully and assessed properly are of great value in recognising the condition. A consideration of the factors which would favour the occurrence of hypovitaminosis will assist the clinician to suspect its presence in patients in whom those etiological factors are operative.

They/

They may, broadly, operate in two ways:

(1) An insufficient amount of vitamin may be available.

(2) An excessive utilisation of the vitamin may occur demanding greater supplies than normal. This might well be included in (1) as the ultimate result - deficiency - is the same.

One or several causes may act in any one case.

Deficiency may occur -

(a) Prior to consumption of food by destruction of the vitamin in the preparation of the food. Destruction occurs particularly if heat is applied in the presence of light and fresh air.

(b) Lack of balanced diet. Absolute or partial absence of vitamin C (to be remembered especially with patients on "diet").

(c) Faulty digestion. Vomiting, diarrhoea, etc., resulting in

(d) Poor absorption. Insufficient amount of vitamin absorbed.

(e) Imperfect Utilisation. After absorption the vitamin

presumably/

presumably passes through the liver, hence to the systemic circulation and into the tissues where it plays a vital part in cellular metabolism, as already mentioned. The state of nutrition no doubt depends on the interaction and inter-dependence of all the ultimate food factors and deficiency of one may affect the proper action of the other: the intricacies of cellular chemistry are as yet imperfectly understood but the utilisation of any one food factor may doubtless be affected depending on the state of metabolism present. One person will utilise the available vitamin to the full while another may not do so - hence the undesirability of dogmatism with regard to dosage.

(f) Where metabolism is increased and tissues active, greater quantities of essential food factors will be required. Such a state occurs of course in general pyrexial conditions, hyperthyroidism, etc.

Defective/

Defective "alimentation", it should be remembered, will often mean a deficiency in several essential food substances and it is not infrequently found that "deficiency diseases" co-exist in the same individual (e.g. rickets and scurvy).

What is the incidence of hypovitaminosis C as determined by biochemical analysis of urine and blood? In order to answer this question a very large number of subjects would require to be examined and from all different classes of social life. We would expect to find the higher incidence in the poorer classes. Surveys have been made on subjects of the voluntary hospital classes[76,77] and as many as 70% found to be below standard as judged by urinary excretion tests. Orr[78] has shown that the majority of the population receives less than the reputed optimal allowance of vitamin C.

Much work has been done on vitamin C subnutrition in both normal physiological conditions (pregnancy, lactation) and/

and in many diseases.

During pregnancy the foetus obtains its supply of vitamin C from the placenta - in which vitamin C is stored - and deposits it to some extent in the adrenal gland.[79.] The need for vitamin C is greater in pregnant women and nursing mothers.[80.]

Other workers[34] showed that in infants and children hypovitaminosis C is not uncommonly found in hospital classes. In the dietetic treatment of gastric and duodenal ulcers it has been frequently encountered[76,81,82.] and Platt[83] has even demonstrated the development of scurvy in four cases on prolonged 'gastric' diets.

With regard to infection, Lawrynowicz[84] as far back as 1931 showed that a deficiency of vitamin C led to a loss of the normal resistance against infection.[85] King and Menten demonstrated that the effect of bacterial toxins on guinea-

pigs/

pigs was more marked if these animals were for some time previously, deprived of vitamin C. Other workers[86] found that in many infections and intoxications (e.g. diphtheria) the ascorbic acid content of the adrenal gland of guinea-pigs was definitely diminished. Schroeder[87] also has demonstrated the increased usage of vitamin C in pyrexial states and infections. Evidence of vitamin C deficiency has been reported in the human being in many pyrexial and infective diseases, such as rheumatic fever,[88] and tuberculosis.[88,89.]

The incidence of hypovitaminosis as a concomitant of morbid conditions has led to the use of ascorbic acid as a therapeutic measure in the treatment of many diseases, and many reports claim considerable improvement in the patient's condition following its exhibition. On surveying this wide field of experimentation, one would, I think, be wise in concluding that ascorbic acid has by no means a specific

value/

value but, in virtue of its vital rôle in cellular metabolism, the possibility of a deficient supply (due to any of the favouring circumstances already mentioned) should be constantly and carefully considered. Unless this be done, a serious deficiency and even frank scorbutic symptoms may appear.

In my own observations I employed mainly the method of blood plasma analysis[40] as already described. In order to perform this analysis accurately and become accustomed to a definite end-point, I was obliged for a considerable time to practise repeated titrations.[90] In conjunction with Wilson however a standard stable solution was prepared which was used for comparison with the solution to be tested and this proved of great assistance in providing a definite depth of colour with which to match that solution.

Urinary analysis was also carried out in some cases, the 24-hour specimen being collected for examination.

Göthlin's/

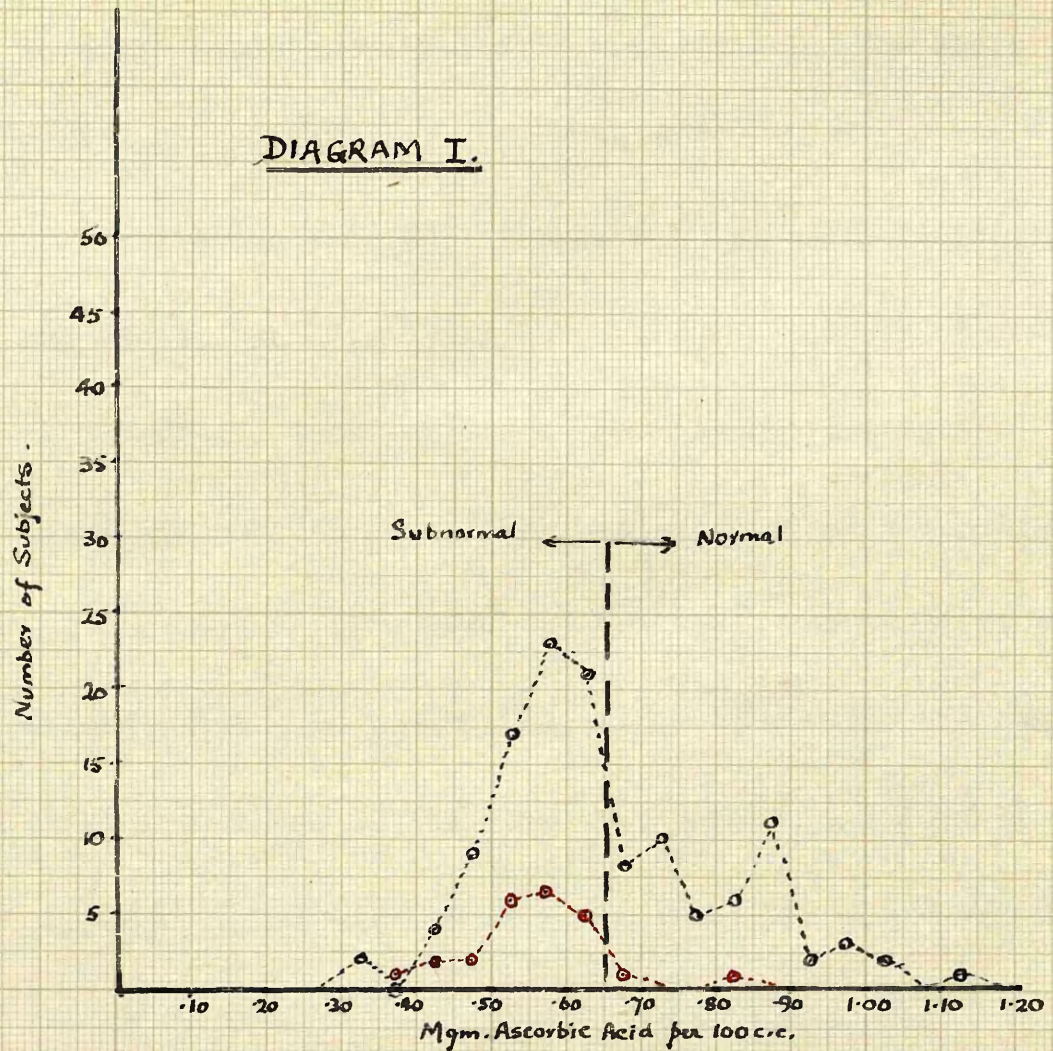
Göthlin's capillary resistance test[66] and the intra-cutaneous test of Rotter[70] were tried but discarded as leading to no more valuable information than was obtained by biochemical procedures and as being non-specific.

My observations were carried out during the two years I was attached to the staff of a general hospital. Patients were mostly of the "poorer classes." The results obtained are therefore to be considered as applying to this class of patient. I could not determine the general incidence of hypovitaminosis C but I set myself rather to find out that if such a condition did exist, was it to be found frequently enough to be of importance from the clinical standpoint.

I first examined the fasting blood of 10 young healthy adult males (students and doctors). The lowest reading was .78 mgm. ascorbic acid % and the highest 1.03 mgm. ascorbic acid %. The average reading was .89 mgm. ascorbic acid %.

The generally accepted minimum normal level for blood ascorbic/

DIAGRAM I.



BLOOD ASCORBIC ACID IN 8 PARTURIENT WOMEN AND THEIR INFANTS.

CASE.	MOTHER. Mgm. Ascorbic Acid %	CHILD. Mgm. Ascorbic Acid %	
1.	6·93	14·72	
2.	6·86	13·18	
3.	6·25	11·63	
4.	5·76	12·76	
5.	5·72	12·44	
6.	5·56	13·28	
7.	5·50	11·46	
8.	5·38	11·60	

ascorbic acid is approximately .65 mgm. %. The above readings were all well over this level.

Following upon this I examined 123 patients (children and adults) choosing them at random as they were admitted to hospital. These subjects had a large variety of miscellaneous diseases. The highest reading was 1.12 mgm.% and the lowest .33 mgm.%. Of the total number 61.8% were below the minimum normal figure of .65 mgm.%. Diagram I (blue ink) shows in the form of a graph the number of subjects falling into each grade starting at .30 mgm.% and rising by intervals of .05 mgm.% to 1.15 mgm.%.

Diagram I (red ink) shows a similar type of graph in respect of 25 infants and children all under two years of age (the younger ones all bottle-fed with cow's milk). 92% of those were found to have a blood ascorbic acid content below .65 mgm.%.

Finally/

Finally I tested the blood of 8 parturient women and their newly-born babies (the blood being obtained from the umbilical cord). (The results are shown in the table).

The conclusionsthat may be drawn from these results are:

(a) a wide range is met with in the amount of ascorbic acid in the blood.

(b) high readings are found in the newly-born infant who appears to obtain his supply from the placenta.

(c) the percentage of 'subnormal' levels in the poorer class hospital patient is high and apparently markedly so in the early years of life (where artificial feeding exists).

I would again point out that my results apply only to the subject requiring medical attention. I can draw no conclusion as to the general incidence of hypovitaminosis C in/

SHOWING THE RELATION BETWEEN
BLOOD ASCORBIC ACID CONTENT AND THE 24-HOUR EXCRETION OF ASCORBIC ACID
AS ANALYSED ON TWO CONSECUTIVE DAYS.

CASE.	BLOOD PLASMA ASCORBIC ACID CONTENT.	1ST DAY. 24 HR. EXCRETION.	2ND DAY. 24 HR. EXCRETION.
1	·48 mgm%	9 mgm.	11 mgm.
2	·46 mgm%	6 mgm.	6 mgm.
3	·47 mgm%	10 mgm.	12 mgm
4	·58 mgm%	12 mgm	13 mgm
5	·53 mgm%	13 mgm.	11 mgm.
6	·68 mgm%	14 mgm.	14 mgm.
7	·66 mgm%	16 mgm.	14 mgm
8	·83 mgm%	19 mgm.	21 mgm.
9	·92 mgm%	23 mgm.	21 mgm.
10	·88 mgm%	24 mgm.	25 mgm.

in the general population.

I found the measurement of the daily (24-hr.) excretion of ascorbic acid of value in confirming some of my results. I performed it on five patients with subnormal blood ascorbic acid and on five patients with normal readings. As will be seen, the excretion figures (given to the nearest mgm.) for those with normal blood readings were higher than in those cases with lower plasma ascorbic acid content.

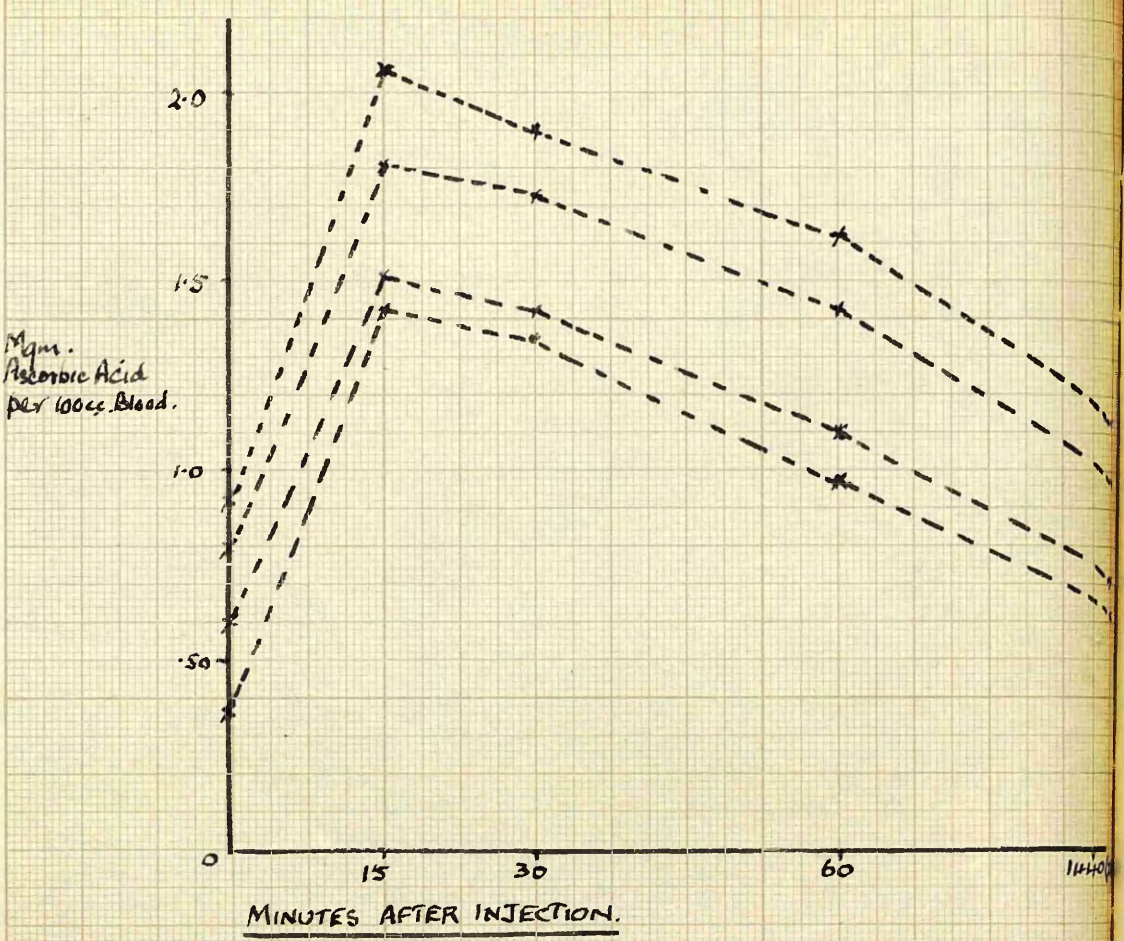
A consideration of the diseases with which those subjects were afflicted provided me with no information that any one disease was more frequently associated with hypovitaminosis C than another. The relatively small number of cases precluded this. Amongst them I had 21 children and adolescents with acute rheumatism of whom 18 had subnormal blood ascorbic acid readings. The other three, however, one of them a young girl with high fever, had readings above .7 mgm.%. Normal and subnormal results were found also in such an assortment of ailments/

ailments as pulmonary phthisis, pneumonia, acute nephritis, chorea, pyogenic urinary infections, duodenal ulcer, chronic heart disease, disseminated sclerosis, diabetes, cerebral thrombosis and chronic bronchitis. Rickets was prevalent among the young children and of nine cases eight of them had a subnormal ascorbic acid content. It is of course recognised that the association of one deficiency with another is not infrequent and my eight subjects with rickets and a subnormal ascorbic acid content in the blood illustrates this.

I repeated the analysis of the blood in 25 subjects after three weeks' rest in hospital, no restriction being placed on the dietary requirements of any one case. No significant rise or fall in the ascorbic acid level occurred in any of the 25 subjects: no reading varied more than plus or minus .18 mgm. ascorbic acid %.

I/

DIAGRAM III.


2·0
1·5
1·0
·50
0
Mgm.
Ascorbic Acid
per 100cc Blood.
15
30
60
MINUTES AFTER INJECTION.

I administered vitamin C in the form of synthetic ascorbic acid (Redoxon, Roche) tablets to many of these subjects, sometimes in massive doses (1000 mgm. daily for 7 days). No ill effects due to massive or long-continued dosage were ever noted, nor was any clinical improvement which could be directly ascribed to vitamin C except in the cases reported later in this paper. Following upon the exhibition of vitamin C the blood content of ascorbic acid rose and the urinary output of ascorbic acid increased, until 'saturation' occurred when a more or less constant excretion took place. (This merely confirms the work done by many other investigators).

The effect of intravenous injection of a large dose of ascorbic acid on the blood ascorbic acid content is shown in Diagram III in the form of a blood curve. The diagram shows the results produced on four subjects. In all, the blood

ascorbic acid rose rapidly, the peak being probably immediately after the injection, and then fell progressively until at twenty-four hours the level of ascorbic acid in the blood had returned to a figure somewhat higher than the initial level. The rapid initial rise is of course due to the introduction directly into the blood stream of a large dose of ascorbic acid, and the rapid fall thereafter must indicate a removal of ascorbic acid from the blood stream by the body tissues and also by the kidneys into the urine. As already mentioned Faulkner and Taylor[57] claim that there is a renal threshold of about 1.4 mgm. % for ascorbic acid.

It is as might be expected, very difficult to assess the value of ascorbic acid therapy in those subjects with subnormal blood ascorbic acid content. It is important also to remember that the extent of vitamin utilisation and/

and need probably varies from person to person and that it cannot be said with any certainty that a level of ascorbic acid in the blood below the average amount necessarily indicates a definite poor vitamin C nutrition in that particular subject.

Blood analysis, in my opinion, is as simple and as correct a method for gauging vitamin C body content as any other procedure but I hesitate to draw up any fixed level to divide the 'normal' from the 'subnormal'. I think it is useful to have a recognised minimum normal figure provided that one is not prepared to classify every subject with a lower figure as being definitely subscorbutic: to put it briefly, a figure over .65 mgm. ascorbic acid % indicates that no serious shortage of vitamin C exists in the body tissues; a figure below .65 mgm. ascorbic acid % may indicate some deficiency of the vitamin, the probability increasing as the figure decreases, and the possible value

of/

of vitamin C therapy should always be considered. Administration of ascorbic acid to such subjects may not and certainly does not in many instances lead to any obvious clinical improvement which can be attributed to such therapy but the withholding of the vitamin may be a serious factor in delaying the recovery of the patient.

Vitamin C deficiency will tend to occur perhaps most commonly and most obviously where diet is defective. It is unusual nowadays to see a case of frank scurvy whether in child or adult but I had the opportunity of examining a patient who, in my opinion and from the result of the therapeutic test, was a victim of vitamin C deficiency.

Male aged 64 years. Unmarried, a labourer, and had been living alone for the past seven years.

His complaint was that during the past few weeks he noticed that he was unusually fatigued at the end of his day's work, that he was breathless and that his legs were weak/

weak. The symptoms became progressively worse and he was eventually admitted to hospital as he was unable to walk more than twenty to thirty yards.

He was a sallow-complexioned, rather emaciated man. Examination revealed a red pigmented skin over both lower limbs, soft spongy gums with a few carious stumps, and a marked anaemia. Hb 50%, R.B.C. 2,860,000, W.B.C. 5,200 and reticulocytes .3%.

Examination of heart, lungs and nervous system revealed no abnormality. Liver and spleen were not enlarged. Urine examination excluded the presence of albumin, sugar, pus or red cells.

I examined the blood ascorbic acid content and found it to be .44 mgm. %. His 24-hour output of ascorbic acid in the urine was 7.2 mgm. (1st day) and 7.8 mgm. (2nd day).

I did not do a saturation test as I wanted to watch his/

his response to ascorbic acid therapy. Anaemia being the most marked clinical feature I paid particular attention to the blood examination.

I administered 100 mgm. ascorbic acid t.i.d. A reticulocyte response occurred on the 3rd day and by the 6th day was 6%; thereafter the reticulocytes gradually decreased in number. The Hb readings and the blood ascorbic acid content are shown below:-

Initial Values:	Mgm. Ascorbic Acid % .44.	Haemoglobin 50%.
After 3 days	.72	52%
After 6 days	1.06	57%
After 10 days	1.44	62%
After 14 days	1.92	66%
After 21 days	2.56	76%
After 28 days	3.80	80%

Apart from vitamin C no other treatment was given or required. Ordinary hospital diet was offered although for the/

the first 10 days appetite was very poor.

This patient admitted to having eaten no fruit or green vegetables for a "good many years" (his words).

His recovery was uneventful and the reticulocytosis with resulting and progressive improvement in his anaemia on using ascorbic acid was most interesting. The clinical improvement was undoubtedly due to the use of vitamin C. It led me to consider the value of ascorbic acid in anaemia, if at the same time a hypovitaminosis existed (as shown by low ascorbic acid content of the blood).

It is, of course, known that cases of frank scurvy frequently show an anaemia. Different types of blood picture, normocytic and orthochromic,[91,92.] hypochromic,[92,] and macrocytic[92,93] have been reported.[93] It has been suggested that the stage of development of the erythron affected by vitamin C lack may depend on the extent of the vitamin deficiency. That vitamin

C/

Relation between Plasma Ascorbic Acid Content and Haemoglobin Content in 66 cases.

Haemoglobin %	Number of cases with Subnormal Plasma Ascorbic Acid Content.	Number of cases with Normal Plasma Ascorbic Acid Content.
Over 80%	4	16
71 - 80%	11	9
61 - 70%	13	4
Under 61%	9	-

C plays some important rôle in normal red cell maturation has long been recognised and recently from observations made on guinea-pigs and on cases of anaemia in infants, Rohmer, Bezssonoff and others[94] have concluded that ascorbic acid does play a definite part in normal blood formation. The case just reported would appear to illustrate this.

Pursuing this question further I analysed the blood of patients with different haemoglobin values. I selected twenty subjects with haemoglobin of 80% or over, twenty with haemoglobin of 71 - 80%, seventeen with Hb 61 - 70% and nine with Hb under 60%. The results obtained proved interesting and are shown on the opposite page. The main feature to be gathered is that as the haemoglobin percentage decreases the tendency to a lowering of the vitamin content of the blood increases. We may express this in another way - namely that the greater the degree of anaemia the more likely are we to find/

find a low vitamin content in the blood. This is not at all unexpected in the case of nutritional anaemias where a coincident vitamin deficiency no doubt will often exist. In my own cases I found that the anaemia (apart from cases of pernicious anaemia) was usually accompanied by an infective illness which in most cases was the reason for the patient's admission to hospital. This was most markedly so in children and infants - a rheumatic manifestation (carditis, arthritis, chorea, tonsillitis, subcutaneous nodules) or a respiratory or acute digestive upset was invariably present in any case I examined and it could quite safely be assumed that the anaemia was aggravated by and in many cases largely due to the acute infective process present. Of the 66 cases, fifty-one were of this type, five were cases of pernicious anaemia and the remaining ten were microcytic hypochromic anaemias (5) and post-haemorrhagic anaemias (5). Of the nine cases below 60% Hb, five were infants and young children with/

with rickets (2), enteritis (1) and rheumatism (2), two were adults with an anaemia following a severe haemoptysis (1) and haematemesis (1) and the remaining two were pernicious anaemia (1) and microcytic hypochromic anaemia (1).

This small series of cases was sufficient to convince me of the frequency of low blood ascorbic acid content in association with a low haemoglobin content. This was the sole purpose of the investigation. It seemed reasonable to assume that one and the same etiological factor - namely some interference with nutrition in its fullest sense - was responsible for the subnormal values present.

In order to restore the patient to health after the acute stage of the illness was over, iron therapy would in most cases be indicated in order to raise the haemoglobin content of the blood. Similarly ascorbic acid would be required to speed recovery from the defective state of vitamin C nutrition.

The/

The importance of vitamin C in blood formation being recognised I decided to try and discover its possible value in the occasional subject met with in whom an anaemia proves somewhat recalcitrant to iron therapy.

I found that administration of vitamin C alone in doses of 300 mgm. daily produced no change in the haemoglobin value in a series of twelve cases of anaemia in infants just recovered from acute respiratory and digestive upsets. I was only fortunate enough to find one case of an infant where iron therapy alone seemed inadequate in raising the haemoglobin value to normal. (This case is reported later). In four cases in adults I was able to try the value of ascorbic acid therapy when iron was producing no marked improvement in the blood picture.

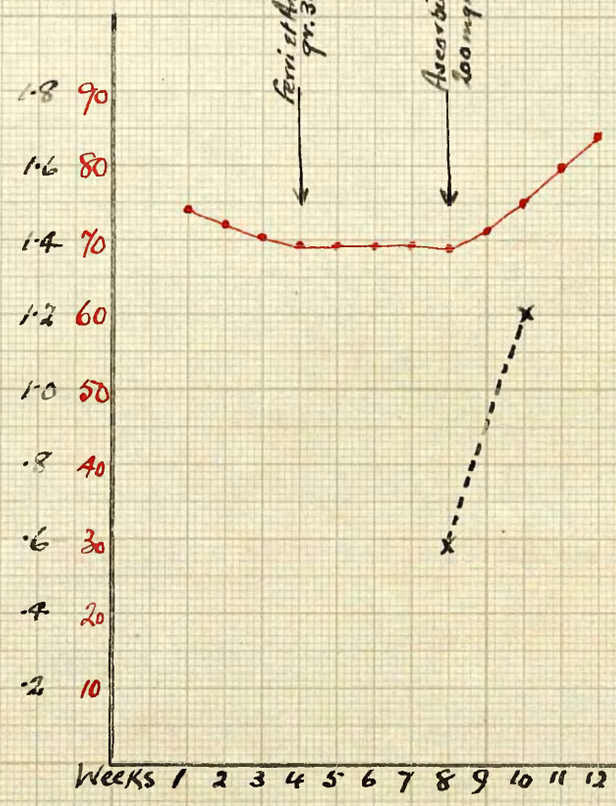
I shall now report these 5 cases (one in an infant).

Case I.

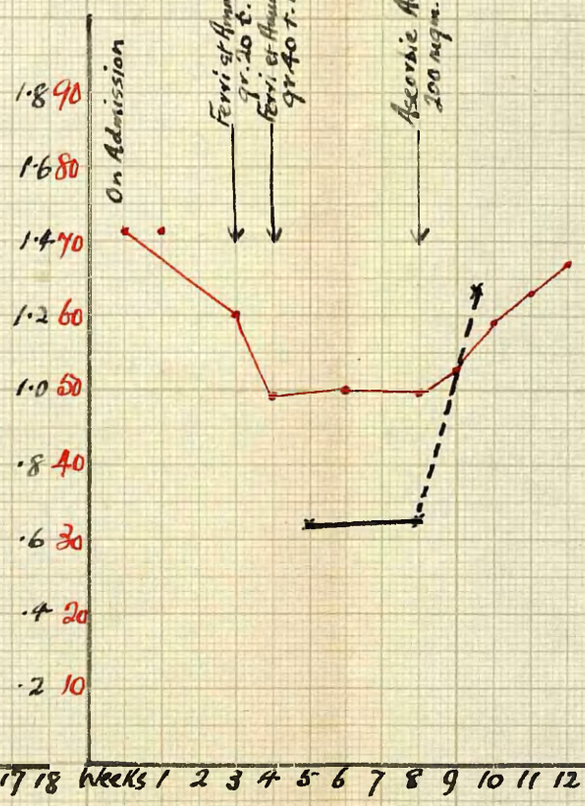
Female aged 35 years.

Admitted/

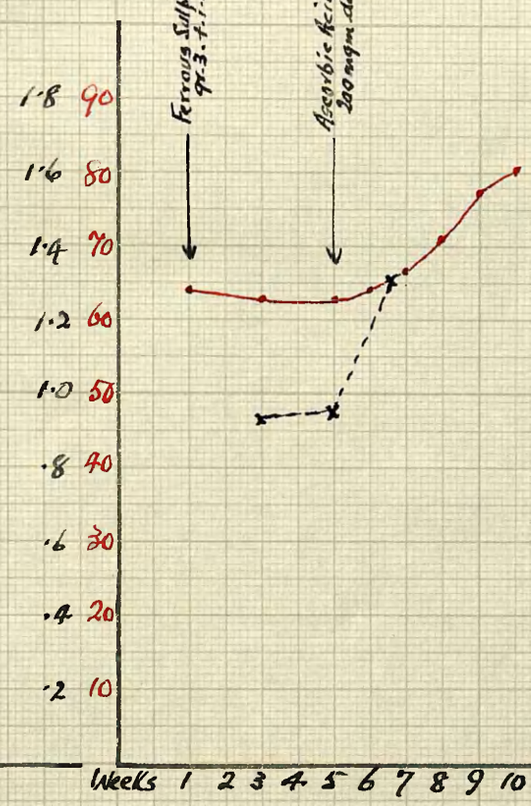
CASE I.



CASE II.



CASE III.



—●— Haemoglobin %

x----x Ascorbic Acid per 100 cc. Blood Plasma

Admitted with pyuria. Treated with alkalis and sulphanilamide. Urine sterile to repeated catheter specimens after four week's treatment. Iron therapy (Ferri et Ammon. Cit. grs.30 4 i.D) was then started. After four weeks of such treatment there was no improvement in the Haemoglobin reading. The ascorbic acid content of the blood was found to be .58 mgm. %. Ascorbic acid (300 mgm. daily) was commenced the iron being continued. The haemoglobin reading thereafter rose steadily.

Case II.

Male aged 33 years.

Admitted with diagnosis of Pulmonary Tuberculosis. During the first month of his stay in hospital he had several slight attacks of haemoptysis. No further haemoptysis occurred after twenty-five days following admission.

Ferri et Ammon. Cit. gr.20 t.i.d. was commenced after three weeks in hospital and after four weeks the dose was increased/

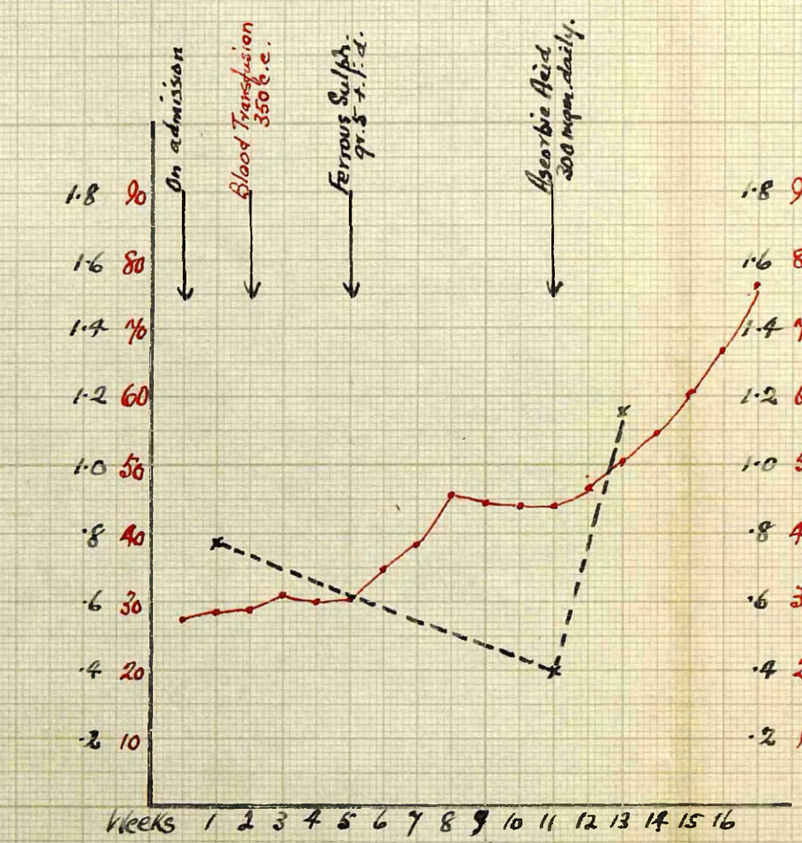
increased to gr.40 t.i.d. No improvement in the blood occurred until ascorbic acid was prescribed after a further four weeks (in doses of 200 mgm. daily). The improvement resulting from this additional therapy is shown in the chart.

Case III.

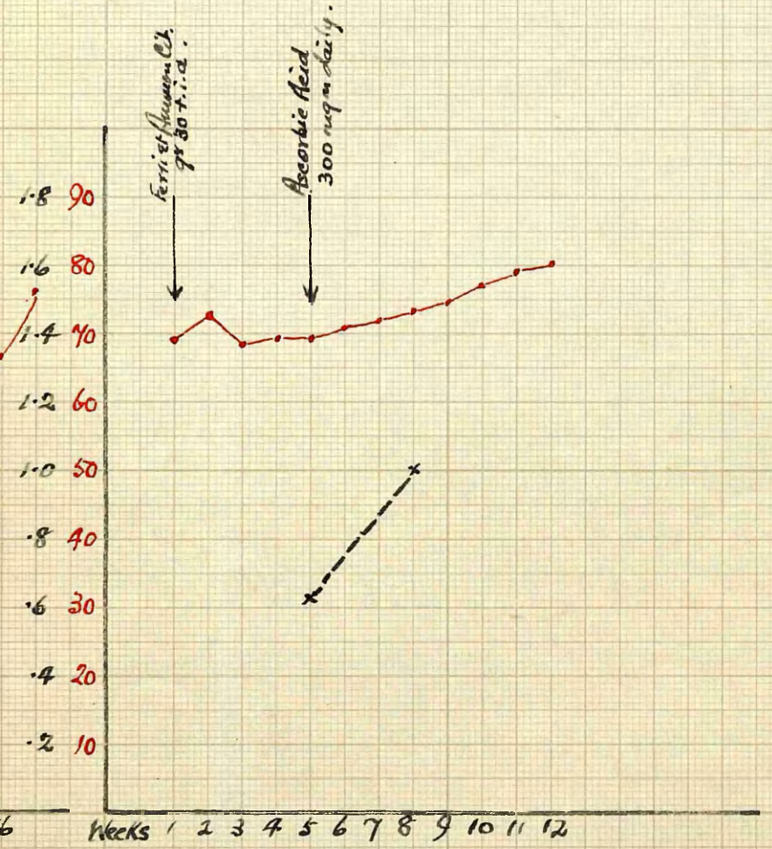
Male aged 2 months.

Bottle-fed since birth. No infective process present. Haemoglobin 66%. Ferrous sulphate in doses of 3 grains T.I.D. was begun. After three weeks there was no improvement in the haemoglobin reading and so a blood ascorbic acid analysis was performed. The result showed the child to have a blood content of .92 mgm. %. This seemed quite a satisfactory level so no ascorbic acid was administered, but after a further two weeks, since no rise in haemoglobin had occurred, it was decided to try the value of ascorbic acid. 200 mgm. was given orally every day, the ferrous sulphate being continued. The haemoglobin/

CASE IV.



CASE V.



Haemoglobin %

x----x Ascorbic Acid gm. 100 cc. Blood Plasma

haemoglobin immediately began to rise.

Case IV.

Male aged 54 years.

Patient had recurring symptoms of Duodenal Ulcer during the past 10 years. He was admitted to hospital following a severe haematemesis. He was, after initial treatment, put on to Sippy diet. A blood transfusion was carried out at the end of a fortnight. Iron therapy (Ferrous Sulph. gr.V. T.I.D.) was commenced after a further three weeks and this was continued for the following eleven weeks. The blood ascorbic acid value shortly after admission was .78 mgm.%. It was tested again at the commencement of the eleventh week when it had fallen to .40 mgm.%. This was presumably due to the lack of vitamin C in the diet during the preceding eleven weeks. At the commencement of the eleventh week ascorbic acid was administered (200 mgm. t.i.d.).

The/

The haemoglobin which had remained at 40% - 45% during the preceding four weeks now began to rise steadily and by the sixteenth week was up to almost 80%.

Case V.

Male aged 24 years.

Bilateral pulmonary Tuberculosis. Cough and sputum for the past three months.

Iron was administered (Ferri et Ammon. Cit. gr.30.t.i.d.) from the end of the first week, and continued thereafter. Ascorbic acid was given from the end of the fifth week onwards and a steady rise in haemoglobin occurred.

A consideration of these cases does, in my view, point out the necessity of recalling the frequency of lowered blood ascorbic acid and the possible value of vitamin C in some anaemic subjects, as an adjuvant to iron therapy. Case III had an ascorbic acid content of .92 mgm.% and yet the use of ascorbic/

ascorbic acid raised the haemoglobin content of the blood. The reason for this is unknown. The utilisation of vitamin C may however have been defective at the level of .92 mgm.% and required a higher blood level to produce an effect. Each individual no doubt varies in his or her requirements of any essential food factor. The importance of dietetic treatment in the production of a hypovitaminosis is illustrated in Case IV.

The value of vitamin C in the anaemia of scurvy is not disputed and I would suggest that its value, as an adjuvant to other treatment, in anaemias where a co-existing hypovitaminosis exists, should not be overlooked.

The results of disease are evidence of defective nutrition whatever the factors causing this, and deficiency must in many cases apply to more than one of the essential food substances. Vitamin C plays an important part in cellular metabolism and also in blood formation and where a deficiency exists good

health/

health cannot obtain. To treat the individual rather than the disease seems to be of prime importance.

During my work on vitamin C I carried out another small investigation.

It had been reported[95,96] that ascorbic acid plays an important part in muscle activity especially in relation to glycogen metabolism. With increased muscular activity there is a greater demand for vitamin C.[95.]

Abelin and Spichtin[96] showed that there was a marked reduction of total creatinine in the skeletal muscle of hyperthyroid rats. Sure and Theis[97] reported that in hyperthyroid rats there is a definite deficiency of vitamin C in the adrenals, thymus, liver and kidneys. Harris and Ray[98] had previously reported an extreme loss of vitamin C in the adrenals/

adrenals of scorbutic guinea-pigs.

Hirsch[99] and Altenburger[100] have stressed the value of vitamin C in hyperthyroidism, as under thyroxin therapy the disappearance of muscle glycogen is restrained by treatment with ascorbic acid. Hirata and Suzuki[101] showed that in progressive muscular dystrophy vitamin C raises the low glycogen and phospho-creatine values of muscle. Berg[102] demonstrated that the excessive breakdown of phospho-creatine in heart-muscle during the action of thyroxin is greatly reduced by large doses of vitamin C.

Fischer and Oehme[103] reported that large doses of vitamin C reduced the creatinuria produced by experimental hyperthyroidism in the rodent. v.Plehwe[104] attempting a self-experiment, took a meat-free vitamin-rich diet in an attempt to get a constant protein intake. During a period of three weeks he measured the creatinuria. It was 10 mgm. $\pm$ 14.4, similar to that found

by/

105.
by Taylor and Chew who investigated the creatinuria of fifteen healthy young men on a meat-free diet. Then he administered to himself thyroxin which caused an increase in the creatinuria. The administration of vitamin C effected a definite reduction in the output of creatine although the raised B.M.R. and pulse rate persisted. The same investigator also found that ascorbic acid diminished the creatinuria in two cases of hyperthyroidism. His general impression was that ascorbic acid effected only a partial improvement in the patient's condition.

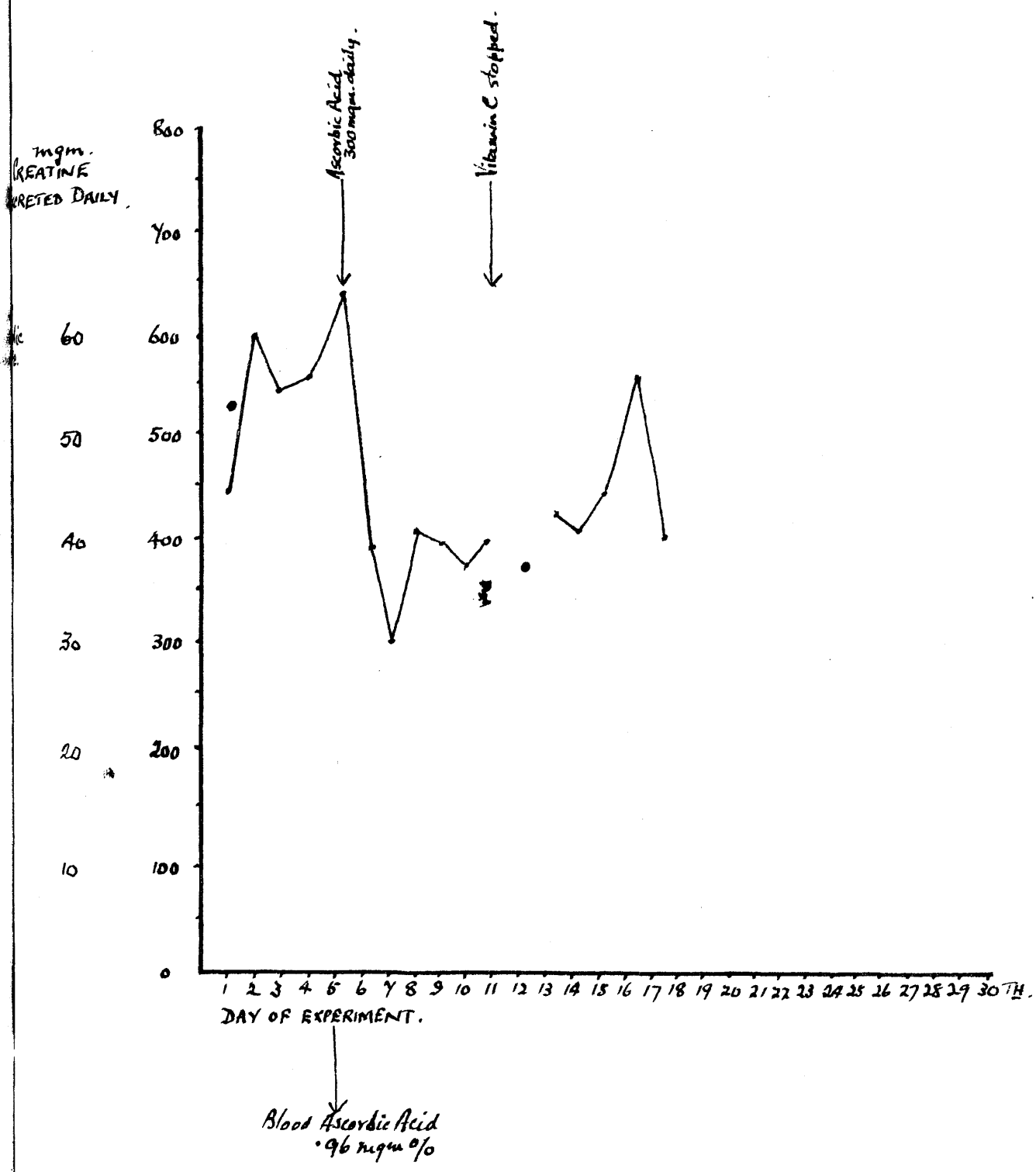
I studied four cases of hyperthyroidism, and determined the output of creatine in the urine prior to and during the administration of ascorbic acid (300 mgm. daily (orally)). In them all I found that the creatinuria was lessened during vitamin C therapy.

Case I.

Female aged 62 years.

Adenomatous/

CASE T.


Ascorbic Acid 300 mgm. daily.
Vitamin C stopped.
mgm. CREATINE CRETED DAILY.
800
700
600
500
400
300
200
100
0
60
50
40
30
20
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 TH.
DAY OF EXPERIMENT.
Blood Ascorbic Acid .96 mgm %

Adenomatous goitre. Exophthalmos +. Tremor +.

Auricular Fibrillation present (confirmed by electro cardiogram). Evidence of right-sided cardiac failure was present (oedema, albuminuria, venous congestion, etc.).

B.M.R. +52.2% (no doubt partly due to cardiac failure).

The creatinuria was measured for five days, the patient having been on a protein-free diet for a week previous to the commencement of the experiment. Ascorbic acid was then given in doses of 300 mgm. daily for one week. During this period the output of creatine was markedly diminished (see Diagram). Vitamin C was then withdrawn and the output of creatine began to rise once more (no measurement was made on the twelfth day of the experiment as the urine was accidentally contaminated).

Apart from the lessening of the creatinuria the patient's condition improved during the time of the experiment and the basal metabolic rate fell to + 36.98%. Rest and sedative treatment however no doubt accounted for the improvement to

a/

a large extent so that it is difficult to draw any conclusion about the value of ascorbic acid apart from the diminution of the creatine loss.

Case II.

Female aged 38 years.

Diffuse goitre. Slight exophthalmos. Tremor +; pulse rate averaging about 96 per min.

B.M.R. was not estimated.

Ascorbic acid was given on the sixth day of the test and a fall in the output of creatine occurred (see Diagram).

Case III.

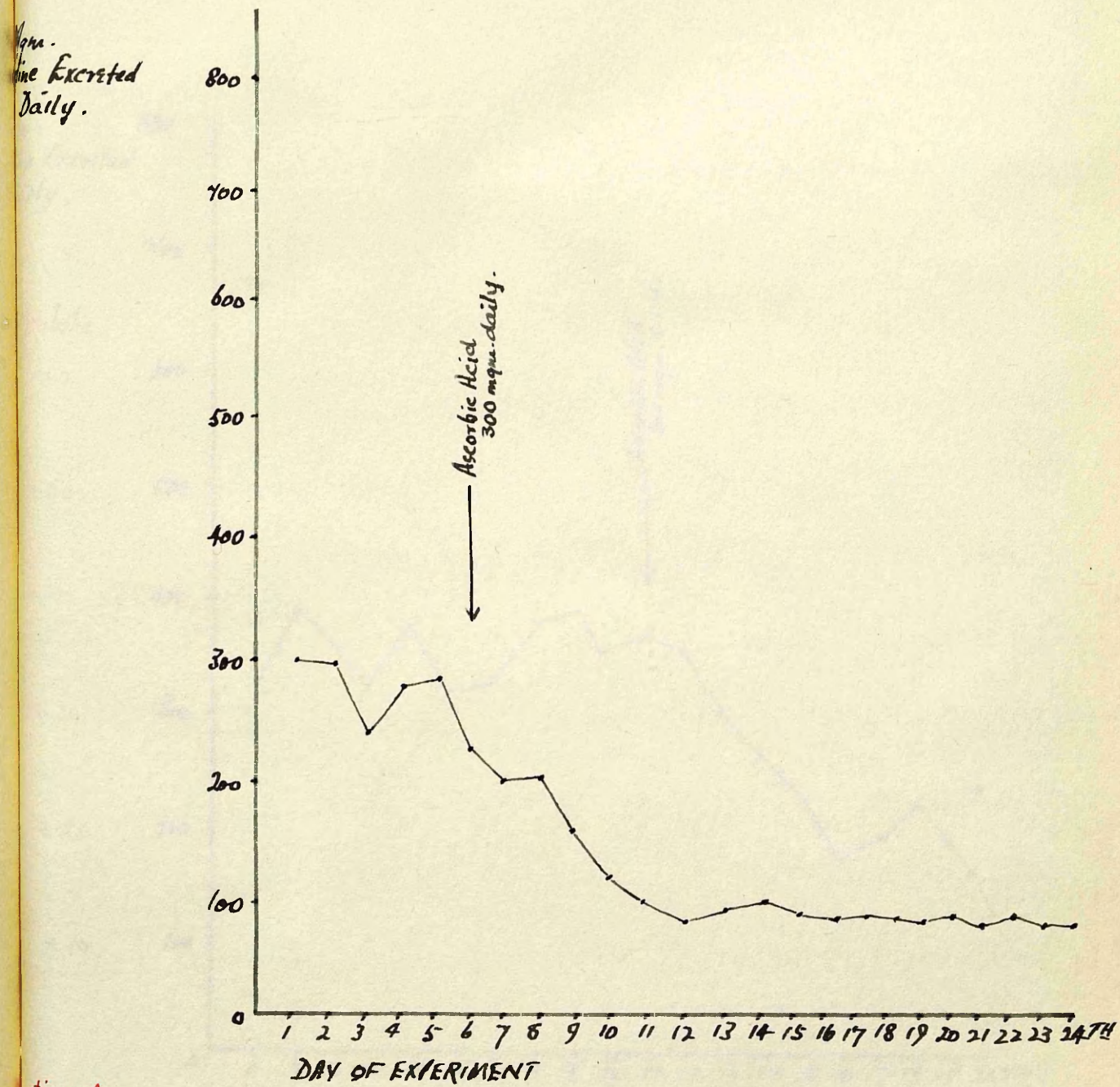
Female aged 36 years.

Diffuse goitre. No exophthalmos. Tremor +; pulse rate about 104 per minute.

B.M.R. + 38.3%.

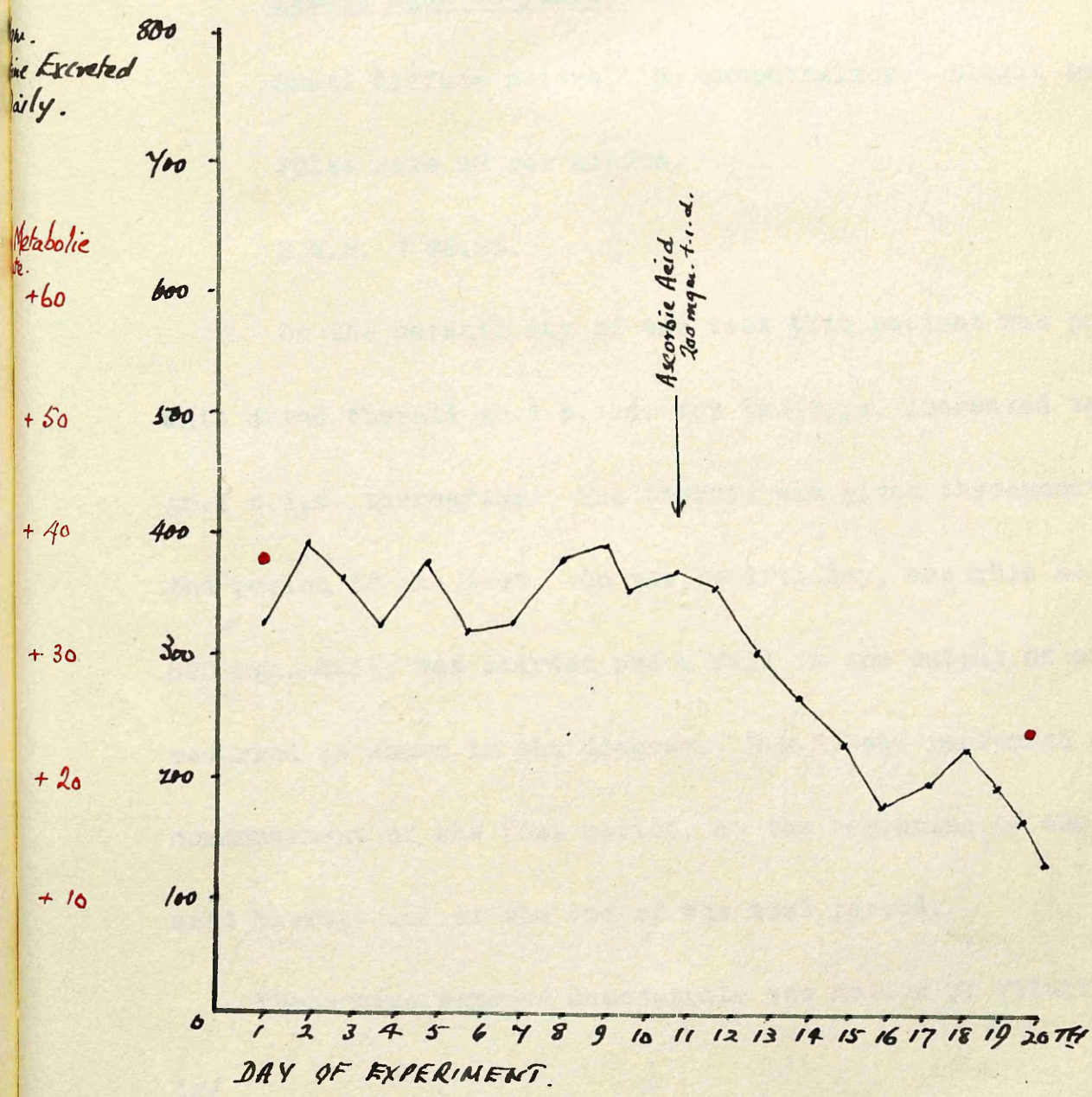
Creatinuria was measured daily and on the eleventh day ascorbic acid was given in doses of 200 mgm. t.i.d. A fall/

CASE II.



ation of
Basal Metabolic Rate not performed.

CASE III.


ine Excreted
ily.
800
700
Metabolic
te.
+60
600
+50
500
+40
400
+30
300
+20
200
+10
100
0
Ascorbic Acid
200 mgm. t.i.d.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20TH
DAY OF EXPERIMENT.

fall in creatinuria occurred (see Diagram). B.M.R. also fell to + 26.6%.

Case IV.

Female aged 33 years.

Small diffuse goitre. No exophthalmos. Slight tremor.

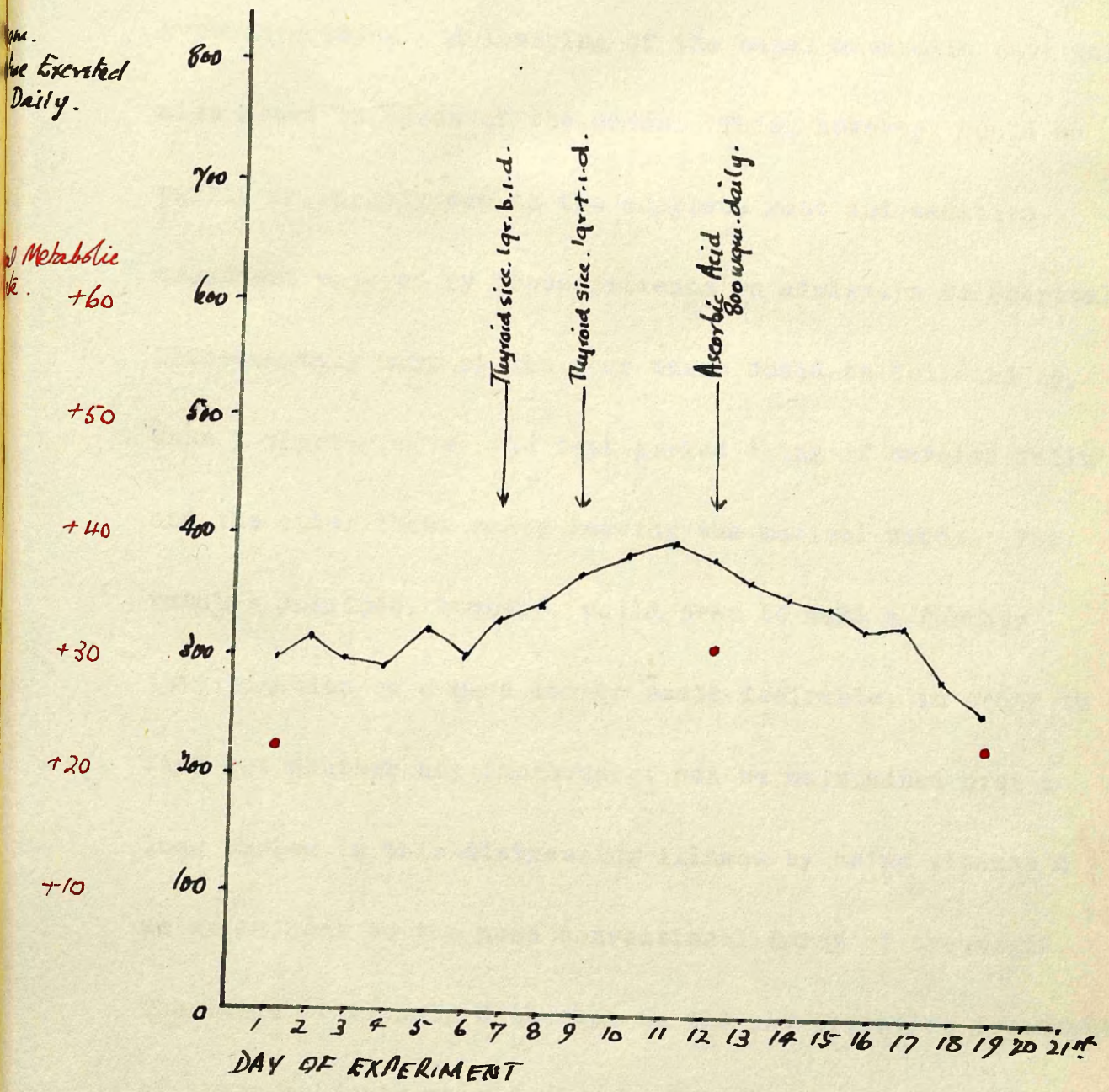
Pulse rate 92 per minute.

B.M.R. + 22.8%.

On the seventh day of the test this patient was put onto dried thyroid gr.i b.i.d. for two days, increased to gr.i t.i.d. thereafter. The thyroid was given throughout the period of the test. On the twelfth day, ascorbic acid 800 mgm. daily was started and a fall in the output of creatine occurred as shown in the diagram. B.M.R. was performed at the commencement of the test period, at the beginning of ascorbic acid therapy and at the end of the test period.

These case reports demonstrate the action of vitamin C in/

CASE IV.


gm.
ine Excreted
Daily.
d Metabolic
te.
+60
+50
+40
+30
+20
+10
800
700
600
500
400
300
200
100
0
Thyroid sicc. 1gr. b.i.d.
Thyroid sicc. 1gr. t.i.d.
Ascorbic Acid
800 mgm. daily.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21st
DAY OF EXPERIMENT

in diminishing the output of creatine in the urine and therefore in lessening the destruction of muscle tissue in hyperthyroidism. A lowering of the basal metabolic rate was also noted in three of the cases. This, however, could be partly or largely due to the complete rest and sedative treatment enjoyed by these patients on admission to hospital. Unfortunately none of the four cases could be followed up, Case I shortly after the test period dying of cardiac failure, and the other three cases leaving the medical wards. The results obtained, however, would seem to make a further investigation on a much larger scale desirable, in order to find out whether any improvement can be maintained over a long period in this distressing illness by using vitamin C as an adjunct to the more conventional forms of treatment. The value of vitamin C therapy in the pre-operative treatment of these subjects should also be considered.

VIII./

VIII. SUMMARY AND CONCLUSIONS.

1. The successive stages of vitamin C research have been described.

2. The importance of adequate vitamin C nutrition and the subject of hypovitaminosis C have been discussed.

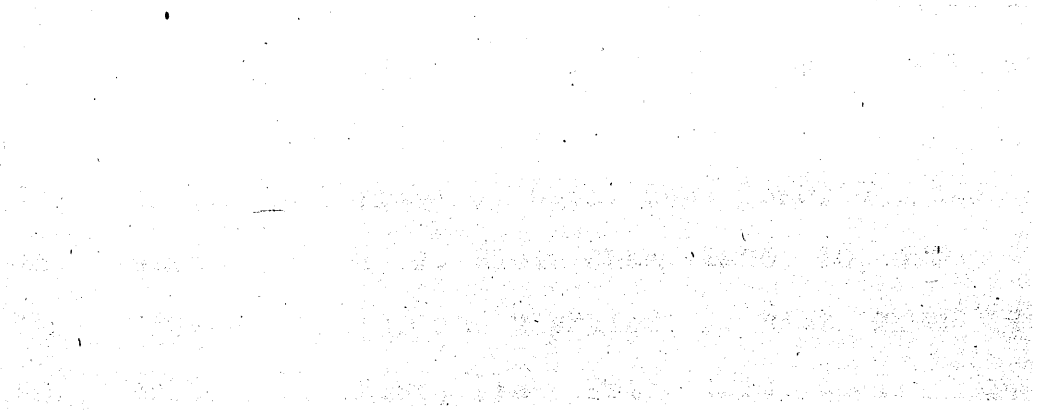
3. Personal observations in children and adults have been reported, and the importance of recognising the hypovitaminotic state stressed.

4. The possible value of vitamin C as a remedial agent should be remembered and some cases of anaemia and hyperthyroidism illustrating this have been detailed.

In conclusion I would affirm that from my own observations I do not doubt that a hypovitaminosis or sub-clinical scorbutic state does exist and is not readily diagnosed. Health depends on good nutrition and this is especially important in infancy and childhood when growth is rapid.

The/

The individual variation in vitamin requirements no doubt exists but there is no proof that any ill results from 'too much' vitamin: too little vitamin can and does lead to interference with nutrition, growth and health. A balanced diet is required to maintain health; during ill health we must make sure that a sufficient quantity of the ultimate essential food factors is available: it is the clinician who has the opportunities to apply this knowledge and convince himself that it is carried out in practice.



BIBLIOGRAPHY.

1. Glisson, Treatise on Rickets (English Translation of 2nd Edition) 1651, 249.
2. Bachstrom, Observations circa scorbutum, (1734).
3. Ingerslev, Hospital-stidende, 1871, 121.
4. Cheadle, Lancet, 1878, 685.
5. Barlow, Trans. Med. Chir. Soc. Lond. 1883, 66, 159.
6. Szent-Györgyi, Biochem. J. 1928, 22, 1387.
7. Tillmans, Hirsch and Hirsch, Ztschr. f. untersuch. d. lebensmittel, 1932, 63, 1.
8. Waugh and King, Science, 1932, 75, 357.
9. Svirbely and Szent-Györgyi, Biochem. J. 1932, 26, 865.
10. Waugh, J. Chem. Educ. 1934, 11, 69.
11. Szent-Györgyi, Nature, 1933, 131, 225.
12. McKinnis and King, J. Biol. Chem. 1930, 87, 615.
13. Zilva, Biochem. J. 1934, 28, 663.
14. Barron, De Meio and Klemperer, J. Biol. Chem. 1936, 112, 625.
15. Pijoan and Klemperer, J. Clin. Invest. 1937, 16, 443.
16. Ray, Biochem. J. 1934, 28, 996.
17. Brown and Morris, J. Chem. Soc. 1890, 57, 458.
18. Szent-Györgyi, J. Biol. Chem. 1930, 90, 385.
19. Tauber, Kleiner and Mishkind, J. Biol. Chem. 1935, 110, 211.
20. Wolbach and Howe, Arch. Path. 1926, 1, 1.
21. Wolbach, Menkin and Menkin, Am.J.Path. 1934, 10, 569.

22. Fish and Harris, Philos. Trans. Ser. B. 1934, 223, 489.

23. Lindow, Elvehjem and Peterson, J. Biol. Chem. 1929, 82, 465.

24. Tillmans, Ztschr. f. untersuch. d. Lebensmittel, 1930, 60, 34.

25. Barron, J. Biol. Chem. 1932, 97, 287.

26. Westergaard, Biochem. J. 1934, 28, 1212.

27. Levine, Proc. Soc. Exper. Biol. and Med. 1936, 35, 231.

28. Svirbely and Szent-Györgyi, Biochem. J. 1933, 27, 279.

29. Harris and Ray, Biochem. J. 1933, 27, 303.

30. Emmerie, Biochem, J. 1934, 28, 268.

31. Emmerie and van Eekelen, Biochem. J. 1936, 30, 25.

32. van Eekelen, and Heinemann, J. Clin. Invest. 1938, 17, 293.

33. Johnson and Zilva, Biochem. J. 1934, 28, 1393.

34. Harris and Ray, Lancet, 1935, 1, 71.

35. van Eekelen, Emmerie, Josephy and Wolff, Klin. Woch. 1934, 13, 564.

36. Hopkins and Millikan, Biochem. J. 1935, 29, 2803.

37. Musulin and King, J. Biol. Chem. 1936, 116, 409.

38. Bersin, Köster and Jusatz, Z.f.physiol. Chem. 1935, 235, 12.

39. Fujita and Iwatake, Biochem. Z. 1935, 277, 293.

40. Ingalls, J. Paediatrics, 1937, 10, 577.

41. Höjer, Acta Paediatr. 1924, 3, Suppl. 8.- 278.

42. Höjer, Brit. J. exp. Path. 1926, 7, 356.

43. Lund, Spur and Fridericia, Biochem. J. 1934, 28, 1825.

44. Birch, Harris and Ray, Biochem. J. 1933, 27, 590.

45. Boas, Fixsen and Roscoe, Nutr. Abstr. & Rev. 1938, 7, 823.

46. Harris, Ray and Ward, Biochem. J. 1933, 27, 2011.

47. van Eekelen, Emmerie, Josephy and Wolff, Nature, 1933, 132,315.

48. Emmerie and van Eekelen, Biochem. J. 1937, 31, 2125.

49. van Euler and Klussman, Ztschr.f.Physiol.Chem. 1933, 219, 215.

50. Farmer and Abt, Proc. Soc. Exper. Biol. and Med. 1935, 32, 1625

51. Hess, Am. J. Dis. Child. 1916, 12, 152.

52. Comby. Bull, Soc. Med. Hôp de Paris, 1921, 45, 288.

53. Stefánsson, J. Am. Med. Ass. 1918, 71, 1715.

54. McCarrison, Lancet, 1931, 1, 1151.

55. Guldager and Poulsen, Hospitalstid. 1935, 78, 1029.

56. Wright, Lillienfeld, and MacLenathen, Arch. Int. Med. 1937, 60, 264.

57. Faulkner and Taylor, J. Clin. Invest. 1938, 17, 69.

58. Mirsky, Swadesh and Soskin, Proc. Soc. Exper. Biol. and Med. 1935, 32, 1130.

59. Stephens and Hawley, J. Biol. Chem. 1936, 115, 653.

60. Harris, and Abbasy, Lancet 1937, 2, 1429.

61. Widenbauer, Klin. Woch. 1936, 15, 815.

62. Laurin, Acta paediatr. 1938, 20, 352.

63. van Eekelen, Biochem, J. 1936, 30, 2291.

64. Wortis, Liebmann and Wortis, J. Am. Med. Ass. 1938, 110, 1896.

65. Schroeder, Deutsch.Med. Woch, 1938, 64, 469.

66. Göthlin, Skand, Arch. f. Physiol. 1931, 61, 225.

67. Schultzer, Acta med. Skand, 1934, 81, 113.

68. Schultzer and Griis, Acta med. Skand, 1935, 85, 563.

69. Schultzer, Acta med. Skand, 1936, 88, 317.

70. Rotter, Nature, 1937, 139, 717.

71. Portnoy and Wilkinson, Brit. Med. J. 1938, 1, 328.

72. Poncher and Stubenrauch, J. Am. Med. Ass. 1938, 111, 302.

73. Göthlin, Nature, 1934, 134, 569.

74. van Eekelen and Wolff, Acta brev. Neerl. 1936, 6, 12.

75. Parsons, Lancet, 1938, 1, 65.

76. Harris, Abbasy, Yudkin and Kelly, Lancet, 1936, 1, 1488.

77. Dyke, Brit. Med. J. 1937, 2, 692.

78. Orr, Food, Health and Income, 1936.

79. Neuweiler, Klin, Woch, 1935, 14, 1040.

80. Neuweiler, Klin. Woch. 1935, 14, 1793.

81. Archer and Graham, Lancet, 1936, 2, 364.

82. Lazarus, Brit. Med. J. 1937, 2, 1011.

83. Platt, Lancet, 1936, 2, 366.

84. Lawrynowicz, J. de Physiol. et de Path. Gén. 1931, 29, 270.

85. King and Menten, J. Nutrition, 1935, 10, 129.

86. Harde, Rothstein and Ratish, Proc. Soc. Exper. Biol. and Med. 1935, 32, 1088.

87. Schroeder, Klin. Woch. 1935, 14, 484.

88. Abbasy, Gray Hill and Harris, Lancet, 1936, 2, 1413.

89. Hasselbach, Deutsch, med. Woch. 1936, 62, 924.

90. Wilson, Lancet, 1938, 1, 667.

91. Wood, Lancet, 1935, 2, 1405.

92. Parsons and Smallwood, Arch. Dis. Child, 1935, 10, 327.

93. Jennings and Glazebrook, Brit. Med. J. 1938, 2, 784.

94. Rohmer, Bezssonoff, Schneegans-Hoch and Sacrez, (Compt.rend.) Soc. de Biol. 1938, 127, 1279.

95. Hamel, Klin. Woch, 1937, 2, 1105.

96. Abelin and Spichtin, Biochem. Z. 1930, 228, 250.

97. Sure and Theis, Proc. Soc. Exper. Biol. and Med. 1938, 37, 646.

98. Harris and Ray, Biochem. J. 1933, 27, 303.

99. Hirsch, Biochem, Z. 1936, 287, 126.

100. Altenburger, Klin. Woch. 1936, 15, 1129.

101. Hirata and Suzuki, Klin. Woch, 1937, 16, 1019.

102. Berg, Arch. f. Exp. Path, 1937, 185, 359.

103. Fischer and Oehme, Klin. Woch, 1937, 16, 1453.

104. v. Plehwe, Deutsch. Arch. f. Klin. Med. 1938, 182, 145.

105. Taylor and Chew, Am. J. Med. Sc. 1936, 191, 256.