

VITAMIN-C REQUIREMENT OF HUMAN ADULTS

EXPERIMENTAL STUDY OF VITAMIN-C DEPRIVATION IN MAN

A PRELIMINARY REPORT BY THE VITAMIN C SUBCOMMITTEE OF THE ACCESSORY FOOD FACTORS COMMITTEE, MEDICAL RESEARCH COUNCIL*

THERE is no agreement on the requirement of vitamin C in man. The League of Nations Technical Commission on Nutrition (1938) estimated the daily requirement of human adults at 30 mg., whereas the U.S. National Research Council's Committee on Food and Nutrition (1943) recommended an allowance of 75 mg. Some authorities put the daily requirement much below 30 mg. (see Zilva 1944). All these are rough estimates.

To obtain information more accurate than that available, on which an assessment of the requirement could be based, a trial was undertaken on human volunteers. It was planned to deprive one group of vitamin C and to give graded doses of the vitamin when signs of deficiency had developed. Other groups were to have supplements of vitamin C from the start as protective doses. The trial was carried out at the Sorby Research Institute, Sheffield, under the supervision of Prof. H. A. Krebs; it lasted from October, 1944, to February, 1946.

Nineteen men and one woman, aged 21–34, volunteered for the experiment. They lived a normal life without strenuous physical work. The basal diet was designed to be as low as possible in vitamin C but complete in every other respect. It was sufficiently varied to be reasonably acceptable. It included milk aerated at 60°C after addition of 10⁻⁶ parts of copper sulphate, and a number of items, such as dehydrated meat, potatoes, and carrots, selected because they could be purchased in bulk, which were cooked in a special way to remove vitamin C. Plum jam was given to meet any possible criticism that factors included under the term "vitamin P" were omitted. A representative daily intake for a volunteer was: protein 104 g., fat 130 g., carbohydrate 340 g., calories 2900, calcium 1.2 g., iron 17.8 mg., vitamin A (exclusive of any carotene in the diet) 4800 i.u., vitamin D 900 i.u., aneurine 1.1 mg., riboflavin 2 mg., and nicotinamide 13 mg. From chemical analyses it was calculated that on the average a volunteer obtained not more than 1 mg. of vitamin C daily from the diet.

To obtain base-line data the experiment began with a preliminary period, in most cases of 6 weeks, on a complete diet including about 70 mg. of vitamin C daily. At the end of the period all the volunteers were given the basal deficient diet and divided into three groups. Ten had no supplements, seven received 10 mg. of vitamin C daily, and three received 70 mg. of vitamin C daily. The volunteers did not know to which group they belonged, nor did the physicians responsible for the clinical investigation. All the volunteers were daily given seven supplementary tablets of identical taste and appearance, some containing vitamin C, the others being dummies. The investigations, made on the volunteers at regular intervals, included general clinical examinations, chemical analyses of blood and urine, hematological examinations, capillary-fragility tests, capillaroscopy, measurements of capillary-filtration rate, radiography, electrocardiography, studies of fatigue, and studies of experimental wounds.

This paper represents the main results of the experiment in summary form; full details of the evidence on

which the conclusions are based, and full references to the literature, will be published elsewhere.

OCCURRENCE OF SIGNS OF DEFICIENCY

In Volunteers Receiving no Supplement

The clinical examinations, by inspection and physical methods, revealed no definite changes during the first 17 weeks of deprivation, beginning on Nov. 13, 1944. The first changes which retrospectively were recognised as significant were enlargement and keratosis of the hair follicles in one volunteer, particularly on the outer aspect of the upper arm. After 21 weeks six of the ten deprived volunteers had developed follicular changes, and after 26 weeks all had done so. In all of them except one the enlarged hair follicles eventually became haemorrhagic. The various stages of development, observed by the skin microscope, were as follows:

The initial change was the plugging of a few follicles by horny material in which the hair was coiled or looped. The number of enlarged hair follicles increased in the ensuing weeks, the main areas affected being the upper arms, back, buttocks, backs of thighs, calves, and shins. A few weeks later the enlarged follicles turned red. Microscopically this redness presented itself as congestion and proliferation of the blood-vessels round the hair follicles; it gradually increased, and within another week or two the enlarged hair follicles became haemorrhagic, the red colour turning dark purple and no longer disappearing on compression; with the microscope many red cells could be seen outside the vessels.

By May, 1945, after 26 weeks of deprivation, six of the ten volunteers, and 9 weeks later nine of the ten, had numerous haemorrhagic follicles. In general it was on the legs that the follicles showed the greatest tendency to become haemorrhagic. No subjective sensations accompanied the appearance of the haemorrhages.

As the development of enlarged and haemorrhagic hair follicles progressed, five of the ten deprived volunteers showed a very pronounced exacerbation of the acne present in a mild form at the start of the experiment. The papules became more numerous after about 22 weeks; they increased in size and later became bright red and eventually haemorrhagic. The other five deprived volunteers who had no acne at the start remained free throughout the experiment.

The second change generally noted during the period of deprivation was in the gums. The earliest signs were tiny haemorrhages in the tips of the interdental papillæ and swelling, seen first in May, 1945, after 26 weeks of deprivation. By the end of July, nine of the ten deprived volunteers had developed abnormalities of the gums. In two cases the changes were gross: the gums were purplish, much swollen, and spongy. In parts the tissue became necrotic, and there was some bleeding. In five others the gum changes, all located in the interdental papillæ, were less advanced but beyond question; they consisted of small haemorrhages, swelling, and purplish discoloration. In two more of the men swelling and haemorrhages developed but to a less extent, and their scorbutic origin was less certain; one of these subjects was edentulous. In general the teeth and gums were in good condition at the start of the experiment, but the two volunteers who developed the most severe gum changes showed evidence of gingivitis and parodontal disease at the start of the deprivation.

Another striking observation, in agreement with the old accounts of scurvy, was recorded from June, 1945, onwards in six of the ten deprived volunteers, affecting the behaviour of the scars of the experimental wounds (see below). Scars of wounds made between February and May, whose healing had proceeded normally, became red and livid. New wounds made at the stage of pronounced haemorrhagic scurvy showed a reduced tendency to heal.

Some important abnormalities were observed in single cases. One man developed effusions into both knee-joints and ecchymoses of the leg in June, 1945, after a

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long walk. Another was taken ill in July, 1945, nineteen hours after heavy physical exercise. He had severe pain in the lower sternal region, and he became dyspnoeic and cyanosed. The pulse was rapid and the blood-pressure low. The clinical picture was that of an acute cardiac emergency. He was immediately admitted to hospital and dosed with vitamin C. The lower sternal pain, which at first had increased in intensity, passed off after nine hours. The electrocardiogram showed high ST levels in leads I and II. A radiogram of the chest showed no abnormality. Eighteen days later another deprived volunteer complained of a sudden constrictive pain in the chest. Physical examination revealed a systolic murmur which had not been heard before, and the electrocardiogram showed a partial heart-block, the P-R interval being 0.28 sec. Before the experiment the electrocardiogram had been normal with a P-R interval of 0.20 sec. It was thought necessary to treat this volunteer immediately with large doses of vitamin C. The chest pain and the systolic murmur disappeared within twenty-four hours, but during the following months the P-R interval showed variable periods between 0.20 and 0.28 sec., depending on posture, breathing, administration of drugs, and other factors. These subsequent electrocardiographic observations raised the question whether the heart-block in this case had any connexion with the deficient diet, especially in view of recent observations on Service personnel (see Manning and Stewart 1945, Hall et al. 1942, Holmes and Weill 1945).

A modification of the "agility" test (Frankau 1943), which had been used to demonstrate the acceleration of coöordinated muscular effort in human subjects given niacinamide, was used to measure objectively the incidence of fatigue in the volunteers. Interruption in the consecutive sequence of the tests, caused by the infliction of the experimental wounds, interfered seriously with the manifestation of any clear-cut trends. In all three groups, however, accuracy of coöordinated movement was unaltered throughout the trial; in the totally deprived group there appeared first a variability, and later a small but significant increase, in the time taken to perform the test. Both these observations indicate increased fatigue. On the pulse-rate the effect of the "all-out" effort demanded by the agility test was to cause a rise followed by a fall in the 3 min. immediately after the test, both of which were significantly greater in the group receiving 70 mg. than in the groups receiving 10 mg. or none.

There was no evidence in any of the volunteers of serious psychiatric disturbances connected with the deprivation of vitamin C; neither the character of the diet, with the restrictions entailed by adhering to it, nor the somewhat abnormal conditions of life proved unduly irritating or difficult. The appearance of clinical signs of scurvy was followed by a wave of instability, introspection, and curiosity about the composition of the groups. This phase was only transitory and was followed by no psychiatric disturbance. An "attention" test was introduced as an objective check on the alleged occurrence of apathy in scorbutic subjects. No evidence of deterioration in performance was found.

Measurements of capillary-filtration rate were made by a method modified from that of Landis and Gibbon (1933); no significant changes were found in the deficient group as the experiment progressed.

Some other negative findings are worth recording. There was no significant change in body-weight, no increased incidence of infection, and no change in the appearance of the capillaries of the nail bed and of the conjunctivæ. Dark-adaptation, as measured by the Wald-Stevens-Bartley apparatus and by rod scotometry, remained normal. Subjectively there were no complaints of general pains or weakness.

Special steps were taken to look for haemorrhages elsewhere than in the skin and mouth but none were

found. The urine never contained red cells. There was no occult blood in the stools of the two subjects who were tested at the height of their scorbutic state. There was no epistaxis, and no conjunctival haemorrhages were seen on slit-lamp examination.

The capillary-resistance tests of Hess (1920) and of Göthlin (Falk et al. 1932) showed no consistent trends throughout the period of deprivation. Other capillary tests, made by Dr. Harold Scarborough by his special method, will be described in a separate publication.

Hæmoglobin concentration, red-cell count, total and differential leucocyte-counts, platelet-count, and bleeding-time showed no significant changes during the course of the depletion. The results of chemical tests on the blood in deprived and in non-deprived subjects are considered below.

In Volunteers Receiving 10 mg. of Vitamin C Daily

In the seven volunteers receiving a supplement of 10 mg. of vitamin C daily no abnormalities were noted during the first 160 days of the experimental period. It was then decided that four of the volunteers should continue with the 10 mg. supplement and three of them be deprived of it, the object being to ascertain whether signs of deficiency would develop quickly on withdrawal of the supplement.

Three of the four volunteers to receive 10 mg. continued for another 264 days, and one abandoned the experiment after another 92 days. No abnormalities were recorded. Wound healing, as judged by the appearance of the excision scar on inspection, proceeded normally, and, in contrast with the deprived group, there were no hemorrhages into the scar tissue.

The second group of three volunteers had no supplement for 71 days, broken in one case by a 26-day period on a supplement of 10 mg. Towards the end of the period of 71 days two of these volunteers showed a few keratotic, but not haemorrhagic, hair follicles; this abnormality disappeared when large doses of vitamin C were given, and it was probably connected with the deficient diet. The third man of this group showed no changes at the end of 71 days. At this stage of the experiment (July, 1945) two totally deprived volunteers showed the cardiac disturbances already mentioned. It was therefore decided to restore the supplement to the extent of 5 mg. daily. On this dose the three men continued for another 125 days. Two of them showed a slight increase in the number of keratotic hair follicles but no other clinical changes.

In Volunteers Receiving 70 mg. of Vitamin C Daily

This group of three volunteers served as positive controls for 300, 326, and 331 days. No changes worthy of note were recorded.

EFFECTS OF VARIOUS DOSES ON DEPRIVED SUBJECTS AND ON THOSE ALREADY GIVEN SUPPLEMENTS

Three of the ten volunteers who developed scurvy on the unsupplemented diet were lost for trials with graded doses, because sudden emergencies demanded their immediate treatment with large doses of the vitamin. Two of these three, as already mentioned, were dosed because they had signs of acute cardiac complications. A third had shortness of breath and pain in the chest in May, 1945. Radiography revealed spondylitis and a paravertebral abscess, and spinal tuberculosis was diagnosed. A review of earlier radiograms indicated that this man's spine was not healthy at the beginning of the experiment. At the time when he developed symptoms his skin and gums showed the most advanced scorbutic changes seen in the volunteers. In view of his serious state he was at once given a large dose of vitamin C. The three volunteers dosed with large amounts showed striking improvement within a few days.

The remaining seven all showed unequivocal signs of scurvy in multiple skin haemorrhages and gum lesions.

It was desired to select the minimum dose likely to produce a cure within a reasonable time but to aim too low rather than too high, since the dose could be increased later if necessary. A daily dose of 10 mg. was chosen and given to six of the seven volunteers. The seventh received 20 mg. because this volunteer was not available for long.

Result of Dosing with 10 mg. of Vitamin C Daily

The response to the dose of 10 mg. followed the same pattern in all six cases. Within a week haemorrhages into the perifollicular region ceased, and within 1 or 2 weeks the older haemorrhages began to lose their dark purple colour and gradually faded. Within a month the hair in most of the follicles uncoiled, lifting out the plug. The dilatation and congestion of the capillaries round the hair follicles disappeared, and within 7-9 weeks the skin appeared normal except for a slight brown pigmentation at the site of the former haemorrhages.

The liability to haemorrhage in the wound tissue and the failure of the wounds to heal disappeared as the follicular eruptions regressed. The wound haemorrhages disappeared within 2 months, the original blue and purple colour gradually giving way to a pure red, pink, and finally pale brown, and changes in the appearance of the wounds indicated improved healing.

The acneiform papules likewise regressed to the pre-experimental state, though in most cases somewhat more slowly than the other skin signs. The initial state was regained within 10-18 weeks.

The gum lesions did not respond to dosing as promptly as did the follicular skin lesions. When improvement began, the first sign was a change from livid blue to bright red, followed by the normal pink. Slowly the swelling decreased and the consistency of the gums improved, restoration being complete within 10-14 weeks.

Result of Dosing with 20 mg. of Vitamin C Daily

One volunteer, as already stated, received 20 mg. of vitamin C daily at the end of the depletion period. Both the skin and gum lesions were slight, consisting of a limited number of haemorrhages. Complete restoration was achieved within 3 weeks.

Five of the six volunteers who had been treated with 10 mg. of vitamin C daily, and cured of clinical scurvy, received subsequently a daily dose of 20 mg. of vitamin C for 47-92 days. The appearance of skin, gums, and wounds showed no further changes.

VITAMIN-C CONTENT OF THE BLOOD

Vitamin-C determinations were made on blood taken from the subjects in a fasting condition. Vitamin C in the plasma and in the white cells of the blood (Butler and Cushman 1940) was estimated by the dye titration method at weekly or fortnightly intervals throughout the trial.

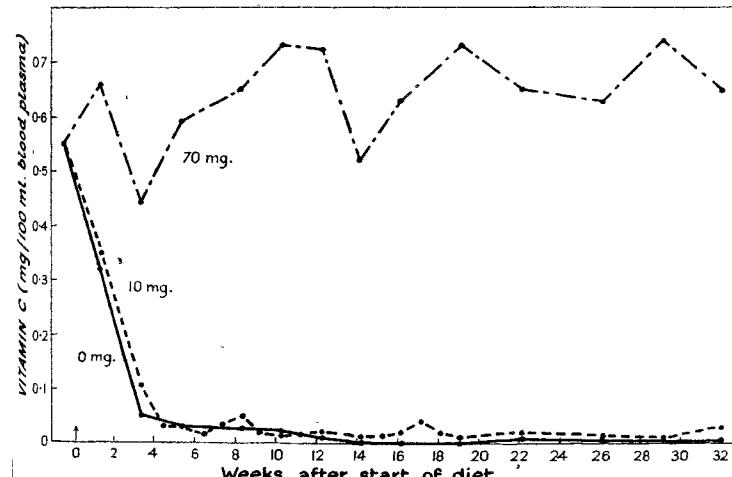


Fig. 1.—Average vitamin-C content of blood plasma, estimated with dichlorophenolindophenol, of the groups of volunteers receiving daily 70, 10, or 0 mg. of ascorbic acid as supplement to the basal diet, which contained about 1 mg.

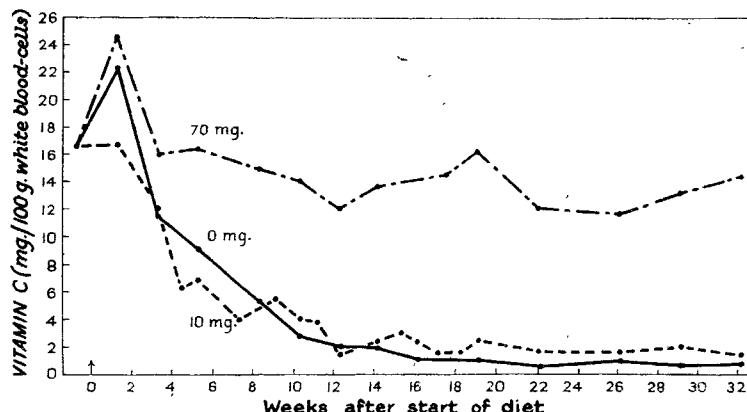


Fig. 2.—Average vitamin-C content of white blood-cells, estimated with dichlorophenolindophenol, of the groups of volunteers receiving daily 70, 10, or 0 mg. of ascorbic acid as supplement to the basal diet, which contained about 1 mg.

The average values for the concentration of vitamin C in the plasma and white blood-cells are shown in figs. 1 and 2.

Vitamin-C Content of Plasma.—The initial average value for all the volunteers, at the end of the 6-week preliminary period with an intake of about 70 mg. daily, was 0.55 mg. per 100 ml. of plasma, and this value was maintained throughout the period of the experiment by those receiving 70 mg. daily. In the totally deprived volunteers the average value was 0.03 mg. per 100 ml. after 37 days, and remained between 0 and 0.03 mg. per 100 ml. for the rest of the deprivation period. In the volunteers receiving a supplement of 10 mg. of vitamin C daily the average value was 0.03 mg. after 31 days and fluctuated between 0.01 and 0.05 mg. per 100 ml. during the experimental period. Owing to the shortcomings of the analytical method no significance can be attached to the slight differences in the concentration of vitamin C below 0.05 mg. per 100 ml. of plasma.

Vitamin-C Content of White Blood-cells.—The initial average value of the white-cell concentration for all volunteers was 16.6 mg. per 100 g., and was maintained at this level throughout in those receiving 70 mg. of vitamin C daily. In the totally deprived group it fell to 1 mg. per 100 g. in 113 days, and remained below this value for the remainder of the period. For concentrations below 2 mg. per 100 g. of white cells the method is not very accurate, and differences between 0 and 2 mg. per 100 g. are of doubtful significance. The average concentration of the vitamin C in the white cells of the volunteers receiving a supplement of 10 mg. of vitamin C daily attained a value between 1.5 and 3 mg. per 100 g. in 86 days, and from the 109th day onwards remained roughly 1 mg. per 100 g. above the figure of the deficient group.

Relation between Clinical Signs and Vitamin-C Content of Blood.—About 100 days elapsed between the virtual disappearance of vitamin C from the plasma and the first clinical signs of scurvy. On the other hand, the concentration of vitamin C in the white cells reached its lowest value only 3-6 weeks before clinical scurvy appeared.

Effect of Dosing with Vitamin C on Blood Concentration.—When the deficient volunteers were dosed with 10 mg. of vitamin C daily, the concentration of the vitamin in the plasma and white cells showed a small but distinct rise towards the end of a dosing period of 101-157 days. The average concentration of the plasma rose from 0.016 to 0.06 mg. per 100 ml., and that of the white cells from below 1 mg. to 2.7 mg. per 100 g. Increasing the dose to 20 mg. daily produced no change in the vitamin-C concentration of the plasma, and a slight rise in that of the white cells to 3.6 mg. per 100 g.

Relation between Blood Concentration and Vitamin-C Intake.—It is remarkable that an intake of 10 mg. daily above the basal level (estimated at about 1 mg.) hardly affected the concentration of the vitamin in plasma

and white cells. There seemed to be a significant difference between the average value for the deficient group and the average value for the group receiving a supplement of 10 mg. of vitamin C daily, but it is doubtful whether a single blood determination could differentiate the concentration of vitamin C in plasma or white cells of persons on a prolonged intake of about 1 mg., which in nine cases out of ten produced scorbutic haemorrhages after 6-8 months, from that of persons on an intake of about 11 mg., which over a period of 14 months prevented the appearance of the clinical signs of scurvy.

To obtain further data correlating the vitamin-C intake with its concentration in the plasma and white blood-cells, two volunteers who had received 70 mg. of vitamin C daily for 326 and 331 days were given 50 mg. daily for 66 and 61 days. Towards the end of these periods the vitamin-C concentration in the plasma had fallen from 0.64 and 0.52 mg. to 0.32 and 0.29 mg. per 100 ml., and in the white cells from 12 and 10 mg. to 9.6 and 7.6 mg. per 100 g.

Synopsis of Data on Vitamin-C Content of Blood in Relation to Vitamin-C Intake.—The accompanying table summarises the data on the concentration of vitamin C in the plasma and in the white-cell layer at different levels of intake (see also Thysell 1939).

VARIOUS OTHER BLOOD EXAMINATIONS

The following chemical tests on the blood plasma gave no significant variations from normal values, relative to the vitamin-C intake: plasma-protein, albumin and globulin ratio, urea, and phosphatase.

EXPERIMENTS ON WOUND REPAIR

Experiments were undertaken to extend the work of Lund and Crandon (1941), Pijoan and Lozner (1944), and Farmer (1944) on wound healing in vitamin-C deficiency in man.

Procedure.—Preliminary tests indicated the suitability of a linear incision 3 cm. long and a stab wound 1 cm. long, both on the outer aspect of the upper thigh. The linear incisions were made to the depth of the fascia lata and were sutured with three gut stitches, removed after 4 days, and covered with a pad, which was removed after 10 or 21 days, when a swab was taken to test sterility and the scar was excised. The gap was sutured and left to heal. The excised material was cut into several pieces to be examined histologically, and for breaking-strain. The stab wounds were made by pushing a scalpel 1 cm. wide to a depth of 1 cm. The wound was covered with 'Elastoplast' without suture and was excised for histological examination after 10 or 21 days. In all, 72 wounds were made on 19 volunteers.

Appearance of Wounds on Inspection.—Reference to the appearance of the wound scars on inspection has already been made. These statements refer to the wounds left after excision of the first incision or stab. They do not refer to the scars, whose physical and histological properties are described below. Since the latter were covered with a dressing throughout they could not be observed; when they were seen at the time of excision no abnormality was observed except in one instance which is noted below. In the groups receiving a 10 or a 70 mg. supplement no abnormalities were ever seen in the excision wounds, but in the deprived group at the height of the depletion the excision wounds had a reduced tendency to heal, and older wounds which had begun to heal normally showed haemorrhages into the scar and surrounding tissues.

Histological Observations.—The main histological criteria for assessing wound repair were union of epidermis, quantity of collagen, quantity of reticulin, maturity of fibroblasts, and appearance of blood-vessels. According to Wolbach (1933) "wounds on completely depleted scorbutic guineapigs show adequate fibroblastic proliferation but no reticulin formation." On low doses of ascorbic acid, however, Danielli et al. (1945) found profuse reticulin formation but no maturation to collagen.

VITAMIN-C CONTENT OF PLASMA AND WHITE CELLS IN RELATION TO VITAMIN-C INTAKE

Vitamin-C intake additional to about 1 mg. in basal diet (mg. daily)	No. of subjects	Duration of dose (days)	Average vitamin-C concentration in—	
			Plasma (mg./100 ml.)	White cells (mg./100 g.)
0	10	205-269	<0.03	<1.0
5	3	125	<0.05	2.0
10	6	101-157	<0.10	2.7
20	5	47-92	<0.10	3.6
50	2	61-66	0.31	8.6
70	3	300-336	0.55	10.0
About 600	15	8-11	1.02	17.0

The data refer to average values found towards the end of a period on the dose specified in the first column. The figures recorded in the bottom line of the table are the averages of the highest values observed in each of the 15 volunteers who were given a "saturation" test at the end of the experiment, when they received a dose of 10 mg. of vitamin C per kg. of body-weight for 8-11 days. All data were obtained in the fasting state.

According to unpublished work of Penney and Balfour on guineapig wounds there may, in complete deficiency, be also decreased vascular and fibroblastic proliferation.

It was found that 10 days was too short a period to show up major differences in healing, especially in collagen formation, and after preliminary tests this period was abandoned in favour of one of 21 days. The findings in the linear and stab wounds excised after 21 days resembled one another sufficiently to justify their being considered together. To classify the observations, the wound responses were placed in two main grades: (1) normal responses; and (2) gross abnormalities of the type seen in wounds made on scorbutic guineapigs. Wounds showing macroscopic haemorrhages or infection were omitted from the series.

All wounds from subjects belonging to the groups receiving either 70 or 10 mg. of vitamin C came within the first or normal grade, except those from one man whose skin showed definite endarteritis and should therefore be excluded as abnormal. In the group of totally depleted subjects seven 21-day wounds were made after 5-8 months' deficiency. Of the seven wounds four showed scorbutic lesions and three showed none; one of the wounds showed good healing though it was made when clinical signs of scurvy were well developed.

Six further wounds on these subjects, made after they had been "saturated" with vitamin C, showed considerable variation. In three cases healing was of the first or normal grade, but in the other three it was poor and an unusually large amount of degenerated collagen was present at the side of the wound track.

Breaking-strain.—The data discussed in this paragraph all refer to wounds excised after 21 days. There was no difference as regards breaking-strain of the wounds between the groups supplemented with 10 and 70 mg. of vitamin C daily; values between 12 and 31 kg. per sq. cm. were recorded for each. At the height of depletion three acceptable readings were obtained from deficient subjects, all of which gave lower values (6.0-8.8 kg. per sq. cm.) than those of the supplemented groups (12-31 kg. per sq. cm.). In three volunteers the breaking-strain was still low (7.2-10.0 kg. per sq. cm.) after saturation. The correlation between low tensile strength and histological evidence of poor healing was not close. Of nine wounds made either before or after saturation, in which the breaking-strain might be considered low (less than 11 kg. per sq. cm.), healing was histologically satisfactory in four. The low tensile strength in these cases may have been due to non-scorbutic abnormalities whose cause and nature are not clear.

Conclusions.—Judged by the criteria available, a dose of 10 mg. of vitamin C daily was sufficient to maintain the normal healing power of the skin up to 11 months. In the non-supplemented group severe defects in wound healing occurred similar to those recorded in scorbutic guineapigs. These defects were encountered only when, and not before, clinical signs of scurvy had appeared, i.e., after 6 months' depletion.

DISCUSSION

Course and Signs of Depletion

No attempt is made here to discuss the literature on scurvy. The course of the development of scurvy was fairly uniform in the ten volunteers and very similar to that in the case described by Crandon et al. (1940). The general course was as follows: for about 17 weeks no clinical signs; after 17–21 weeks the first sign was hyperkeratosis of the hair follicles (see Wiltshire 1919); after 26–34 weeks perifollicular haemorrhages; and after 30–38 weeks swelling and haemorrhages of the gums. Exacerbation of acne, not apparently hitherto recognised as a sign of scurvy, began after 22 weeks.

Like all the other single clinical signs of scurvy, neither hyperkeratosis nor congestion of the hair follicles is a specific sign, and the occurrence or gradual development of either of them in a person does not necessarily indicate lack of vitamin C. They occur in many people "saturated" with vitamin C. Deficiency in this vitamin is only one of a variety of causes which can evoke them. In the present trial the appearance and disappearance of the skin changes strictly reflected the intake of vitamin C, and this proved beyond doubt that they were the early stages of the typical haemorrhagic spots of scurvy.

The gum lesions appeared always after the skin lesions. Though this may not always be true of scurvy, it might nevertheless be a useful diagnostic pointer in deciding on the cause of gum lesions of doubtful origin.

Many signs listed as scorbutic in the classical description of the disease—e.g., pallor, dryness of the skin, anaemia, and night-blindness—were not observed. It is probable that classical scurvy was often a multiple deficiency.

Intake in Relation to Level in Plasma and White Cells

So long as the diet contained no more than 20 mg. of vitamin C daily, the average plasma level remained below 0·10 mg. per 100 ml. At higher levels of intake the concentration of the vitamin in the plasma rose. A concentration of about 0·30 mg. per 100 ml. corresponded to an intake of 50 mg. daily, and of about 0·55 mg. per 100 ml. to an intake of 70 mg. daily (compare Thysell 1939). When the vitamin was withdrawn from the diet, the plasma level began to fall almost at once. In contrast, the vitamin-C level in the white cells fell much more slowly on the withdrawal of the vitamin. Nevertheless, as the table shows, the level in the white cells did to some extent reflect the dietary intake. In general the level in the white cells was about 25 times that in the plasma.

For assessing the state of vitamin-C nutrition it appears that, in a fasting person, a plasma value below 0·10 mg. per 100 ml. indicates an average daily intake in the region of 20 mg. If, therefore, in a doubtful case of scurvy the plasma level is 0·10 mg. per 100 ml. or more, the existence of scurvy is very improbable, since the intake of 20 mg. daily, necessary to maintain a plasma level of 0·10 mg. per 100 ml., was found to be an adequate curative dose. On the other hand, a plasma level of below 0·10 mg. per 100 ml., though an accompaniment of scurvy, is not proof of scurvy or of imminent scurvy. At present, therefore, the main clinical use of the plasma value for vitamin C is to exclude rather than to confirm the diagnosis, and this is likely to remain so as long as the technique does not distinguish more accurately than at present between levels of 0 and 0·10 mg. per 100 ml.

The determination of vitamin C in the white cells is of somewhat greater diagnostic value, because it shows more definite differences between the intake levels of 20 mg., 10 mg., and less than 5 mg. daily. A concentration below 2 mg. per 100 g., especially when confirmed on repeated analyses, indicates severe depletion and supports the diagnosis of scurvy. Eventually, with some further improvement in the technique, it may be possible to assess the dietary intake from the result of vitamin-C determinations in the white cells.

Requirement of Vitamin C

The term requirement is here used to mean the amount of a dietary essential which must be eaten to maintain full health. In using the facts obtained in the present trial for an assessment of the human requirement the diet and the mode of life of the volunteer must be kept in mind. The main facts relevant to the assessment of the requirement are as follows:

(1) A supplement of 10 mg. cured clinical scurvy in all six cases examined.

(2) A supplement of 10 mg. protected seven volunteers throughout the period of observation, which, in the case of three of them, extended to 424 days.

(3) When a 10 mg. supplement was withdrawn from three volunteers after 160 days and was followed by a period of 195 days during which the intake varied slightly, but for which the average intake was 3·2, 3·2, and 4·5 mg. of vitamin C daily, no definite clinical signs of scurvy—i.e., no haemorrhages—appeared.

These facts suggest that in the group under test the "minimal protective dose" of vitamin C, as measured by the criteria of the presence of scurvy, was in the region of, perhaps somewhat below, 10 mg. daily. On the other hand, the tests of physical fatigue, though not producing conclusive results, leave some doubt whether 10 mg. was an optimal dose, since the statistical analysis revealed small differences in favour of the group receiving 70 mg. against the group receiving 10 mg. It would not be unexpected that the prevention and even cure of clinical scurvy should require a smaller dose than the attainment of maximal efficiency under conditions of stress such as those produced by the "agility" test.

Distinct from the minimal protective dose for a particular group of people, in this case a few normal young adults leading a life without strenuous physical work, is "the larger figure which shall cover the requirement of normal adults with their own inherent variability enhanced by the variety of their activities and environment and ensure for them the margin of protection at which it is decided to aim" (Hume and Krebs 1948). To satisfy these ill-defined additional needs and to allow a margin of safety it does not therefore seem too generous to treble the minimal protective dose of 10 mg., which prevents clinical scurvy, and thereby confirm the figure of 30 mg. of vitamin C daily recommended by the League of Nations Technical Commission on Nutrition (1938) for the requirement of a normal human adult.

Any assessment is, at the present state of knowledge, a matter of judgment and must be regarded as provisional. The present assessment has a firmer basis than previous estimates in that it rests on the determination of the minimal protective dose for a group of human beings. The new estimate is considerably below the allowance of 75 mg. recommended by the U.S. National Research Council's Committee on Food and Nutrition (1943), which is essentially the amount necessary to maintain "saturation"; but, so long as there is no evidence to support the view that an intake of more than 30 mg. daily has beneficial effects, there is no basis for recommending an intake greater than that amount.

When this figure is used, for whatever purpose, it should be borne in mind how it was assessed. It is obvious that intakes much below the recommended figure, which are reflected in a plasma concentration of vitamin C not

distinguishable from a scorbutic one, are not necessarily detrimental to health.

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RESIDUAL SYMPTOMS IN GRAVES'S DISEASE AFTER THYROIDECTOMY

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PATIENTS who have undergone thyroidectomy for Graves's disease (primary thyrotoxicosis) not infrequently retain symptoms such as nervousness, palpitations, sweating, tremor, and lability of the pulse-rate on emotion or exertion, besides some of the eye signs of thyrotoxicosis. Joll (1932) admitted the frequency of these postoperative symptoms: "If complete and permanent disappearance of all signs and symptoms of the disease is to be the necessary criterion for success (of thyroidectomy) comparatively few patients would pass the test." The symptoms are often loosely termed "residual signs of toxicity," but their origin has never been clearly demonstrated, though various causes have been suggested.

Warthin (1928) and Moschcowitz (1930) postulated a "Graves's constitution," or background of nervous instability to the disease, which could not be eradicated by thyroidectomy and persisted as a cause of post-operative symptoms. Rasmussen (1937) observed similar symptoms which remained in cases of Graves's disease after surgical, X-ray, or medical treatment. He termed them "the psychoneurotic syndrome of Basedow's disease" and considered that they were acquired as part of the disease. Rundle (1941) attributed post-operative symptoms to a permanent canalisation of their responsible nervous pathways during the active course of Graves's disease. Finally, Moschcowitz and Bernstein (1944) suggested that neurocirculatory asthenia (effort syndrome or cardiac neurosis) not only provided the background of Graves's disease but also remained to cause symptoms after thyroidectomy.

Thus, though the published work provided nothing stronger than conjectures about the origin of the post-operative symptoms, it did at least offer alternatives to residual thyrotoxicosis as a cause. It appeared, therefore, that a careful analysis of the postoperative symptoms in cases of Graves's disease, particularly as regards their time-relationship with the course of the disease and treatment, might enable the various suggestions to be tested. As a working hypothesis, it seemed that the post-operative symptoms might have the following origins:

(1) *Constitutional*.—Symptoms of this kind would have been present before the onset of Graves's disease and would have persisted after thyroidectomy.

(2) *Residua of Graves's Disease*.—These would have arisen during the active course of the disease and would have persisted afterwards.

(3) *Effect on the Patient of having had Graves's Disease and Thyroidectomy*.—These symptoms would have arisen after thyroidectomy.

(4) *Causes Unconnected with Graves's Disease or Thyroidectomy*.—Symptoms of this kind might have existed before or arisen after thyroidectomy but would be unconnected with it or with constitutional symptoms.

METHODS AND MATERIAL

It was obviously essential to examine patients whose postoperative thyroid function was normal, to avoid the hazard of symptoms caused by hypothyroidism or residual thyrotoxicosis. Accordingly 33 patients were selected in whom thyroid function appeared to be normal, as judged clinically and by basal metabolism estimations when possible. All patients had undergone thyroidectomy for Graves's disease (primary thyrotoxicosis) confirmed histologically. At follow-up the intervals after thyroidectomy ranged from two and a half to ten years. Of the 33 patients, 22 were women. The average age of the women at thyroidectomy was 30 and of the men 38. Graves's disease had been mild in 5 cases, moderate in 13, and severe in 15.

Detailed inquiry was made at examination into any remaining symptoms, and the time of their onset was determined. It was thus possible to assign them to one or other of the categories set out above.

INCIDENCE OF SYMPTOMS

Of the 33 cases only 2 (1 mild and 1 severe) had no residual symptoms at all, a striking confirmation of Joll's dictum. The remaining 31 cases had the following symptoms: nervousness (18), eye signs (16), palpitations (14), dyspnoea on exertion (13), lassitude (12), tremor (11), sweating or dislike of hot weather (10), disturbed sleep (7), headaches (5), phobias (4), left submammary pain (3), and diarrhoea (1). All these symptoms can exist in active Graves's disease, and any of them in combination with persistent eye signs may give an erroneous impression of residual thyrotoxicosis after thyroidectomy. In this inquiry, apart from the eye signs, which alone constituted the symptoms in category (2), the symptoms were distributed among the other three categories without any discernible pattern. The following analysis of symptoms in the various categories simply aims at showing how they arose and does not attempt to estimate their frequency.

(1) *Constitutional Symptoms*

In the analysis of constitutional symptoms inquiry was first made into the physical state and personality of the 33 patients before the onset of Graves's disease. They fell into three broad groups:

(a) *Normal*.—13 cases. These were apparently normal and stable persons, of whom 7 had had no symptoms before the onset of Graves's disease, and 6 had had "symptoms" which were scarcely pathological, such as lifelong dislike of hot weather, mild phobias of crowds or of confined places, and occasional distress on exertion.