VITAMIN C REQUIREMENT OF HUMAN ADULTS

An Experimental Study of Vitamin C Deprivation in Man

INTRODUCTION

THE work described in this Report was undertaken with the object of gaining further knowledge of the vitamin C requirement of human adults. There is no general agreement on even an approximate value for the requirement. The League of Nations Health Organisation (1938) through their Technical Commission on Nutrition estimated the daily requirements of adults at 30 mg., whereas the National Research Council (1948) in the United States recommended an allowance of 75 mg. Some authorities put the daily requirement much below 30 mg. (Rietschel, 1940; Zilva, 1941, 1944), and others suggest quantities even above 75 mg. (Ralli, Friedman and Sherry, 1939; Todhunter and Robbins, 1940; Linghorne, McIntosh, Tice, Tisdall, McCreary, Drake, Greaves and Johnstone, 1946; Scheunert, 1948–9). The divergences in the estimates arise from the different standards used in assessing the requirement. Most of the high values are based on studies of the saturation of the body with vitamin C, while the lower values are inferred from observations of the prevention and cure of the clinical manifestations of scurvy.

Uncertainty about the requirement was often felt when questions of food policy and the need for dietary supplements were raised during the Second World War. It was, therefore, planned to carry out a trial on volunteers on the same lines as the experiment on vitamin A requirements (Hume and Krebs, 1949). A number of volunteers were to be given a diet deficient in vitamin C but otherwise adequate until signs of scurvy appeared. The minimum amount of vitamin C which would cause the signs of deficiency to disappear was then to be ascertained. Other volunteers were to receive the same basal diet with prophylactic doses of vitamin C given daily from the start of the experiment.

A subsidiary aim of the trial was to study the clinical signs and symptoms of the early stages of vitamin C deficiency and to correlate them with laboratory findings; such a correlation would, for example, provide a practical basis for the interpretation of the concentration of vitamin C in blood in terms of vitamin C intake. It was intended to pay special attention to the question of how far the healing of wounds is affected by the milder degrees of deficiency. Wound healing is known to be retarded in severe scurvy, but whether minor degrees of deficiency interfere with healing and whether administration of large doses of vitamin C after surgical operations, as practised by some surgeons, is warranted must be regarded as uncertain.

Special thanks are due to the volunteers who, in addition to their many other sacrifices, allowed incisions to be made on their legs, and the scars to be removed for examination.

The presentation of the results follows that of the Report on vitamin A requirements published in this Series. The Report is in three Sections. Section I contains a brief account of the main aspects of the trial. Section II elaborates a number of special aspects which might interest a general reader but are not an essential part of the main theme. Section III contains a description of the techniques and some of the full experimental particulars.

The editorial work of Miss E. M. Hume in connexion with the Report is gratefully acknowledged.

As in the Vitamin A report, it has proved impossible to include all the detailed results of the experiment. A list is given below of Tables which have had to be omitted but which were considered to be worth making available. They have been deposited as microfilms at the Library of the National Institute for Medical Research, Mill Hill, London, N.W.7, and at University Libraries in this country, where they can be consulted. Copies in the form either of microfilms or photographic enlargements (size 9 in. × 7 in.) may also be obtained on application to H.M. Stationery Office, P.O. Box 569, London, S.E.1, price 3d. net per frame (microfilms) with a minimum of 10 consecutive frames or 1s. 0d. net per print (enlargements) excluding postage.

Table A Haematological data
B Monthly average of body weights
C Average body temperatures
D Resting pulse rates
E Pulse rates in exercise tolerance tests
F Final rod thresholds
G Cone-rod transition times
H Rod scotometry.

A preliminary report of the trial has been published in *The Lancet* (Vitamin C Sub-Committee: Medical Research Council, 1948).

Certain conventions have been adopted for the presentation of the results. In connexion with analytical determinations, "Cambridge" refers to analyses carried out by L. W. Mapson with technical assistance by A. Ward, Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council. "Oxford" refers to analyses carried out by J. R. P. O'Brien and G. Higgins, Department of Biochemistry, The Radcliffe Infirmary, University of Oxford. "Sheffield" refers to analyses carried out at the Sorby Research Institute and under the direction of H. A. Krebs, Department of Biochemistry, University of Sheffield.

The Subcommittee much regrets the decease of Sir Leonard Parsons while the Report was being prepared.

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HISTORICAL ACCOUNT OF EXPERIMENTS ON VITAMIN C DEPRIVATION IN MAN

SEVERAL reports have been made about the effects of experimental deprivation of vitamin C on man. Although often confined to the study of a single adult subject, the experiments have given results of value in a preliminary assessment of the vitamin C requirement of human beings.

Early experiments were those of van Eekelen (1936) and of Rietschel (Rietschel and Mensching, 1939; Rietschel and Schick, 1939). They demonstrated that man could subsist on diets deficient in vitamin C for many days without presenting serious signs of ill health. Van Eekelen restricted himself to a diet deficient in vitamin C for 84 days during which his plasma concentration of vitamin C fell from 1·5 to 0·19 mg. per 100 ml. Except for some fatigue and irritability, he remained well; his body weight and capillary resistance were unchanged. At the end of the experiment, he needed 3·2 g. vitamin C to saturate his body tissues. From the results of his experiment, van Eekelen considered 60 mg. vitamin C as adequate for the daily requirement of a 70 kg. man. Rietschel and his colleagues obtained similar results in experiments on two subjects who lived on a vitamin C deficient diet, one for 100 days and the other for 160 days.

In 1940 Crandon, Lund and Dill (1940) published their excellent study of the effects of vitamin C deficiency on a single subject who, for 6 months, ate a diet free of sources of vitamin C, such as milk, vegetables and fruits. Within 41 days, the concentration of vitamin C in the plasma fell to zero, and after 122 days the vitamin C content of the white cells in the blood reached zero too. During the first 4 months he presented no signs of vitamin C deficiency except a slight loss of weight and a feeling of easy fatigue. After 134 days, he developed perifollicular, hyperkeratotic papules on his buttocks and calves, and, on the 161st day, when the vitamin C content of his plasma had been zero for 120 days and that of his white cells for 39 days, he showed perifollicular haemorrhages on the lower limbs. The haemorrhages gradually replaced the hyperkeratotic papules, and, at the end of the experiment, were most abundant on the thighs. These signs provided the most positive evidence for scurvy in this subject; his other symptoms and signs were not so striking. At the end of the experiment he showed unsatisfactory healing of an experimental wound and a diminished capacity for work; he had no gross changes in the gums, no anaemia, and no significant changes in capillary resistance. All signs and symptoms of vitamin C deficiency quickly disappeared with the institution of vitamin C therapy. From the amount, 4 to 6 g., of vitamin C needed for saturation and the time taken to produce the first signs of scurvy, Crandon and his colleagues estimated the daily requirement of man for vitamin C to be between 30 and 45 mg.

The experiment of Crandon and his colleagues provided a guide for that of Farmer (1944) on twelve young men who, for 7 months, ate a basal diet which contained minimum amounts of the vitamin B complex and was inadequate in vitamin C. Of the twelve, two, who acted as positive controls, received supplements of vitamin C and of the vitamin B complex, five consumed the basal diet supplemented with the vitamin B complex, and five ate only the basal diet. Within 70 days the men of the depleted groups had no vitamin C in their plasma, and within 5 months, none in their white cells, yet none of them showed the striking changes in the skin and gums which are associated with scurvy. There were, however, certain changes attributable to vitamin C deficiency. At the end

of the experiment, studies in wound healing revealed that the wound tissue of the depleted subjects was deficient in collagen and reticulin, and its tensile strength was in inverse ratio to the degree of depletion as measured by saturation tests. The men showed also a measurable decrease in work output, and in the last 2 months of vitamin C deprivation complained of severe fatigue. Neurological and psychological tests pointed to a deterioration in health. In agreement with the finding of Crandon, Lund and Dill, Farmer noted that capillary resistance measured by the usual methods was an unreliable index of vitamin C deficiency.

A striking feature of Farmer's experiment is the absence of definite signs of vitamin C deficiency. Except for the histological changes and weakened strength of the wound tissue, typical signs of vitamin C deficiency did not develop in the course of the 7 months of the experiment although vitamin C had disappeared from the blood by the 5th month of the experiment. The possibility of an occasional provision by the basal diet of from 5 to 10 mg. of vitamin C a day is worthy of note. Considered in the light of the present Report, the amounts may have been enough to ward off the appearance of severe signs of deficiency.

Pijoan and Lozner (1944b) sought to establish the minimum daily requirement of ascorbic acid necessary to protect against scurvy, and to determine the period of protection afforded by saturation with ascorbic acid. They were guided by the consideration that steady decline in the vitamin C content of the white cells or the actual appearance of scurvy is a good index that a diet is deficient in vitamin C. They studied a single subject maintained on a vitamin C deficient diet. After a depletion period of 70 days, vitamin C was absent from the plasma and the content in the white cells was beginning to fall. Doses of 5 and 10 mg. of vitamin C given daily for 50 and 30 days, respectively, did not prevent the fall in the white cell content. Increase of the daily dose to from 18 to 23 mg. vitamin C produced a change in the white cell content to 26 from 23 mg. per 100 g. By continuing the same dose, the concentration in the white blood cells was maintained from the 7th to the 22nd month of the experiment. Throughout the 22 months the subject showed no signs of vitamin C deficiency and experienced no subjective symptoms. An experimental wound made in the 21st month healed normally. Pijoan and Lozner concluded from their study that 25 mg.of vitamin C or less suffice to protect against scurvy. In another experiment, they studied six subjects, who, before being given a vitamin C deficient diet were saturated with the vitamin. With the exception of one who withdrew, all the subjects showed petechial and perifollicular haemorrhages at the end of about 5 months. Pijoan and Lozner concluded that a period of from 5 to 6 months is needed to deplete individuals after saturation with vitamin C and that 4 months of this can be regarded as a period of protection. About 2 g. vitamin C were needed to saturate the depleted subjects at the end of the experiment.

Najjar, Holt and Royston (1944) kept seven men aged from 17 to 21 years on a daily intake of 25 mg. vitamin C for 18 months without observing any signs of scurvy. They concluded that the minimum daily requirement is certainly no greater than 25 mg.

Taken together, these experiments give the characteristic features of experimental vitamin C deficiency in man. A normal healthy man has sufficient reserves of vitamin C to enable him to exist on a diet wholly or almost wholly deficient in vitamin C for from 160 to 200 days without any overt ill effects. More prolonged deprivation produces scurvy. The first scorbutic sign is a follicular keratosis, usually of the lower limbs, which is later replaced or accompanied by perifollicular and petechial haemorrhages distributed widely over the lower

limbs. These clinical changes are associated more closely with the decline in the vitamin C content of the white cells than of the plasma. From the beginning of deprivation, the plasma value falls quickly and reaches zero within from 70 to 80 days, but the white cell content falls only slowly; it reaches low or zero concentration within from 160 to 180 days, and signs of scurvy are by then definite. The concentration of vitamin C in the white cells may thus provide a useful guide to the state of vitamin C nutrition, a view recently supported by Lowry, Bessey, Brock and Lopez (1946). In contrast with naturally occurring scurvy, the gum lesions in experimental scurvy are unexpectedly mild, and are not noticeable till depletion is sufficiently severe to produce scurvy. To ward off gingivitis of scurvy but not that of other conditions, 25 mg. of vitamin C seems adequate (Pijoan and Lozner, 1944 a, b; Najjar *et al.*, 1944; Adamson, Jolliffe, Kruse, Lowry, Moore, Platt, Sebrell, Tice, Tisdall, Wilder and Zamecnik, 1945; Linghorne, McIntosh, Tice, Tisdall, McCreary, Drake, Greaves and Johnstone, 1946). Another feature of natural scurvy, imperfect healing of wounds, has been seen in experimental human scurvy at the end of long depletion periods, but not in the early stages of depletion. The degree of deficiency that leads to unsatisfactory healing of wounds has not been definitely settled. Anaemia, lowered resistance to infection, and abnormal capillary resistance are not prominent features of experimental scurvy. Prolonged deficiency may reduce a man's capacity for work, and may be responsible for neurological and psychological reactions, but such information helps little towards a decision on the amount of vitamin C necessary for the maintenance of health.

On the important matter of the daily requirement of vitamin C by man, the results of the trials just described do not allow a decision to be reached. Calculations based on the amounts of vitamin C needed to saturate depleted subjects or on the intakes needed to preserve a certain white cell content of vitamin C yield values ranging from 25 to 60 mg. a day. The weakness of these values is that most of them are derived from the study of a single subject. The general harmony of the results, as far as they go, is, however, gratifying and remarkable. The results of experiments on twenty volunteers described in the present Report confirm and extend the chief findings of previous trials and allow a more definite recommendation for the daily requirement of man to be made.

I. CONCISE ACCOUNT OF THE EXPERIMENT

A. General Plan

THE experiment began in October 1944 and lasted until February 1946. Nineteen men and one woman, aged 21 to 34, volunteered; particulars concerning them and their management are given on page 56. They lived a normal life without strenuous physical work.

DIET

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 70°C. after addition of 1 part per million of copper sulphate, and a number of items such as dehydrated meat, potatoes and carrots selected because they could be purchased in bulk. The dehydrated vegetables were cooked in a special way to remove vitamin C. Plum jam, containing negligible amounts of vitamin C, 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 carotene) 4,800 I.U., vitamin D 900 I.U., vitamin B_1 1·1 mg., riboflavin 2 mg., and nicotinamide 13 mg. From chemical analyses it was calculated that on the average each individual obtained not more than 1 mg. of vitamin C daily from the diet (see page 66).

Full details of the composition of the diet, of the treatment of special foods, and various samples of the individual food intakes are found in Section III pages 56–67.

GROUPS OF VOLUNTEERS

To obtain base-line data the experiment began with a preliminary period, in most cases of 6 weeks, of a complete diet including about 70 mg. vitamin C daily. At the end of the period all the volunteers were given the basal deficient diet and divided into three groups (Table 1), ten having no supplements, seven having 10 mg. vitamin C daily, and three having 70 mg. vitamin C daily.

TABLE 1

Initial grouping of the 20 volunteers according to the supplements of vitamin C given

Volunteers receiving daily vitamin C supplement of:		
70 mg.	10 mg.	0
Bartley	Golding	Drake
Garling	Jackson	Hudson
Hill	Parry	Milburn
	Proctor	Robinson
	Way	Sanderson
	Whinfield	Tridgell
	Woodhouse	Williams, D.
		Williams, H.
		Wodeman
		Another

TABLE 2

Nature and frequency of the investigations made on the volunteers

Nature of investigation	Investigator	Approximate frequency of examination
General clinical	J. Pemberton A. E. Barnes	Every 4 weeks; more often in special cases
Skin, special	H. R. Vickers	Every 4 weeks
Charting of affected skin areas	W. Bartley J. Pemberton	Every 4 weeks
Teeth and gums, special	G. L. Roberts	Every 4 to 6 weeks
Capillaroscopy	W. Bartley, J. H. Fox	About every week
Mapping of dark-adaptation curve and scotometry	W. Bartley, G. Drake and G. Sanderson	Every month
Audiometry	J. L. Burn	Four times during the trial
Capillary strength	J. Pemberton	Every 4 weeks
Capillary strength	A. E. Barnes	Every 4 weeks
Capillary strength	H. Scarborough	Every month
Capillary strength	S. Yudkin, G. A. Smart	Every month from February 1945
Slit-lamp examination of eyes and gums	W. J. W. Ferguson	Every 6 weeks
Exercise tolerance	W. Bartley and volunteers	Every month
Fatigue	I. M. Frankau	About every 4 weeks
Psychological studies	I. M. Frankau A. Heim	About every 4 weeks Once
Radiography of chest and long bones	J. L. Grout, X-Ray Department, Sheffield Royal Hospital	Four times during trial
Teeth	G. L. Roberts	Three to five times during trial
Pulse rate	Volunteers	Twice daily
Body temperature	Volunteers	Twice daily
Body weight	Volunteers	Twice weekly
Haematological examination: haemoglobin, red cells, white cells, platelets, differential white-cell count, sedimentation rate	W. Bartley, G. Drake	Every 4 weeks or oftener
Bleeding time	G. Higgins	Every 4 weeks
Wasserman reaction	Department of Bacteriology, Sheffield University	Once
Urine analysis: protein, reducing sugar, deposits	W. Bartley, G. Way	Every 4 weeks
Vitamin C content of urine	Cambridge team Oxford team	Variable
Occult blood in faeces	W. Bartley	Special, occasional
Vitamin C content of blood:		
Plasma	Oxford team Sheffield team	Every 2 weeks
Whole blood	Oxford team	Every 2 weeks
White-cell layer	Oxford team	Every 2 weeks
Blood urea, plasma proteins, plasma phosphatase	Oxford team	Every month
Experimental wounds, surgical aspects, histology, breaking strength	G. L. Roberts J. Pemberton B. S. Platt J. Waterlow B. Balfour W. Bartley R. Milburn H. Cairns	Irregular

The volunteers did not know to which group they belonged, nor did the physicians responsible for the clinical investigations. All the volunteers were given each day 7 supplementary tablets of identical taste and appearance, some containing vitamin C, others being dummies (p. 67).

The group receiving a supplement of 70 mg. was intended to serve as a positive control and the group receiving 10 mg. was to be used for a prophylactic test. The dose of 10 mg. was chosen for the latter because it seemed to be near the minimum dose capable of preventing clinical scurvy (Zilva, 1944). The original scheme of dosing had to be somewhat modified later in the experiment when it was realized that even early stages of vitamin C deficiency might be more dangerous to life than had been thought at the start (p. 10). The need for such emergency modifications was responsible for some seemingly erratic variations in the size of the supplements given to some of the volunteers. Full details of the vitamin C supplements given to each volunteer throughout the experiment are shown in Table 30, p. 58.

INVESTIGATIONS

The investigations made on the volunteers at regular intervals included general clinical examinations, chemical analyses of blood and urine, haematological examinations, tests of capillary strength, capillaroscopy, radiography, electrocardiography, dark-adaptation tests, audiometry, studies of fatigue and studies of experimental wounds. Details of the investigations, their frequency and the investigators are shown in Table 2.

B. Effects of Deficient Diet

VOLUNTEERS RECEIVING NO SUPPLEMENT

The clinical examination, by inspection and physical methods, revealed no definite changes during the first 7 weeks of deprivation, beginning on November 13, 1944.

Effect on the Skin

The first changes, the significance of which was uncertain when the observations were first recorded but which were recognized retrospectively as significant, were enlargement and keratosis of the hair follicles in one volunteer (Drake); the main site at that stage was the outer aspect of the upper arm. After 21 weeks six of the ten deprived volunteers (Drake, Milburn, Robinson, Sanderson, D. Williams, Wodeman) had developed follicular changes, and after 26 weeks all had done so. In all except one (Another) the region of the enlarged hair follicles eventually became haemorrhagic.

The various stages of development, as observed with the skin microscope, were briefly 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. Under the microscope the redness resolved itself into a congestion and proliferation of the blood-vessels round the hair follicles. The colour gradually deepened and within another week or two, when the enlarged hair follicles became haemorrhagic, it changed to a dark purple and no longer disappeared on compression; at this stage many red cells could be seen outside the vessels.

By May 1945, after 26 weeks of deprivation, six of the ten volunteers (Milburn, Robinson, Sanderson, D. Williams, H. Williams, Wodeman) and 9 weeks later nine of the ten, the exception being Another, had haemorrhagic follicles. In general it was on the legs that the follicles showed the greatest tendency to become haemorrhagic; there were no accompanying subjective sensations. A fuller account of the changes in and around the hair follicles is given on p. 31.

As the development of the enlarged and haemorrhagic follicles progressed, six of the ten deprived volunteers (Drake, Hudson, Milburn, Sanderson, Tridgell, D. Williams) 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 from 16 to 30 weeks; they increased in size and later became bright red. The other four deprived volunteers who had no acne at the start remained more or less free throughout the experiment. The acneiform eruption became haemorrhagic at about the same time as the hyperkeratotic hair follicles did so (see further, p. 33).

Effect on the Gums

Other changes generally noted during the period of deprivation were in the gums. At the beginning of the experiment, the teeth and gums of most volunteers were in good condition, but the two who developed the most severe gum changes showed evidence of gingivitis and para-odontal disease at the start of the deprivation period (see further, p. 34 and Tables 40, 41, 45, pp. 119, 120, 136). The earliest signs of deficiency were reddening, swelling and tiny haemorrhages in the tips of the interdental papillae, seen first in D. Williams after 23 weeks of deprivation. By the end of July nine of the ten deprived volunteers had developed abnormalities of the gums, the exception being Tridgell. In two (Robinson, Wodeman) the changes were gross; the gums were purplish, much swollen and spongy. Part of the tissue became necrotic and there was some bleeding. In five other volunteers (Drake, Hudson, Sanderson, D. Williams, H. Williams) the gum changes, all located in the interdental papillae, were less advanced but beyond question; they consisted of small haemorrhages, swelling and discoloration. In two more of the men (Milburn, Another) swelling and haemorrhages developed but to a less extent and their scorbutic origin was doubtful; one of the subjects (Another) was edentulous.

Effect on Wound Healing

Another striking observation, in agreement with older accounts of scurvy, was recorded from June 1945 onwards in six of the ten deprived volunteers (Drake, Hudson, Robinson, Sanderson, Tridgell, Wodeman). It concerned the behaviour of the scars where the experimental wounds had been excised.* In scars made between February and May 1945, healing had proceeded normally, but subsequently, as deprivation progressed, they became red and livid as a result of haemorrhages into the scar tissue and surrounding skin. New wounds made at the stage of pronounced scurvy failed to heal at the normal rate (see for example the case history of Sanderson, p. 82).

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* Reference here is not to the behaviour of the experimental wounds themselves, but to the wound or scar left after the experimental wound and the tissue surrounding it had been excised for examination.

Special Incidents

Some abnormalities occurred only in single cases. One man (Drake) developed effusions into both knee-joints and ecchymoses of the leg in June 1945, aggravated by a long walk. Another man (Milburn) was taken ill in July 1945, 19 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 increased in intensity, passed off after 9 hours. The electrocardiogram showed high ST levels in Leads I and II. Radiography of the chest showed no abnormalities. Within 24 hours the patient appeared normal.

Eighteen days later another deprived volunteer (Another) 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 0·32 seconds. Before the experiment the electrocardiogram had been normal with a P-R interval of 0·20 seconds. It was thought necessary to treat this volunteer immediately with large doses of vitamin C. The chest pain and the systolic murmur disappeared within 24 hours but during the following months the P-R interval showed variable periods between 0·13 and 0·32 seconds, depending on posture, breathing, administration of drugs and other factors. The observations on this volunteer are more fully discussed in his case history (p. 87). In view of recent observations on partial heart block in healthy Service personnel (Manning and Stewart, 1945; Hall, Stewart and Manning, 1942; Holmes and Weill, 1945), it might be questioned whether the heart block in this case had any connexion with the vitamin C deficiency. The observations are certainly compatible with the occurrence of a local haemorrhage followed by fibrosis, interfering with the auriculo-ventricular conducting system. A scorbutic haemorrhage would offer an explanation also for Milburn's cardiac attack.

Muscular Exertion

A modification of the agility test (Frankau, 1943), which had previously been used to demonstrate the acceleration of co-ordinated muscular effort in human subjects given nicotinamide, was applied to measure objectively the incidence of fatigue in the volunteers. Interruption in the 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, precision of co-ordinated 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 observations indicated increased fatigue. The "all-out" effort demanded by the agility test caused differences in the rise and fall of the pulse rate in the 3 minutes immediately after the test; both effects were significantly greater in the group receiving 70 mg. vitamin C daily than in those receiving 10 mg. or none. The pulse rate results of an exercise tolerance test were somewhat different since they at no time showed significant differences among the three main groups of volunteers (p. 46).

Psychiatric Disturbances

None of the volunteers presented any evidence 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, but the phase was transitory and was not followed by any 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.

Capillary Strength

Capillary strength was tested by two methods, the positive-pressure and negative-pressure techniques (p. 48). Neither method revealed any change in the average strength of the capillaries of the deprived group which could be correlated with deprivation (Tables 21 and 23, pp. 49 and 51) but one man (Hudson) showed a decreased capillary strength during the period of depletion by both methods, and an increased strength after dosing; in another (Tridgell) only the negative-pressure method showed a decreased capillary strength on depletion and an increased resistance after dosing.

Other capillary tests, made by Dr. Harold Scarborough by his special method, are described in Appendix B, p. 154.

Although the conventional tests of capillary strength failed to show correlation with the state of vitamin C depletion, an unexpected phenomenon bearing on the behaviour of capillaries was noted after application of the positive-pressure test in all the deprived volunteers except Another and H. Williams. About half an hour after the test, an increase in the number and size of the perifollicular haemorrhages was noted on the arm, above and below the area where the cuff had been applied. This occurred at the stage of deprivation when haemorrhagic hair follicles had appeared (p. 51).

Miscellaneous Observations

Some other negative findings are worth recording. There was no significant change in body weight (p. 42 and Table B, see p. 2). There was no increased incidence of infection though there was some suggestion that colds tended to last longer in the deprived group (p. 44). There was no change in the appearance of the conjunctivae (p. 46) or of the capillaries of the nail bed (p. 29). Capacity for dark adaptation, as measured with the Wald-Steven-Bartley apparatus and by rod scotometry, remained normal (p. 26). There was no change in hearing as measured by audiometry (p. 26).

Special steps were taken to look for haemorrhages elsewhere than in the skin and mouth but none were found. Red cells were never detected in the urine. There was no occult blood in the stools of two volunteers (Drake, Wodeman) who were tested at the height of their scorbutic state. There was no increased epistaxis, and no conjunctival haemorrhages were seen on slit-lamp examination.

The haemoglobin concentration, red cell count, total and differential leucocyte counts, platelet count, and bleeding time, showed no significant changes during the course of the depletion.

As far as subjective symptoms were concerned it is worthy of note that pains in the back, joints and limbs of the type often mentioned in the older literature on scurvy (Smith, 1904) were reported with increasing frequency by the depleted volunteers as the signs of scurvy developed. They disappeared within 4 weeks of dosing (p. 44).

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 (Golding, Parry, Proctor, Woodhouse) should continue with the 10 mg. supplement and three of them (Jackson, Way, Whinfield) 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 who received 10 mg. continued to do so for another 264 days, but the fourth (Parry) continued only for another 92 days. No abnormalities were recorded. Wound healing, judged by the appearance of the excision scar on inspection, proceeded normally, and, in contrast with the deprived group, there were no haemorrhages into the scar tissue.

The second group of three volunteers had no supplement for 71 days, broken in one case (Way) by a 27-day period on a supplement of 10 mg. (see Table 30). No significant changes occurred during the period. It was at this stage of the experiment (July 1945) that two of the totally deprived volunteers showed the cardiac disturbances already mentioned. To avoid undue risks it was therefore decided to restore the supplement to the extent of 5 mg. daily. On that dose the three men continued for another 125 days. Two of them (Jackson, Way) showed a slight increase in the number of hyperkeratotic hair follicles, one (Way) developed four follicular haemorrhages, and the other (Jackson) some congested follicles. These abnormalities disappeared when large doses of vitamin C were given, and were thus probably connected with the deficient diet.

VOLUNTEERS RECEIVING 70 MG. OF VITAMIN C DAILY

This group of three volunteers (Bartley, Garling, Hill) served as positive controls for 299, 325 and 329 days, respectively. No changes worthy of note were recorded (see case histories, p. 73).

SYNOPSIS OF THE COURSE OF DEPLETION

The course of the development of scurvy was fairly uniform in the volunteers and very similar to that in the case described by Crandon, Lund and Dill (1940). The general sequence was as follows: for about 17 weeks no clinical signs; after 17 to 21 weeks the first sign, hyperkeratosis of the hair follicles (see Wiltshire, 1919); after 26 to 34 weeks perifollicular haemorrhages; and after 30 to 38 weeks swelling and haemorrhages of the gums. Exacerbation of acne, not apparently hitherto recognized 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 an individual does not necessarily indicate lack of vitamin C. They occur in many people saturated with vitamin C. Deficiency of the 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 reflected the intake of vitamin C, this proving beyond doubt that they were the early stages of the typical haemorrhagic spots of scurvy.

The gum lesions always appeared after the skin lesions. The sequence may not always be true of scurvy, but might 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, such as abnormal pallor or dryness of the skin, anaemia, and night blindness, were not observed. It is probable that classical scurvy was often a multiple deficiency.

C. Effects of Dosing

GENERAL CONSIDERATIONS

Three of the ten deprived volunteers who developed scurvy were lost for trials with graded doses because sudden emergencies demanded their immediate treatment with large doses of the vitamin. Two of the three (Milburn, Another), as already mentioned, were dosed because they had signs of acute cardiac complications. A third (D. Williams) complained of shortness of breath and pain in the chest in May 1945. Radiography revealed spondylitis and a paravertebral abscess, and spinal tuberculosis was diagnosed. When earlier radiograms taken for the chest were critically examined, it was possible to detect that this man's spine had not been healthy at the beginning of the experiment (see case history, p. 84). At the time when he developed symptoms of chest disease, 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. This volunteer had also had an attack of benign tertian malaria in January 1945 (see case history, p. 84). It is of interest that a man suffering from sub-chronic infectious disease developed signs of vitamin C deficiency earlier than any other volunteer, and the observation may be related to the finding that saturation with vitamin C requires larger doses in infections (Abbasy, Harris and Hill, 1937; Abbasy, Harris and Ellman, 1937; Harris, Passmore and Pagel, 1937).

The three volunteers dosed with large amounts showed striking improvements of the skin within a few days.

Six of the remaining seven depleted volunteers (Drake, Hudson, Robinson, Sanderson, Tridgell, Wodeman) showed unequivocal signs of scurvy, in multiple haemorrhages and skin lesions. The seventh (H. Williams) showed changes which were not so clear-cut. In choosing the dose the intention was to give the smallest one 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. To begin with a daily dose of 10 mg. was chosen and given to six of the seven volunteers. The seventh (H. Williams) received 20 mg. because she was not available for long.

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, and lifted out the plug. The dilatation and congestion of the capillaries round the hair follicles disappeared, and within 7 to 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 to heal disappeared as the follicular eruptions regressed. The haemorrhages disappeared within 2 months, the original blue and purple colour gradually giving way to red, pink and finally pale brown, and changes in the appearance of the wounds indicated improved healing.

The acneiform papules likewise regressed, though usually somewhat more slowly than the other skin signs. The initial state was regained within 10 to 18 weeks, except in the case of Drake. This volunteer showed considerable improvement but restitution was not completed until after the daily dose had been raised to 20 mg.

The gum lesions did not respond as promptly to dosing as 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 to 14 weeks.

DOSING WITH 20 MG. OF VITAMIN C DAILY

One volunteer (H. Williams), 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 to 92 days. The appearance of skin, gums and wounds showed no further changes, except in the case of Drake (see above).

D. Vitamin C Content of the Blood

Vitamin C estimations 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 approximately fortnightly intervals throughout the trial. Furthermore in the later stages of the trial (from February 1945 onwards) the dinitrophenylhydrazine method of Roe and Kuether (1943) was applied to the estimation of vitamin C in the whole blood. All the data are given in Table 38, p. 90. The average values for the concentration of vitamin C in the plasma and white cells are shown in Figs. 1 and 2.

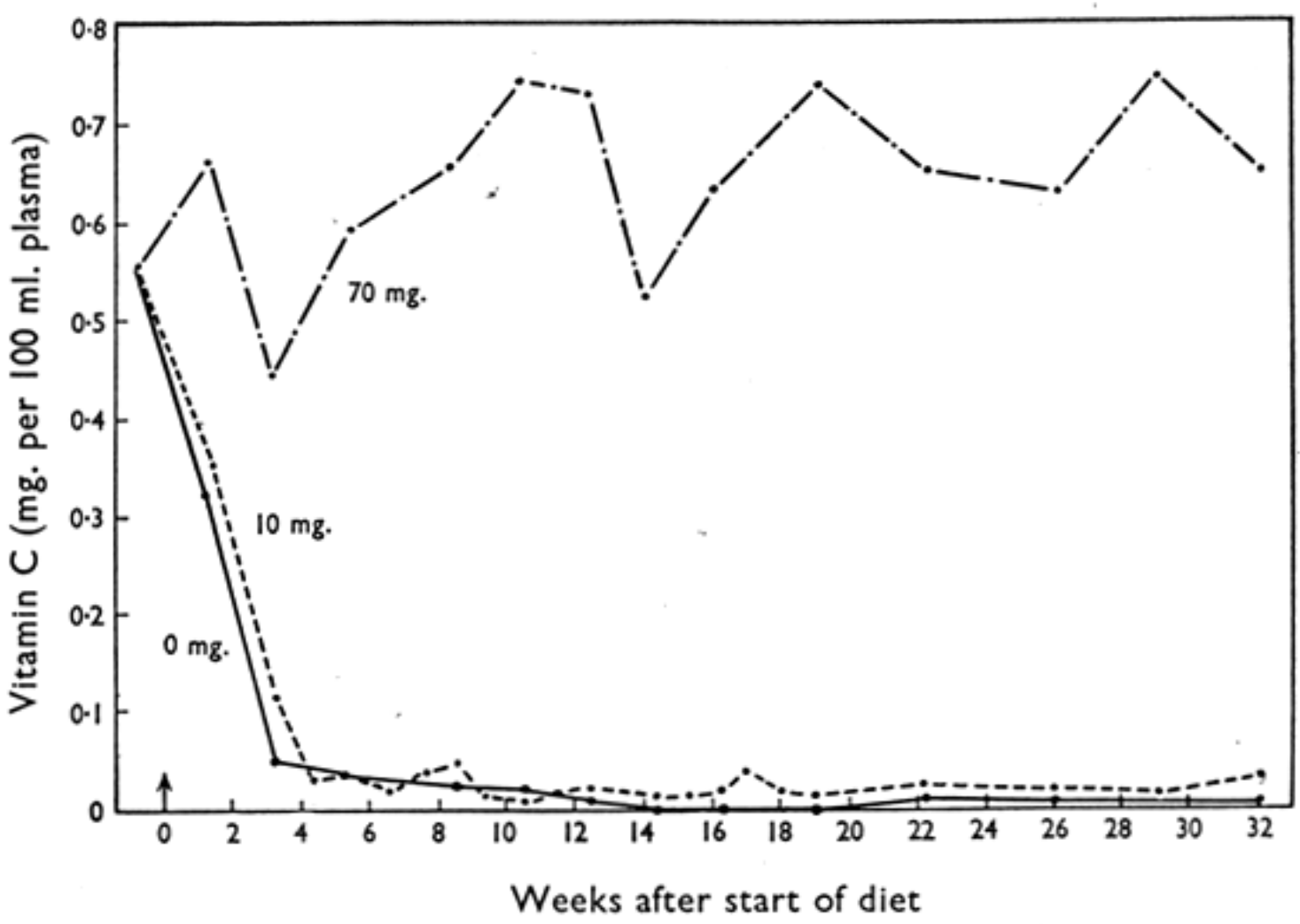

FIG. 1. Average vitamin C content of blood plasma, estimated with dichlorophenolindophenol, of the groups of volunteers receiving daily 70, 10, or 0 mg. of ascorbic acid as supplement to the basal diet, which contained about 1 mg.

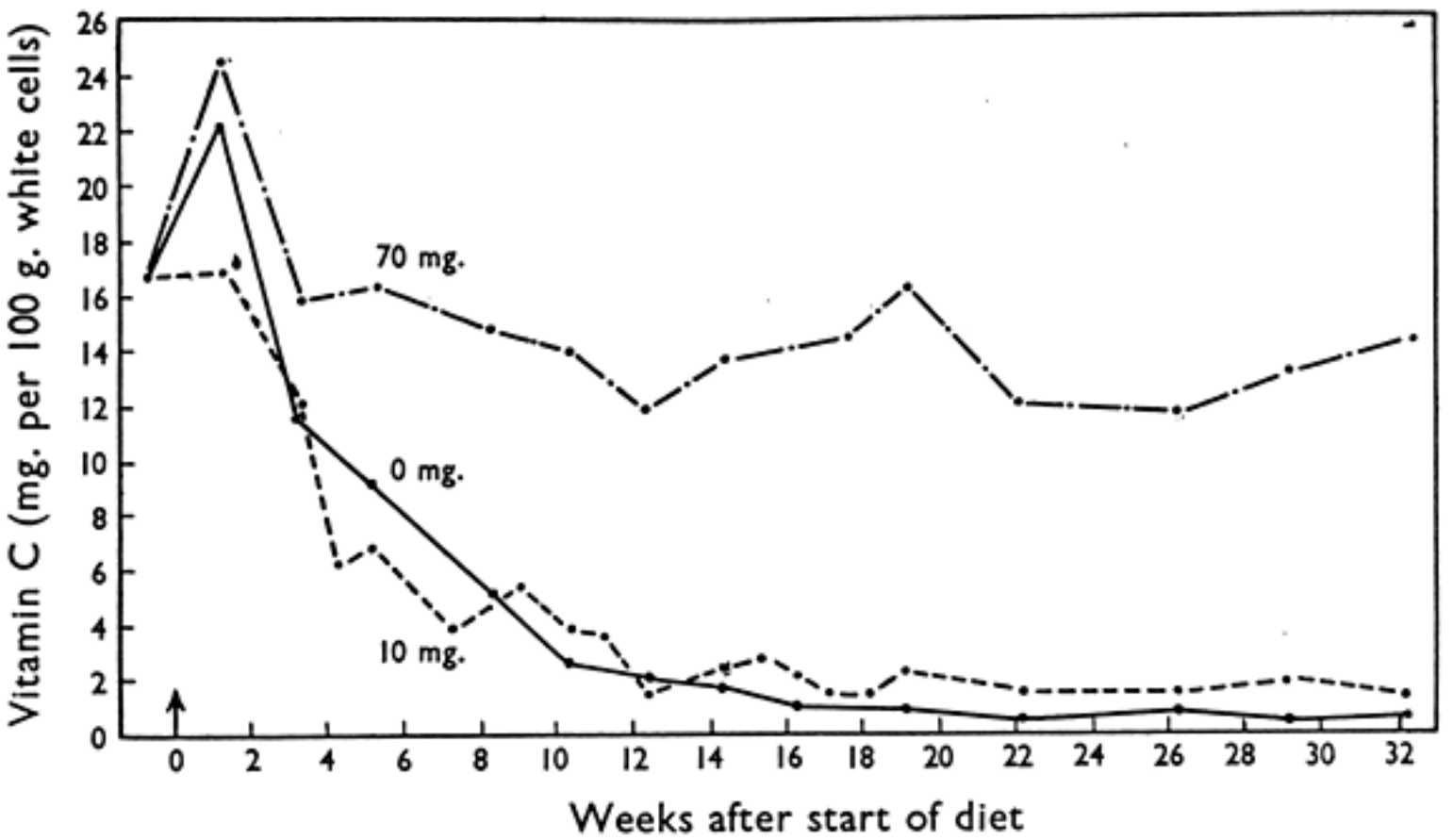

FIG. 2. Average vitamin C content of white blood cells, estimated with dichlorophenolindophenol, of the groups of volunteers receiving daily 70, 10, or 0 mg. of ascorbic acid as supplement to the basal diet which contained about 1 mg. (Figs. 1 and 2 are reproduced by kind permission of *The Lancet*.)

PLASMA

The initial average value of vitamin C in plasma for 16 of 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. plasma, and it was more or less well 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. 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 differences in the concentration of vitamin C below 0·05 mg. per 100 ml. plasma.

WHITE BLOOD CELLS

The initial average value for the concentration of vitamin C in the white cells for all the 20 volunteers was 15·6 mg. per 100 g., and varied little throughout the experiment 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 vitamin C in the white cells of the volunteers receiving a supplement of 10 mg. 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.

WHOLE BLOOD

The dinitrophenylhydrazine method of Roe and Kuether (1943), which determines the sum of dehydroascorbic acid and ascorbic acid, gave values for

the vitamin C content of whole blood which were in general a little higher than those obtained for plasma by titration with dichlorophenolindophenol (Table 3). For example, in the group receiving 70 mg. the average value for whole blood for two periods was 0·88 and 0·89 mg. per 100 ml. against 0·60 and 0·66 mg. per 100 ml. for plasma. In other groups, where the values were lower, the differences were smaller, but definite. Results obtained with the method of Roe and Kuether were more consistent than with the dye titration method at low vitamin C levels, especially at the range below 0·5 mg. per 100 ml., which is of great practical importance.

TABLE 3

Comparison of the vitamin C content of whole blood by the method of Roe and Kuether (1943) *and of blood plasma by the indophenol dye titration method*

Vitamin C supplement (mg. daily)	Date	Whole blood (Roe and Kuether)			Plasma (Dye titration)		
		No. of analyses	Vitamin C (mg./100 ml.)		No. of analyses	Vitamin C (mg./100 ml.)	
			Range	Average		Range	Average
70	Feb. 18, Mar. 3, 1945	6	0·76–1·08	0·88	12	0·48–0·74	0·60
10	Feb. 18, Mar. 3, 1945	14	0·04–0·20	0·11	28	<0·01–0·06	0·02
0	Feb. 18, Mar. 3, 1945	20	<0·01–0·12	0·06	40	<0·01–0·06	0·01
70	June–July 1945	6	0·75–1·03	0·89	12	0·43–0·94	0·66
10	May–July 1945	14	0·07–0·18	0·11	28	<0·01–0·08	0·03
0	(most depleted stage)	20	0·01–0·11	0·05	38	<0·01–0·05	0·02

RELATION BETWEEN CLINICAL SIGNS AND THE VITAMIN C CONTENT OF THE BLOOD

About 100 days elapsed between the virtual disappearance of vitamin C from the plasma and the appearance of 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 to 6 weeks before clinical scurvy appeared.

EFFECT OF DOSING WITH VITAMIN C ON THE CONCENTRATION IN THE BLOOD

When the deficient volunteers were dosed with 10 mg. of vitamin C daily, the concentration of the vitamin in the plasma, whole blood and white cells showed a small but distinct rise towards the end of a dosing period of 101 to 157 days. The average concentration of the plasma rose from 0·016 to 0·06 mg. per 100 ml., that of whole blood from 0·05 to 0·08 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 definite change in the vitamin C concentration of the plasma and whole blood, and a slight rise in that of the white cells to 3·6 mg. per 100 g. (Table 38, p. 90).

DISCUSSION

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, whole blood and white cells. There seemed to be a 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, whole blood 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 to 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, whole blood 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. The data, together with others correlating vitamin C intake with vitamin C blood levels, are assembled in Table 4. The figures are, throughout, lower than comparable ones recently published by Johnstone, Drake, Tisdall and Harvie (1946) and by Dodds and MacLeod (1947).

TABLE 4

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.)	whole blood (mg./100 ml.)
0	10	205–269	0·02	<1·0	0·05
5	3	125	0·07	2·0	0·06
10	6	101–157	0·03	2·4	0·08
20	5	47–92	0·07	3·4	0·10
50	2	61–66	0·38	9·2	0·41
70	3	300–336	0·69	11·1	0·86
about 600	15	8–11	1·02	17·9	1·13

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, at the end of the experiment, when a saturation test was made in which they received a dose of 10 mg. vitamin C per kg. body weight for 8 to 11 days. All data were obtained in the fasting state.

As long as the diet contained no more than 20 mg. of vitamin C daily, the average plasma level was 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 0·4 mg. per 100 ml. plasma corresponded to an intake of 50 mg. daily, and of about 0·7 mg. per 100 ml. to one of 70 mg. daily.

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 of 20 mg. or less. 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. Similar considerations apply to the interpretation of results obtained for whole blood by the method of Roe and Kuether.

The determination of vitamin C in the white cells is of somewhat greater diagnostic value, because it shows more definite differences between the daily intakes of 20 mg., 10 mg., and less than 5 mg. 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 estimations on the white cells.

E. Various other Chemical 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 (p. 39 and Tables 14 and 38). Observations on the vitamin C content of urine and on saturation tests are presented on p. 22.

F. Experiments on Wound Repair

Experiments were undertaken to extend the work of Lund and Crandon (1941), Pijoan and Lozner (1944a, b), 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, on the outer aspect of the upper thigh. The linear incisions were made to the depth of the fascia lata; they were sutured with three stitches, 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 stitches were removed after 4 days. The gap was sutured and left to heal. The excised material was cut into several pieces for histological examination and for determination of the 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. In the groups receiving a 10 or 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 (for details see p. 45).

HISTOLOGICAL OBSERVATIONS

The main histological criteria adopted 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, Fell and Kodicek (1945) found profuse reticulin formation but no maturation to collagen. According to Penney and Balfour (1949) working with guineapig wounds, there may, in complete deficiency, be also decreased vascular and fibroblastic proliferation (see also Campbell, Ferguson and Garry, 1950; Bunting and White, 1950).

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 macroscopical haemorrhages or infection were omitted from the series.

All wounds from subjects belonging to the groups receiving 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 subjects receiving no supplement of vitamin C, seven 21-day wounds were made after 5 to 8 months' deficiency. Of the seven wounds, four (Milburn, Robinson, Sanderson, Wodeman) showed scorbutic lesions and three (Drake, Hudson, Tridgell) showed none; one of the wounds (Hudson) 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 (Hudson, Milburn, Tridgell), healing was of the first or normal grade, but in the other three (Robinson, Sanderson, Wodeman) it was poor and an unusually large amount of degenerated collagen was present at the side of the wound track.

BREAKING STRENGTH

The data, which are fully presented and discussed in Section II, p. 52, indicate that at the height of depletion the breaking strength of wound scar tissue was considerably diminished, and they suggest that immediately after saturation those volunteers who had undergone depletion had not yet recovered their normal capacity for wound repair. The number of reliable data at hand is, however, too small to warrant definite conclusions.

SUMMARY

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 for up to 11 months. In the unsupplemented group severe defects in wound healing occurred similar to those recorded in scorbutic guineapigs. The defects were encountered only when, and not before, clinical signs of scurvy had appeared, that is to say after 6 months' depletion.

G. 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 volunteers 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 individuals tested.

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

(3) When a 10 mg. supplement was withdrawn from three volunteers after 160 days, and a period of 196 days followed, during which the intake varied slightly, but in which the average respective intakes were 3·2, 3·2 and 4·5 mg. of vitamin C daily, no definite clinical signs of scurvy appeared.

These facts suggest that in the group under test the "minimum protective dose" of vitamin C, as measured by the criteria for the presence or absence 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 optimum dose, since the statistical analysis of those results 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 maximum efficiency under conditions of stress such as those produced by the agility test.

Distinct from the minimum 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 that shall cover the requirement of normal adults with their own inherent variability, enhanced by the variety of their activities and environment, and that shall ensure for them the margin of protection at which it is decided to aim" (Hume and Krebs, 1949, p. 41). To satisfy these ill-defined additional needs and to allow a margin of safety it does not, therefore, seem too generous to treble the minimum 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 Health Organisation (1938) for the requirement of a normal human adult.

Any assessment is, in 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 minimum protective dose for a group of human beings. The new estimate is considerably below the allowance of 75 mg. recommended in the United States by the National Research Council (1948), which is essentially the amount necessary to maintain saturation but, as 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. It is true that claims have been made recently, for instance by Scheunert (1948–9), that larger doses of vitamin C reduce the incidence of illness, but the evidence presented can hardly be accepted as conclusive proof.

When the figure of 30 mg. daily 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. It is obvious also, since 6 months elapsed before even the earliest skin changes appeared in the volunteers, that periods of deprivation shorter than this may be, and probably are, undergone without any detrimental effect being detected.

II. ELABORATION OF SPECIAL ASPECTS

A. Saturation Tests

WHEN all signs and symptoms of vitamin C deficiency had disappeared in the deprived volunteers through dosing with 10 or 20 mg. vitamin C daily, 4 groups of individuals were available whose different intakes of vitamin C had been known for comparatively long periods. Their respective intakes towards the end of the experiment after various changes had been made were 5, 10, 20 and 50 mg. daily above the vitamin C content of the basal diet. The minimum time on the same dose had been 47 days, the maximum time 424 days. Full details of the histories of the vitamin C intakes are given in Table 30, p. 58.

There was thus an opportunity for making saturation tests on subjects whose dietary history was known to an unusually precise degree. The tests were carried out in the following way. For the 24 hours from 8·0 a.m. preceding the first dose of the saturation test, the urine was collected in a dark bottle containing 50 g. of metaphosphoric acid. On the first day of the test, at 8·0 a.m., the volunteer emptied his bladder, weighed himself and was given a dose of 10 mg. vitamin C per kg. body weight. It was dissolved in water and drunk, and all the urine was collected for 24 hours in the same manner as on the previous day. The same procedure was usually repeated daily for 10 days. Throughout the test the volunteers continued to eat the basal diet.

The urines were examined for vitamin C by 5 different workers using 3 different methods, (1) direct titration with dichlorophenolindophenol, (2) the formol method of Lugg (1942 a, b) as modified by Mapson (see Appendix A), (3) the dinitrophenylhydrazine method of Roe and Kuether (1943). The last two methods, being more specific, gave consistently lower values than the first but all three agreed in showing at the same time the rise of the vitamin C content in the urine on dosing.

Blood plasma, whole blood and the white cell layer were examined for vitamin C. The blood was collected from the fasting subject before 8·0 a.m. The full data are given in Table 42, p. 122.

VITAMIN C CONTENT OF URINE

The number of daily doses which produced a sharp rise in the vitamin C content of the urine was taken as the criterion of saturation (Table 5). It will be seen that the two subjects whose intake had been 50 mg. vitamin C daily showed a marked rise in the vitamin C output on the first day of dosing; they were therefore fully saturated in accordance with the criterion adopted. The subjects whose intake had been 20 mg. or less required 4 or more doses, but no sharp differences were noticeable between the volunteers whose intakes had been 20, 10 or 5 mg., respectively, the doses being from 4 to 6 for intakes of 20 mg., from 4 to 6 for intakes of 10 mg. and from 5 to 7 for intakes of 5 mg. It should be pointed out that certain differences in the dietary history within the 20 mg. group, involving Milburn on the one hand and the rest on the other, were not reflected by differences in response. Milburn, for reasons explained elsewhere, had received large doses of vitamin C three months before the saturation tests, while the intakes of the others had been very much lower before the 20 mg. period. The more remote history of vitamin C intake had, thus, no appreciable effect on the response to the saturation test. Volunteers whose history of vitamin C nutrition had been identical over a period of 378 days showed a considerable variation in the number of doses required, 5, for instance, in the case of Jackson and 7 in the case of Whinfield.

TABLE 5

Number of doses of vitamin C needed to produce saturation in subjects whose previous intake of vitamin C was known (The subjects were dosed with 10 mg. per kg. body weight per day given in one portion at 8 a.m. each day.)

Name	Dose ascorbic acid just before test		Dose ascorbic acid before period stated in third column		No. of test doses after which a marked rise in vitamin C content of urine occurred
	Amount (mg./day)	Duration (days)	Amount (mg./day)	Duration (days)	
Garling ..	50	66	70	323	1
Hill ..	50	61	70	328	1
Drake ..	20	59	10	124	5
Hudson ..	20	55	10	101	4
Milburn ..	20	92	100	41*	4
Sanderson	20	47	10	115	4
Robinson ..	20	47	10	115	5
Wodeman	20	92	10	101	6
Golding ..	10	424	normal diet		4
Proctor ..	10	424	normal diet		5
Woodhouse	10	424	normal diet		4
Tridgell ..	10	157	0	264	6
Jackson ..	5	125	0	72	5
Way ..	5	125	0	36	6
Whinfield ..	5	125	0	72	7

* Before the period of 41 days during which the dose was 100 mg./day, a dose of 6 g. had been given within 6 days (see case history, p. 80).

The main conclusion, then, is that, while the saturation test can differentiate between intakes above 20 mg. or thereabouts, it is not a method for differentiating between intake levels of 20 mg. and below, which are those of the greatest practical importance. An intake of 10 or 20 mg., sufficient to cure and prevent scurvy, gives about the same result as an intake of 5 mg. which is probably below the safety level.

VITAMIN C CONTENT OF BLOOD

The blood plasma values obtained by the dinitrophenylhydrazine and dichlorophenolindophenol methods show fairly good agreement. In those volunteers whose vitamin C intake had been low, very little of the saturation doses remained in the plasma during the first 4 or 5 days. In the plasma the rise of level began slightly before the rise of level in the urine and became marked at about the same time. In the whole blood, and especially in the white cells, the rise of the vitamin C level began at once after dosing but was more gradual, and continued until the urinary output indicated saturation (Fig. 3).

A remarkable feature of the values for vitamin C in the plasma and in the white cells, if compared with the data published by other workers, is their relatively low level after saturation. An analysis of the plasma and white cell levels observed after the rise of the vitamin C excretion in the urine had started is given in Table 6. The range for the plasma was 0·45 to 1·50 mg. per 100 ml.,

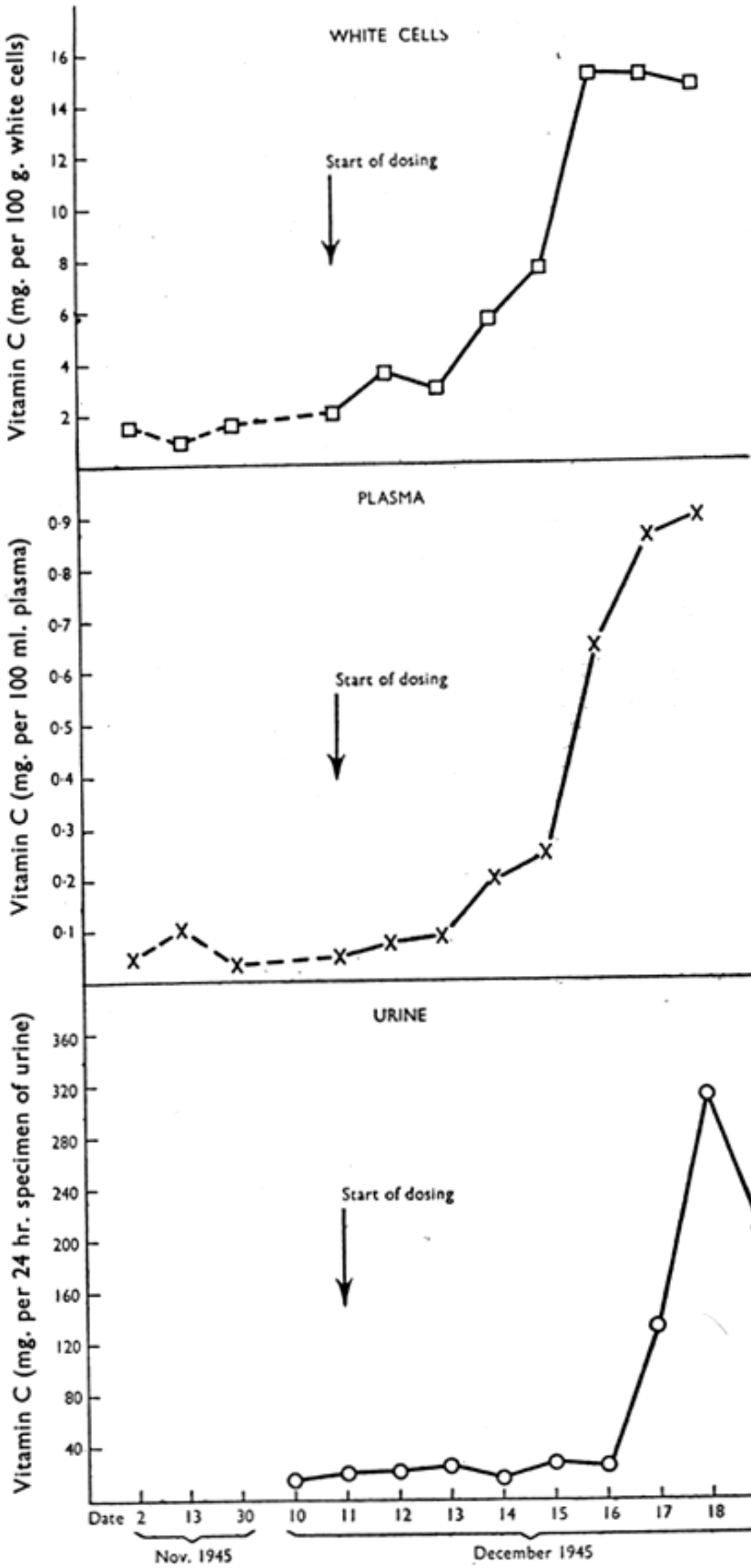

FIG. 3. Graphs showing rise of vitamin C content of white cells, plasma and urine (Whinfield) during saturation test.

TABLE 6

Range of values for vitamin C in the plasma and white cells during the later stages of the saturation test, after the rise of the urinary output of vitamin C

Blood fraction	Method of estimation	No. of observations	Ascorbic acid, range of values	No. of values above 1·1 mg./100 ml.
Plasma	Dinitrophenylhydrazine	68	0·45–1·32 mg./100 ml.	5
Plasma	Indophenol	74	0·5 –1·50 mg./100 ml.	4
White cells	Indophenol	74	7·6 –21·6 mg./100 g.	

TABLE 7

Data assembled from the literature for the content of vitamin C in the plasma, whole blood and white blood cells of various types of subjects

Authors	Subjects		Ascorbic acid in:		
	Type	No.	plasma (mg./100 ml.)	whole blood (mg./100 ml.)	white cells (mg./100 g.)
Farmer and Abt (1934–5)	Medical students	6	0·69–2·3	—	—
Pijoan and Klemperer (1937)	Normal adults	150	0·65–2·0	—	—
Goldsmith and Ellinger (1939)	Adults, fasting	22	0·05–1·38	—	—
	saturated	22	0·12–2·99	—	—
Butler, Cushman and MacLachlan (1943)	Normal adults	12	—	0·2–1·4	—
		3	—	—	22·8–36
Rourke, MacLachlan and Butler (1946)	Normal adults, fasting	Not stated	0·4 –1·0	—	25–40
Lubschez (1945)	Children 2–14 years	48	—	—	6–58
	Normal adults	Not stated	—	—	13–38
Wilson and Lubschez (1946)	Children 2–14 years, 2–3 hours after a vitamin C-free breakfast	76	0·1 –2·1	—	8–54
Hawk, Oser and Summerson (1947)	Not stated	Not stated	0·8 –2·4	—	—
Sinclair (1947)	Not stated	Not stated	0 –1·7	—	—
King (1946)	Not stated	Not stated	0·3 –1·3	—	—
Present investigation: indophenol method	Volunteers, after saturation	15	0·5 –1·50	—	7·6–21·6
dinitrophenyl-hydrazine method		15	0·45–1·32	—	—

with only 9 results out of 142 exceeding 1·1 mg. per 100 ml. The range of the vitamin C level in the white cells was from 7·6 to 21·6 mg. per 100 g. The results include those for two men (Hill, Garling) whose vitamin C intake had been high, 50 mg. or more daily, for 13 months. The range of plasma values given by other workers (Table 7), with the exception of those by Rourke, MacLachlan and Butler (1946), contains considerably higher figures at the top end of the range. It is possible that the fasting of the subjects in the present series, and the fact that the last dose of vitamin C was taken 24 hours before the blood analysis, is responsible for the discrepancy. The subjects used by Rourke and his colleagues and those by Goldsmith and Ellinger (1939) were the only ones among those listed in Table 7 who were fasting at the time of the blood collection. Whether the low values found in the white cell layer can be explained by fasting alone is doubtful. It is more probable that differences in technique are responsible. It is obviously difficult to standardize the separation of the white cells from the plasma, and inclusion of a larger proportion of plasma would account for lower values.

B. Capacity for Dark Adaptation

It has been suggested by Stewart (1939, 1941) and denied by Yudkin (1941) that lack of vitamin C is a factor in the aetiology of nutritional night blindness.

Throughout the present trial the capacity for dark adaptation was tested at approximately monthly intervals. The dark-adaptation curve was measured with the Wald–Steven–Bartley adaptometer. Livingston's (1944) rod scotometry method was used also. The conditions of testing were as previously described (Hume and Krebs, 1949). There was no significant change in the capacity for dark adaptation measured by these methods throughout the experiment. The average values for the cone-rod transition time, final rod threshold and rod scotometry measurements for the deprived group, at the start of the experiment, at the time of maximum depletion and after recovery are given in Table 8.

TABLE 8

Summary of the mean values for capacity for dark adaptation in the deprived group at three stages of the experiment

Criterion of capacity for dark adaptation	Average value for deprived group		
	At start	Immediately before dosing	After treatment
Rod scotometry* (sq. cm.)	12·5	12·9	11·2
Cone-rod transition time (min.)	7·5	7·2	7·5
Final rod threshold (log μmlambert)	1·72	1·74	1·78

* For units used see Hume and Krebs (1949).

The complete values for the final rod threshold, the cone-rod transition time and the scotometry measurements are given in Tables F, G and H (see p. 2).

C. Summary of Audiometry Measurements by J. L. Burn

Acuity of hearing was measured five times during the course of deprivation by means of the pure tone audiometer (Hume and Krebs, 1949). The average

decibel loss of the deprived subjects at the start of the trial was 13·6, and at the time when signs of vitamin C deficiency were most severe it was 11·1. These differences are not significant.

D. The Breakdown of Tyrosine in the Body on Deprivation of Vitamin C

Several reports in the literature indicate that vitamin C plays a role in the metabolism of tyrosine in the mammalian body. Sealock and Silberstein (1940) showed that the ingestion of 0·5 g. a day of tyrosine by scorbutic male guinea-pigs weighing about 300 g. caused an increase in excretion of phenolic compounds in the urine, as measured by the method of Folin and Ciocalteu (1927). Between 50 and 80 per cent of the tyrosine supplements were recovered from the urine as phenolic compounds, consisting mainly of homogentisic acid, *p*-hydroxy-phenylpyruvic acid and *p*-hydroxyphenyllactic acid. Addition of vitamin C to the diet prevented the excretion of these substances. A supplement of L-phenyl-alanine to scorbutic guineapigs had similar effects (Sealock, Perkinson and Basinski, 1941), and again vitamin C prevented the excretion of abnormal metabolites. Painter and Zilva (1947) found that doses below 0·1 g. tyrosine per 300 g. body weight did not cause an increase of phenolic substances in the scorbutic guineapig.

Levine, Marples and Gordon (1939) reported analogous findings on human subjects. They found that premature infants on a diet of cow's milk showed an increased excretion of the keto- and hydroxy-derivatives of tyrosine and phenyl-alanine and that the effect was prevented by vitamin C. Full-term infants did not show the increase on a diet of cow's milk but did so if the diet was supplemented by 0·2 to 2 g. tyrosine or phenylalanine per kg. body weight. When vitamin C was given at the same time no abnormal metabolites were found in the urine (Levine, 1946–7; see also Levine, Barnett, Bierman and McNamara, 1951; Painter and Zilva, 1947; Rogers and Gardner, 1949; Rienits, 1950).

No information seems to be available on whether tyrosine metabolism is abnormal in adult human subjects depleted of vitamin C. Experiments to study the point were made on two dèpleted volunteers (Wodeman and Hudson), with a third, non-depleted one (Garling), serving as a control. The three volun-teers had received the basal diet for 211 days when the tyrosine test started. Wodeman showed numerous skin haemorrhages and scorbutic gum lesions. Hudson too showed some skin haemorrhages, but no gum changes. In both men the plasma value for vitamin C was near zero and the white cells contained less than 1 mg. per 100 g. Garling's diet had been supplemented throughout by 70 mg. vitamin C daily and he was in every respect normal. His plasma value for vitamin C was 0·8 mg. per 100 ml. and his white cell value 14·6 mg. per 100 g.

On the first day of the test the total urine output was collected while the diet was the usual one. On the second day the three men each consumed 20 g. L-tyrosine prepared from silk waste, divided into 4 lots of 5 g., one lot being eaten with each meal during the day. No unpleasant effects ensued. Faeces, as well as urine, were collected from Wodeman in order to test whether tyrosine was excreted unchanged. The urine and faeces were analysed by the method of Folin and Denis (1915) as modified by Folin and Ciocalteu (1927) and by Medes (1932). The faeces were mixed thoroughly with 2*N*HCl and the total volume was made up to 500 ml. The mixture was filtered and the clear filtrate was treated in the same way as the urine. Total phenols only were estimated. The results of the urine analyses are shown in Table 9. It will be seen that the

supplement of tyrosine failed to produce an appreciable increase of the urinary excretion of phenolic substances. Wodeman's 48-hour faeces sample covering the second and third days contained only 0·54 g. tyrosine, showing that the bulk of the 20 g. tyrosine eaten was not excreted. Whether it was absorbed or destroyed by the flora of the alimentary canal is an open point.

TABLE 9

Amount of phenolic substances excreted in the urine before and after ingestion of tyrosine by two subjects deprived of vitamin C and by one non-deprived one

Name of subject	Body weight (kg.)	State of vitamin C nutrition	Urine			
			Volume (ml.)		Total content of phenolic substances, expressed as tyrosine (g.)	
			Before ingestion	After ingestion	Before ingestion	After ingestion
Garling	64	Receiving daily supplement of 70 mg.	1,435	1,840	1·08	1·36
Hudson	67	Deprived for 211 days	1,150	1,520	1·25	1·33
Wodeman	81		—	2,625	—	1·58

The total dietary protein of the volunteers was about 112 g. per day of which 77 g. was animal protein. It follows that the daily intake of tyrosine was about 5·6 g. and of phenylalanine about 6·3 g. The supplement of tyrosine thus increased the tyrosine intake from about 5·6 g. to 25 g. It should be noted that the tyrosine supplement given in the test was from 3 to 4 times greater than the combined tyrosine and phenylalanine content of any probable normal diet. The tyrosine supplement was about 0·25 to 0·3 g. per kg. body weight, which was lower, on a weight-for-weight basis, than in the guineapig experiments of Sealock and Silberstein (1940), but within the range of the experiments on infants reported by Levine (1946–7). It would thus appear that the capacity of the human adult to deal with tyrosine may not be as readily affected by vitamin C deficiency as that of infants.

E. Capillaroscopy in Vitamin C Deficient Subjects

In view of the abnormal behaviour of the capillaries in scurvy as demonstrated by the occurrence of capillary haemorrhages, efforts were made to study the capillaries of the deprived subjects from various aspects. The studies included observations on the morphology of the capillaries as seen under the microscope. The technique developed by Müller (1922) was used to examine the capillaries of the conjunctivae and gums, and the vessels of the retina were examined ophthalmoscopically.

SKIN CAPILLARIES

Three different areas of the skin were examined at about weekly intervals: (1) the capillary loops of the nail bed, (2) the capillaries of the inner aspect of the forearm and (3) the capillaries adjacent to the hair follicles in various parts

of the body. The instrument used was the Leitz Ultrapak microscope which incorporates a lighting system giving vertical surface illumination. For record purposes photographs were taken, mainly when abnormal findings were encountered; the instrument used was a Reichert metallurgical microscope to which a camera was attached. Kodak 0 100 plates with a green filter were used, the exposure was from 5 to 6 seconds with a stop F16. For both visual observation and photographic recording the epidermis was made transparent by a drop of cedarwood oil. Points to which special attention was paid were the shape of the loops, whether smooth or tortuous, degree of branching, presence of anastomoses, width of capillaries, rate of blood flow (stasis) and presence of red cells outside blood vessels.

Nail bed. At the start of the experiment it could be seen that the capillary loops were tortuous to the usual varying extent (Plate VII, A and B) in all the subjects except Robinson whose capillaries were much wider and showed more branching and more anastomoses (Plate VII, C). It is of interest that Robinson, at the start of the experiment, showed an abnormally high fragility with both positive- and negative-pressure methods of measuring capillary fragility.

No changes in the appearance of the nail-bed capillaries were observed in any subject throughout the experiment.

Forearm. The capillaries were in general of the same shape as in the nail bed and Robinson showed the same variation. No changes were seen throughout the experiment.

Hair follicles. During the early stages of deprivation, while the microscopical appearance of the hair follicles was normal, only the usual capillary loops of the skin, arranged perpendicularly to the surface, were seen and no changes were noticed within the first 17 weeks. Thereafter the microscopical changes developed roughly parallel with the macroscopical changes of the hair follicles. The first microscopical change was the formation of new capillaries which grew to form a circular system of vessels round the hair follicles (Plate VIII, A). Gradually the network of capillaries forming this ring became closer and the individual capillaries wider; capillary buds developed, and many anastomoses between the capillaries could be seen. Macroscopically this stage was recorded as "congested" hair follicles. A few days or weeks later red blood cells were seen lying in increasing numbers outside the blood vessels and macroscopically the bright red colour changed to dark purple (Plate VIII, B). No definite rupture of any capillaries was seen.

Simultaneously with the changes in the capillaries, abnormal processes took place in the hair follicles, by which deposition of horny material within the follicle caused dilatation and keratosis of the follicle and coiling of the hair. The deposition of this material preceded the formation of new capillaries by 2 to 3 weeks.

Shortly after the start of dosing with 10 mg. vitamin C daily the number of extravascular erythrocytes ceased to increase and the red cells already present in the tissue disintegrated leaving a brown pigment behind. The capillary loops gradually disappeared and within 2 to 3 weeks after the beginning of treatment all abnormalities had disappeared.

The time course of the macroscopical and microscopical observations are summarized in Table 10. The sequence of events was the same in all the volunteers deprived of vitamin C. Differences concerned the speed at which the abnormalities developed and the number of hair follicles affected.

TABLE 10

General scheme of the sequence of the vascular changes in and around the hair follicles

Time	Macroscopic changes	Microscopic changes
0–16 weeks after start of deficient diet	None	None
17–26 weeks after start of deficient diet	First appearance, or increase in number, of (hyperkeratotic) follicles	Formation of new capillaries beginning 1–3 weeks after appearance, or increase in number, of hyperkeratotic follicles
2–4 weeks after appearance of elevated follicles	Congestion of follicles (bright red colour, disappearing on pressure)	Formation of more new capillaries and buds, dilatation of vessels, stasis
1–4 weeks after beginning of congestion	Haemorrhagic spot (dark purple colour persisting on pressure)	Many red cells outside vessels
1–2 weeks after dosing with 10 mg. vitamin C daily	No change	No new extravasation of red cells, crenated and disintegrated red cells, localized deposits of brown pigment
2–4 weeks after dosing with 10 mg. vitamin C daily	Colour changing from dark purple to light brown, elevation disappearing	Disappearance of capillaries except the normal skin vessels, deposits of brown pigment

DISCUSSION

The changes in the capillaries seen in the course of the depletion were of two kinds: (1) proliferation of the capillaries around the hair follicles; (2) diapedesis of red blood cells into the surrounding tissues.

It might be argued that the appearance of new capillaries might in fact be an opening up of existing vessels rather than a new formation, but the skin of normal subjects, after being warmed sufficiently to cause sweating, showed no capillaries running parallel to the surface like those seen round the hair follicles of the deprived subjects; it is, therefore, probable that the capillaries were newly formed. The fact that the deposit of keratin in the hair follicles preceded the formation of capillaries suggests that the proliferation of the capillaries was perhaps not directly due to lack of vitamin C but was a response to the abnormal developments within the hair follicles. On the other hand the increased permeability of the capillaries to red cells might be a direct effect of the lack of vitamin C because administration of a small dose of 10 mg. of vitamin C daily promptly prevented any further leakage of red cells.

The question arises why diapedesis of the erythrocytes took place in certain capillaries of the body, such as those round the hair follicles, but not in other areas of the skin. Abell (1946) has shown that newly formed capillaries were

more permeable to the dye T 1824 (Evans blue) than older vessels, and that their greater permeability lasted for from 2 to 3 months. The only explanation, therefore, which can be offered at present is that the age of the capillaries determines the site of the petechiae, their location round the keratotic hair follicle and in fresh scar tissue being due to the presence of young capillaries.

F. General Account of the Changes in and around the Hair Follicles

The following account is based on numerous macroscopical and microscopical examinations, including skin biopsies. Apart from the regular inspections, a special intensive investigation was carried out on a limited area of one volunteer (Sanderson) when the signs of depletion were most severe. Two circular skin areas, about 3 cm. in diameter, were marked out, one just below the antecubital fossa of the right elbow and the other on the extensor aspect of the thigh. These areas were inspected three times weekly with the aid of a lens and skin microscope.

CONDITION AT THE START OF THE EXPERIMENT

Scattered elevated hair follicles with irregular distribution were seen at the start in eight volunteers (Bartley, Garling, Golding, Jackson, Proctor, Sanderson, Way, Wodeman) and acneiform lesions in seven (Drake, Hudson, Jackson, Milburn, Sanderson, Tridgell, D. Williams). An example of the elevated hair follicles as seen at the start, is given in Plate IX, A. The appearance of these follicles is very similar to those seen in "gooseflesh", and in order to avoid confusion between the temporary elevation of gooseflesh and follicular enlargement proper, all examinations were carried out in a warm room.

OBSERVATIONS IN THE GROUPS RECEIVING SUPPLEMENTS

The volunteers of the two groups receiving vitamin C supplements showed no progressive skin changes during the trial. Occasional hyperkeratotic follicles were recorded; their number fluctuated and they rarely reached the erythematous stage. No follicular haemorrhages were observed.

The two volunteers (Golding, Way) who had follicular abnormalities at the start showed them with fluctuating intensity during the trial but with no definite tendencies of development. Golding had raised keratotic follicles on the arms which had been under observation for the 2 years preceding the trial (see Hume and Krebs, 1949). Throughout the trial Way had keratotic and slightly erythematous follicles on the external aspects of the upper arm and on the backs of the thighs.

OBSERVATIONS IN THE DEPRIVED GROUP

Changes in the Individual Hair Follicles

After about 17 to 24 weeks of deprivation raised hair follicles, about 0·5 mm. in diameter, began to appear, usually first on the external aspect of the upper arm (Plate IX, B). A few weeks later raised follicles appeared also on the legs. At the early stage of development the skin microscope revealed no changes in the area of the follicles, apart from the elevation. As in the normal skin (Plate IX, A and C), the pin-point mouth of the follicle invested the hair closely. The elevation slowly increased in size reaching a diameter of about 1 mm. at the base within 1 or 2 weeks. The mouths of some of the new follicles gradually widened and became plugged with a mass of keratin of a soft cheesy consistency which

could be easily pressed out. The appearance of a hair follicle at this stage, magnified 40 times, is shown in Plate X, A. The hair is seen protruding from the mouth of the follicle which is widened by the deposit of keratin.

Sooner or later the portion of the hair outside the mouth of the follicle broke off (Plate X, B), and the part inside the follicle became caught in the keratinous mass which gradually hardened. The growing hair, having no outlet, then began to coil within the follicle as seen in Plate XI, A. It was often possible at this stage to remove the plug with a needle; the hair then became released. The outline of the follicular plug was often marked by a deposit of dirt adhering to the keratin.

About 5 to 8 weeks after the first appearance of the elevated follicles, the area round the follicles became erythematous and the changes in the capillaries described in Section II E (p. 28) began to take place. The macroscopical appearance of the congested follicle is shown in Plate XI, B. When, after 24 to 33 weeks, the follicle became haemorrhagic, the hair was usually no longer emerging from it, but could be seen in longitudinal sections of the follicle (Plate XII, A). At this stage the follicle appeared macroscopically as a red or purple spot, with a diameter of from 1 to 3 mm. and in some cases of up to 4 mm. Material from a skin biopsy showing a hair follicle at this stage is given (Plates XII, A and B). The pathologist (J. H. Barrie) reported "The epidermis and the sebaceous and sweat glands appear normal. Around the follicle there is a zone of haemorrhage. The red cells are scattered evenly in an oedematous stroma and some are fragmented, a few phagocytosed. There are a few small haemosiderin crystals, showing that the haemorrhage was of fairly long standing. Further away from the follicles the red cells are more clumped and form lines parallel to the capillaries. Everywhere in the section the capillaries show reduplication of their walls and pericapillary dilated empty spaces. The latter are most obvious at the base of the follicles. Here too, the hyperplasia of the capillary walls is most obvious; there are numerous mitoses and the cellular capillary walls blend into the increased number of fibroblasts in the oedematous stroma."

The appearance of the blood vessels round the hair follicles at this stage is described elsewhere (section on capillaroscopy p. 28 , Plate VIII, A and B).

Once the hair follicles had started to enlarge, new follicles became involved at irregular intervals throughout the state of depletion, but even at the stage of severe depletion, such as is seen in Plate XVIII, A, the proportion of enlarged follicles was small. For example, per sq. cm. of the thigh, three or four abnormal follicles at most were seen, usually less. A search of the literature produced no figure for the total number of hair follicles normally occurring per sq. cm. in that area but it might well be over a hundred.

Not all the follicles which showed enlargement and keratosis suffered any further pathological change, but the majority of those that became congested developed haemorrhages. The rate of progress through enlargement to keratosis, congestion and haemorrhage varied with the site of the lesion. On the arms and trunk the rate was much slower than on the legs, and a smaller proportion of the lesions developed beyond the stage of congestion on the arms than on the legs, so that though the first changes were noted on the arms, the number and size of the haemorrhages in the later stages of depletion were much greater on the legs (Sanderson, Plates III, A; XVIII, A; XIX). On the arms, the enlargement and keratosis of some follicles became stationary or even regressed while the depletion was continuing, but haemorrhages connected with follicles were not seen to regress without vitamin C treatment.

Effect of Treatment

On dosing with 10 mg. vitamin C daily, changes could be seen in the hair follicles after a few days. The purple colour became paler, and disappeared within from 2 to 4 weeks, leaving a pale brown spot. The formation of keratin by the follicles ceased within a week or two and after 4 weeks the hair usually uncurled, and lifted out the plug of keratin blocking the mouth of the follicle. The lumen of the follicle then contracted to the normal size and invested the hair closely.

G. General Account of the Changes in the Acneiform Lesions

Six volunteers had acneiform eruptions at the start of the experiment (Drake, Hudson, Milburn, Sanderson, Tridgell, D. Williams). They were fairly severe in Drake, very mild in Tridgell and intermediate in the others. In all six the main site was on the shoulders and the scapular regions; two, Hudson and Milburn, had some acne also on the face.

The changes which were observed during the period of depletion may be placed under three headings:

1. The area of the skin affected by acne.
2. The number of papules.
3. The size and appearance of the individual papules.

From the original site on the shoulders the areas affected increased to involve the back, buttocks, chest, upper arms and waist, in the order given. At the height of the depletion Drake and Sanderson were affected in all the named areas; in the case of Hudson the original area expanded relatively little and in the remaining three only some of the areas mentioned above were affected. It is noteworthy that on the face the severity of the acne fluctuated only a little. Acne did not develop on the faces of the four men who had been free from it at the start.

The increase in the number of papules generally progressed with the extension of the site.

The sequence of changes in the individual lesions was as follows: The earliest stage was a small dome-shaped erythematous papule, centred on a follicle. It gradually increased in size by extending over neighbouring follicles and was a vivid reddish yellow differing distinctly from the usual dull purplish colour of common acne. Later the red colour of some of the lesions no longer faded on pressure, indicating that it was due to haemorrhage. A biopsy of a haemorrhagic lesion was made by J. H. Barrie who reported: "Serial sections through this biopsy show severe acne with enlarged sebaceous glands and dilated follicles full of keratin and amorphous material. There are also widespread recent small extravasations of blood in the corium along the course of the congested capillaries. The haemorrhages are most numerous and appear oldest in a saucer-shaped zone deep to a small acneiform pustule."

Definite deterioration of the acne began in D. Williams after 16 weeks, in Drake and Milburn after 20 weeks, in Hudson and Tridgell after 23 weeks and in Sanderson after 30 weeks of depletion. In Sanderson and Tridgell some expansion of the acne was already seen after 8 weeks but it remained doubtful whether the increase merely represented a fluctuation in severity or was the result of deprivation. The haemorrhages into the acneiform eruptions were noted at about the same time as the haemorrhages into the keratotic hair follicles.

Only a few of the acneiform papules arising through depletion showed secondary pustulation, which was in any case much less than in ordinary, well established common acne.

Treatment with 10 mg. vitamin C daily caused the colour of the lesions to fade to a light brown-purple within 1 or 2 weeks. At the same time the size of the eruptions decreased. The area affected gradually diminished in the reverse order to that in which it had increased until only the areas affected at the start remained. The complete regression to the initial stage lasted for up to 6 months, on a dose of 10 mg. vitamin C daily, and thus happened more slowly than the disappearance of the hyperkeratotic follicular lesions.

H. Changes in the Mouth, especially in the Gums

The mouth was inspected by several observers at intervals throughout the experiment. X-ray pictures of the teeth and bones of the jaw were made three times. Photographs were taken when possible of any noteworthy changes.

STATE OF THE MOUTH AT THE START OF THE EXPERIMENT

The mouths of the majority were in good condition at the start of the experiment. The result of the first examination is summarized in Table 45, p. 136.

CHANGES IN THE GROUPS RECEIVING VITAMIN C SUPPLEMENTS

Lesions recorded in the groups receiving supplements were: bleeding of the gums, spontaneous or traumatic; fluctuating gingivitis; aphthous ulcers; tenderness and pain in the gums; small non-progressive haemorrhages into the gums. Details of the incidence of these changes are given in Table 40, p. 119. There was no progressive deterioration or production of typical scorbutic gum lesions, such as swelling and haemorrhage in the interdental papillae, in any of the volunteers receiving supplements.

CHANGES IN THE DEPRIVED GROUP

The changes described in the previous paragraph were recorded also in the deprived group. In addition typical scorbutic changes were seen. Details are given in Table 41, p. 120.

A change, subsequently recognized to be the start of the scorbutic gum lesions, was a slight reddening and swelling at the tips of the interdental papillae; it appeared after about 30 weeks' deprivation (see case history of Sanderson, p. 82, Plate XVII, A (a)), and was followed, a week or so later, by a small haemorrhage into the tip of the papilla, gradually increasing. The gums appeared bright red and haemorrhagic. If two adjacent papillae were affected the swellings became confluent. The whole of the swollen area then changed to a purplish colour and later the surface layer of the gum degenerated, producing a slimy whitish film and some ulceration. The gum became loose and sagged away from the teeth. The time taken for the later changes to appear, after the appearance of the first lesion, was variable, being most rapid in those volunteers whose gums had been in a poor condition originally. None of the volunteers showed severe ulceration.

CHANGES IN THE DEPRIVED GROUP AFTER DOSING WITH VITAMIN C

Dosing with 10 mg. vitamin C daily caused the colour of the gums to change rapidly within 1 to 2 weeks from purple to red (see case history of Wodeman,

p. 85). The swelling gradually subsided, persisting longest in the tips of the interdental papillae. The colour gradually faded from red to pink. The gums took longer than the skin to recover completely, needing about 12 weeks. Recovery was slow even when the lesion had gone no further than the small haemorrhage into the tip of the papilla.

COMPARISON OF THE CHANGES NOT TYPICALLY SCORBUTIC WHICH OCCURRED IN THE DEPRIVED AND NON-DEPRIVED GROUPS

Bleeding of the gums was reported in all groups. In four of the deprived group, Hudson, Sanderson, Tridgell and Wodeman, bleeding did not occur until scorbutic signs were obvious; it disappeared after dosing and it seems clear that it was the result of vitamin C deficiency. Frequency of bleeding was not directly connected with the severity of the scorbutic gum lesions. Wodeman, who showed the most marked gum changes, reported bleeding only on the 2 days immediately before dosing whereas Hudson reported bleeding without having typical scorbutic lesions.

Gingivitis of varying degrees of severity occurred in all but one of the deprived group and in five of the group given supplements. Tridgell already had haemorrhagic follicles in the skin, when gingivitis was first noted.

Aphthous ulcers were noted on twelve occasions in the supplemented group and on eleven in the deprived group; their occurrence was thus not related to the vitamin C intake. Tenderness and pain also were independent of the diet.

Non-typical haemorrhages into the gums, small extravasations without swelling in places other than the interdental papillae, were observed in four of the supplemented group and in five of the deprived group. In the deprived group they occurred at the height of the depletion which suggests that they were a result of vitamin C deficiency.

I. Vitamin C Content of the Urine

The trial offered an opportunity of determining the vitamin C content of urine from subjects with a controlled vitamin C intake. Determinations of vitamin C in urine were made at intervals by three different methods. The procedures are described in Section III, p. 68 and Appendix A, p. 145.

Direct Titration with 2:6–Dichlorophenolindophenol

This method, which is widely used in connexion with saturation tests, is known to be relatively unspecific, since reducing substances other than ascorbic acid react with the dye and are, therefore, included in the values for vitamin C. All the data obtained by this method are given in Table 39, p. 110.

It will be seen from Table 39 that great variations occurred in the same individual during a constant intake of vitamin C. The most remarkable feature was the apparent presence of considerable amounts of vitamin C in the urine of the volunteers after several months of deprivation when the true vitamin C content must have been near zero. The values never fell below 13 mg. a day in the deprived group and sometimes reached 48 mg. a day; they give an indication of the amount of vitamin C which was simulated by other reducing substances reacting with the dye. The average figure for the deprived group was 24 mg. a day, but it should not be taken as a standard value, since it is likely that it would depend on the nature of the diet. It follows that, in the subjects of the present experiment, individual vitamin C values of, say, 20 to 40 mg. a day, if obtained by the dye titration method were not significant.

To test whether group differences could be distinguished when a large number of results were considered, all results from the Oxford and Cambridge laboratories for volunteers having the same vitamin C intake for the same period were grouped together and the average values over a number of 4-weekly periods were calculated. The results are shown in Table 11. It will be seen that the average values for the group supplemented with 70 mg. were generally somewhat higher than those for the two other groups, but between the two other groups there was no consistent difference, and the average value did not change in the deprived group as depletion proceeded.

TABLE 11

Average daily excretion of apparent vitamin C in the seven 4-week periods from November 1944 until May 1945
(Dye titration method)

4-week period	Av. amount apparent vitamin C daily in the urine of group receiving: vitamin C, 70 mg. daily (mg.)	vitamin C, 10 mg. daily (mg.)	no supplement (mg.)
First	23·9	24·9	20·0
Second	41·1	24·5	34·8
Third	26·4	23·1	20·7
Fourth	—	—	20·5
Fifth	—	—	18·9
Sixth	—	24·0	19·0
Seventh	33·4	25·0	22·7
Average of all throughout	33·2	24·3	24·0

Dinitrophenylhydrazine Method of Roe and Kuether (1943)

Analyses by Roe and Kuether's method began in January 1945. Four-weekly averages were calculated in the same way as for the dye titration results. They are shown in Table 12. The values were very much lower than by dye titration,

TABLE 12

Average daily excretion of apparent vitamin C in the four 4-week periods between December 1944 and May 1945
(Method of Roe and Kuether, 1943)

4-week period*	Av. amount apparent vitamin C daily in the urine of group receiving: vitamin C, 70 mg. daily (mg.)	vitamin C, 10 mg. daily (mg.)	no supplement (mg.)
Second	—	4·4	—
Third	11·9	4·3	3·8
Sixth	—	4·4	—
Seventh	10·4	5·4	4·3

* The times stated correspond to those given in Table 11.

confirming Roe and Kuether's claim for the relatively high specificity of their method, but, even so, individual volunteers of the deprived group still appeared to excrete between 0·6 and 6·8 mg. vitamin C in 24 hours after 5 months of deprivation. Most of it can hardly have been true vitamin C. The average value for the daily excretion of the group supplemented with 70 mg. was distinctly higher than the corresponding values for the two other groups, between which there was little difference.

Titration with 2:6–Dichlorophenolindophenol in the Presence of Formaldehyde

This method was used from January 1945 onwards. Average values obtained for two representative 4-weekly periods are given in Table 13. For the groups receiving 10 mg. or no supplement the values were even lower than those obtained by the method of Roe and Kuether, but those for the group supplemented with 70 mg. were much the same as those given by the method of Roe and Kuether. Again there was no significant difference between those deprived and those receiving a supplement of 10 mg. After 5 months of depletion, daily quantities of up to 5·8 mg. of apparent vitamin C were sometimes excreted even by individuals in the deprived group, which means that the true value for the quantity excreted by volunteers supplemented with 70 mg. is about 5 to 8 mg. These results confirm those obtained by the method of Roe and Kuether.

TABLE 13

Daily excretion of apparent vitamin C in two representative 4-week periods (Dye-formaldehyde method)

4-week period*	Amount of apparent vitamin C daily in the urine of group receiving:					
	vitamin C, 70 mg. daily		vitamin C, 10 mg. daily		no supplement	
	average (mg.)	range (mg.)	average (mg.)	range (mg.)	average (mg.)	range (mg.)
Third	10·8	4·5–13·3	0·8	0·0–3·6	0·7	0·0–2·2
Seventh	10·4	9·7–11·0	3·3	1·7–4·7	2·7	0·6–5·8

* The times stated correspond to those given in Table 11.

Comparative Evaluation of the three Methods

Its lack of specificity makes the simple dye titration method useless for estimating a daily output in the urine of less than about 50 mg. of vitamin C. Since the daily output of inactive reducing substances averaged 24 mg. it is obvious that at least an equal amount of active substances must be present to give the results even a qualitative value. Use of the method is justified in saturation tests where the quantities are considerably above the 50 mg. level. Both the dinitrophenylhydrazine and the dye-formaldehyde method are great improvements on the simple dye titration method in specificity but interfering substances simulating vitamin C are still not completely eliminated. Urine seems to contain more of such substances than other body fluids. A further examination of the value of these two methods in connexion with clinical routine and dietary surveys would be of interest.

TABLE 14

Average plasma protein concentration of volunteers at different times of the trial (Tot. = total protein, Alb. = albumin, Glob. = Globulin, Fib. = fibrinogen. The individual data for all the volunteers are given in Table 38.)

Date	Time of estimation	Average plasma protein concentration in group receiving: vitamin C, 70 mg. daily					vitamin C, 10 mg. daily					vitamin C, 10 mg. initially then 5 mg.					no supplement initially				
		No. of subjects	Tot. (g./100 ml.)	Alb. (g./100 ml.)	Glob. (g./100 ml.)	Fib. (g./100 ml.)	No. of subjects	Tot. (g./100 ml.)	Alb. (g./100 ml.)	Glob. (g./100 ml.)	Fib. (g./100 ml.)	No. of subjects	Tot. (g./100 ml.)	Alb. (g./100 ml.)	Glob. (g./100 ml.)	Fib. (g./100 ml.)	No. of subjects	Tot. (g./100 ml.)	Alb. (g./100 ml.)	Glob. (g./100 ml.)	Fib. (g./100 ml.)
Oct. 1944	Before controlled diet	(**) 3	6·46	4·15	1·80*	0·246*	(**) 3	6·70	4·58	1·90	0·183	(**) 4	6·20	4·08	1·86*	0·216†	(**) 9	6·63	4·47	1·88	0·239
Jan. 1945	About 70 days on controlled diet	3	6·30	3·83	2·20	0·254	3	6·20	4·03	2·02*	0·225*	3	6·20	4·10	1·83	0·231	7	6·18	3·87	1·96‡	0·240*
Mar. 1945	About 114 days on controlled diet	3	6·31	3·73	2·33	0·207	3	6·31	4·03	2·07	0·200	4	6·31	3·95	2·03†	0·226†	9	6·07	3·93	1·88	0·241
May 1945	About 185 days on controlled diet	3	6·51	4·03	2·30*	0·192*	3	6·51	4·10	2·50*	0·205*	4	6·50	4·10	2·15	0·250	9	6·28	3·74§	2·34†	0·245†
July 1945	Controlled diet continued with supplements for deprived volunteers	2	6·62	3·95	2·45	0·210	2	6·97	3·90	2·80	0·250	—	—	—	—	—	5	6·31	3·83	2·18	0·294
Sept. 1945		2	6·12	3·55	2·35	0·220	3	6·03	3·56	2·25	0·220	3	6·16	3·68	2·23	0·203	6	6·38	3·56	2·60	0·209
Nov. 1945		2	6·48	3·65	2·55	0·222	3	6·46	3·83	2·35*	0·226*	3	6·38	3·76	2·38	0·212	7	6·62	3·80	2·55	0·254
Feb. 1946	After saturation test	2	6·45	4·40	1·80	0·250	3	6·50	4·38	1·83	0·270	3	6·51	4·48	1·76	0·270	7	6·66	4·33	2·07	0·263
Sept. 1946	After 7 months on a normal diet	3	6·68	4·21	2·27	0·181	3	6·58	4·06	2·30	0·192	3	6·93	4·18	2·53	0·215	7	6·68	4·18	2·27	0·217

(**) In some cases the average number of subjects differed from that given in this column; this is indicated by reference marks:
* = average of 2; † = average of 3; ‡ = average of 6; § = average of 7.

J. Biochemical Investigations on the Blood

BEHAVIOUR OF THE PLASMA PROTEIN CONTENT

Changes in the amount of the plasma proteins are not commonly mentioned as a feature of scurvy. When a low value for plasma proteins, particularly for albumin, has been noted in severe scurvy (Vilter, Woolford and Spies, 1946) it is more likely to have been the result of an ill balanced diet deficient in protein than of vitamin C deficiency.

In the present investigation a deficiency of vitamin C produced no change in the plasma content of proteins. In Table 14 the mean values of the plasma proteins are given for the groups of volunteers. The total protein values and the ratios of albumin to globulin for all the groups fall within the limits recorded for large groups of normal subjects.

The figures of Table 14 are of interest in showing the course of the fluctuations in the concentration of the plasma proteins over a continuous long period of 17 months, during which the daily intake of protein was about 100 g. When allowance is made for the experimental error of the method of analysis, the fluctuations during the period were not great. The groups receiving vitamin C supplements gave rather low values in September 1948, but it is not possible to decide whether the fall was due to a seasonal variation or other causes. Evidence for a seasonal variation in plasma proteins is conflicting; some have noted a rise in amount in summer (Dyson, 1945) and others in winter (Peters and Eisenman, 1933; Trevorrow, Kaser, Patterson and Hill, 1941–2).

PHOSPHATASE ACTIVITY OF THE PLASMA

Plasma phosphatase values about half the normal have been recorded in scorbutic infants (Smith and Maizels, 1932; Smith, 1933; Shwachman, 1941), and in guineapigs with severe scurvy (Scoz, Cattaneo and Gabbrielli, 1937; Todhunter and Brewer, 1940; Gould and Shwachman, 1941–2). With the institution of vitamin C therapy, the plasma phosphatase activity rapidly reached a normal value in both the infants and the guineapigs.

TABLE 15

Average phosphatase activity of the plasma of the three groups of volunteers at different dates (The mean value determined for 100 *normal subjects by J. R. P. O'Brien and G. Higgins was* 7·9, *with range* 4 *to* 14, *King-Armstrong units.)*

Date	Duration of period on basal diet (days)	Average value for group receiving: vitamin C, 70 mg. daily (King-Armstrong units)	vitamin C, 10 mg. daily (King-Armstrong units)	no supplement (King-Armstrong units)
October 1944 ..	0	13·0	12·0	11·5
January 1945 ..	68	5·5	5·0	5·0
March 1945 ..	114	8·0	6·3	7·7
June 1945.. ..	205	4·6	4·5	5·5
September 1945..	303	5·0	5·0	4·8
November 1945..	366	7·5	5·0	5·1
February 1946 ..	After saturation tests	6·3	5·6	6·0
September 1946..	After 7 months' full normal diet	7·6	7·0	7·0

In the present trial, deprivation of vitamin C produced no significant change in the value for plasma phosphatase (see Table 15). Compared with those for the groups which received 70 mg. or 10 mg. vitamin C daily, the mean values for the group deprived of the vitamin show no difference. After a depletion period of 205 days, when signs of scurvy were definite, the values of the deficient group did not differ from those of the non-deprived groups, and remained unchanged when the signs of scurvy were most striking. A change common to all groups was a fall from a mean value of about 12 King-Armstrong units per 100 ml. plasma before the start of the experiment, to one of about 5 at the first estimation after the experiment had begun; it may have been due to the change from the ordinary diet of the volunteers to the basal diet, whether supplemented or not, but it is not possible to be sure. It is to be noted that subsequent consumption of an ordinary diet for 7 months after the end of the experiment produced no great change in the phosphatase values.

K. Haematological Investigations

GENERAL SURVEY

The haemoglobin concentration, the number of red cells, white cells and platelets, the bleeding time and the erythrocyte sedimentation rate were determined at about monthly intervals. All the results are presented in Table A (see p. 2), average figures in Table 16. No striking changes in the blood picture related to the vitamin C intake were noted. It should be specially emphasized that at the time when the scorbutic signs were most severe, the volunteers all showed normal values for haemoglobin and cell counts, except H. Williams, the only woman in the trial, and D. Williams, who had malaria from the start of the experiment and who developed a tuberculous spondylitis.

Anaemia has often been regarded as a feature of scurvy. The present results raise the question whether factors other than vitamin C deficiency, notably iron deficiency, were in previous observations responsible for the "scorbutic" anaemia.

HAEMOGLOBIN CONCENTRATION AND NUMBER OF ERYTHROCYTES

On two separate occasions the haemoglobin concentration of Garling was as low as 85 per cent and 89 per cent; iron therapy produced normal values. The number of erythrocytes paralleled the haemoglobin concentration.

WHITE-CELL COUNT

At the start of the experiment, the white-cell count of the volunteers was normal (range 4·92 to 8·68 $\times$ 10^3) with the exception of Wodeman who had an unexplained leucocytosis of 15·46 $\times$ 10^3 which later disappeared. No major change in the counts of the two groups receiving vitamin C supplements occurred during the experiment. Of the deprived group, Drake, Hudson, Sanderson, Tridgell and Another showed to varying degrees a fall in the counts, without alteration in the differential picture. The mean value of the counts of these five men fell from 8·05 $\times$ 10^3 at the beginning of the experiment to 5·3 $\times$ 10^3 at the time when their deficiency signs were manifest. In two cases (Hudson and Sanderson) a rise followed the administration of vitamin C.

PLATELET COUNT

The variations found cannot be regarded as significant.

TABLE 16

Average values at intervals throughout the experiment for the content of haemoglobin, red cells, white cells, and platelets and for the erythrocyte sedimentation rate of groups of volunteers receiving different supplements of vitamin C

Approximate* period on basal diet (days)	No. of subjects	Supplement of vitamin C (mg. daily)	Haemoglobin (Haldane, per cent) Mean	Haemoglobin (Haldane, per cent) Range	Red cells (millions per c.mm.) Mean	Red cells (millions per c.mm.) Range	White cells (thousands per c.mm.) Mean	White cells (thousands per c.mm.) Range	Platelets (hundred thousands per c.mm.)	Sedimentation rate (Westergren) (mm.) 1 hr.	Sedimentation rate (Westergren) (mm.) 2 hr.
0	3	70	104	86–116	5·69	5·02–6·47	6·78	5·24– 8·26	2·76	2·5	5·5
66	3	70	113	109–120	5·67	5·44–6·03	6·57	6·40– 6·72	2·72	3·0	7·0
105	3	70	107	100–119	5·45	4·81–5·86	8·36	7·28–10·40	2·64	3·5	9·0
205	3	70	105	102–113	5·87	5·50–6·61	8·03	7·20– 9·50	3·52	3·5	8·5
258	3	70	109	91–123	5·19	5·00–5·37	8·73	6·60–10·40	5·39	—	—
353	2	50	102	99–115	5·10	4·81–5·39	8·65	6·80–10·50	5·34	2·0	4·5
0	3	10	100	95–106	5·39	5·11–5·59	5·54	4·92– 6·04	2·64	3·0	6·5
65	3	10	97	86–106	5·11	4·81–5·17	6·16	5·00– 7·60	4·28	2·5	5·5
104	3	10	107	100–117	5·58	5·45–5·61	6·78	4·08–10·00	2·91	3·0	7·5
215	3	10	98	95–101	5·20	4·69–5·40	5·90	4·80– 7·20	3·03	2·5	6·0
253	3	10	106	95–120	5·24	5·21–5·40	5·97	5·10– 6·60	—	—	—
352	3	10	104	99–109	5·29	4·85–5·87	6·34	5·10– 7·60	2·12	5·5	10·0
32	4	10	108	103–111	5·42	4·35–5·94	6·75	5·40– 5·80	2·25	6·0	11·5
65	4	10	110	99–115	5·26	4·64–6·09	7·60	5·40– 9·60	2·10	3·5	8·5
170	4	10	104	99–107	5·63	5·50–5·83	7·40	5·50–10·30	2·39	4·5	9·5
218	4	0	102	92–111	5·46	4·95–6·57	7·65	6·60– 9·70	4·39	—	—
315	3	5	110	97–120	5·16	4·83–5·19	5·86	4·40– 7·40	2·58	6·5	14·0
0	9	0	103	87–122	5·51	4·96–6·02	6·66	5·40–15·46	2·99	2·5	6·5
72	9	0	110	83–120	5·46	4·96–6·42	5·66	4·60– 9·60	3·15	4·0	9·0
100	9	0	106	90–114	5·65	5·36–6·15	6·77	5·40– 9·80	2·41	5·0	12·0
205†	9	0	107	95–119	5·67	4·78–6·45	6·42	3·90–13·50	2·58	5·0	12·0
252†	6	0	110	99–110	5·54	5·19–5·85	6·25	4·70– 7·20	4·16	—	—
352	6	10	106	96–123	5·65	5·07–6·55	6·18	5·20– 7·20	4·79	3·0	6·5

* As the tests were not all carried out on the same day, an approximate number of days' depletion is given.
† At this time signs of deficiency had become definite in the group receiving no supplement of vitamin C.

SEDIMENTATION RATE

A raised sedimentation rate was occasionally observed in some subjects c all groups, Bartley, Proctor and Golding of the supplemented groups, an Robinson, Hudson and H. Williams of the deprived group.

D. Williams and Milburn (deprived group) and Whinfield (supplemente group) had persistently raised sedimentation rates throughout the experimen In the case of D. Williams the raised rate was no doubt related to the tuberculou lesion of his spine; no explanation can be offered for the values of the other tw Saturation with vitamin C had no effect on the rate in these three volunteers.

L. Various Clinical Aspects

BODY WEIGHT

Each volunteer weighed himself without clothing, twice weekly, befor breakfast. There was no significant change in any group during the experimen The average weights of the unsupplemented group at the start of the experimen at the time of greatest depletion and after dosing, are given in Table 17, togethe with the average weights at comparable times of the other two groups. Th monthly average weights of all the volunteers throughout the experiment ar given in Table B (see p. 2).

TABLE 17

Average body weight of volunteers at different stages of the experiment

Stage of experiment	Average body weight of group receiving:					
	vitamin C, 70 mg. daily		vitamin C, 10 mg. daily		no supplement	
	(lb.)	(kg.)	(lb.)	(kg.)	(lb.)	(kg.)
Beginning	135·5	61·6	135·5	61·6	132·5	60·2
Greatest depletion of unsupplemented group	134·4	61·1	134·5	61·1	130·4	59·3
After dosing and saturation ..	134·8	61·3	133·5	60·7	132·2	60·1

PULSE RATE

The pulse rate was measured twice daily, before rising in the morning and a least 5 minutes after going to bed at night. The pulse was always counted for full minute.

In all three groups there was a slight increase in the average rate during th experiment, but there were no differences or changes in it which could be corr lated with the vitamin C intake. A summary of the pulse rates of the grou throughout the experiment is given in Table D (see p. 2).

BODY TEMPERATURE

INCIDENCE AND DURATION OF COLDS

Throughout the experiment the volunteers recorded the number and duration of the colds they experienced. These data are presented in Table 44 (p. 134). At a glance they seemed to indicate that the average number of colds in the deprived and non-deprived groups did not differ markedly, but that they lasted longer in the deprived group. The material was accordingly submitted to a statistician (C. H. Jowett) for analysis, who summarized his investigation as follows:

> "The lengths of all colds were subjected to the transformation $y = 20 \log x$. It was considered that this would make the various distributions on which the statistical tests depended more close to 'normal'. The data analysed consisted of the following:
>
> Colds of members of deprived group before dosing.
> Colds of members of supplemented group up to and including July 1945.
>
> Assuming for the moment that time of year had no appreciable effect, the following conclusions emerged from the analysis of variance given in Table 18.

TABLE 18

Analysis of variance of transformed lengths of colds

Source of variation	Sum of squares	Degrees of freedom	Mean square	
Difference between groups	431·8	1	431·8	(1)
Differences between individuals within a group ..	1092·1	10	109·2	(2)
Variation of length of cold within an individual ..	1365·8	27	50·6	(3)

The ratio of the mean squares (2)/(3) fell just short of the 5 per cent level of significance, but since it almost attained that level, and moreover for *a priori* reasons, it was considered that differences between individuals did in fact exist; the mean square (1) was accordingly tested against (2), the ratio (1)/(2) being equal to 3·95. This value lies between the 5 per cent level and the 10 per cent level of significance, and hence there is no conclusive evidence of a difference between mean transformed length of colds from supplemented to deprived groups. Such evidence as there is, however, definitely confirms the hypothesis that the absence of vitamin C tended to cause colds to last longer.

The geometric mean length of colds of non-deprived subjects = 3·3 days (4)
The geometric mean length of colds of deprived subjects = 6·4 days (5)

As a further check the seasonal difference in colds was investigated; differences between season lengths were not marked, and the numbers of winter and summer colds did not differ seriously from group to group. It may safely be concluded that the difference between the means (4) and (5) was not a manifestation of the seasonal effect.

In illustration of the seasonal differences, the geometric mean lengths of colds for the 'winter' months and the 'early summer' months are given in Table 19.

TABLE 19

Seasonal incidence and duration of colds

Season	Geometric mean length in group:		No. of colds in group	
	supplemented (days)	deprived (days)	supplemented	deprived
November–February	4·0	7·6	11	8
April–July	3·2	7·2	8	8

Conclusion

The data support the hypothesis that colds of deprived subjects lasted longer, but do not establish it."

In connexion with this result mention should be made of the observation of Glazebrook and Thomson (1942) who studied the incidence and duration of infectious diseases in groups of adolescents living in an institution where the dietary level of vitamin C was very low. The incidence of the common cold and tonsillitis or the average duration of illness due to the common cold was not affected by vitamin C supplements, but the average duration of illness due to tonsillitis was longer in the unsupplemented group.

SUBJECTIVE PAINS IN THE BACK, JOINTS AND LIMBS

The following analysis refers to records of subjective pains made by the volunteers themselves. Pains due to experimental wounds or gross pathological changes such as the effusion into the knee joints suffered by Drake were omitted. The number of days on which pains in the back, joints and limbs were reported are tabulated in Table 20. It shows a rise in the number of days on which pain was reported, starting about 8 weeks before dosing and reaching a maximum just before dosing. After dosing with 10 mg. vitamin C daily the incidence of pain fell again and remained low throughout the experiment. The groups receiving supplements did not show a rise at the corresponding time.

At the time when the increase of pain was reported it was obvious to the deprived subjects that they had scurvy. It might, therefore, be argued that the pains were due to auto-suggestion. The impression of the clinicians was that they were not. There was no objective evidence of pain in any of the volunteers.

Detailed information on the precise nature and duration of the pains, as recorded, is scanty, but the following points emerge:

1. The pains were nearly always aching pains.
2. Pains in the back were often associated with, or intensified by, deep breathing, coughing, sneezing or sudden movement.
3. Pains in the limbs were more or less continual and not associated specifically with movement.
4. The onset of pain was often preceded by complaints of stiffness and feeling of tiredness in the legs.

The pains closely resembled those recorded in the classical description of scurvy (Lind, 1757; Smith, 1904).

TABLE 20

Number of days, during 4-week periods, on which pains in the back, joints and limbs were reported by six deprived subjects and seven receiving supplements, all of whom completed the experiment

Six deprived subjects		Seven subjects receiving supplements	
Period	No. of days on which pain occurred	Period	No. of days on which pain occurred
32–28 weeks before dosing	1	Comparable with the deprived subjects	0
28–24 ,, ,, ,,	1		0
24–20 ,, ,, ,,	1		11 *
20–16 ,, ,, ,,	0		13 *
16–12 ,, ,, ,,	0		8 *
12–8 ,, ,, ,,	1		1
8–4† ,, ,, ,,	13		1
4–0 ,, ,, ,,	57		0
Dosing begins			
0–4 weeks after dosing‡	20		1
4–8 ,, ,, ,,	0		0
8–12 ,, ,, ,,	2		4
12–16 ,, ,, ,,	4		0
16–20 ,, ,, ,,	0		1

* The bracketed figures are those reported by Garling. Apart from these only 8 days were reported by the subjects receiving supplements.

† In the period from 8 to 4 weeks before dosing the presence of scurvy had become obvious to the volunteers concerned.

‡ All pains disappeared within a week of dosing with 10 mg. vitamin C daily.

INSPECTION OF THE WOUNDS LEFT AFTER EXCISION OF THE SCARS

The wounds referred to in this section are those resulting from the excision of the linear or stab wounds made for the examination of the histological and physical properties of the scar tissue. The linear and stab wounds themselves were not under observation between the dates of incision and excision as they were covered with "Elastoplast".

The gap left after excision was closed with three or four sutures which were removed after 10 days. At the time when the stitches were removed, all wounds showed signs of healing with normal granulation tissue and normal epithelialization. Healing progressed normally in the groups receiving supplements and also in the deprived group during the first 5 months of deprivation, but later the deprived volunteers showed haemorrhages at the edge of the wound scar, which gradually proceeded towards the centre at the time when the hair follicles became haemorrhagic. Once haemorrhages into the wound tissue had started, healing made little or no progress; the granulation tissue did not become organized and haemorrhages continued, causing discoloration of the surrounding skin area (see, for example, case history of Sanderson, p. 82).

Wounds which had been made during the earlier stages of deprivation and which had healed to give a normal scar usually showed haemorrhages soon after the hair follicles had become haemorrhagic. The pink scar tissue became red and later purple and the neighbouring area showed brownish yellow discoloration

(see case history of Wodeman, p. 85). Complete disintegration of the scar tissue was not observed.

After dosing with 10 mg. vitamin C daily, haemorrhages into the wound tissue ceased to occur within 1 or 2 weeks. The colour of the scar changed to a light brown shade and later to pink, within about 8 weeks.

Defective healing and breakdown of wound scars have, of course, often before been reported in scorbutic patients. Lind wrote of scorbutics (1757, p. 119) "It is not unusual at this time for such persons as have had ulcers formerly healed up to have them break out afresh". Smith stated (1904, p. 594) "An old scar, a recent wound . . . may become the focus of a rapidly spreading scorbutic ulcer".

OPHTHALMOSCOPIC AND SLIT-LAMP EXAMINATIONS

(Summary of Report of W. J. Wellwood Ferguson)

Ophthalmoscopic examination of the retina and slit-lamp examination of the eye as well as of the gums was carried out at regular intervals. A map of the limbic plexus on the exposed areas of the conjunctiva was made in each case, and the condition of the gums as seen with the slit-lamp and corneal microscope was noted also. Sketches were made showing the degree of vascularization of the gums and of the limbic plexus for each volunteer. No variations in the condition of the conjunctivae or the limbic plexus were noted during the period of deprivation, nor were there any variations in the number of visible corneal nerves; in no case did any vascularization of the cornea develop.

The capillaries of the gums showed no changes before the macroscopical appearance of lesions.

ELECTROCARDIOGRAMS

Electrocardiograms were taken for all the volunteers, after the illnesses of Milburn and Another, in August 1945 and February 1946. Theirs were the only abnormalities reported; the detailed findings are reported in their case histories.

M. Exercise Tolerance Tests

The test consisted in stepping on and off a chair about 1 ft. 6 in. high at the rate of once every 2 seconds for 2 minutes, after which the subject lay on a couch and the pulse rate was taken immediately, at one-minute intervals for 6 minutes, at two-minute intervals from 6 to 20 minutes and at four-minute intervals from 20 to 52 minutes or until the pulse returned to the resting rate before the test, whichever was shorter. The resting pulse rate, before the test, was taken after the subject had been reclining for at least 5 minutes.

Owing to the presence of the experimental wounds which partially incapacitated the subject, it was not possible to carry out the tests at regular intervals.

The data obtained are shown in Table E (see p. 2). They suggest that the increase of the pulse rate after exercise diminished in some of the volunteers in the course of the experiment, and that this "training effect" was smaller in the deprived group. The point was statistically examined by C. H. Jowett, Department of Mathematics, University of Sheffield, who reported as follows:

> "The irregular way in which these data were collected renders it impossible to take all of them into consideration in any one analysis, and the task had to be faced of selecting data for analysis in such a way as to give the most sensitive comparison; this resulted in the choice of two analyses, discrimination between which is not possible on *a priori* grounds.

First analysis

$$\text{Variable} = \frac{\text{exercised pulse/resting pulse (4th test)}}{\text{exercised pulse/resting pulse (1st test)}}$$

The fourth test was chosen since for the deprived group it was the last test to take place at roughly the same time for all subjects, and therefore there was good *a priori* reason to justify the assumption that the training effect had affected each subject to roughly the same extent. In the case of the supplemented group, there was considerable variation in the time at which the test was carried out, but the effect of this would be to attenuate the training effect, since in some cases the test was carried out much later than for the deprived subjects. If this fact introduced any bias into the test, such bias would tend to favour the hypothesis 'no difference in response between groups'.

The analyses of variance were as follows: Analysis of variance IA refers to the pulse rate immediately after the exercise; analysis of variance IB to the pulse rate after 2 minutes.

Analysis IA

(Immediate; 4th test)

	Sum of squares	Degrees of freedom	Mean square	Variance ratio
Between groups	0·0047	1	0·0047	0·19
Within groups	0·4251	17	0·0250	

The mean of supplemented group is 0·879 (standard error 0·045) and of the deprived group is 0·910 (standard error 0·058).

Analysis IB

(After 2 min.; 4th test)

	Sum of squares	Degrees of freedom	Mean square	Variance ratio
Between groups	0·0415	1	0·0415	1·73
Within groups	0·4084	17	0·0240	

The mean of the supplemented group is 0·914 (standard error 0·064) and that of the deprived group is 1·007 (standard error 0·023).

Conclusion

No evidence of any significant difference between groups is found in either analysis or in the whole.

Second analysis

$$\text{Variable} = \frac{\text{exercised pulse/resting pulse (June test)}}{\text{exercised pulse/resting pulse (1st test)}}$$

In this case the June test was chosen because the maximum effect of deprivation might have been expected to be observed in the deprived group. Unfortunately the number of subjects in the deprived group had diminished from the previous case, so that there was an accompanying decline in the power of the test, and also a bias in favour of the hypothesis of "no effect" since the missing subjects were likely to be the most seriously affected.

The analyses are given as before (Analyses of Variance IIA and IIB).

Analysis IIA

(Immediate; June test)

	Sum of squares	Degrees of freedom	Mean square	Variance ratio
Between groups	0·0195	1	0·0195	1·07
Within groups	0·2183	12	0·0182	

The mean of the supplemented group is 0·810 (standard error 0·037) and that of the deprived group is 0·888 (standard error 0·077).

Analysis IIB

(After 2 min.; June test)

	Sum of squares	Degrees of freedom	Mean square	Variance ratio
Between groups	0·0545	1	0·0545	2·00
Within groups	0·3275	12	0·0273	

The mean of the supplemented group is 0·860 (standard error 0·057) and that of the deprived group is 0·990 (standard error 0·067).

In neither case is there significant evidence of the existence of an effect. The probability of obtaining values for the variance ratio as great as the two obtained under the hypothesis of 'no effect' lay between 0·10 and 0·05.

Hence, in this case too, the effect cannot be regarded as proved.

Summary of conclusions

There is no conclusive evidence that the improvement in performance of the exercise due to training is less in the deprived group than in the supplemented group. Such evidence as there is suggests this, but is not conclusive."

N. Tests of Capillary Strength

Many attempts have been made to relate capillary strength to nutritional status in respect of vitamin C. They date from 1914 when Hess and Fish (1914) made measurements of capillary strength on scorbutic patients by the application of positive pressure.

Another test has been used for the same purpose, introduced by Hecht (1907), in which the resistance of the skin capillaries to suction is measured. The literature has been reviewed recently and the conclusion reached that no consistent relationship has been observed between vitamin C nutritional status and capillary strength as measured by the positive-pressure test of Hess or the negative-pressure test of Hecht (Munro, Lazarus and Bell, 1947–8). Both types of test were used in the present trial.

POSITIVE-PRESSURE TEST

The number of capillary haemorrhages visible to the naked eye in a previously marked circle, one inch in diameter, situated in the antecubital fossa, one inch below the sphygmomanometer cuff, were counted after positive pressure of 100 mm. Hg had been applied for 5 minutes. Determinations were made at intervals of about 4 weeks.

The results are given in Tables 21 and 46, p. 138. The individual results (Table 46) show the degree of variation and its effect on the mean values. In the groups receiving supplements, no consistent change was apparent throughout the course of the experiment. Three subjects receiving supplements showed

occasional increases in the number of petechiae produced by the test, Garling, on three successive occasions, Proctor on two, and Parry on one.

TABLE 21

Average values for capillary resistance, measured by a positive-pressure method for the groups of volunteers at different stages of the experiment
(The values are averages of the number of petechiae produced in the groups.)

Stage of experiment	Average number of petechiae in group receiving:		
	vitamin C, 70 mg. daily (3 volunteers)	vitamin C, initially 10 mg. daily (7 volunteers)	no supplement initially (8 volunteers)
First 2 months	1·0	3·5	10·2*
The 2 months when the unsupplemented group was most depleted	2·7	2·3	12·6*
After dosing, last 2 months of experiment	1·8	1·5	13·0*

* The high average values for the deprived group, whether before or after dosing, are due almost entirely to the behaviour of one subject, Robinson, whose capillaries had always had an abnormal appearance.

In the group without supplement no correlation of capillary strength with vitamin C status was apparent, with one possible exception, Hudson. Three volunteers, Tridgell, Another and Wodeman, showed little change in their response to the tests throughout the experiment and one, Sanderson, an increased number of petechiae on one occasion during the period when he was being dosed with vitamin C. Robinson, whose capillaries were shown by the skin microscope to be singularly tortuous, developed many petechiae in almost all the tests. His figures are responsible for the high mean values of the group without supplement. At the time when his signs of scurvy were definite, his responses to the positive-pressure test were notably inconsistent. At one test he produced 100 petechiae, and at the next 2. Variable responses to the test were seen in Drake, H. Williams and Milburn. In Hudson alone was the increase in petechiae maintained during the time of greatest depletion with a decline in response on dosing, but the number increased again at the end of the experiment.

NEGATIVE-PRESSURE TEST

Suction was applied to the skin of the front of the forearm, just below the elbow in the midline, through a glass cup 4 cm. in diameter with a rounded edge 4 mm. thick. Before the test the front of the forearm was warmed for 5 minutes with an electrically heated pad. Suction was applied for 30 seconds at a time, with intervals of 15 seconds; during each interval the pressure in the cup was reduced by 25 mm. Hg. After each application, the skin was examined for the presence of petechiae with the aid of a constant source of light. The pressure in the cup at the first application was 25 mm. Hg below atmospheric pressure and the highest pressure at which at least 10 petechiae were seen was the crucial reading. Results were recorded in mm. Hg below atmospheric pressure. Measurements were made at intervals of 3 to 4 weeks.

The results of the negative-pressure test are given in Tables 22 and 23.

TABLE 22

Measurements of capillary resistance by a negative-pressure method for all the volunteers individually
(The results are expressed as the number of mm. Hg by which atmospheric pressure had to be lowered in order to produce at least 10 petechiae. Measurements by S. Yudkin and G. A. Smart.)

Date of test	Capillary resistance expressed as mm. Hg in the group receiving: vitamin C, 70 mg. daily			vitamin C, 10 mg. daily				vitamin C, 10 mg. initially, then 5 mg.			no supplement initially									
	Bartley	Garling	Hill	Golding	Parry	Proctor	Woodhouse	Jackson	Way	Whinfield	Drake	Hudson	Milburn	Robinson	Sanderson	Tridgell	Williams, D.	Williams, H.	Wodeman	Another
February 12, 1945 ..	175	200	150	125	—	75	100	225	75	200	150	175	150	100	150	275	125	300	175	250
March 13, 1945 ..	175	225	200	200	225	150	225	200	125	175	175	175	75	125	200	200	200	275	100	175
April 10, 1945	175	150	250	150	225	100	225	150	125	175	175	125	125*	75	125	225	100*	250	100	225
May 1, 1945	150	150	250	225	100	150	175	100	75	150	200	125	150*	75*	125	150	75*	250	100*	350
May 29, 1945	175	200	175	375	200	250	175	175	150	125	225*	250	125*	100*	200	200	—	175	125*	175
June 19, 1945	175	225	275	300	175	125	125	150	125	150	175*	150*	100*	75*	125*	200	—	250	50*	200
July 10, 1945	175	175	275	225	200	125	250	125	125	200	150*	100*	75*	125*	100*	175*	—	150	150*	300
July 23, 1945	150	150	250	250	175	125	50	75	125	150	175*	125*	—	100*	125*	125*	—	125	175*	325
August 14, 1945 ..	175	200	275	—	—	150	150	100	125	175	200	225	—	50*	125	150*	—	275	200	—
September 14, 1945 ..	—	200	200	—	—	225	125	100	125	150	175	175	150	100	150	250	—	—	125	—
October 16, 1945 ..	125	200	—	375	—	100	225	150	125	150	200	200	50	75	150	275	—	—	150	125
December 18, 1945 ..	175	200	275	350	—	125	225	175	75	200	150	175	125	100	100	150	—	—	175	—
January 1, 1946 ..	150	200	300	350	—	175	150	225	175	175	100	275	200	150	175	325	—	—	175	—
February 19, 1946 ..	175	200	225	—	—	150	250	225	125	200	200	175	150	—	125	175	—	—	—	—

* Signs of clinical scurvy (skin haemorrhages) present.

TABLE 23

Average values for capillary resistance, measured by a negative-pressure method, for the groups of volunteers, derived from the individual values given in Table 22 (The results are expressed as the number of mm. Hg by which atmospheric pressure had to be lowered in order to produce at least 10 petechiae. Measurements by S. Yudkin and G. A. Smart.)

Date of test	Capillary resistance expressed as mm. Hg in the group receiving:			
	vitamin C, 70 mg. daily	vitamin C 10 mg. daily	vitamin C, 10 mg. daily initially, then 5 mg.	no supplement initially
February 12, 1945 ..	175	100	166	185
March 13, 1945 ..	200	192	186	170
April 10, 1945 ..	192	158	166	152
May 1, 1945 ..	183	183	106	160
May 29, 1945 ..	183	266	162	175
June 19, 1945 ..	225	183	150	147*
July 10, 1945 ..	208	200	162	147*
July 23, 1945 ..	183	141	131	159*
August 14, 1945 ..	217	150	133	175†
September 14, 1945	200	175	125	161†
October 16, 1945 ..	163	233	141	153†
December 18, 1945	217	233	150	139†
January 23, 1946 ..	217	225	192	200†
February 19, 1946 ..	200	200	183	165†

* Deficiency signs definite in unsupplemented group.
† Unsupplemented group receiving vitamin C.

Of the subjects receiving supplements, Bartley, Garling and Hill, who received 70 mg. vitamin C daily, and Golding, Proctor and Woodhouse, who received 10 mg., showed no obvious change in capillary strength throughout the experiment. Of the other four subjects receiving vitamin C, Jackson alone showed a definite fall in capillary strength.

Of the group without supplement, only Hudson, Tridgell and H. Williams showed a fall in capillary resistance. In H. Williams the fall continued when she was dosed with 20 mg. vitamin C daily and improved clinically; in the other two a rise in capillary strength occurred when they were dosed with 10 mg. vitamin C daily. The results as a whole, however, are not sufficiently consistent to suggest any definite reflection of the state of vitamin C nutrition.

DELAYED HAEMORRHAGES AFTER POSITIVE-PRESSURE TESTS

While the standard positive-pressure tests used thus yielded inconclusive results, an abnormal behaviour of the capillaries was noted after these tests had been made on deprived subjects who already had haemorrhagic hair follicles. About half an hour after the test new follicular haemorrhages were visible both above and below the area where the cuff had been applied, and existing haemorrhages became larger. The phenomenon was noticed in June 1945 in all deprived volunteers, except Another, but not in those receiving any supplement. Unfortunately insufficient attention was paid to it at the critical time and no systematic study was made. It should be noted that the standard capillary-strength tests

employing very brief periods of examination record only the rupture of vessels. The haemorrhages observed as after-effects were probably not due to rupture, but to slow leaking of red cells from the perifollicular capillaries. The study of the scorbutic haemorrhages (Section II F) indicates that they, too, were not caused by the rupture of vessels, but by abnormal diapedesis of the red cells. The application of the pressure cuff in scorbutic subjects thus seemed to bring out the latent abnormality, for reasons which are not clear.

DISCUSSION

In the present trial the results of positive- and negative-pressure tests showed a similar variability. Neither test yielded clear-cut results varying with the vitamin intake of the volunteers. Only one volunteer (Hudson) showed a fall in capillary strength by both methods; two others (Tridgell, H. Williams) showed a fall by the negative-pressure method only. The results are in agreement with those of other authors in similar human experiments (Crandon, Lund and Dill, 1940; Farmer, 1944), where capillary strength likewise failed to show general deterioration in vitamin C deficiency.

In the light of the occurrence of delayed haemorrhages, it is perhaps not surprising that the positive- and negative-pressure tests showed no correlation with the vitamin C intake. It is suggested that future work on the abnormal behaviour of capillaries in scurvy should employ tests examining the slow diapedesis of red cells rather than the rupture of capillaries.

O. Breaking Strength of Wound Tissue

The term, breaking strength, here refers to the ability of the tissue to withstand rupture on the application of tension. It is measured by the weight in grammes required to rupture a wound scar 1 cm. long under standard conditions, which are described in Section III, p. 71. Breaking strength thus defined is different from tensile strength which is measured by the weight per unit of area required to rupture a material. From the practical point of view the breaking strength of the scar tissue is of more immediate interest than its tensile strength because a thin layer of epithelium or tissue may possess a high tensile strength but owing to its thinness the scar as a whole may rupture easily. Breaking strength depends not only on the tensile strength of the scar tissue but also on the amount of tissue formed.

TABLE 24

Breaking strength of normal skin (for procedure see p. 71)

Name	Thickness of skin sample (mm.)	Weight required to rupture 1 cm. of skin sample (g.)
Bartley ..	3·0	15,300
Golding ..	2·0	22,900
Hill ..	3·0	19,600
Milburn ..	3·5	22,200
Parry ..	3·0	13,300
Proctor ..	3·5	13,000
Robinson ..	2·0	15,900
Sanderson ..	3·0	20,700
Woodhouse	3·0	16,700
Average ..	*2·9*	*17,300*

Data on the breaking strength of normal skin are given in Table 24. Considerable variations were found from individual to individual but the values were all of the same order of magnitude. They were not directly correlated with the thickness of the skin, but, presumably, with the collagen content.

In the earlier stages of the experiment wound scars were excised after 10 days. Data collected with the material thus obtained are given in Table 25. The breaking strength of the tissue was very low, amounting to only a few per cent of that of normal skin. No correlation is shown between vitamin C intake and breaking strength, the average values for the different groups of volunteers being of the same order of magnitude.

TABLE 25

Breaking strength of wound scar excised 10 *days later*

Name	Date of excision	Daily supplement vitamin C (mg.)	Period on controlled diet (days)	Weight required to rupture 1 cm. of scar tissue (g.)	Range (g).
Bartley ..	April 17, 1945	70	155	224	
Hill ..	March 2, 1945	70	109	100*	100–300
Hill ..	May 8, 1945	70	176	300	
Golding ..	April 3, 1945	10	141	410	
Parry ..	April 17, 1945	10	155	202	
Proctor ..	April 3, 1945	10	141	628	202–628
Woodhouse	April 3, 1945	10	141	510	
Woodhouse	May 8, 1945	10	176	225	
Drake ..	May 8, 1945	0	176	222	
Milburn ..	May 1, 1945	0	169	325	
Robinson ..	May 1, 1945	0	169	440	222–530
Sanderson ..	March 20, 1945	0	127	225	
Tridgell ..	May 1, 1945	0	169	530	
Wodeman	June 15, 1945	0	214	306	

* Subject bled exceptionally easily.

TABLE 26

Average breaking strength of wound scars, excised 21 *days later, of groups of volunteers at different periods of the experiment*

(*Derived from individual data in Table* 27)

Group of volunteers	Period of experiment	Weight required to rupture 1 cm. scar tissue (g.)
Initially unsupplemented	Time of greatest depletion	1,660
	After deprivation and dosing with 10 mg. vitamin C daily for between 13 and 100 days	2,500
	After saturation (omitting Milburn) ..	2,400
Supplemented with 10 mg. throughout	Before saturation	4,500
All those initially supplemented	After saturation	3,890

TABLE 27

Breaking strength of wound scars excised 21 days later

Name	Date of excision	Vitamin C intake (mg. daily)	Weight required to rupture 1 cm. scar tissue (g.)
Garling	June 19, 1945 ..	70 for 245 days	4,710
	January 26, 1946	70 for 325 days; 50 for 68 days; saturation test ending December 19, 1945	5,370
Hill ..	November 16, 1945	70 for 329 days, 50 for 30 days ..	2,180
	January 26, 1946	70 for 329 days; 50 for 64 days; saturation test ending December 19, 1945	5,300
Golding	July 3, 1945 ..	10 for 232 days	3,200
	February 9, 1946	10 for 422 days; saturation test ending January 18, 1946	3,290
Jackson	May 28, 1945 ..	10 for 161 days	8,000
	January 26, 1946	10 for 161 days; 5 for 125 days; saturation test ending December 19, 1945	3,090
Proctor	July 3, 1945 ..	10 for 232 days	2,970
	October 26, 1945	10 for 347 days	3,200
	February 9, 1946	10 for 422 days; saturation test ending January 18, 1946	3,300
Way ..	June 26, 1945 ..	10 for 161 days	3,770
	January 26, 1946	10 for 188 days; 5 for 125 days; saturation test ending December 20, 1945	3,420
Whinfield	May 28, 1945 ..	10 for 161 days	4,370
	January 26, 1946	10 for 161 days; 5 for 125 days; saturation test ending December 19, 1945	3,470
Hudson	February 2, 1946	0 for 260 days; 10 for 111 days; 20 for 46 days; saturation test ending January 13, 1946	3,780
Milburn	November 3, 1945	0 for 250 days; saturation after illness (see case history p. 80) then 20 for 41 days	4,220
	February 2, 1946	20 for 92 days; saturation test ending January 26 , 1946	4,430
Robinson	July 3, 1945 ..	0 for 233 days	692
	October 26, 1945	0 for 256 days; 10 for 94 days ..	2,260
	February 2, 1946	0 for 256 days; 10 for 115 days; 20 for 46 days; saturation test ending January 16, 1946	1,900
Sanderson	May 28, 1945 ..	0 for 197 days	1,500
	November 3, 1945	0 for 256 days; 10 for 100 days ..	2,540
	February 9, 1946	0 for 256 days; 10 for 115 days; 20 for 46 days; saturation test ending January 15, 1946	2,370
Tridgell	August 17, 1945 ..	0 for 265 days; 10 for 13 days ..	3,090
	November 3, 1945	0 for 265 days; 10 for 91 days ..	2,500
	February 9, 1946	0 for 265 days; 10 for 157 days; saturation test ending January 20, 1946 ..	2,040
Wodeman	August 17, 1945 ..	0 for 224 days; 10 for 54 days ..	2,130
	February 2, 1946	0 for 224 days; 10 for 101 days; 20 for 92 days; saturation test ending January 17, 1946	1,920
Another	July 3, 1945 ..	0 for 233 days	2,790

After 10 days the scar consisted mainly of granulation tissue containing little or no collagen. It is thought that vitamin C is concerned with the formation of collagen, so it was decided to excise the scar tissue at a later stage when the organization of the wound tissue and the formation of collagen might be expected to have reached a more advanced state, and a period of 21 days was chosen. Data on the breaking strength of wound scars after 21 days are shown in Tables 26 and 27. The values obtained for the groups supplemented with 10 or 70 mg. vitamin C were all of the same order, ranging from 2,180 to 8,000 g. Variations which occurred could not be correlated with the diet, and there was no improvement of the average breaking strength after saturation with vitamin C.

Only three deprived volunteers (Robinson, Sanderson, Another) had wounds inflicted during the time of greatest deprivation, and the average breaking strength of the scars was less than half that of the supplemented groups. Seven of the originally deprived volunteers (Hudson, Milburn, Robinson, Sanderson, Tridgell, Wodeman, Another), and seven of those receiving supplements from the start (Garling, Hill, Golding, Jackson, Proctor, Way, Whinfield), had wounds made within a month after saturation with vitamin C. The average breaking strength of the scars of the latter group was at this time twice as great as the value for the seven volunteers who had previously undergone deprivation, which suggests that normal capacity for wound healing was not restored immediately after saturation in those who had undergone prolonged deprivation.

III. EXPERIMENTAL DETAILS

A. Particulars and Management of Volunteers

Particulars

The details of the ages, weights, heights, occupations and period of participation in the trial of the twenty volunteers are given in Table 28. They all lived in the Sorby Research Institute. Ten of them had participated in the vitamin A trial which preceded the present experiment.

Management

The general management of the volunteers was along the lines described in the Report of the vitamin A trial (Hume and Krebs, 1949). The volunteers could be relied upon to co-operate in every respect. They assisted in the laboratory work, keeping of records, and the general running of the Institute. They were fully aware of the nature of the experiment and were kept informed of the decisions of the Committee. However, for the first 6 months they did not know to which group they belonged, whether totally deprived of vitamin C, or supplemented with it, with the exception of two, Bartley and Garling, who for reasons arising from the organization of the trial, had to be kept informed. During the later stages, beginning in May 1945, the grouping became obvious from the appearance of the signs of scurvy.

B. Diet

DIETARY HISTORY OF THE VOLUNTEERS BEFORE THE TRIAL

The ten volunteers who had taken part in the vitamin A trial had consumed 50 mg. vitamin C daily throughout the experiment, which finished 2 to 6 months before the present trial (Table 29). In the interval they had an unrestricted diet. No relevant information was available on the dietary history of the other ten volunteers.

TABLE 29

History of vitamin C intake, before the beginning of the trial, of the 10 *volunteers who took part in the previous experiment with vitamin A* (*Hume and Krebs*, 1949)

Name	Dates for intake	
	Controlled diet supplemented with 50 mg. vitamin C daily	Unrestricted diet
Bartley	16.8.42 to 10.7.44	11.7.44 to 30.9.44
Drake	23.3.43 to 30.6.44	1.7.44 to 30.9.44
Garling ..	10.8.42 to 15.6.44	16.6.44 to 30.9.44
Golding ..	10.8.42 to 1.6.44	2.6.44 to 30.9.44
Proctor ..	10.8.42 to 29.7.44	30.7.44 to 30.9.44
Tridgell ..	13.8.42 to 30.6.44	1.7.44 to 30.9.44
Williams, D. ..	10.8.42 to 1.4.44	2.4.44 to 30.9.44
Wodeman ..	10.8.42 to 17.4.44	18.4.44 to 30.9.44
Woodhouse ..	10.8.42 to 11.5.44	12.5.44 to 30.9.44
Another ..	10.8.42 to 2.7.44	3.7.44 to 30.9.44

TABLE 28

Personal details of volunteers and period on experiment

Name	Sex	Age at start of experiment		Weight at start of experiment		Height		Occupation		Period on experiment (days)
		(years)	(months)	(lb.)	(kg.)	(ft. in.)	(cm.)	Before experiment	During experiment	
Bartley ..	M	28	10	147	67	5 8½	174	Laboratory technician	Laboratory technician	341
Drake ..	M	32	5	115	52	5 2	157	Commercial traveller, S.R.I.*	S.R.I.	470
Garling ..	M	29	5	137	63	5 4	163	Civil servant, S.R.I.	S.R.I.	444
Golding ..	M	34	8	117	53	5 6½	169	Insurance clerk, S.R.I.	Probation officer	474
Hill	M	28	10	134	61	5 5	165	Draughtsman	Draughtsman	445
Hudson ..	M	21	2	144	65	5 8½	174	Land worker	S.R.I.	469
Jackson ..	M	26	2	138	62	5 8½	174	Shipping clerk	S.R.I.	387
Milburn ..	M	21	4	153	69	5 10½	179	Student	S.R.I.	471
Parry ..	M	17	6	116	53	5 5	165	Various	Laboratory technician	262
Proctor ..	M	33	4	124	56	5 5½	166	Baker, S.R.I.	S.R.I.	474
Robinson ..	M	31	9	91	41	5 2	157	Various, S.R.I.	S.R.I.	451
Sanderson ..	M	22	10	140	64	5 9	175	Land worker	S.R.I.	471
Tridgell ..	M	23	2	136	62	5 9	175	Clerk, S.R.I.	S.R.I.	476
Way	M	26	0	144	66	5 9	175	Male nurse	S.R.I.	379
Whinfield ..	M	33	1	150	68	5 11	181	Various	S.R.I.	394
Williams, D.	M	24	5	141	64	5 8	173	Land worker, S.R.I.	Male nurse	227
Williams, H.	F	22	5	124	56	5 6	168	Various	Various	298
Wodeman ..	M	34	7	173	79	6 0	183	Various, S.R.I.	S.R.I.	473
Woodhouse ..	M	26	1	146	67	5 11½	182	Clerk	Clerk	474
Another ..	M	27	7	134	61	5 9	175	Architect's clerk, student	Student	309

* S.R.I. indicates volunteer in the full-time service of the Sorby Research Institute.

DIET DURING THE PRELIMINARY PERIOD

Base line data for fifteen of the volunteers were collected for a preliminary period of 6 weeks, during which they consumed an ordinary diet containing about 60 mg. vitamin C daily from natural sources. Apples were used to adjust the vitamin C intake to that level. The other five volunteers joined the team a few weeks later and they were given, for the preliminary period, the basal diet with a supplement of 70 mg. vitamin C daily. Details are given in Table 30.

TABLE 30

Duration of the preliminary period, and the periods of administration and amounts of vitamin C supplements received by the volunteers

Name	Period on preliminary diet* (days)	Vitamin C supplement (mg. daily)	Period on supplement	
			Dates	No. of days
Bartley ..	42	70	13.11.44 to 7. 9.45	299
Drake ..	42	0	13.11.44 to 3. 7.45	233
		10	4. 7.45 to 4.11.45	124
		20	5.11.45 to 3. 1.46	60
		510	4. 1.46 to 14. 1.46	11
Garling ..	42	70	13.11.44 to 3.10.45	325
		50	4.10.45 to 10.12.45	68
		630	11.12.45 to 19.12.45	9
Golding ..	42	10	13.11.44 to 8. 1.46	422
		570	9. 1.46 to 18. 1.46	10
Hill	42	70	13.11.44 to 7.10.45	329
		50	8.10.45 to 10.12.45	64
		630	11.12.45 to 19.12.45	10
Hudson ..	42	0	13.11.44 to 30. 7.45	260
		10	31. 7.45 to 18.11.45	111
		20	19.11.45 to 3. 1.46	46
		670	4. 1.46 to 13. 1.46	10
Jackson ..	14	0	11.12.44 to 17.12.44	7
		10	18.12.44 to 27. 5.45	161
		0	28. 5.45 to 7. 8.45	71
		5	8. 8.45 to 10.12.45	125
		630	11.12.45 to 19.12.45	9
Milburn ..	42	0	13.11.44 to 20. 7.45	250
		6,000	21. 7.45	1
		100	22. 7.45 to 31. 8.45	41
		Uncontrolled intake	1. 9.45 to 3.10.45	33
		20	4.10.45 to 3. 1.46	92
		670	4. 1.46 to 15. 1.46	12
Parry ..	10	10	18.12.44 to 26. 8.45	252

TABLE 30 (*continued*)

Name	Period on preliminary diet* (days)	Vitamin C supplement (mg. daily)	Period on supplement	
			Dates	No. of days
Proctor ..	42	10	13.11.44 to 8. 1.46	422
		560	9. 1.46 to 18. 1.46	10
Robinson ..	21	0	13.11.44 to 26. 7.45	256
		10	27. 7.45 to 18.11.45	115
		20	19.11.45 to 3. 1.46	46
		420	4. 1.46 to 16. 1.46	13
Sanderson ..	42	0	13.11.44 to 26. 7.45	256
		10	27. 7.45 to 18.11.45	115
		20	19.11.45 to 3. 1.46	46
		660	4. 1.46 to 15. 1.46	12
Tridgell ..	42	0	13.11.44 to 4. 8.45	265
		10	5. 8.45 to 8. 1.46	157
		620	9. 1.46 to 20. 1.46	12
Way	11	10	18.12.44 to 27. 5.45	161
		0	28. 5.45 to 4. 6.45	8
		10	5. 6.45 to 1. 7.45	27
		0	2. 7.45 to 7. 8.45	37
		5	8. 8.45 to 10.12.45	125
		670	11.12.45 to 20.12.45	10
Whinfield ..	20	0	11.12.44 to 17.12.44	7
		10	18.12.44 to 27. 5.45	161
		0	28. 5.45 to 7. 8.45	71
		5	8. 8.45 to 10.12.45	125
		670	11.12.45 to 19.12.45	10
Williams, D.	42	0	13.11.44 to 16. 5.45	185
		500	17. 5.45 to ?	?
Williams, H.	42	0	13.11.44 to 4. 6.45	204
		20	5. 6.45 to 26. 7.45	52
Wodeman ..	42	0	13.11.44 to 24. 6.45	224
		10	25. 6.45 to 3.10.45	101
		20	4.10.45 to 3. 1.46	92
		830	4. 1.46 to 17. 1.46	14
Woodhouse ..	42	10	13.11.44 to 8. 1.46	422
		640	9. 1.46 to 18. 1.46	10
Another ..	42	0	13.11.44 to 6. 8.45	267

* A mixed diet containing about 50 to 70 mg. vitamin C daily, natural or synthetic.

BASAL DIET

Permitted Foods

The choice of the ingredients was determined by the availability of foods in Britain at the time of the trial and by the amount of the vitamin C which they

contained. The number of food items which are almost free from vitamin C is rather small, and in order to avoid monotony means were devised to reduce the vitamin C content of milk, potatoes and vegetables to so low a level that the inclusion of these foods in the basal diet would be possible. A number of items whose vitamin C content was known to be variable were analysed before they were included in the diet. Dehydrated meat, dehydrated potatoes, dehydrated carrots, plum jam, synthetic lemon curd and specially treated milk were obtained in bulk by courtesy of the Ministry of Food and commercial firms. This simplified the control of the diet and the check on the vitamin C content.

A list of the foods which were permitted is given in Table 31.

TABLE 31

Experimental basal diet deficient in vitamin C: foods of low vitamin C content from which selection was permitted

Bread
Flour
Rice
Pearl barley
Macaroni
Semolina
Oatmeal
Cornflour
Breakfast foods (wheat flakes etc.)
Soya flour

Meat, special dried pork and beef, canned

Oxo
Bovril
Marmite
Bacon

Fish, herring, fresh
cod, frozen
haddock, kipper, smoked
paste
tinned, not in tomato sauce

Potatoes, dehydrated
Carrots, dehydrated
Peas and beans, dried
} specially cooked

Lemon curd, synthetic
Plum jam, yellow
Plum jam, greengage
Toffee
Golden syrup
Chocolate, plain, non-fortified

Milk, processed, fresh, whole
dehydrated
Eggs, fresh or dried
Butter and margarine
Cheese

Sultanas
Raisins
Prunes
Dates
Figs

Sauces, various, tested for absence of vitamin C

Sugar
Lard
Coffee
Cocoa
Tea
Mustard
Salt
Custard powder
Baking powder
Peanut butter
Curry powder
Peanuts
Mineral waters
Vinegar
Pepper
Cashew nuts
Volatile oil of mint
Volatile oil of onion
} made by steam distillation

Special Treatment of Foods to reduce Vitamin C Content

Potatoes. The dehydrated potato strips, as received, were put in a large pan with about 12 volumes of cold water, which was brought to the boil and allowed to stand without further heating for about 1½ hours. The supernatant liquid was

TABLE 32

Content of vitamin C, vitamin B_1, riboflavin and nicotinic acid of some of the items in the basal diet (Vitamin C was estimated by indophenol titration alone (dye), and by indophenol titration in conjunction with formol treatment (formol). The values for reconstituted foods refer to the material as served.)

Food	Vitamin C content before treatment (mg./100 g.)	Treatment	Vitamin C content after treatment by: dye method (mg./100 g.)	Vitamin C content after treatment by: formol method (mg./100 g.)	Vitamin B_1 (µg./100 g.)	Riboflavin (µg./100 g.)	Nicotinic acid (µg./100 g.)
Potatoes, dehydrated	17	Reconstituted as described in text (p. 61)	0	—	4	7·5	262
							—
Meat, dehydrated	—	None	—	—	61	470	1,110
Carrots, dehydrated	15	Reconstituted as described in text (p. 62)	0	—	17	18	220
Dates, dried	—	None	4	0	—	—	—
Muscatel raisins	—	None	5·8	0	—	—	—
Currants, dried	—	None	5·5	0	—	—	—
Prunes, dried	—	None	7·1	0	—	—	—
Sultanas, dried	—	None	5·5	0	—	—	—
Figs, dried	—	None	16·8	0	—	—	—
Walnuts, peanuts	—	None	0	—	—	—	—
Jam, greengage, from old sulphited pulp	—	None	1·4	—	—	—	—
Jam, yellow plum, from old sulphited pulp	—	None	1·2	—	—	—	—
Lemon curd, synthetic	—	None	0·9	0	—	—	—
Milk, spray-dried, whole, copper-treated (1)	—	As described in text (p. 62)	0·8	0	158	1,230	580
(2)			0·9	0	—	—	—
Fish, frozen cod, smoked, various	—	None	0	—	—	—	—
Soya flour	—	None	0	—	—	—	—

poured off and replaced by an equal quantity of cold water. The mixture was again poured off and the potatoes were mashed hot by means of a wire masher. Pan and contents were kept hot for half an hour before serving. During the early part of the experiment a sample was analysed for vitamin C before consumption was allowed, but detectable quantities of vitamin C (more than 0·02 mg. per 100 g.) were never found, so the daily examination of the potatoes was, after some months, replaced by occasional checks.

Carrots. The procedure was the same as that described above except for the omission of mashing; the whole cooked strips were kept hot for half an hour. Samples were tested as for the potatoes.

Dried peas and beans. Vitamin C can be formed during germination, so the dry pulses were plunged into a large quantity of boiling water to kill the seeds. They were then left to soak overnight. The following morning the supernatant liquid was poured away and the pulses were cooked in plenty of water with the addition of sodium bicarbonate.

Milk. After various preliminary experiments on the destruction of vitamin C in fresh milk the following method was adopted. Copper sulphate was added to fresh milk to produce a final concentration of 1 part copper in a million parts of milk. The milk thus treated was heated to 70° C. and aerated for half an hour, which reduced the vitamin C content of the milk to below 0·04 mg. per 100 ml. The milk was treated in bulk in this manner by United Dairies, Ltd. and spray dried. The dried product contained no measurable amount of vitamin C.

During the early part of the trial, when there was a break in the delivery of the bulk supply, aeration was carried out for a short time in the laboratory.

Vitamin Content of Certain Foods used in the Basal Diet

To supplement the information available in food tables a number of items were specially tested for vitamin C, vitamin B_1, riboflavin and nicotinic acid. The results are given in Table 32. The data were used in computing the vitamin content of the diet.

SUPPLEMENTS

In order to ensure a satisfactory intake of vitamins A and D, each volunteer was given 8 "Adexolin" capsules weekly, supplying about 5,000 I.U. vitamin A and 1,000 I.U. vitamin D daily. On February 5, 1945 it was decided to give all volunteers 50 mg. anhydrous ferrous sulphate daily, so that iron deficiency could be excluded as a cause of any anaemia which might develop. One volunteer, Whinfield, later dropped the iron supplement because he was liable to gastric pain after taking it.

ADEQUACY OF THE DIET

At three different stages of the experiment the amounts of food consumed by the volunteers were measured for a period of a week. From the data, which are given in Table 33, the average food intake per head daily was calculated (Table 34). It will be seen that the values of the different nutrients are near to those of the recommended allowances of the National Research Council (1948) of the United States.

TABLE 33

Gross amounts of foods entering kitchen for consumption in the basal diet of the volunteers in three sample weeks of 1944–5

Food	Amount of food in one week: for 17 people (November 1944)		for 20 people (March 1945)		for 18 people (June–July 1945)	
	(lb.)	(kg.)	(lb.)	(kg.)	(lb.)	(kg.)
Sugar	13	5·99	16	7·35	17	7·82
Cheese	14	6·45	16	7·35	15	6·90
Tea	3	1·37	2½	1·15	2½	1·15
Margarine	8	3·7	7½	3·45	7	3·22
Bread	70	32·2	68	31·3	70	32·2
Lard	6	2·6	7	3·22	4½	2·07
Wheat flakes ..	1	0·46	—	—	4½	2·07
Dried egg	3¾	1·72	4	1·84	—	—
Barley flakes ..	6	2·6	7	3·22	1½	0·69
Haricot beans ..	4½	2·07	6	2·60	—	—
Rice	1½	0·69	—	—	—	—
Sultanas	4½	2·07	—	—	6	2·6
Prunes	1	0·46	4	1·84	—	—
Dates	2	0·92	—	—	1½	0·69
Figs	½	0·23	—	—	1½	0·69
Tinned salmon ..	¾	0·34	—	—	—	—
Spam, tinned meat ..	4½	2·07	2½	1·15	2¼	1·03
Flour	12	5·52	11	5·0	10	4·6
Frozen cod	14	6·45	—	—	—	—
Bacon	5	2·3	5	2·3	3¾	1·72
Dehydrated meat ..	4½	2·07	7(?)	3·22	8½	3·91
Dehydrated carrots	2¼	1·03	2¼	1·03	1½	0·69
Dehydrated potatoes	21	9·66	20	9·2	12¾	5·87
Chicken and ham roll	3	1·37	—	—	—	—
Butter	2½	1·15	2½	1·15	2½	1·15
Dried milk ..	10	4·6	17½	8·05	16¼	7·46
Lemon curd ..	7	3·22	9	4·15	5½	2·53
Jam	4¼	1·95	4½	2·07	4½	2·07
Raisins	—	—	9	4·15	—	—
Salt beef	—	—	5	2·3	—	—
Biscuits	—	—	2	0·92	4	1·84
Fish paste	—	—	½	0·23	¾	0·34
Peas	—	—	4	1·84	4½	2·07
Syrup	—	—	6½	2·99	—	—
Eggs, shell ..	—	—	2½	1·15	—	—
Mackerel, tinned ..	—	—	3	1·38	—	—
Oxo	—	—	½	0·23	—	—
Salt pork	—	—	—	—	4½	2·07
Smoked haddock ..	—	—	—	—	7	3·22
Kippers	—	—	—	—	4	1·84
Herrings	—	—	—	—	4	1·84
Suet	—	—	—	—	½	0·23
Tinned beans ..	—	—	—	—	9	4·15
Semolina	—	—	—	—	1	0·46
Macaroni	—	—	—	—	1	0·46
Cocoa	—	—	—	—	¼	0·11

TABLE 34

Average daily nutritive value per head, calculated from Food Tables, of the basal diet (edible portion) consumed by the volunteers in three sample weeks (see Table 33), compared with the standards of requirements set up by the National Research Council (1948) of the United States and by the League of Nations Health Organisation (1938) (The supplements of vitamin A and of iron are not included in the computations.)*

Nutrient	Average daily nutritive value calculated for food intake in:			Requirements of moderately active man weighing 70 kg.	
	1st sample week November 1944 (average weight of the 17 volunteers = 57·6 kg.)	2nd sample week March 1945 (average weight of the 20 volunteers = 61·4 kg.)	3rd sample week June–July 1945 (average weight of the 18 volunteers = 61·6 kg.)	Standards of National Research Council (1948)	Standards of League of Nations Health Organisation (1938)
Calories	2,980	2,910	2,870	3,000	3,000
Protein (g.)	96	105	99	70	70
"Available" carbohydrate (as starch, g.)	371	362	355	—	—
Fat (g.)	120	122	121	—	80–125
Calcium (g.)	1·2	1·3	1·3	1·0	0·75
Iron (mg.)	17	17	17	12	10
Vitamin B_1 (mg.)	1·2	1·1	1·1	1·5	0·9
Riboflavin (mg.)	1·8	2·0	1·9	1·8	—
Nicotinic acid (mg.)	13·6	10·3	13·1	15	—
Vitamin A potency†(I.U.) ..	5,510	5,520	4,130	5,000	2,000–4,000

* For the special dehydrated foods the values were taken from Table 32; otherwise War Memorandum No. 14 (Medical Research Council, 1945) was used, and for nicotinic acid, the Food Composition Tables for the Commonwealth of Australia Council for Scientific and Industrial Research (Marston and Dawbarn, 1944).

† Calculated by adding together the values for preformed vitamin A and for one third of the carotene value. Preformed vitamin A contributed 2,290–2,680 I.U.; total carotene 5,500–8,770 I.U.

TABLE 35

Quantities of food consumed by two volunteers when they were showing scorbutic signs on the 7 days, June 27–July 3 1945

Food	Amount consumed by: Wodeman (g.)	Drake (g.)
Porridge	3,465	160
Bacon	45	50
Cornflakes	84	83
Dried egg	58	42
Curried macaroni	140	—
Potatoes	1,257	930
Meat	455	808
Beans and raisins	40	—
Peas	255	—
Peas and beans	—	447
Carrots	126	86
Date batter	106	—
Cakes	409	535
Fried herring	179	—
Bread	2,037	1,590
Sugar	304	137
Butter and margarine	153	201
Jam	236	110
Rice and currants	923	—
Figs	15	—
Toast and margarine	20	—
Sardines	24	—
Bakewell tart	140	—
Macaroni cheese	232	80
Custard	226	—
Spam	40	—
Spam and potato cake	144	—
Cheese	94	97
Dried egg on fried bread	74	—
Haddock	110	120
Suet pudding, figs, lemon curd	144	—
Lemon curd tart	18	—
"Vegetable salad"	450	350
Cheesecakes	47	—
Kipper	72	—
Meat suet pudding	230	250
Bread and fruit pudding	284	—
Dried egg, beans on toast	34	—
Date shortbread	32	—
Barley kernel pudding	—	246
Semolina	—	110
Egg, fresh	—	48
Milk, reconstituted from dried powder	2,287 (ml.)	3,500 (ml.)

In the vitamin A trial good agreement was found between the food values calculated and the food values directly determined by analysis (Hume and Krebs, 1949, Table 36), and it was, therefore, thought unnecessary to analyse the food directly.

The adequacy of the diet was confirmed by the fact that the volunteers who acted as controls by consuming the basal diet supplemented with 70 mg. vitamin C daily remained in normal health throughout the trial.

Further samples of the food intake were collected from two volunteers, one, Wodeman, a consistently large eater, the other, Drake, a consistently small eater, when they were showing severe signs of depletion. The data are given in Tables 35 and 36; they show that the average food consumption was unchanged when frank scurvy had appeared.

TABLE 36

Average daily nutritive value per head of the diet set out in Table 35 (*The food values were calculated from the Tables for the edible portion* (*Medical Research Council*, 1945). *For the special dehydrated foods, the values for vitamin* B_1, *riboflavin and nicotinic acid were obtained by direct analysis. The carotene content of the diet was divided by* 3 *to give the total vitamin A potency.*)

Nutrient	Calculated nutrient value:	
	Wodeman	Drake
Calories	3,362	2,594
Protein (g.)	129	124
Carbohydrate (g.)	421	261
Fat (g.)	132	118
Calcium (g.)	1·2	1·0
Iron (mg.)	29·3	23·3
Vitamin B_1 (mg.)	1·2	0·9
Riboflavin (mg.)	2·0	1·6
Nicotinic acid (mg.)	13·5	11·6
Vitamin A potency (I.U.)	4,041	3,617

VITAMIN C POTENCY OF THE DIET

None of the foodstuffs consumed by the volunteers, with the exception of jam, contained measurable amounts of vitamin C (Table 32). The jam was not analysed by the formol method and its true vitamin C content is therefore uncertain. The figure obtained on the assumption that the whole indophenol titre of the jam represented vitamin C is thus the maximum vitamin C content of the jam.

The average weekly consumption of jam per head was 110 g. On the basis of an average figure of 1·3 mg. vitamin C per 100 g., the weekly intake of vitamin C from this jam was 1·43 mg., and the daily intake 0·20 mg. Other items, though they contained no measurable amounts of vitamin C may have contained traces. With the limits of accuracy of the analyses in mind, it may be safely assumed that the basal diet contained daily less than 1·0 mg. vitamin C and most probably even less than 0·5 mg.

VITAMIN C SUPPLEMENTS

Vitamin C supplements were given in the form of tablets containing 5 or 10 mg. ascorbic acid. It was desired that the volunteers should not know to which group they belonged so each day all were given 7 tablets identical in size, appearance and taste, some containing vitamin C and others being dummies. The latter consisted of 5 mg. tartaric acid, 94·5 mg. lactose, 22 mg. starch, 3 mg. sugar, 5 mg. talc and 0·5 mg. stearic acid.

C. Biochemical Methods

ESTIMATION OF VITAMIN C

Blood

Collection of Samples

Between 7·0 a.m. and 8·0 a.m., 25 ml. of blood were drawn from the cubital vein of the fasting subject. Sodium oxalate was added as the anticoagulant. Six ml. of the blood were set aside for estimation of vitamin C, and the remainder was immediately centrifuged to separate the plasma, the white cell layer and the red cell layer. Determination of the vitamin C content of the plasma and white cells was begun as soon as possible. The determinations on whole blood were done at Oxford on the following day. During the saturation tests additional specimens of blood and plasma were despatched to Oxford on the morning of collection, and determination of the vitamin C content by the method of Roe and Kuether was done in the evening.

Plasma: Procedure of the Oxford Team

Estimations of vitamin C in the plasma were made independently by the Oxford team and the Sheffield team. Two methods were used by the Oxford team.

Titration with 2:6-*dichlorophenolindophenol.* Three ml. of plasma were slowly added to 12 ml. of metaphosphoric acid solution freshly prepared; the product was well mixed, centrifuged, and filtered in the dark through a 9 cm. Whatman No. 42 filter paper. Duplicate samples of 5 ml. of the filtrate were then titrated with the dye, freshly dissolved and standardized against pure vitamin C, and of such a strength that 100 ml. was equivalent to 2 mg. vitamin C. The titrations were made with a Conway microburette, and the end point was taken as a faint pink colour persisting for 30 seconds. The reading was corrected for a blank value. The figures reported are the averages of those duplicate estimations which agreed within 0·04 mg. per 100 ml. plasma.

Method of Roe and Kuether (1943). In this procedure vitamin C is converted into dehydroascorbic acid by treatment with charcoal; the dehydroascorbic acid is treated with 2:4-dinitrophenylhydrazine and the product, probably a 2:4-dinitrophenylosazone, is estimated colorimetrically in sulphuric acid solution. The method estimates the sum of ascorbic acid and dehydroascorbic acid.

Five ml. of the plasma were added drop by drop to 15 ml. trichloroacetic acid and shaken well. After it had stood for 5 minutes, the mixture was centrifuged; about 0·75 g. of washed "Norite" was added to it, and it was again shaken, and left to stand. The mixture was centrifuged and filtered through a 9 cm. Whatman No. 42 filter paper. Duplicate samples of 4 ml. of the filtrate were placed in two Evelyn colorimeter tubes, and 2 drops of 10 per cent thiourea solution in alcohol were added to each. To one of the tubes 1 ml. of 2 per cent 2:4-dinitrophenylhydrazine in 9*N* H_2SO_4 was added, and the tube was incubated for 3 hours at

37° C. The tube was then cooled in ice water, and 5 ml. of 85 per cent sulphuric acid were added slowly, the temperature being kept low. Similar amounts of dinitrophenylhydrazine solution and sulphuric acid were then added to the other tube. After 30 minutes the optical density of the incubated tube was determined by means of an Evelyn photo-electric colorimeter, the tube not incubated providing the blank. Recovery experiments indicated that the results were accurate to within 0·05 mg. per 100 ml. plasma.

Results obtained from the same plasma by the two methods are given in Table 42 (p. 122).

Plasma: Procedure of the Sheffield Team

The method used was a modification of the dye titration procedure. Four ml. of oxalated plasma, 2 ml. of water and 4 ml. of 20 per cent metaphosphoric acid, freshly prepared, were thoroughly mixed and filtered through a 7 cm. filter paper. A little more than 5 ml. of filtrate was obtained, and duplicate determinations were made on two 2·5 ml. samples. One hundred ml. of the dye solution was equivalent to 1 mg. of ascorbic acid, hence the volume in ml. of the dye solution required was numerically identical with the concentration of vitamin C in mg. in 100 ml. of the plasma. The method requires twice the amount of plasma usually used but the accuracy is greater, duplicates agreeing to within 0·03 mg. per cent.

Whole Blood

The estimations of vitamin C in whole blood were made by the Oxford team, who used the method of Roe and Kuether.

White Cells

The estimations of vitamin C in white cells were made by the Oxford team who used the method of Butler and Cushman (1940). After the blood had been centrifuged for 15 minutes at 15,000 r.p.m. and the plasma had been removed, the buffy layer was carefully transferred by means of a wide-bore Pasteur pipette to a tube similar to a Wintrobe haematocrit tube, having internally a length of 110 mm. and a diameter of 4 mm. It was then centrifuged for 30 minutes at 4,000 r.p.m. The white cells were thus packed into a clearly defined column which, after the supernatant plasma had been carefully removed, could be taken up with a pipette, transferred to a small centrifuge tube and weighed. To disintegrate them, 0·5 ml. of distilled water and 1·5 ml. of 10 per cent metaphosphoric acid were mixed with the cells. The mixture was centrifuged for 10 minutes at 2,500 r.p.m., and the whole of the clear supernatant fluid was used for determining the vitamin C content by titration with dichlorophenol-indophenol. The content was expressed in mg. per 100 g. white cells.

Urine

Collection of Samples

Urine was collected for 24-hour periods beginning at 8 a.m. It was placed in dark-coloured bottles containing 40 g. of metaphosphoric acid, sufficient to give a final concentration of about 2 per cent. Analyses were made independently at Oxford and Cambridge, the specimens reaching their destination 10 to 24 hours after the end of the collecting period. The extent of the loss of reducing substances during transit can be gauged from the figures in Table 37. For the saturation tests members of the Oxford and Cambridge teams were stationed at Sheffield and determinations by the dye titration methods were made independently.

TABLE 37

Effect of the delay caused by transit on the apparent vitamin C content of samples of urine sent from Sheffield to Oxford as estimated by titration with 2:6-dichlorophenol indophenol

Apparent vitamin C content of 24-hour specimen of urine:	
estimated in Sheffield at end of 24-hour period of collection (mg.)	estimated in Oxford 10 to 24 hours after end of collection (mg.)
17·8	15·8
19·2	17·3
27·0	25·0
28·8	25·8
36·4	32·4
35·4	34·4
43·5	43·5
72·5	71·0
90·0	85·8
194	176

Methods. Three methods were used:

(1) Titration with 2:6-dichlorophenolindophenol (Harris, Ray and Ward, 1933). The method is widely used for clinical purposes but is known to be rather unspecific and to include a variety of reducing substances in the estimation.

(2) Titration with 2:6-dichlorophenolindophenol in the presence of formaldehyde. The procedure, an elaboration of Lugg's (1942, a, b) method, is described in Appendix A.

(3) The colorimetric method of Roe and Kuether (1943) with 2:4-dinitrophenylhydrazine.

Methods (2) and (3) are known to be more specific than method (1).

Food

The method described by Mapson (1943) was used. It is based on titration with 2:6-dichlorophenolindophenol at different acidities in the presence of formaldehyde.

ESTIMATION OF B VITAMINS

Vitamin B_1 was estimated by the method of Harris and Wang (1941), nicotinic acid and nicotinamide by the method of Kodicek (1940), and riboflavin by the method of Wang and Kodicek (1943) (see Table 32).

ESTIMATION OF BLOOD CONSTITUENTS OTHER THAN VITAMIN C

Constituents of blood other than vitamin C were estimated by the Oxford team.

Plasma Proteins

The micro-Kjeldahl method was used to determine total protein, albumin and fibrinogen nitrogen. Globulin was calculated by difference. The sodium sulphate method (Howe, 1921) was used to separate albumin and globulin. For the digestion the quantities used were:

For total protein, 1 ml. samples of plasma diluted 1 in 10.

For albumin, 5 ml. samples of the globulin-free sodium sulphate filtrate.

For fibrinogen, the fibrin clot formed by the addition of calcium to 1 ml. of plasma.

The samples were digested in concentrated sulphuric acid for 3 hours. One drop of hydrogen peroxide was added to complete the digestion if the solutions were not clear after 2½ hours' heating, and the heating was then continued for another half hour. The factor of 6·25 was used for converting grammes of nitrogen into grammes of protein.

Plasma Alkaline Phosphatase

The method of King and Armstrong (1934) was used.

Blood Urea

The method used was that of Archer and Robb (1925).

D. Haematological Methods

Haemoglobin

Haemoglobin was estimated by the Haldane method with the following precautions to ensure accuracy (see Macfarlane, 1945).

1. All pipettes were calibrated at the National Physical Laboratory.
2. The colour standard was checked at the National Physical Laboratory.
3. The diluting tube was calibrated by the mercury method.
4. The standard background used for comparison was provided by a ground glass screen illuminated by the light from a "daylight" bulb reflected from a magnesium oxide screen.
5. All the estimations were carried out by one person (W. Bartley) whose accuracy in manipulations and colour matching was determined photometrically at the National Physical Laboratory.

The blood used was venous blood, and was part of the sample drawn for the vitamin C analyses, except occasionally when it was collected by finger puncture. The samples were numbered, not named, and the key to the numbers was not revealed until all the estimations had been completed. All determinations were made within 2 hours of the collection of the blood.

The sample was placed in a small specimen tube and mixed thoroughly by rotating the tube. The pipette was filled to the mark, care being taken not to draw the blood more than 1 mm. above the mark. The level was adjusted exactly to the mark by touching the tip of the pipette with dry filter paper. The tip of the pipette was cleaned, the blood column drawn up into the pipette, and the tip and outside of the pipette thoroughly cleaned with moist filter paper. The blood was then allowed to run out slowly into the bottom of the diluting tube which contained ammoniacal water approximately to the 40 per cent mark. The pipette was lifted to the clear supernatant layer and washed several times by sucking up the liquid gently and allowing it to run out. The pipette was then slowly raised and drained. The blood in the diluting tube was gassed by bubbling coal gas through it with a fine glass capillary. To prevent foaming a small drop of octanol was added. After 5 minutes the capillary was withdrawn after draining it. The blood was diluted with the scale hidden until its colour was just appreciably lighter than the standard. The scale was read and 2 per cent was subtracted. The photometric checks at the National Physical Laboratory had shown that this

method of matching gave the best results with the particular investigator who made all the tests. Duplicate determinations agreed within 2 per cent of haemoglobin.

Red Cells

The standard technique with a Thoma counting chamber was used. Blood was obtained by finger puncture.

White Cells

The whole field of a Thoma chamber was counted and the average of 3 separate counts on different samples was obtained. At the same time a blood film was made for a differential white cell count.

Platelets

The first drop of blood obtained by finger puncture was collected in a wax-lined tube containing isotonic sodium citrate together with brilliant cresyl blue. The dilution was arranged to give a field of 20 to 40 red cells under a $\frac{1}{6}$ inch objective. The red cells and platelets were counted in successive fields until 50 platelets were seen.

Erythrocyte Sedimentation Rate

The method of Westergren was used.

Bleeding Time

The standard ear-puncture method was used.

E. Method of Determining the Breaking Strength of Wounds

Preliminary Experiments on Scars in Situ

In the first experiments on the breaking strength of scar tissue an attempt was made to rupture the scar *in situ*. It was thought that in these conditions the state of the scar would be more natural than after excision. Wounds 3 cm. long were made in twelve subjects on the outer aspect of the upper thigh extending to the depths of the fascia. A suture parallel to the incision was inserted on each side of the wound for applying traction and the edges of the wounds were drawn together with "Elastoplast". After 10 days the "Elastoplast" was removed and traction was applied to the sutures. The method proved unsatisfactory, owing to the transmission of the tension to the tissue surrounding the wound scar, and the procedure was abandoned. It was then decided to excise the wound scar before applying tension.

Apparatus used for Measuring the Breaking Strength of Excised Scars

The apparatus for measuring breaking strength consisted essentially of two clamps which held the tissue to be tested between them. The lower clamp was capable of being loaded to exert tension on the tissue. It is shown in Fig. 4. The apparatus consisted of a rectangular iron framework with a crosspiece I from which was suspended the clamp A which freely pivoted about the pin P. A brass tube C, slotted to half its length, was held below the slot K by a clamp attached to the iron frame. The lower clamp B slid freely in a vertical direction inside C, but was prevented from rotating by the pin H, which was fastened to B. The long brass pointer L, pivoted at D, acted as a counterpoise to the clamp B and the large thick-walled glass tube F and scale pan S attached

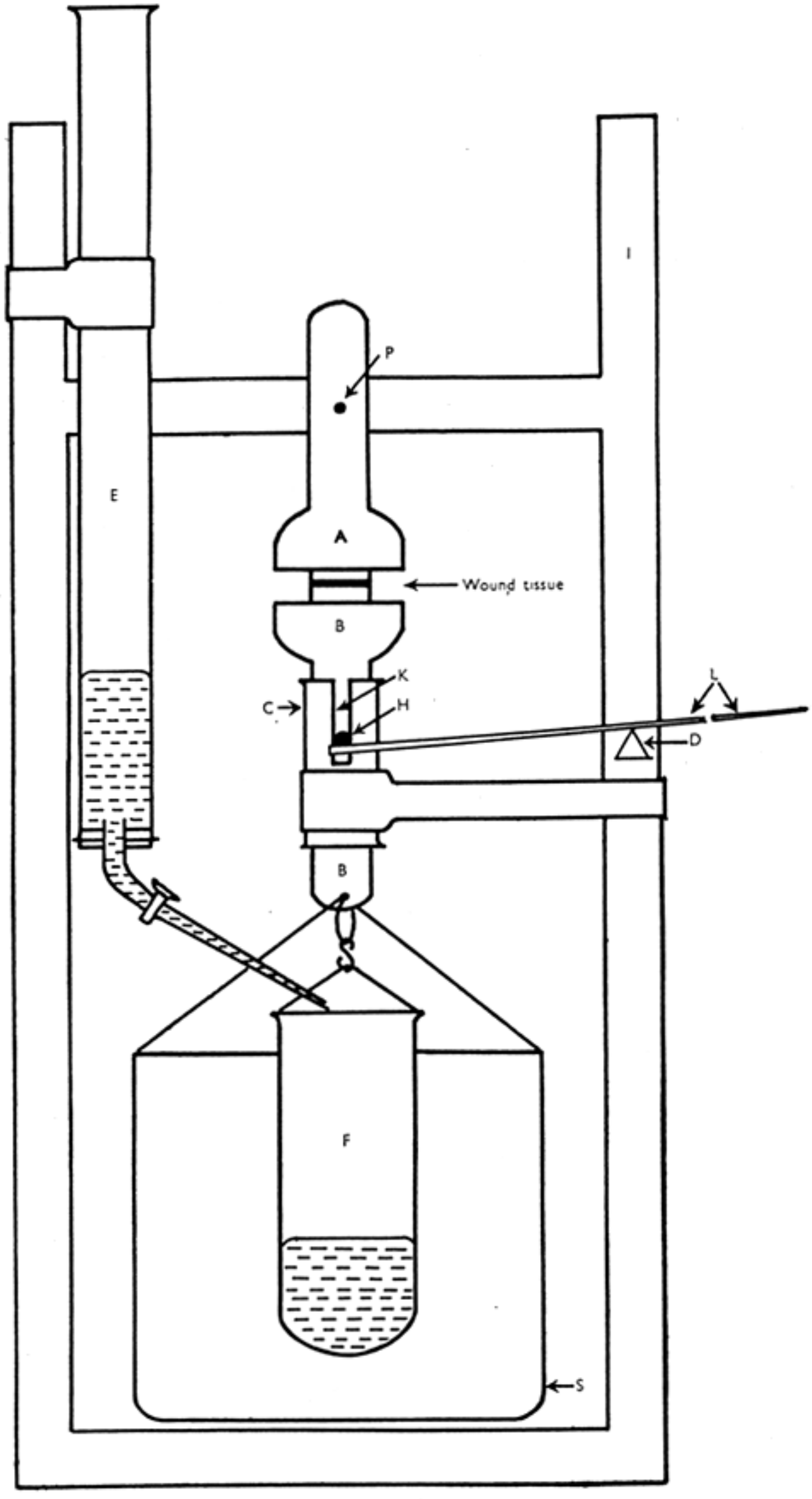

FIG. 4. Apparatus for measurement of breaking strength of wounds. For key see text.

to it, by producing an upthrust on the pin H. The burette E was fastened to the iron framework in such a position that mercury could be run from it into the glass tube F without disturbing the remainder of the apparatus.

The burette which contained 1,000 g. of mercury was graduated so that the weight of mercury delivered could be read directly to within 5 g. The weight of the clamp B and its attachments was slightly less than the upthrust exerted by the lever L. Perfect balance was obtained by addition of small weights to the scale pan.

Procedure for Measuring the Breaking Strength of Excised Scars

The wound scar and skin surrounding it to a distance of about 1 cm. was excised under procaine anaesthesia to the depth of the fascia. The scar tissue and skin were cut transversely with a sharp scalpel to obtain a piece of scar about 5 mm. long; the remainder of the wound was used for histological examination. The lower clamp was carefully counterpoised by adding weights to the scale pan and the piece of tissue was clamped in A with the scar parallel to the jaws of the clamp. Clamp B was then raised and attached to the lower side of the tissue. Owing to the elastic properties of skin, the clamps could be tightened on the edge of the tissue to give a satisfactory fastening without producing strain on the scar tissue.

Mercury was slowly added to the glass tube, a brief pause being made after every 50 g. Onset of rupture of the wound was indicated by an increased movement of the pointer L. When this occurred mercury was added in lots of 5 g. until the scar ruptured. If more than 1,000 g. was required the mercury tube was emptied, a 1,000 g. weight was put on the scale pan, and mercury was added again as before. The determinations were done as rapidly as possible to prevent the tissue drying. The whole time between excision of the wound and breaking of the scar was less than 20 minutes. The length and thickness of the scar tissue were measured in mm. both before and after the rupture, and the weight required for rupture per cm. wound scar was calculated. Breaking of the wound was sudden and involved only scar tissue except in one case (Jackson, wound of May 28, 1945) when a small portion of normal skin was involved.

F. Case Histories

The case histories are condensed accounts from the observations of the various investigators and the records of the volunteers themselves. Only observations which seem to be relevant to the main theme are included. Omitted are data reported elsewhere, on colds (p. 43), febrile attacks (p. 42), haematology (Table A), body weight (Table B), temperature (Table C), biochemical analysis of blood and urine (pp. 90, 110, 122) breaking strength of wounds (p. 52), capillaroscopy (p. 28), tests of the capillary strength (pp. 48, 138) saturation tests (pp. 22, 122), pulse rate (Table D), exercise tolerance (Table E), and capacity for dark adaptation (Tables F, G, H). (For location of Tables A–H, see note on p. 2.)

The case histories are arranged according to the initial grouping (see Table 1, p. 6), beginning with the group receiving a daily supplement of 70 mg. vitamin C, followed by that receiving 10 mg., and the deprived group. Within the groups the histories are arranged alphabetically.

Bartley

Past history. Scarlet fever 1930. Took part in vitamin A experiment but was not deprived.

Period of controlled diet. 13.11.44: start of deficient diet supplemented with 70 mg. vitamin C daily; slight gingivitis which persisted throughout the experiment. 5.9.45: (296 days on 70 mg. vitamin C) severe attack of influenza lasting about 10 days. 8.9.45: start of unrestricted diet. Enlargement and congestion of follicles on buttocks, thighs and legs of varying severity was seen throughout the period of observation.

Summary. No signs of clinical scurvy developed during a period of 299 days on a vitamin C intake of 70 mg. daily. Slight gingivitis and follicular enlargement present at the start remained unchanged throughout the experiment.

Garling

Past history. Pneumonia 1922. Took part in vitamin A experiment, deprived of vitamin A from 4.8.42 to 19.5.44.

Period of controlled diet. 13.11.44: start of deficient diet supplemented with 70 mg. vitamin C daily. 17.2.44 to 11.6.44: (96 to 210 days on 70 mg. vitamin C) pain in back reported. 4.10.45: (324 days on 70 mg. vitamin C) dose of vitamin C reduced to 50 mg. daily. 11.12.45: (68 days on 50 mg. vitamin C).

Summary. No signs of clinical scurvy developed during a period of 324 days on a vitamin C intake of 70 mg. daily or subsequently during a period of 68 days on a vitamin C intake of 50 mg. daily.

Hill

Past history. Pneumonia 1920.

Period of controlled diet. 13.11.44: start of deficient diet supplemented with 70 mg. vitamin C daily. 4.10.45: (324 days on 70 mg. vitamin C) dose reduced to 50 mg. vitamin C daily. 11.12.45: (68 days on 50 mg. vitamin C) saturation test started. Epistaxis occurred before and during the experiment, slight gingivitis throughout the period of observation.

Summary. No signs of clinical scurvy developed during a period of 324 days on a vitamin C intake of 70 mg. daily, or subsequently during a period of 68 days on a vitamin C intake of 50 mg. daily.

Golding

Past history. No serious illnesses. Took part in vitamin A trial, deprived of vitamin A from 2.8.42 until 2.10.43. Towards the end of this period showed impaired capacity for dark adaptation.

Period of controlled diet. 13.11.44: start of deficient diet supplemented with 10 mg. vitamin C daily; slight enlargement and hyperkeratosis of follicles present on backs of arms, which had already been under observation for 2 years and found to fluctuate (Hume and Krebs, 1949). 17.1.45: (65 days on 10 mg. vitamin C) enlargement and hyperkeratosis of follicles on arms increased and present also on volar aspect of forearms and on thighs and legs where many follicles were congested. 22.3.45: (129 days on 10 mg. vitamin C) follicles of arms examined with skin microscope were normal but large, with their orifices more or less covered with a layer of epithelium in which the top of the hair was embedded: instead of standing erect the hair formed a loop; follicles not surrounded by a ring of capillaries. 21.4.45: (159 days on 10 mg. vitamin C) no change. 17.5.45: (185 days on 10 mg. vitamin C) hyperkeratosis and enlargement of follicles on arms decreased; no change on lower limbs. 24.7.45: (233 days on 10 mg. vitamin C) microscopic appearance of follicles as before. 25.10.45: (345 days on 10 mg. vitamin C) enlarged follicles on arms; enlarged

hyperkeratotic follicles on backs of thighs and legs, some congested. 11.12.45: (393 days on 10 mg. vitamin C) skin condition unchanged except for absence of congested follicles. 9.1.46: (422 days on 10 mg. vitamin C) skin condition unchanged.

Summary. No signs of clinical scurvy developed during a period of 422 days on a vitamin C intake of 10 mg. daily. Follicular hyperkeratosis on the arms and the backs of the thighs and legs, present at the beginning, fluctuated in severity but never progressed to a haemorrhagic state. The enlarged follicles differed from those seen in the deprived volunteers in that there was no horny plug in the mouth of the follicles. The orifices were covered with a sheet of epithelium. Furthermore, the hair had partly emerged with the tip remaining attached to the epithelium forming a loop, whilst in the deprived subjects the hair remained completely coiled within the plugged follicles (see Section II F, p. 31). No abnormalities were seen in gums or wound scars.

Jackson

Past history. Scarlet fever complicated by nephritis in 1928.

Period of controlled diet. 18.12.44: start of deficient diet supplemented with 10 mg. vitamin C daily. 10.4.45: (113 days on 10 mg. vitamin C) a few follicular papules on arms and thighs. 1.5.45: (134 days on 10 mg. vitamin C) slight acne on back, otherwise no major change. 27.5.45: (160 days on 10 mg. vitamin C) daily supplement of 10 mg. vitamin C withdrawn from this date. 28.5.45: (1 day's depletion) skin condition essentially unchanged: a few keratotic, but no congested, follicles on backs of arms, thighs and calves; no changes in gums. 3.7.45: (37 days' depletion) hair follicles on backs of thighs and legs slightly congested. 26.7.45: (60 days' depletion) slight acne still present on back; no change otherwise. 8.8.45: (73 days' depletion) daily supplement of 5 mg. vitamin C begun. 4.9.45: (27 days on 5 mg. vitamin C) no significant change in skin, gums or wounds. 6.10.45: (59 days on 5 mg. vitamin C) acne on back increasing, now extending from shoulders to buttocks; keratotic follicles on thighs and buttocks and calves more numerous, some congested. 17.11.45 to 10.12.45: (101 to 124 days on 5 mg. vitamin C) no change. 11.12.45: saturated with vitamin C. No definite changes in the skin subsequently.

Summary. No definite clinical signs of scurvy developed during a period of 160 days on an intake of 10 mg. vitamin C daily. The mild skin changes observed during the period were of doubtful significance. The withdrawal of the supplement for 73 days produced no major change. During a subsequent period of 124 days on a 5 mg. supplement of vitamin C, the skin condition (follicular changes and acne) seemed to deteriorate slightly, suggesting that a dose of 5 mg. daily was below the minimum requirement. The changes were, however, too slight to allow definite conclusions.

Parry

Past history. Pneumonia 1940.

Period of controlled diet. 18.12.44: start of deficient diet, supplemented with 10 mg. vitamin C daily. During period of observation (December 1944 to August 1945, 251 days on 10 mg. vitamin C) no abnormalities seen; slight acne of back on one occasion; slight folliculitis of buttocks and thighs on some occasions.

Summary. No signs of clinical scurvy developed during a period of 251 days on a vitamin C intake of 10 mg. daily.

Proctor

Past history. No serious illnesses. Took part in vitamin A trial, deprived of vitamin A from 27.7.42 to 17.1.44.

Period of controlled diet. 13.11.44: start of deficient diet supplemented with 10 mg. vitamin C daily. 20.11.44: (7 days on 10 mg. vitamin C) slight folliculitis of buttocks and thighs which appeared and disappeared throughout the experiment; slight gingivitis which persisted throughout the period of observation. 8.1.46: (421 days on 10 mg. vitamin C) last dose of 10 mg. vitamin C daily.

Summary. No signs of clinical scurvy developed during a period of 421 days on a vitamin C intake of 10 mg. daily. Slight folliculitis of buttocks and thighs, and some gingivitis, present at the start, showed some variation during the period of observation, but was not affected by the vitamin C intake.

Way

Past history. No serious illnesses.

Period of controlled diet. 18.12.44: start of deficient diet supplemented with 10 mg. vitamin C daily; mild hyperkeratosis of upper arms and thighs, which was diagnosed as "keratosis pilaris"*. The lesions differed from scorbutic ones in that they were confined mainly to the extensor aspect of the arms and thighs; they were grouped in patches within which a large proportion of the follicles was involved in contrast with the small proportion involved in scurvy; they failed to show microscopically the typical capillary arrangement described in Section II E (p. 29): they did not become haemorrhagic. 10.4.45: (113 days on 10 mg. vitamin C) hyperkeratosis of legs also. 1.5.45: (134 days on 10 mg. vitamin C) some congested follicles on backs of arms, buttocks, thighs and legs. 20.5.45: (153 days on 10 mg. vitamin C) enlarged keratotic follicles of arms becoming more numerous and pigmented. 28.5.45: (160 days on 10 mg. vitamin C) skin as before. Supplement of 10 mg. vitamin C daily withdrawn. 5.6.45: (7 days' depletion) skin as before. Given 10 mg. vitamin C daily. 2.7.45: (26 days on 10 mg. vitamin C) few acneiform papules on back. Slight enlargement and congestion of follicles on arms, backs of thighs and backs of legs. Supplement of 10 mg. vitamin C withdrawn. 8.8.45: (37 days' depletion) skin as before. Given 5 mg. vitamin C daily. 18.8.45: (10 days on 5 mg. vitamin C) enlarged hyperkeratotic and congested follicles now also on buttocks. 6.10.45: (59 days on 5 mg. vitamin C) numerous keratotic follicles on thighs, some congested. 10.11.45: (94 days on 5 mg. vitamin C) acne of back fading; gums and wounds normal. Similar abnormal follicles (keratosis pilaris) on medial aspects of knees. 10.12.45: (124 days on 5 mg. vitamin C) skin lesions distributed more extensively and slightly more severe; enlarged, hyperkeratotic and congested follicles now also below umbilicus; on back of thighs four haemorrhagic follicles. 11.12.45: saturated with vitamin C. 21.12.45: start of unrestricted diet; some of the congested follicles and the four haemorrhagic follicles no longer visible.

Summary. No definite signs of clinical scurvy developed during a period of 160 days when the intake was 10 mg. daily, or subsequently during the 37 days

* Keratosis pilaris is described by Andrews (1946, p. 681) as a follicular disease, probably of congenital origin, in which the horny accretions of about pinhead size give the skin a stippled appearance resembling gooseflesh. It is most pronounced during the winter months in those with dry skins. It is found mainly on the extensor surfaces of the arms and thighs. The individual lesions are follicular papules of pinpoint to pinhead size. They may or may not be erythematous. The keratotic plug is composed of epithelial cells and inspissated sebum collected about a hair shaft which at times is found coiled and wrapped up in them. The lesions tend to be arranged in poorly defined groups dotting the otherwise normal skin in a fairly regular pattern (Plate XIII, A).

when the supplement was withdrawn. Follicular changes during this period may be regarded as normal fluctuations of the keratosis pilaris at the beginning. After a further period of 114 days on an intake of 5 mg., four haemorrhagic follicles developed. During the period on an unrestricted diet after saturation with vitamin C, the skin lesions showed little change except for the disappearance of the four haemorrhagic follicles and some lessening in the congested ones. Throughout the experiment the gums and wounds were normal. The few haemorrhagic follicles which developed when the vitamin C intake was 5 mg. daily suggest that this dose was below the minimum requirement.

Whinfield

Past history. No serious illnesses.

Period of controlled diet. 11.12.44: start of deficient diet supplemented with 10 mg. vitamin C daily; old healed acne. 27.5.45: (167 days on 10 mg. vitamin C) no changes in skin, gums or wounds. Withdrawal of supplement. 7.8.45: (72 days' depletion) no change. 8.8.45: supplement of 5 mg. vitamin C daily. 10.12.45: (124 days on 5 mg. vitamin C) no change.

Summary. No signs of clinical scurvy developed during a period of 167 days when the intake was 10 mg. daily, during the 72 days when the supplement was withdrawn, or during the subsequent period of 124 days on a daily intake of 5 mg. daily.

Woodhouse

Past history. No serious illnesses. Took part in vitamin A experiment, deprived of vitamin A from 27.7.42 to 23.3.44.

Period of controlled diet. 13.11.44: start of deficient diet supplemented with 10 mg. vitamin C daily; slight gingivitis which persisted throughout the experiment. 23.11.44: (10 days on 10 mg. vitamin C) prominent follicles on upper arms, outer thighs and buttocks. 7.2.45: (86 days on 10 mg. vitamin C) slightly enlarged follicles on left upper arm. 30.4.45: (168 days on 10 mg. vitamin C) some enlarged follicles on backs of arms and front of thighs, hyperkeratotic follicles on buttocks and backs of legs; congested follicles on backs of thighs. 14.5.45: (182 days on 10 mg. vitamin C) skin normal. 23.5.45: (191 days on 10 mg. vitamin C) slight congestion of follicles on backs of arms, thighs, legs and buttocks. 20.6.45: (219 days on 10 mg. vitamin C) hair follicles normal. 17.7.45: (246 days on 10 mg. vitamin C) a few scattered enlarged follicles on backs of arms and buttocks. 6.9.45: (297 days on 10 mg. vitamin C) slight enlargement and congestion of follicles on arms, shoulders and buttocks. 11.10.45: (332 days on 10 mg. vitamin C) no change. 20.12.45: (402 days on 10 mg. vitamin C) no change. 8.1.46: (421 days on 10 mg. vitamin C) last dose of 10 mg. vitamin C daily. 9.1.46: start of saturation test; no change in skin after saturation.

Summary. No signs of clinical scurvy developed during a period of 421 days on a vitamin C intake of 10 mg. daily. Some slight follicular skin changes present at the start varied in magnitude, and did not progress. No change in the skin condition was produced by saturation with vitamin C.

Drake

Past history. No serious illnesses.

Period of depletion. 13.11.44: start of deficient diet, skin normal except for acne on back; gums normal. 2.3.45: (110 days' depletion) a few keratotic follicles on external aspect of upper arm. 13.3.45: (121 days' depletion) acne increased.

10.4.45: (149 days' depletion) acneiform eruption on shoulders and back as far as buttocks. 24.4.45: (163 days' depletion) severe pains in shoulders reported. 30.4.45: (169 days' depletion) acneiform eruption spreading from back round sides of thorax to abdomen; enlarged, hyperkeratotic and congested follicles now also on buttocks; slight gingivitis. 26.5.45: (195 days' depletion) pain in legs, getting worse until dosing. 28.5.45: (197 days' depletion) small haemorrhages into tip of interdental papilla between left lower lateral incisor and canine; acne very severe, almost all back, shoulders and part of abdomen involved, lesions bright red, congested, some haemorrhagic; enlarged hyperkeratotic, congested follicles on buttocks and thighs. 12.6.45: (206 days' depletion) lesions as before; experimental wound scar of 8.5.45 purplish with a yellowish margin. 16.6.45: (216 days' depletion) greenish-blue discoloration round left knee joint with swelling, effusion and haemorrhage which became more severe after a long walk. 26.6.45: (226 days' depletion) acne profuse and some lesions haemorrhagic; follicular eruption on buttocks, backs and fronts of thighs and legs with many perifollicular haemorrhages; gum haemorrhage larger; wound of 8.5.45 reddish-blue in colour, infiltrated and surrounded by bruise (Plate I, A). 30.6.45: (230 days' depletion) left knee much swollen; small effusion also in right knee. 3.7.45: (233 days' depletion) acneiform and follicular eruptions widespread and haemorrhagic (Plates I, B and XIV, A); condition of wound and gums unchanged; haemorrhagic patch behind knee joints (Plate I, C).

Dosing. 4.7.45: dosing with 10 mg. vitamin C daily begun. 6.7.45: (3 days' dosing) swelling of left knee reduced. 8.7.45: (5 days' dosing) no more pains in legs. 17.7.45: (14 days' dosing) acneiform lesions fading (Plate XIII, B), older lesions turning brown, no new lesions; follicular eruption on lower limbs largely disappeared, follicular haemorrhages turning brown (Plate XIV, B); gum haemorrhages reduced in size; effusion into left knee further reduced but still present; wound scar becoming pink. 20.7.45: (17 days' dosing) acneiform lesions still widespread and congested; perifollicular haemorrhages gone from legs, keratosis still on thighs, keratotic plugs coming out of follicles; wound pink. 8.8.45: (36 days' dosing) lesions paler; gum haemorrhages still just perceptible, wound healing normally and effusion into knee joint almost gone. 4.9.45: (63 days' dosing) acne much improved, slight follicular lesions on back of thighs only; wounds almost normal in colour, and effusion into left knee joint gone. 5.11.45: (125 days' dosing) acne still fading (Plate I, D); gum lesion gone; dose of vitamin C increased to 20 mg. daily. 24.11.45: (144 days' dosing) follicular eruptions completely disappeared; acne greatly improved but persisting in the mild form in which it was present at the beginning of the experiment. Saturation with vitamin C produced no further improvement.

Summary. A diagnosis of scurvy was possible after about 28 weeks of depletion when haemorrhages in the gums, skin and experimental wound were observed. Three weeks later an effusion into the left knee joint was observed. In this volunteer, who was very hairy, a pre-experimental mild acne became very severe and many of the acneiform papules became haemorrhagic (see Section II G, p. 33).

Dosing with 10 mg. of vitamin C daily produced some reduction of the effusion in the knee joint within 2 days, and a disappearance of the perifollicular haemorrhages within a fortnight. The improvement in the gum lesion and the acne was not so rapid. After 5 weeks of dosing with 10 mg. vitamin C daily the gum haemorrhage was still recognizable and the acne was less severe but still marked. After 9 weeks of dosing the effusion of the knee joint had completely

disappeared, the wounds had assumed a normal colour and the acne was still present. The gum lesion and keratosis disappeared completely after 16 weeks' dosing. The acne did not return to its original state during the 125 days of treatment with 10 mg. vitamin C daily, but did so within 19 days when the dose was raised to 20 mg. daily.

In this volunteer the severity of the acne was remarkable. He was the only one into whose joints effusions occurred.

Hudson

Past history. Diphtheria at the age of 11.

Period of depletion. 13.11.45: start of deficient diet; acne of face which fluctuated but persisted throughout the experiment. 5.1.45: (54 days' depletion) acne of face worse. 18.4.45: (157 days' depletion) a few prominent keratotic follicles on arms. 30.4.45: (169 days' depletion) erythematous hair follicles on backs of arms and thighs. 15.5.45: (184 days' depletion) acneiform papules on shoulders and upper part of back; keratotic follicles more numerous on backs of arms and thighs. 28.5.45: (197 days' depletion) acne increased, some on upper chest; several haemorrhagic follicles on back of left forearm. 19.6.45: (219 days' depletion) acne spread to back of neck; enlarged erythematous follicles on backs of arms, right forearm, backs and fronts of thighs and legs, also some follicular hyperkeratosis on backs of thighs and legs; two petechial haemorrhages on medial aspect of left knee; haemorrhage into hair follicles under sphygmomanometer cuff after capillary fragility test. 13.7.45: (247 days' depletion) more follicular haemorrhages on backs and fronts of lower limbs; wound scars of February and June purplish, one showing signs of deep haemorrhage. 18.7.45: (252 days' depletion) pains in legs. 31.7.45: (261 days' depletion) upper arm covered with numerous erythematous and haemorrhagic keratotic follicles; enlarged, hyperkeratotic and erythematous follicles on thighs (Plate I, E), legs, knees and dorsum of feet; minute haemorrhage in upper gum; all three wound scars purplish, two with yellow margins; capillary test caused follicular haemorrhages; pain in back, joints and legs more or less continuous since 18.7.45.

Dosing. 31.7.45: dosing with 10 mg. vitamin C daily begun. 2.8.45: (3 days' dosing) follicles of upper arm less congested. 4.8.45: (5 days' dosing) pains gone. 8.8.45: (9 days' dosing) acneiform eruption gone from chest but still present on back; follicular haemorrhages gone from thighs and legs; gum lesion healed. 14.8.45: (15 days' dosing) acne of face and body faded and nearly gone; enlarged and slightly congested follicles still present on arms, and backs and fronts of thighs; fronts of lower limbs clear; wound scars improving. 4.9.45: (36 days' dosing) acne of body gone. 16.10.45: (78 days' dosing) slight enlargement and congestion of follicles persisting on backs of thighs only (Plate I, F). 10.11.45: (103 days' dosing) dose of vitamin C increased to 20 mg. daily. 15.1.46: (169 days' dosing) no major change; slight acne of face as at the start of the experiment; skin clear; wound scars brown.

Summary. After a period of depletion of 219 days, clinical scurvy was definitely diagnosed from the presence of haemorrhagic and hyperkeratotic follicles. Skin haemorrhages later became very numerous. A minute haemorrhage occurred in the upper gum, and major haemorrhages in the scars of the experimental wounds. Mild acne present at the start increased greatly in severity. Dosing with 10 mg. vitamin C daily checked the haemorrhages. The follicular haemorrhages and the gum lesion disappeared within 8 days and the acne

started to improve also at that time. After a fortnight's treatment the discoloration of the wound scars began to fade. Within 30 days the acne regressed to its original state. The follicular lesions disappeared from the arms after 60 days' dosing, and almost completely from the lower limbs after about 130 days. Increase of the dose of vitamin C to 20 mg. daily had no perceptible effect.

Milburn

Past history. Scarlet fever 1935.

Period of depletion. 13.11.44: start of deficient diet; mild acne on back and face, some enlarged follicles on back of upper arm. 6.3.45: (114 days' depletion) acne on back; follicular hyperkeratosis on back of arms and slight on chest. 10.4.45: (149 days' depletion) acne more definite; follicular hyperkeratosis on arms extending to trunk, buttocks, thighs and medial aspects of knees. 18.4.45: (151 days' depletion) keratotic follicles on upper arm becoming congested (Plate II, A). 30.4.45: (169 days' depletion) acne and follicular lesions more severe; many congested follicles; swollen interdental papillae. 13.4.45: (182 days' depletion) acneiform rash spreading, some haemorrhagic follicles on the arms; some gingivitis. 28.5.45: (197 days' depletion) skin condition severe; acneiform rash on shoulders, chest and back; many papules congested; keratosis of arms, of area between navel and thighs, on thighs and calves; perifollicular haemorrhages on arms, and particularly on thighs and calves. 19.6.45: (219 days' depletion) acne and keratosis more severe; scar of a wound of 1.5.45, which had split 2 mm. on removal of stitches and had oozed, now healing. 28.6.45: (228 days' depletion) gum haemorrhage behind top right upper molar. 17.7.45: (247 days' depletion) acne of back profuse; marked enlargement, hyperkeratosis and congestion of follicles, arms, abdomen and lower limbs; follicular haemorrhages on buttocks and backs and fronts of lower limbs; spontaneous bleeding of gums; haemorrhages at apices of dental papillae; scars of wounds, purplish; bruising of arm after a capillary fragility test. 20.7.45: (250 days' depletion) at 8 a.m. agility test; on same day B.P. 104/72; at 5 a.m. next day severe pain in lower sternal region, gradually becoming worse; when seen at 9 a.m., was sitting up, breathing with difficulty and in great pain; pulse rapid; at 10.30 a.m. in great distress and appearing critically ill; grey face, breathing slow, deep and painful; pulse 85; temperature 99·2; B.P. 80/40; heart sounds not easily audible because of dyspnoea; no distention of cervical veins; given morphia and removed to hospital in an ambulance. As it seemed likely that the condition was the result of vitamin C deficiency, an intravenous injection of 1 g. vitamin C was given with 6 g. vitamin C by mouth at 11.20 a.m. Oxygen also was administered. Within 3 hours the patient was feeling considerably better and made an uninterrupted recovery.

A radiograph of the chest on 23.7.45 showed the heart to be normal in position and size. 2.8.45: skin lesions almost gone; brown pigmented spots left on sites of follicular haemorrhages; gums normal. 22.8.45: received 100 mg. vitamin C daily for the next 9 days. 4.10.45: resumed basal diet and received 20 mg. vitamin C daily. 31.10.45: slight acne on back; folliculitis of beard area, arms and front of thighs near wounds. This condition remained substantially unchanged until the end of the experiment.

Summary. The diagnosis of clinical scurvy became definite on 19.6.45, the 219th day of the period of depletion, through the occurrence of haemorrhages into the hair follicles of the forearms and lower limbs. Nine days later a haemorrhage behind the top right upper molar was observed and 28 days later

haemorrhages of the interdental papillae. Acne, present at the start of the experiment in a mild form, grew worse as the period of depletion lengthened and became very profuse. On 21.7.45 a sudden acute illness occurred presenting the clinical picture of acute cardiac emergency. After a dose of 6 g. vitamin C there was recovery within a few hours.

While no definite statement about the cause of the cardiac attack can be made it is not improbable that it was due to a scorbutic haemorrhage.

Robinson

Past history. No serious illnesses.

Period of depletion. 13.11.44: start of deficient diet; slight gingivitis. 9.1.45: (58 days' depletion) papilla between lower left incisor and canine red, tender and slightly swollen. 6.2.45: (82 days' depletion) enlarged follicles on back of upper arms. 6.3.45: (114 days' depletion) enlarged follicles also on front and back of thighs. 10.4.45: (149 days' depletion) enlarged follicles on front and back of thighs, inside knees and back of legs. 24.4.45: (163 days' depletion) keratotic follicles on arms, back, abdomen, thighs and calves, some erythematous. 1.5.45: (170 days' depletion) skin lesions more extensive; seborrhoeic papules on chest; erythematous follicles on thighs. 3.5.45: (172 days' depletion) some haemorrhagic follicles on legs; haemorrhage into experimental wound noted. 28.5.45: (197 days' depletion) seborrhoeic papules on chest; many keratotic follicles on backs of upper arms and forearms; keratotic, erythematous and some haemorrhagic follicles on backs and fronts of thighs, fronts of legs and calves. No change in gums since 9.1.45. 12.6.45: (206 days' depletion) gum condition deteriorating, swollen papilla between lower lateral incisor and canine. 26.6.45: (226 days' depletion) many follicular haemorrhages; wound infiltrated and haemorrhagic; pain in back first noted. 10.7.45: (237 days' depletion) gingivitis worse, interdental papillae of lower jaw swollen (Plates II, B and XV, A (a)). 17.7.45: (247 days' depletion) keratotic follicles on upper arms (less numerous); numerous hyperkeratotic, erythematous and haemorrhagic follicles on backs and fronts of thighs and legs; a few keratotic follicles on chest; interdental papillae swollen, congested and sagging (Plate XV, A (b)); gums bleeding readily; 8 petechiae in upper jaw; wound scars haemorrhagic and surrounded with yellow stain (Plate XV, B); condition deteriorating till time of dosing. 26.7.45: (246 days' depletion) pains in back, joints and limbs continuing and getting worse since first reported on 26.6.45.

Dosing. 27.7.45: dosing with 10 mg. vitamin C daily begun. 1.8.45: (6 days' dosing) no change in skin condition (Plate XVI, A); gums deteriorated, now bright red, swollen and sagging (Plate II, C). 8.8.45: (13 days' dosing) haemorrhagic follicles brown, no longer purple; haemorrhages in upper gums almost gone, but papillae of lower gum still swollen, boggy and sagging (Plate II, D); pains in back gone. 15.8.45: (20 days' dosing) enlarged hyperkeratotic and erythematous follicles only on thighs and legs; haemorrhagic spots fading; gums less swollen; wound scars less purple. 4.9.45: (40 days' dosing) area of follicular haemorrhages now stained brown (Plate XVI, B); hairs growing freely from follicles; gums reddish and much less swollen. 5.10.45: (71 days' dosing) slightly enlarged follicles on arms, and some enlarged and hyperkeratotic follicles on legs and thighs persisting till end of experiment; wounds almost normal in colour. 14.11.45: (111 days' dosing) gums almost normal; still some marginal gingivitis but no swelling (Plate II, E). 19.11.45: (116 days' dosing) dose of vitamin C increased to 20 mg. daily; no definite changes subsequently.

Summary. After a period of depletion of 197 days, clinical scurvy could be definitely diagnosed from the presence of haemorrhagic follicles. After 250 days there were numerous perifollicular haemorrhages on the lower limbs. Within the next 50 days the skin condition deteriorated, the gums became swollen and congested and the experimental wounds haemorrhagic. The gums of this volunteer were in a poor condition at the start of the experiment and they deteriorated to a greater extent than in any other volunteer. Dosing with 10 mg. vitamin C daily did not immediately check the progress of the haemorrhages in skin and gums, but after 8 days of dosing fresh haemorrhages into follicles and gums had ceased; after 15 days, abnormal follicles were confined chiefly to the thighs and legs and the haemorrhagic spots were fading, but the gums were still swollen. Improvement continued until clinical cure was achieved in 3 months. Increase of the dose of vitamin C to 20 mg. daily had no observable effect. At the end of the experiment on 15.1.46, after saturation with vitamin C, there were still a few enlarged hyperkeratotic and congested follicles, the wounds were normal in colour, and the gums were in the same condition as at the beginning of the experiment.

Sanderson

Past history. No serious illnesses.

Period of depletion. 13.11.44: start of deficient diet; mild acne on back and shoulders. 24.4.45: (163 days' depletion) prominent and keratotic follicles on backs of arms, buttocks, thighs, calves, a small area involved on the front of the arm in the elbow region and some on the fronts of the thighs. 30.4.45: (169 days' depletion) acne on shoulders, keratotic follicles becoming slightly congested. 15.5.45: (184 days' depletion) further extension of acne on back. 20.5.45: (189 days' depletion) keratotic follicles on back of upper arm more numerous and congested. 28.5.45: (197 days' depletion) acne on back extending from neck to waist, on front covering the shoulders; keratotic follicles much more widely distributed on backs of upper arm, thighs and calves, fronts of thighs and legs and on loins; perifollicular haemorrhages present on thighs and calves. 6.6.45: (206 days' depletion) acne more widespread on back and becoming haemorrhagic; perifollicular haemorrhages on the arm after the Hess test. 12.6.45: (212 days' depletion) acne progressing; scar of wound excision of 28.5.45 very haemorrhagic, black scab in centre surrounded by a dark purple area, the adjacent skin being purplish-pink; scar only partly covered by very thin epithelium (Pate II, F). 24.6.45: (224 days' depletion) pains in legs first reported. 3.7.45: (232 days' depletion) acne severe on whole of back and present also on chest; enlarged hyperkeratotic, congested and haemorrhagic follicles on arms, buttocks, thighs and legs; minute haemorrhages at apices of interdental papillae between lower incisors and lower left canine; wound scar unchanged. 18.7.45: (248 days' depletion) pains in back, chest and ribs; haemorrhages in gums more definite (Plate XVII, A (a)). 24.7.45: (254 days' depletion) acne and follicular haemorrhages very severe and widespread; gums and wound the same as on 3.7.45 (see Plates III, A; XVII, B (a); XVIII, A; XIX; XX).

Dosing. 27.7.45: dosing with 10 mg. vitamin C daily begun. 31.7.45: (5 days' dosing) colour of haemorrhagic hair follicles and wound scar changed from dark purple to pink-purple; no further pains reported; haemorrhages into interdental papillae less marked. 8.8.45: (13 days' dosing) follicular haemorrhages brown, purple colour of wound fading. 15.8.45: (20 days' dosing) acne lesions less red; brown staining from haemorrhages disappearing; follicular

plugs coming out but hyperkeratotic and congested follicles still present on the legs and thighs. 4.9.45: (40 days' dosing) colour of wound scar becoming normal; gum haemorrhages almost gone; enlarged keratotic follicles on backs of legs and thighs only. 5.10.45: (71 days' dosing) gum haemorrhages completely gone (Plate XVII, A (b)). 23.10.45: (89 days' dosing) acne mild, a few slightly enlarged and hyperkeratotic follicles on legs, wound scar normal in colour. 19.11.45: (116 days' dosing) dose of vitamin C increased to 20 mg. daily. 15.1.46: (173 days' dosing) mild acne on back, a few enlarged keratotic and congested follicles on backs of legs and thighs. (For condition of the skin and gums in the various areas named, as healing improved, see Plates II, B; XVII, B (b); XVIII, B; XXI).

Summary. At the beginning of June, after a period of depletion of 206 days, clinical scurvy could be definitely diagnosed from the presence of haemorrhagic follicles and of haemorrhage at the bases of some of the acneiform papules. Until the beginning of treatment with 10 mg. of vitamin C daily the follicular regions worsened and the acne increased in severity, wound scars became purplish in colour and the interdental papillae had small haemorrhages. The gum lesions never became very severe. Hyperkeratosis and enlargement of follicles preceded haemorrhages by about one month. Dosing with 10 mg. vitamin C daily checked the progress of the changes in the skin and gums within 4 days, and within 12 days caused slow regression of the follicular changes and some improvement in the gums and also healing in the wound scar. Improvement continued until complete clinical cure was achieved within 3½ months. Increase of dose of vitamin C to 20 mg. daily had no observable effect.

Tridgell

Past history. Diphtheria in childhood. Took part in vitamin A trial, deprived of vitamin A from 14.8.42 to 3.12.42.

Period of depletion. 13.11.44: start of deficient diet. 5.1.45: (54 days' depletion) very slight acne on back. 24.4.45: (163 days' depletion) scattered keratotic follicles on backs of upper arms, buttocks, thighs and calves. 1.5.45: (170 days' depletion) acne on back increased; congested hair follicles on buttocks, backs of thighs and legs. 14.5.45: (183 days' depletion) acne of back extended to buttocks and appearing on chest. 4.6.45: (203 days' depletion) severe pain in back first reported. 2.7.45: (232 days' depletion) some increase in acneiform papules; follicular lesions on thighs and calves erythematous. 12.7.45: (242 days' depletion) pain in legs noted and reported every day subsequently. 17.7.45: (247 days' depletion) follicular eruption more severe; perifollicular haemorrhages on backs of thighs and fronts of legs; slight gingivitis round lower wisdom tooth; marginal and alveolar haemorrhage left upper molar and premolar; both wound scars slightly haemorrhagic. 31.7.45: (261 days' depletion) skin changes more extensive; gum margins injected; alveolar haemorrhage 3 days previously; wound scars purplish. 4.8.45: (265 days' depletion) many perifollicular haemorrhages on fronts of legs.

Dosing. 5.8.45: dosing with 10 mg. vitamin C daily begun. 8.8.45: (4 days' dosing) no substantial change in skin or gums; two wound scars still purplish. 9.8.45: (5 days' dosing) most of pains gone. 15.8.45: (11 days' dosing) distinct improvement: skin almost normal, haemorrhagic spots nearly gone, keratotic plugs coming out of follicles; some enlarged, hyperkeratotic and congested follicles on backs of thighs and legs; wound scars more pinkish. 5.9.45: (30 days' dosing) skin clear except for some prominent follicles on arms and legs, no

longer present on 15.1.46. 5.10.45: (61 days' dosing) slight acne reappeared on back but cleared up by January 1946.

Summary. Clinical scurvy could be definitely diagnosed on 17.7.45, 246 days after the beginning of the depletion period, rather later than in any of the other deprived volunteers. The signs then observed were perifollicular haemorrhages, particularly on the lower limbs, and haemorrhages in the gums and experimental wounds. A slight acne which appeared just after 2 months from the start of the depletion period ultimately involved the back to the buttocks and the chest.

Dosing with 10 mg. vitamin C daily caused the disappearance of most of the follicular lesions within 10 days.

Williams, D.

Past history. No previous illnesses. Experimentally infected with benign tertian malaria 2.4.44, prophylactically treated with small doses of "Mepacrine." Took part in vitamin A experiment, deprived of vitamin A from 27.7.42 to 30.1.44.

Period of depletion. 13.11.44: start of deficient diet. 2.1.45: (51 days' depletion) attack of malaria, 4 temperature peaks on 2.1.45, 4.1.45, 6.1.45 and 8.1.45 of 100°, 102°, 103·8° and 103·6° F., respectively. Parasites found in blood film on 8.1.45. The attack of malaria was probably due to the fact that, owing to a misunderstanding, the volunteer had stopped taking the prophylactic dose of "Mepacrine." Temperature normal on 9.1.45 after "Mepacrine" treatment. 16.1.45: (65 days' depletion) a few enlarged hair follicles on arms. 7.2.45: (87 days' depletion) acne on back. 2.3.45: (110 days' depletion) a few keratotic follicles on arms. 10.4.45: (149 days' depletion) erythematous follicles on arms, hyperkeratotic follicles on inner aspect of thigh. 24.4.45: (163 days' depletion) acne spreading over back; a few keratotic follicles on arms; hyperkeratotic follicles on buttocks, thighs, and backs and fronts of legs, many haemorrhagic; many petechial haemorrhages on dorsum of foot; small petechial haemorrhages inside right cheek; interdental papillae swollen; bruise on right arm and left thigh and one on right wrist after negative pressure test. 5.5.45: (174 days' depletion) acne spread to front of chest and to buttocks; follicular haemorrhages on thighs and legs; petechiae on medial aspect of knees as well as on dorsum of foot; interdental papillae more swollen, tender and haemorrhagic; pain in chest and shortness of breath first reported. 17.5.45: (186 days' depletion) numerous follicular and petechial haemorrhages, some on legs confluent (Plate III, C); scars of wound purplish; routine X-ray of chest revealing a paravertebral abscess and an erosion of the upper border of the body of the 8th dorsal vertebra, interpreted as an active tuberculous lesion. The patient was at once admitted to hospital, placed on a normal diet and given large doses of vitamin C. On review, the X-ray films of the chest taken in November 1944 and March 1945 both showed a slight shadow suggesting the existence already, at those times, of a paravertebral abscess. They were originally overlooked because the films, and the attention of the radiologists, had not been focussed on the spine. The onset of the tuberculous process thus preceded the development of scurvy or of clinical malaria.

Dosing. 17.5.45: 100 mg. vitamin C five times a day. 23.5.45: (7 days' dosing) total dose so far about 3 g. vitamin C; skin smoother; haemorrhages on arms gone, and follicles of legs no longer keratotic, congested or haemorrhagic (Plate III, D); wound scar nearly normal in colour. 26.5.45: (10 days' dosing) acne no longer red, rapidly disappearing; legs and gums normal.

Subsequent history. Conservative treatment in hospital until 7.6.46. On 4.7.45 radiography showed the shadow of an abscess situated over the bodies of 7th, 8th and 9th dorsal vertebrae, and the lesion between the bodies of the 7th and 8th vertebrae. In December 1945 there was swelling of the right scrotum, interpreted as tuberculous epidydimitis; it subsided within a few weeks without specific treatment. Remained under observation until October 1949. Since 1947 has been without symptoms of an active process and has been working normally.

Summary. A diagnosis of scurvy could definitely be made on 24.4.45 (163 days' depletion) on the grounds of the numerous perifollicular haemorrhages of the thighs and legs and petechial haemorrhages on the foot. The period was the shortest in which any volunteer developed definite scurvy, and it may perhaps be related to the accompanying tuberculosis and malaria. At the early stages of the experiment, there were signs of healed acne. The acne reappeared on the back on 7.2.45 and eventually spread over the back and involved the buttocks and the chest. The gums also became swollen and haemorrhagic. On 17.5.45 routine radiography of the chest disclosed tuberculosis of the spine, probably present at the start of the experiment. After administration of about 3 g. vitamin C at the rate of 0·5 g. a day, all scorbutic lesions showed complete regression within 10 days. The tuberculous process healed under conservative hospital treatment. Although the process probably began before the deficient diet started it is likely that its development was precipitated by the deprivation.

Williams, H.

Past history. No serious illnesses.

Period of depletion. 13.11.44: start of deficient diet. 1.12.44: (19 days' depletion) bruise, cause unknown, on right leg. 7.2.45: (87 days' depletion) some enlarged follicles on arms. 24.3.45: (132 days' depletion) bruise on right knee. 10.4.45: (149 days' depletion) erythematous follicles on exterior aspect of upper arm; bruise on left ankle. 21.4.45: (160 days' depletion) enlarged follicles on insides of knees. 30.4.45: (169 days' depletion) congested keratotic follicles widely distributed over backs of arms, buttocks, thighs and calves; one or two haemorrhagic follicles. 14.5.45: (183 days' depletion) condition same except for appearance of a few acneiform papules in the sternal region. 20.5.45: (188 days' depletion) pain in chest and back first noted. 28.5.45: (197 days' depletion) a few acneiform papules on back; some keratotic follicles of thighs, legs and calves now haemorrhagic; small apical haemorrhage on interdental papilla. The volunteer wished to discontinue the experiment in July 1945, so dosing began after 205 days' depletion.

Dosing. 5.6.45: dosing with 20 mg. vitamin C daily begun. 25.6.45: (20 days' dosing) less acne; follicular eruptions less prominent. 27.7.45: (52 days' dosing) allowed unrestricted diet and given 100 mg. vitamin C daily.

Summary. The follicular changes and gum lesion were rather slight, but haemorrhages suggested scurvy after a period of depletion of 197 days. Dosing with 20 mg. vitamin C daily caused the haemorrhages to disappear in 3 weeks.

Wodeman

Past history. No serious illnesses. Recurrent eczema on lower leg. Took part in vitamin A trial, deprived of vitamin A from 3.8.42 to 31.1.44.

Period of depletion. 13.11.44: start of deficient diet; some gingivitis clearing up within 3 weeks. 22.12.44: (40 days' depletion) gums normal (Plate III, E).

22.3.45: (130 days' depletion) hyperkeratotic hair follicles on external aspects of upper arms. 10.4.45: (149 days' depletion) keratotic hair follicles also on medial aspects of knees and thighs. 24.4.45: (163 days' depletion) increased number of hair follicles extending to abdomen and buttocks, some congested. 11.5.45: (180 days' depletion) many congested follicles present (Plate III, F), some haemorrhagic. 15.5.45: (184 days' depletion) gums tender; slight swelling of interdental papillae; small eczematous patch on left ankle, not present at start. 30.5.45: (199 days' depletion) fewer keratotic hair follicles on upper arms, but many more on abdomen and legs and many of them haemorrhagic (Plate IV, A); eczematous patch on left ankle red to red-brown with haemorrhages in centre, size of patch $2\frac{1}{2} \times 3$ cm. (Plate IV, C); haemorrhage 1 to 2 hours after a capillary filtration test at the point on the arm where the pressure was applied (Plate IV, B). 2.6.45: (202 days' depletion) pain in back first reported. 7.6.45: (207 days' depletion) gums of lower jaw swollen; interdental papillae from central incisor to canine enlarged and bluish-red; gums spongy, soft (Plate IV, E). 12.6.45: (212 days' depletion) scar of excision of wound of 22.2.45, which had healed normally, reddish-purple and slightly swollen (Plate IV, D); patch of dermatitis also on right ankle, slightly smaller than on left ankle. 16.6.45: (216 days' depletion) legs covered with numerous haemorrhagic follicles (Plate XXIII, A), many also on trunk; wound scars haemorrhagic; six acneiform papules on chest, all haemorrhagic (Plate XXII). 18.6.45: (218 days' depletion) more swelling and discoloration of gums (Plate IV, F); more haemorrhages on legs (Plate V, A). 22.6.45: (222 days' depletion) pains in legs first reported, pain in back noted intermittently since first reported on 2.6.45.

Dosing. 25.6.45: dosing with 10 mg. vitamin C daily begun. 2.7.45: (8 days' dosing) gums less blue but slightly more swollen (Plate V, C); haemorrhages on legs more numerous (Plate XXIII, B). 3.7.45: (9 days' dosing) improvement definite; eczematous patch pale pink (Plate V, B); wound scar of 22.2.45 less purple (Plate V, D) and haemorrhages on legs less bright (Plate VI, A). 4.7.45: (10 days' dosing) perifollicular capillaries disappearing; hair breaking free from plugs, pain in back and limbs gone, extravasated blood absorbed. 10.7.45: (16 days' dosing) fewer haemorrhages on legs, brown in colour (Plate VI, B); gums improved, blue colour gone but still swollen (Plate V, E); wound scar of 22.2.45 pink (Plate VI, C); brown staining on the legs disappearing; for gradual improvements see Plate XXIV. 1.8.45: (38 days' dosing) legs normal except for a few brown stains at site of former haemorrhages and for the patches of dermatitis still reddish; gums a little swollen but colouring almost normal. 29.9.45: (97 days' dosing) a few enlarged follicles on forearms, buttocks and thighs; wound scars brownish. 4.10.45: (102 days' dosing) dose of vitamin C increased to 20 mg. daily; gums normal (Plate V, F). 15.1.46: (205 days' dosing) a few enlarged hyperkeratotic hair follicles on thighs; area of former patch of dermatitis light brown.

Summary. Definite clinical scurvy, with follicular haemorrhages and gum lesions, developed in the middle of May 1945, after deprivation for about 184 days. Follicular enlargement and hyperkeratosis preceded the appearance of haemorrhages by about 2 months. Dosing with 10 mg. vitamin C daily stopped the development of new lesions. Regression of existing lesions was notable after 9 days' dosing. An unusual feature was the haemorrhage into the eczematous patches which were never haemorrhagic before the trial or after it, but were sometimes pigmented; the rapid regression of this lesion on dosing was notable.

Another

Past history. Scarlet fever in childhood. Took part in vitamin A trial, deprived of vitamin A from 27.7.42 to 24.5.44.

Period of depletion. 13.11.44: start of deficient diet; slight marginal gingivitis round remaining stumps of 2 lower canines. 4.6.45: (206 days' depletion) a few scattered erythematous follicles on back. 3.8.45: (264 days' depletion) still a few scattered erythematous follicles. 7.8.45: (269 days' depletion) woke up during the night with a constrictive pain in the chest which was intensified by deep breathing; looked ill but was not in severe pain; temperature was normal; pulse rate 72; blood pressure 90/60; respiration 18; systolic murmur detected on physical examination; electrocardiogram, normal before the experiment with a P-R interval of 0·20 seconds, now showing a P-R interval of 0·32; transferred to hospital and given 3·6 g. vitamin C, the dose being repeated daily for 3 weeks; within 24 hours disappearance of systolic murmur and pain in chest.

In later investigations made by Professor E. J. Wayne, the P-R interval was found to vary according to posture, respiration, administration of drugs and other factors. Professor Wayne reported as follows:

> "A control electrocardiogram taken on 24th May 1944 before the reduction of ascorbic acid intake showed a P-R interval of 0·20 seconds in Lead II and the sitting position. After the attack of pain in the chest on 7th August 1945, the P-R interval was 0·32 seconds recumbent. During the next month it fell from 0·32 seconds to 0·26 seconds recumbent. The ventricular complexes showed no significant abnormality. Observations were made on several occasions about three months after the attack and on every occasion under resting conditions and in the sitting position, the P-R interval was 0·28 seconds ($\pm$ 0·01 seconds). The duration of the interval was the same at heart rates between 75 and 115 beats per minute. The effect of various procedures on the P-R interval was studied.
>
> Pressure on the carotid sinus caused a prolongation of the P-R interval to 0·32 seconds. A similar result was obtained if the breath was held, probably due to temporary reflex vagal stimulation of the heart since it was accompanied by cardiac slowing. No effect on the interval was found during the phases of normal or deep respiration. Atropine sulphate (1·0 mg.) given intravenously reduced the P-R interval to 0·13 seconds during the second minute after injection and the full effect persisted for 6 minutes. Twenty minutes after injection the P-R interval had risen to 0·24 seconds although the heart rate was still greatly increased. Adrenaline hydrochloride (0·5 mg.) was given by intramuscular injection and caused a reduction in the P-R interval to 0·17 seconds. In all these observations there was no correlation between the level of heart rate and the length of the P-R interval.
>
> There is considerable evidence that prolongation of the P-R interval above the accepted upper limit of normal (0·22 seconds) can occur in healthy hearts (Manning and Stewart, 1945; Robinson, 1945). In two series of cases it has been shown that the P-R interval may be much shorter in the upright and sitting postures than when recumbent (Manning and Stewart, 1945; Holmes and Weill, 1945). It has been shown moreover, that even in heart block due to acute rheumatism, atropine may cause a considerable reduction in the P-R interval (Robinson, 1945). From this fact it has been deduced that there is an increase of vagal tone in acute rheumatism.
>
> None of the observations on the present case provide definite evidence that the prolonged P-R interval was the result of a localized lesion of the conducting

tissues. It should however be pointed out that this case differed from the cases with varying P-R intervals in that the P-R interval was remarkably constant in control observations made on several different occasions. Moreover in the recorded cases in which posture has affected the degree of heart block the longest P-R intervals were associated with the recumbent posture. The readings in the present case were taken in the sitting position and would presumably have been still more prolonged if the curves had been recorded when the patient was recumbent.

The history of pre-cordial pain followed by the development of a systolic murmur and a prolonged P-R interval in a person deficient in vitamin C is compatible with the occurrence of a local haemorrhage into the auriculo-ventricular conducting system by fibrosis."

Summary. Unequivocal signs of scurvy did not develop. After 270 days on the deficient diet the volunteer had an attack of pain in his chest, which was associated with a systolic murmur and a prolonged P-R interval of 0·32 seconds. The P-R interval subsequently varied with posture, respiration and other factors, but remained, in general, chronically prolonged. Whether the cardiac symptoms can be attributed to vitamin C depletion remains uncertain.

G. Special Tables

TABLE 38

Content of vitamin C in the whole blood, plasma and white cells, and content of urea, total proteins, albumin, globulin and phosphatase in the plasma of all the volunteers throughout the experiment
(Vitamin C was estimated at Oxford (O) and at Sheffield (S) by the dinitrophenylhydrazine method (DNPH) and by titration with dichlorophenolindophenol (dye)

Dose of vitamin C (mg./day)	Date	Vitamin C content of: plasma (dye) O (mg./100 ml.)	plasma (dye) S (mg./100 ml.)	whole blood (DNPH) O (mg./100 ml.)	white cells (dye) O (mg./100 g.)	Urea content of plasma (mg./100 ml.)	Protein content of plasma: Total proteins (g./100 ml.)	Albumin (g./100 ml.)	Globulin (g./100 ml.)	Phosphatase content of plasma (units/100 ml.)
Bartley										
Normal	1.10.44	—	0·78	—	—	—	—	—	—	—
diet	15.10.44	—	0·92	—	—	—	—	—	—	—
	22.10.44	0·90	0·89	—	—	31·0	6·20	4·20	1·70	11·0
70	5.11.44	0·72	0·67	—	16·0	—	—	—	—	—
	19.11.44	0·67	0·74	—	30·0	—	—	—	—	—
	26.11.44	—	0·70	—	—	—	—	—	—	—
	3.12.44	0·35	0·72	—	18·0	28·0	6·05	3·90	1·65	6·0
	17.12.44	0·67	0·75	—	17·9	—	—	—	—	—
	7. 1.45	0·78	0·82	—	16·7	—	—	—	—	—
	21. 1.45	0·92	0·78	—	14·3	28·0	6·30	3·90	2·10	5·0
	4. 2.45	0·76	0·76	—	12·0	—	—	—	—	—
	18. 2.45	0·56	0·57	0·82	11·5	—	—	—	—	—
	4. 3.45	0·68	0·74	0·79	18·0	35·0	6·40	3·95	2·10	7·0
	25. 3.45	0·76	0·68	0·96	—	—	—	—	—	—
	15. 4.45	0·68	0·71	0·83	—	—	—	—	—	—
	13. 5.45	0·68	0·67	0·92	11·6	28·0	6·45	3·80	2·30	—
	3. 6.45	0·76	0·73	0·86	11·0	—	—	—	—	3·0
	24. 6.45	0·70	0·83	0·92	16·2	—	—	—	—	—
	15. 7.45	0·66	0·75	0·92	12·0	—	—	—	—	—
Normal	12. 8.45	0·60	—	0·90	10·0	33·0	6·00	3·60	2·10	5·0
diet	30. 9.45	0·82	—	1·10	18·0	—	—	—	—	—
(9.9.45)	21.10.45	0·77	—	1·15	—	—	—	—	—	—
	28.10.45	0·98	—	1·15	17·0	—	—	—	—	—
	11.11.45	0·76	—	0·87	17·5	24·0	6·65	3·65	2·70	6.0
	25.11.45	0·66	—	0·75	14·4	—	—	—	—	—
	16.12.45	0·74	—	0·95	16·0	—	—	—	—	—
	7. 1.46	0·81	—	1·14	17·4	—	—	—	—	—
	10. 2.46	0·54	—	0·93	21·2	30·0	6·30	4·05	1·80	5·0

Drake											
Normal	15.10.44	—	0·34	—	—	—	—	—	—	—	—
diet	22.10.44	0·70	0·55	—	—	25·0	6·15	4·40	1·40	12·0	
	5.11.44	0·40	0·39	—	10·0	—	—	—	—	—	
0	19.11.44	0·27	0·24	—	13·0	—	—	—	—	—	
	26.11.44	—	0·04	—	—	—	—	—	—	—	
	3.12.44	0·02	0·09	—	15·6	—	—	—	—	—	
	17.12.44	0·02	0·05	—	11·0	32·0	6·00	3·85	1·90	7·0	
	7. 1.45	<0·02	0·05	—	5·1	—	—	—	—	—	
	21. 1.45	0·02	0·03	—	3·3	26·0	5·96	4·00	1·70	6·0	
	4. 2.45	0·02	0·01	—	1·0	—	—	—	—	—	
	18. 2.45	<0·01	0·01	0·04	1·5	—	—	—	—	—	
	4. 3.45	0·01	0·01	0·03	<1·0	33·0	6·00	3·60	2·10	6·0	
	25. 3.45	<0·10	0·02	0·02	<1·0	—	—	—	—	—	
	15. 4.45	0·03	<0·01	0·05	<1·0	—	—	—	—	—	
	13. 5.45	<0·01	0·03	0·04	<1·0	26·0	6·10	—	—	—	
	3. 6.45	<0·01	0·03	0·01	<1·0	—	—	—	—	6·0	
	24. 6.45	<0·01	—	0·11	<1·0	—	—	—	—	—	
10	4. 7.45	—	—	—	—	—	—	—	—	—	
	15. 7.45	<0·01	0·03	0·06	<1·0	—	—	—	—	—	
	12. 8.45	0·04	—	0·06	—	31·0	—	—	—	—	
	9. 9.45	0·04	—	0·06	2·4	37·0	5·90	3·30	2·40	4·0	
	30. 9.45	0·03	—	0·07	—	—	—	—	—	—	
	21.10.45	0·10	—	0·09	—	—	—	—	—	—	
	28.10.45	0·08	—	0·07	2·6	—	—	—	—	—	
20	5.11.45	—	—	—	—	—	—	—	—	—	
	11.11.45	0·08	—	0·09	3·0	18·0	6·20	3·70	2·20	6·0	
	25.11.45	0·06	—	0·12	3·1	—	—	—	—	—	
	16.12.45	0·02	—	0·10	3·5	—	—	—	—	—	
510 (Saturation test, see Table 42)	4. 1.46	—	—	—	—	—	—	—	—	—	
Normal	15. 1.46	—	—	—	—	—	—	—	—	—	
diet	10. 2.46	0·42	—	0·76	17·2	25·0	6·75	4·47	2·07	6·0	

TABLE 38 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of: plasma (dye) O (mg./100 ml.)	plasma (dye) S (mg./100 ml.)	whole blood (DNPH) O (mg./100 ml.)	white cells (dye) O (mg./100 g.)	Urea content of plasma (mg./100 ml.)	Protein content of plasma: Total proteins (g./100 ml.)	Albumin (g./100 ml.)	Globulin (g./100 ml.)	Phosphatase content of plasma (units/100 ml.)
Garling Normal diet	8.10.44	—	0·42	—	—	—	—	—	—	—
	15.10.44	—	0·70	—	—	—	—	—	—	—
	22.10.44	0·74	0·67	—	—	27·0	6·20	4·10	1·90	15·0
	5.11.44	0·55	0·55	—	17·0	—	—	—	—	—
70	13.11.44	—	—	—	—	—	—	—	—	—
	19.11.44	0·69	0·59	—	22·0	—	—	—	—	—
	26.11.44	—	0·50	—	—	—	—	—	—	—
	3.12.44	0·48	0·56	—	14·0	34·0	6·00	3·70	2·10	5·0
	17.12.44	0·54	0·56	—	15·4	—	—	—	—	—
	7. 1.45	0·64	0·64	—	14·0	—	—	—	—	—
	21. 1.45	0·84	0·63	—	16·6	27·0	6·30	3·70	2·10	5·0
	4. 2.45	0·74	0·82	—	—	—	—	—	—	—
	18. 2.45	0·52	0·70	0·95	14·0	—	—	—	—	—
	4. 3.45	0·74	0·74	1·08	12·6	31·0	6·20	3·80	2·20	7·0
	25. 3.45	0·80	0·67	0·97	15·4	—	—	—	—	—
	15. 4.45	0·69	0·80	1·05	12·0	32·0	6·20	3·80	2·20	—
	13. 5.45	0·64	0·66	0·94	13·5	25·0	6·70	4·30	2·20	14·0
	3. 6.45	0·80	0·77	0·88	14·6	—	—	—	—	7·0
	24. 6.45	0·74	0·94	1·03	16·0	—	—	—	—	—
	15. 7.45	0·70	0·70	0·99	14·2	25·0	6·90	4·20	2·60	9·0
	12. 8.45	0·62	—	0·91	11·0	28·0	—	—	—	—
	9. 9.45	0·50	—	0·77	10·0	31·0	6·20	3·60	2·40	4·0
	30. 9.45	0·64	—	0·95	12·0	—	—	—	—	—
50	4.10.45	—	—	—	—	—	—	—	—	—
	21.10.45	0·58	—	0·75	—	—	—	—	—	—
	28.10.45	0·58	—	0·67	9·2	—	—	—	—	—
	11.11.45	0·44	—	0·55	10·2	21·0	6·55	3·74	2·60	6·0
	25.11.45	0·32	—	0·60	9·6	—	—	—	—	—
630 (Saturation test, see Table 42)	11.12.45	—	—	—	—	—	—	—	—	—
Normal diet	21.12.45	—	—	—	—	—	—	—	—	—
	7. 1.46	0·51	—	0·78	13·6	—	—	—	—	—
	10. 2.46	0·42	—	0·80	15·5	24·0	6·40	4·40	1·80	6·0

Golding Normal diet	1.10.44	—	—	—	—	—	—	—	—	—	—
	8.10.44	—	0·62	—	—	—	—	—	—	—	—
	15.10.44	—	0·30	—	—	—	—	—	—	—	—
	22.10.44	0·44	0·34	—	—	30·0	7·20	5·20	2·00	16·0	
	29.10.44	0·30	0·37	—	10·0	—	—	—	—	—	
10	13.11.44	—	—	—	—	—	—	—	—	—	
	19.11.44	0·22	0·23	—	18·0	—	—	—	—	—	
	26.11.44	—	0·13	—	—	—	—	—	—	—	
	3.12.44	—	0·07	—	—	—	—	—	—	—	
	17.12.44	0·04	0·08	—	8·5	—	—	—	—	9·0	
	7. 1.45	<0·02	0·08	—	4·9	—	—	—	—	—	
	21. 1.45	0·02	0·05	—	2·6	25·0	6·20	4·20	—	—	
	4. 2.45	0·02	0·01	—	1·4	—	—	—	—	—	
	18. 2.45	<0·01	0·04	0·04	1·7	—	—	—	—	—	
	4. 3.45	0·02	0·03	0·11	2·5	29·0	6·15	4·10	1·80	6·0	
	25. 3.45	<0·01	0·02	0·13	3·2	—	—	—	—	—	
	15. 4.45	0·03	0·05	0·15	1·7	—	—	—	—	—	
	13. 5.45	<0·01	0·01	0·09	1·4	28·0	6·65	4·10	2·30	—	
	3. 6.45	<0·01	0·04	0·10	<1·0	—	—	—	—	4·0	
	24. 6.45	0·04	0·03	0·09	<1·0	—	—	—	—	—	
	15. 7.45	<0·01	0·01	0·11	—	—	—	—	—	—	
	9. 9.45	0·01	—	0·09	2·7	31·0	6·00	3·45	2·30	4·0	
	30. 9.45	0·04	—	0·10	—	—	—	—	—	—	
	21.10.45	0·06	—	0·11	—	—	—	—	—	—	
	11.11.45	0·08	—	0·07	4·6	19·0	6·40	4·00	2·20	4·0	
	25.11.45	0·08	—	0·10	2·9	—	—	—	—	—	
	16.12.45	0·05	—	0·06	1·1	—	—	—	—	—	
570 (Saturation test, see Table 42) Normal diet	9. 1.46	—	—	—	—	—	—	—	—	—	
	19. 1.46	—	—	—	—	—	—	—	—	—	
	10. 2.46	0·64	—	0·93	15·3	30·0	6·50	4·40	1·80	6·0	

TABLE 38 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of: plasma (dye) O (mg./100 ml.)	plasma (dye) S (mg./100 ml.)	whole blood (DNPH) O (mg./100 ml.)	white cells (dye) O (mg./100 g.)	Urea content of plasma (mg./100 ml.)	Protein content of plasma: Total proteins (g./100 ml.)	Albumin (g./100 ml.)	Globulin (g./100 ml.)	Phosphatase content of plasma (units/100 ml.)
Hill										
Normal diet	1.10.44	—	—	—	—	—	—	—	—	—
	8.10.44	—	0·29	—	—	—	—	—	—	—
	15.10.44	—	0·24	—	—	—	—	—	—	—
	22.10.44	0·40	0·25	—	—	—	—	—	—	—
	29.10.44	0·36	0·34	—	17·0	—	—	—	—	—
	5.11.44	—	—	—	—	25·0	7·00	4·10	—	—
70	13.11.44	—	—	—	—	—	—	—	—	—
	19.11.44	0·62	0·55	—	21·8	—	—	—	—	—
	26.11.44	—	0·63	—	—	—	—	—	—	—
	3.12.44	0·49	0·56	—	16·0	30·0	6·30	3·40	2·60	7·0
	17.12.44	0·55	0·67	—	15·7	—	—	—	—	—
	7. 1.45	0·53	0·43	—	14·2	—	—	—	—	—
	21. 1.45	0·43	0·31	—	11·0	—	—	—	—	—
	4. 2.45	0·65	0·72	—	12·0	25·0	6·30	3·60	2·40	5·0
	18. 2.45	0·48	0·61	0·76	15·0	—	—	—	—	—
	4. 3.45	0·48	0·55	0·91	12·5	30·0	6·35	3·70	2·40	10·0
	25. 3.45	0·62	0·54	0·83	16·8	—	—	—	—	—
	15. 4.45	0·57	0·62	0·91	12·0	—	—	—	—	—
	13. 5.45	0·56	0·54	0·87	10·0	22·0	6·40	4·00	—	—
	3. 6.45	0·66	0·62	0·84	13·4	—	—	—	—	4·0
	24. 6.45	0·50	0·50	0·78	10·0	—	—	—	—	—
	15. 7.45	0·48	0·43	0·75	10·0	27·0	6·30	3·70	2·30	5·0
	12. 8.45	0·52	—	0·77	12·8	—	—	—	—	—
	9. 9.45	0·44	—	0·87	10·0	26·0	6·00	3·40	2·30	6·0
50	8.10.45	—	—	—	—	—	—	—	—	—
	21.10.45	0·52	—	—	—	—	—	—	—	—
	28.10.45	0·52	—	0·63	10·1	—	—	—	—	—
	11.11.45	0·40	—	0·55	9·4	18·0	6·40	3·60	2·50	9·0
	25.11.45	0·29	—	0·35	7·6	—	—	—	—	—
630 (Saturation test, see Table 42)	11.12.45	—	—	—	—	—	—	—	—	—
Normal	21.12.45	—	—	—	—	—	—	—	—	—
diet	6. 1.46	0·46	—	0·67	11·8	—	—	—	—	—
Normal diet + 50	11. 1.46	—	—	—	—	—	—	—	—	—
Normal	29. 1.46	—	—	—	—	—	—	—	—	—
diet	10. 2.46	0·28	—	0·60	15·3	25·0	7·20	4·20	2·70	8·0

Hudson Normal diet	8.10.44	—	0·43	—	—	—	—	—	—	—	—
	15.10.44	—	0·45	—	—	—	—	—	—	—	—
	22.10.44	0·59	0·51	—	—	27·0	7·10	4·40	2·40	21·0	
	5.11.44	0·24	0·39	—	14·0	—	—	—	—	10·0	
0	13.11.44	—	—	—	—	—	—	—	—	—	
	19.11.44	0·29	0·29	—	31·0	—	—	—	—	—	
	26.11.44	—	0·13	—	—	—	—	—	—	—	
	3.12.44	0·02	0·11	—	18·0	29·0	7·30	4·00	2·90	—	
	17.12.44	0·04	0·04	—	7·0	—	—	—	—	8·0	
	7. 1.45	<0·02	0·08	—	1·8	—	—	—	—	—	
	21. 1.45	<0·02	0·04	—	1·8	24·0	6·10	3·86	2·00	5·0	
	4. 2.45	<0·01	<0·01	—	3·0	—	—	—	—	—	
	18. 2.45	<0·01	0·02	0·10	3·2	—	—	—	—	—	
	4. 3.45	<0·01	<0·01	0·06	<1·0	27·0	5·80	3·70	1·90	8·0	
	25. 3.45	<0·01	0·01	0·07	1·1	—	—	—	—	—	
	15. 4.45	<0·01	0·02	0·06	1·0	—	—	—	—	—	
	13. 5.45	0·01	< 0·01	0·06	<1·0	28·0	5·75	3·50	—	—	
	3. 6.45	0·02	0·03	0·06	<1·0	—	—	—	—	4·0	
	24. 6.45	0·02	0·03	0·07	<1·0	—	—	—	—	—	
	15. 7.45	<0·01	0·04	0·01	<1·0	27·0	6·00	3·75	2·00	6·0	
10	31. 7.45	—	—	—	—	—	—	—	—	—	
	12. 8.45	<0·01	—	0·01	3·5	27·0	5·90	3·75	1·90	—	
	9. 9.45	0·03	—	0·07	2·1	32·0	6·00	3·45	2·35	4·0	
	30. 9.45	0·07	—	0·12	2·9	—	—	—	—	—	
	21.10.45	0·07	—	0·09	—	—	—	—	—	—	
	28.10.45	0·06	—	0·08	3·1	—	—	—	—	—	
	11.11.45	0·04	—	0·10	3·6	20·0	6·30	3·85	2·20	7·0	
20	19.11.45	—	—	—	—	—	—	—	—	—	
	25.11.45	0·02	—	0·10	4·1	—	—	—	—	—	
	16.12.45	0·04	—	0·06	4·2	—	—	—	—	—	
670 (Saturation test, see Table 42)	4. 1.46	—	—	—	—	—	—	—	—	—	
Normal diet	11. 2.46	0·33	—	0·78	18·0	27·0	6·65	4·10	2·25	7·0	

TABLE 38 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of: plasma (dye) O (mg./100 ml.)	plasma (dye) S (mg./100 ml.)	whole blood (DNPH) O (mg./100 ml.)	white cells (dye) O (mg./100 g.)	Urea content of plasma (mg./100 ml.)	Protein content of plasma: Total proteins (g./100 ml.)	Albumin (g./100 ml.)	Globulin (g./100 ml.)	Phosphatase content of plasma (units/100 ml.)
Jackson Normal diet	26.11.44	—	0·43	—	—	—	—	—	—	—
70	27.11.44	—	—	—	—	—	—	—	—	—
	3.12.44	0·32	0·46	—	12·0	37·0	6·40	4·80	1·40	9·0
0	11.12.44	—	—	—	—	—	—	—	—	—
	17.12.44	0·02	0·12	—	10·4	—	—	—	—	—
10	18.12.44	—	—	—	—	—	—	—	—	—
	7. 1.45	0·05	0·09	—	5·7	—	—	—	—	—
	21. 1.45	<0·02	0·03	—	5·7	31·0	6·25	4·25	1·80	—
	4. 2.45	0·02	0·04	—	4·2	—	—	—	—	6·0
	18. 2.45	<0·01	0·04	0·12	3·9	—	—	—	—	—
	4. 3.45	<0·01	0·02	0·12	1·0	30·0	6·45	—	—	—
	25. 3.45	0·01	0·04	0·11	2·9	42·0	—	—	—	—
	15. 4.45	0·01	0·03	0·11	2·2	—	—	—	—	—
	13. 5.45	<0·01	0·03	0·12	2·0	26·0	7·00	4·20	2·50	—
0	28. 5.45	—	—	—	—	—	—	—	—	—
	3. 6.45	<0·01	0·02	0·06	2·3	—	—	—	—	9·0
	24. 6.45	0·04	0·02	0·09	<1·0	—	—	—	—	—
	15. 7.45	0·03	0·01	0·10	1·5	—	—	—	—	—
5	8. 8.45	—	—	—	—	—	—	—	—	—
	12. 8.45	<0·01	—	0·05	<1·0	32·0	6·30	3·65	2·50	—
	9. 9.45	<0·01	—	0·06	2·0	36·0	6·30	3·90	2·10	7·0
	30. 9.45	<0·01	—	0·06	<1·0	—	—	—	—	—
	21.10.45	0·08	—	0·06	—	—	—	—	—	—
	28.10.45	0·03	—	0·10	1·5	—	—	—	—	—
	11.11.45	0·06	—	0·03	2·0	25·0	6·60	3·90	2·50	8·0
	25.11.45	0·06	—	0·07	2·7	—	—	—	—	—
630 (Saturation test, see Table 42)	11.12.45	—	—	—	—	—	—	—	—	—
Normal diet + 50	5. 1.46	—	—	—	—	—	—	—	—	—
	6. 1.46	0·80	—	1·05	13·0	—	—	—	—	—
Normal diet	27. 1.46	—	—	—	—	—	—	—	—	—

Milburn Normal diet 0	8.10.44	—	0·37	—	—	—	—	—	—	—	—
	15.10.44	—	0·65	—	—	—	—	—	—	—	—
	22.10.44	0·80	0·76	—	—	26·0	6·75	4·50	1·90	15·0	
	5.11.44	0·72	0·76	—	—	—	—	—	—	—	
	13.11.44	—	—	—	—	—	—	—	—	—	
	19.11.44	0·39	0·39	—	21·0	—	—	—	—	—	
	26.11.44	—	0·15	—	—	—	—	—	—	—	
	3.12.44	0·04	0·09	—	5·8	36·0	7·20	4·10	2·80	7·0	
	17.12.44	0·07	0·05	—	7·3	—	—	—	—	—	
	7. 1.45	<0·02	0·06	—	4·4	—	—	—	—	—	
	21. 1.45	0·04	0·03	—	3·0	23·0	6·40	3·75	2·30	—	
	4. 2.45	<0·01	0·01	—	2·8	—	—	—	—	6·0	
	18. 2.45	<0·01	0·02	0·06	1·3	—	—	—	—	—	
	4. 3.45	<0·01	0·02	0·07	<1·0	42·0	6·30	4·10	1·80	7·0	
	25. 3.45	<0·01	0·02	0·06	<1·0	—	—	—	—	—	
	15. 4.45	0·02	0·04	0·06	<1·0	—	—	—	—	—	
	13. 5.45	0·01	0·02	0·03	<1·0	25·0	6·60	3·80	2·40	8·0	
	3. 6.45	0·02	0·03	0·04	<1·0	—	—	—	—	6·0	
	24. 6.45	<0·01	0·02	0·05	<1·0	—	—	—	—	—	
	15. 7.45	<0·01	0·04	0·05	<1·0	27·0	7·30	4·10	2·80	8·0	
6000 Normal diet + 100	21. 7.45	—	—	—	—	—	—	—	—	—	
	22. 7.45	—	—	—	—	—	—	—	—	—	
	12. 8.45	0·91	—	1·10	20·8	32·0	6·70	3·60	2·80	6·0	
Normal diet 20	1. 9.45	—	—	—	—	—	—	—	—	—	
	30. 9.45	0·68	—	0·79	13·7	—	—	—	—	—	
	4.10.45	—	—	—	—	—	—	—	—	—	
	21.10.45	0·42	—	0·47	8·7	—	—	—	—	—	
	28.10.45	0·15	—	0·31	—	—	—	—	—	—	
	11.11.45	0·12	—	0·26	6·2	24·0	6·80	3·70	2·90	7·0	
	25.11.45	0·14	—	0·16	—	—	—	—	—	—	
	16.12.45	0·08	—	0·12	4·2	—	—	—	—	—	
670 (Saturation test, see Table 42)	4. 1.46	—	—	—	—	—	—	—	—	—	
Normal diet	3. 2.46	—	—	—	—	—	—	—	—	—	
	10. 2.46	0·51	—	0·78	12·3	30·0	6·35	4·10	1·85	7·0	

TABLE 38 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of: plasma (dye) O (mg./100 ml.)	plasma (dye) S (mg./100 ml.)	whole blood (DNPH) O (mg./100 ml.)	white cells (dye) O (mg./100 g.)	Urea content of plasma (mg./100 ml.)	Protein content of plasma: Total proteins (g./100 ml.)	Albumin (g./100 ml.)	Globulin (g./100 ml.)	Phosphatase content of plasma (units/100 ml.)
Parry										
70	8.12.44	—	—	—	—	—	—	—	—	—
	17.12.44	0·15	0·18	—	15·2	31·0	6·20	3·90	2·10	6·0
10	18.12.44	—	—	—	—	—	—	—	—	—
	7. 1.45	0·11	0·10	—	13·6	—	—	—	—	—
	21. 1.45	0·04	0·03	—	6·3	20·0	6·45	4·10	2·10	—
	4. 2.45	0·06	0·01	—	4·3	30·0	7·00	4·00	2·70	5·0
	18. 2.45	0·02	<0·01	0·12	4·6	—	—	—	—	—
	4. 3.45	0·03	0·03	0·10	2·4	—	—	—	—	—
	25. 3.45	0·02	0·03	0·09	1·9	—	—	—	—	—
	15. 4.45	0·04	0·07	0·06	1·9	—	—	—	—	—
	13. 5.45	0·04	0·04	0·09	2·2	33·0	6·80	4·00	—	8·0
	3. 6.45	0·03	0·04	0·09	2·0	—	—	—	—	5·0
	24. 6.45	0·02	0·07	0·18	2·2	—	—	—	—	—
	15. 7.45	<0·01	0·01	0·09	1·6	29·0	7·00	3·80	3·00	—

Proctor Normal diet	1.10.44	—	1·20	—	—	—	—	—	—	—	—
	8.10.44	1·28	—	—	—	—	—	—	—	—	—
	15.10.44	—	1·30	—	—	—	—	—	—	—	—
	22.10.44	1·28	1·14	—	—	25·0	6·65	4·35	2·10	9·0	
	5.11.44	0·96	0·91	—	23·0	—	—	—	—	—	
10	13.11.44	—	—	—	—	—	—	—	—	—	
	19.11.44	0·56	0·52	—	16·0	—	—	—	—	—	
	26.11.44	—	0·29	—	—	—	—	—	—	—	
	3.12.44	0·15	0·15	—	10·0	31·0	7·00	3·85	2·80	4·0	
	17.12.44	0·02	0·10	—	5·0	—	—	—	—	—	
	7. 1.45	0·12	0·09	—	7·2	—	—	—	—	—	
	21. 1.45	<0·02	0·04	—	4·8	28·0	6·23	3·90	2·10	—	
	4. 2.45	0·04	<0·01	—	1·8	—	—	—	—	5·0	
	18. 2.45	0·01	0·02	0·12	3·2	—	—	—	—	—	
	4. 3.45	<0·01	0·06	0·12	3·3	32·0	7·00	4·10	2·70	7·0	
	25. 3.45	<0·01	0·03	0·08	3·0	—	—	—	—	—	
	15. 4.45	<0·01	0·04	0·10	2·0	—	—	—	—	—	
	13. 5.45	<0·01	0·04	0·10	2·6	24·0	7·00	4·10	2·70	8·0	
	3. 6.45	<0·01	0·05	0·11	3·0	—	—	—	—	5·0	
	24. 6.45	<0·01	0·03	0·12	1·6	—	—	—	—	—	
	15. 7.45	0·03	0·04	0·09	1·8	29·0	6·91	4·60	2·10	5·0	
	12. 8.45	0·03	—	0·09	2·0	—	—	—	—	—	
	9. 9.45	0·08	—	0·09	2·3	33·0	6·10	3·65	2·20	4·0	
	30. 9.45	0·07	—	0·09	2·6	—	—	—	—	—	
	21.10.45	0·02	—	0·11	—	—	—	—	—	—	
	28.10.45	0·07	—	0·08	2·8	—	—	—	—	—	
	11.11.45	0·06	—	0·07	2·6	20·0	6·72	3·85	—	5·0	
	25.11.45	0·08	—	0·07	2·7	—	—	—	—	—	
	16.12.45	0·01	—	0·06	2·6	—	—	—	—	—	
560 (Saturation test, see Table 42) Normal diet + 50 Normal diet	9. 1.46	—	—	—	—	—	—	—	—	—	
	19. 1.46	—	—	—	—	—	—	—	—	—	
	10. 2.46	—	—	—	—	—	—	—	—	—	
	12. 2.46	1·00	—	1·30	—	28·0	6·75	4·45	2·00	5·0	

TABLE 38 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of: plasma (dye) O (mg./100 ml.)	plasma (dye) S (mg./100 ml.)	whole blood (DNPH) O (mg./100 ml.)	white cells (dye) O (mg./100 g.)	Urea content of plasma (mg./100 ml.)	Protein content of plasma: Total proteins (g./100 ml.)	Albumin (g./100 ml.)	Globulin (g./100 ml.)	Phosphatase content of plasma (units/100 ml.)
Robinson Normal diet 0	23.10.44	0·76	0·53	—	—	24·0	6·65	4·40	2·00	12·0
	5.11.44	0·92	0·82	—	15·0	—	—	—	—	—
	13.11.44	—	—	—	—	—	—	—	—	—
	19.11.44	0·37	0·28	—	17·0	—	—	—	—	—
	26.11.44	—	0·14	—	—	—	—	—	—	—
	3.12.44	0·08	0·07	—	7·4	28·0	6·10	3·70	2·10	4·0
	17.12.44	0·04	0·06	—	10·2	—	—	—	—	—
	7. 1.45	0·04	0·14	—	—	—	—	—	—	—
	21. 1.45	<0·02	0·03	—	1·3	25·0	6·10	3·85	1·90	5·0
	4. 2.45	0·01	0·03	—	1·1	—	—	—	—	—
	18. 2.45	<0·01	<0·01	<0·01	1·2	—	—	—	—	—
	4. 3.45	<0·01	0·04	0·06	2·0	28·0	6·15	4·15	1·80	—
	25. 3.45	<0·01	0·01	0·06	1·1	—	—	—	—	—
	15. 4.45	0·01	0·06	0·09	<1·0	—	—	—	—	—
	13. 5.45	<0·01	<0·01	—	<1·0	21·0	6·65	4·10	2·30	—
	3. 6.45	0·01	0·05	0·06	<1·0	—	—	—	—	3·0
	24. 6.45	0·02	0·03	0·07	<1·0	—	—	—	—	—
	15. 7.45	—	—	0·04	—	—	—	—	—	—
10	27. 7.45	—	—	—	—	—	—	—	—	—
	12. 8.45	0·01	0·02	0·03	1·8	24·0	5·90	3·30	2·40	6·0
	9. 9.45	<0·01	—	0·09	1·4	35·0	6·75	3·75	2·75	5·0
	30. 9.45	0·05	—	0·06	2·0	—	—	—	—	—
	21.10.45	0·12	—	0·14	—	—	—	—	—	—
	28.10.45	0·05	—	0·06	2·4	—	—	—	—	—
	11.11.45	0·11	—	0·09	3·0	18·0	6·60	3·68	2·60	5·0
20	19.11.45	—	—	—	—	—	—	—	—	—
	25.11.45	0·08	—	0·10	4·0	—	—	—	—	—
	16.12.45	0·03	—	0·10	4·2	—	—	—	—	—
420 (Saturation test, see Table 42)	4. 1.46	—	—	—	—	—	—	—	—	—
Normal diet + 50	17. 1.45	—	—	—	—	—	—	—	—	—
Normal diet	8. 2.45	—	—	—	—	—	—	—	—	—
	11. 2.45	0·78	—	1·35	19·6	23·0	6·55	4·18	2·10	6·0

Sanderson											
Normal	15.10.44	—	0·39	—	—	—	—	—	—	—	
diet	22.10.44	0·46	0·39	—	—	24·0	6·30	4·50	1·60	7·0	
	5.11.44	0·42	0·45	—	16·0	—	—	—	—	—	
0	13.11.44	—	—	—	—	—	—	—	—	—	
	19.11.44	0·20	0·22	—	23·6	—	—	—	—	—	
	26.11.44	—	0·09	—	—	—	—	—	—	—	
	3.12.44	0·02	0·10	—	10·0	23·0	6·15	4·40	1·50	—	
	17.12.44	0·08	0·03	—	8·0	—	—	—	—	11·0	
	7. 1.45	0·02	<0·01	—	3·5	—	—	—	—	—	
	21. 1.45	0·05	0·02	—	3·1	—	—	—	—	—	
	4. 2.45	<0·01	0·02	—	2·4	44·0	6·00	3·80	—	—	
	18. 2.45	0·01	<0·01	0·09	1·2	—	—	—	—	—	
	4. 3.45	<0·01	0·02	0·12	1·8	29·0	6·00	3·70	2·10	6·0	
	25. 3.45	<0·01	0·02	0·07	<1·0	—	—	—	—	—	
	15. 4.45	<0·01	0·04	0·13	<1·0	—	—	—	—	—	
	13. 5.45	0·02	0·05	0·05	<1·0	30·0	6·00	3·50	2·20	—	
	3. 6.45	0·02	0·05	0·05	<1·0	—	—	—	—	7·0	
	24. 6.45	0·02	0·04	0·05	<1·0	—	—	—	—	—	
	15. 7.45	0·02	0·01	0·05	<1·0	—	—	—	—	—	
10	27. 7.45	—	—	—	—	—	—	—	—	—	
	12. 8.45	0·06	—	0·09	1·0	30·0	5·80	3·50	2·10	10·0	
	9. 9.45	0·01	—	0·04	1·2	29·9	6·00	3·50	2·20	7·0	
	30. 9.45	0·06	—	0·06	1·1	—	—	—	—	—	
	21.10.45	0·10	—	0·10	—	—	—	—	—	—	
	28.10.45	0·08	—	0·06	2·4	—	—	—	—	—	
	11.11.45	0·04	—	0·12	1·6	23·0	7·00	3·84	2·80	5·0	
20	19.11.45	—	—	—	—	—	—	—	—	—	
	25.11.45	0·08	—	0·09	3·0	—	—	—	—	—	
	16.12.45	0·05	—	0·06	2·8	—	—	—	—	—	
660 (Saturation test, see Table 42)	4. 1.46	—	—	—	—	—	—	—	—	—	
Normal diet	16. 1.46	—	—	—	—	—	—	—	—	—	
Normal diet + 50	17. 1.46	—	—	—	—	—	—	—	—	—	
Normal diet	10. 2.46	0·61	—	1·15	16·5	26·0	6·90	4·56	2·04	7·0	

TABLE 38 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of: plasma (dye) O (mg./100 ml.)	plasma (dye) S (mg./100 ml.)	whole blood (DNPH) O (mg./100 ml.)	white cells (dye) O (mg./100 g.)	Urea content of plasma (mg./100 ml.)	Protein content of plasma: Total proteins (g./100 ml.)	Albumin (g./100 ml.)	Globulin (g./100 ml.)	Phosphatase content of plasma (units/100 ml.)
Tridgell Normal diet	8.10.44	—	0·78	—	—	—	—	—	—	—
	15.10.44	—	0·55	—	—	—	—	—	—	—
	22.10.44	0·58	0·54	—	—	29·0	7·40	5·10	2·00	14·0
	5.11.44	0·42	0·41	—	24·0	—	—	—	—	—
0	13.11.44	—	—	—	—	—	—	—	—	—
	19.11.44	0·34	0·25	—	26·0	—	—	—	—	—
	26.11.44	—	0·10	—	—	—	—	—	—	—
	3.12.44	0·04	0·14	—	15·7	33·0	6·30	4·07	2·00	—
	17.12.44	0·02	0·06	—	10·0	—	—	—	—	9·0
	7. 1.45	0·04	0·08	—	5·7	—	—	—	—	—
	21. 1.45	<0·02	0·03	—	3·2	30·0	—	3·90	—	—
	4. 2.45	0·02	0·01	—	1·0	36·0	6·42	4·00	2·10	5·0
	18. 2.45	0·02	0·01	0·06	2·1	—	—	—	—	—
	4. 3.45	0·01	0·03	0·11	1·1	30·0	6·00	4·05	1·60	13·0
	25. 3.45	<0·01	0·01	0·08	1·0	—	—	—	—	—
	15. 4.45	<0·01	0·03	0·05	<1·0	—	—	—	—	—
	13. 5.45	0·02	0·03	0·07	<1·0	31·0	6·80	4·10	—	—
	3. 6.45	<0·01	0·06	0·04	<1·0	—	—	—	—	7·0
	24. 6.45	<0·01	0·03	0·04	<1·0	—	—	—	—	—
	15. 7.45	0·01	0·03	0·04	1·0	29·0	6·65	4·10	2·30	8·0
10	5. 8.45	—	—	—	—	—	—	—	—	—
	12. 8.45	0·04	—	0·03	1·0	32·0	—	—	—	—
	9. 9.45	0·03	—	0·05	1·2	38·0	6·75	3·60	2·90	5·0
	30. 9.45	0·05	—	0·13	2·2	—	—	—	—	—
	21.10.45	0·07	—	0·09	—	—	—	—	—	—
	28.10.45	0·06	—	0·09	2·0	—	—	—	—	—
	11.11.45	0·22?	—	0·12	2·8	23·0	6·60	3·75	2·60	5·0
	25.11.45	0·06	—	0·07	2·5	—	—	—	—	—
	16.12.45	0·05	—	0·08	4·0	—	—	—	—	—
620 (Saturation test, see Table 42)	9. 1.46	—	—	—	—	—	—	—	—	—
Normal diet + 50	21. 1.46	—	—	—	—	—	—	—	—	—
Normal diet	10. 2.45	0·51	—	1·04	22·0	28·0	6·25	4·05	1·87	6·0

Way											
70	7.12.44	—	—	—	—	—	—	—	—	—	—
	17.12.44	0·14	0·07	—	22·0	33·0	6·00	3·80	—	6·0	
10	18.12.44	—	—	—	—	—	—	—	—	—	
	14. 1.45	0·02	0·04	—	10·5	—	—	—	—	—	
	21. 1.45	0·05	0·03	—	5·7	33·0	5·90	4·00	1·60	—	
	4. 2.45	0·01	0·05	—	3·6	—	—	—	—	—	
	18. 2.45	0·02	0·04	0·15	6·0	—	—	—	—	—	
	4. 3.45	0·02	0·02	0·13	5·4	26·0	5·80	3·90	1·60	6·0	
	25. 3.45	0·01	0·07	0·14	2·1	—	—	—	—	—	
	15. 4.45	0·05	0·05	0·15	1·0	—	—	—	—	—	
	13. 5.45	0·03	0·02	0·10	1·0	23·0	6·00	4·00	1·60	8·0	
0	28. 5.45	—	—	—	—	—	—	—	—	—	
	3. 6.45	<0·01	0·05	0·06	<1·0	—	—	—	—	5·0	
10	5. 6.45	—	—	—	—	—	—	—	—	—	
	24. 6.45	0·01	0·05	0·14	<1·0	—	—	—	—	—	
0	2. 7.45	—	—	—	—	—	—	—	—	—	
	15. 7.45	0·02	0·01	0·06	2·0	—	—	—	—	—	
5	8. 8.45	—	—	—	—	—	—	—	—	—	
	12. 8.45	0·04	—	0·08	<1·0	33·0	5·80	3·20	2·40	3·0	
	9. 9.45	0·02	—	0·06	<1·0	34·0	5·90	3·50	2·20	—	
	30. 9.45	0·03	—	0·06	1·7	—	—	—	—	—	
	21.10.45	0·06	—	0·06	—	—	—	—	—	—	
	28.10.45	0·05	—	0·06	2·0	—	—	—	—	—	
	11.11.45	0·07	—	—	2·0	30·0	6·20	3·60	2·30	5·0	
	25.11.45	0·05	—	0·06	2·2	—	—	—	—	—	
670 Saturation test, see Table 42)	11.12.45	—	—	—	—	—	—	—	—	—	
Normal diet	21.12.45	—	—	—	—	—	—	—	—	—	
Normal diet + 50	5. 1.46	—	—	—	—	—	—	—	—	—	
	6. 1.46	0·47	—	0·64	16·0	—	—	—	—	—	
Normal	26. 1.46	—	—	—	—	—	—	—	—	—	
diet	10. 2.46	0·25	—	0·69	11·2	26·0	6·67	4·66	1·76	5·0	

Vitamin C content of:				Urea content of plasma	Protein content of plasma			Phosphatase content of plasma
plasma (dye)		whole blood (DNPH)	white cells (dye)		Total proteins	Albumin	Globulin	
ml.)	S (mg./100 ml.)	O (mg./100 ml.)	O (mg./100 g.)	(mg./100 ml.)	(g./100 ml.)	(g./100 ml.)	(g./100 ml.)	(units/100 ml.)
5	0·35	—	—	—	—	—	—	—
	0·31	—	—	—	—	—	—	—
4	0·32	—	21·0	27·0	6·20	3·75	2·10	4·0
	—	—	—	—	—	—	—	—
1	0·20	—	13·1	—	—	—	—	—
	—	—	—	—	—	—	—	—
1	0·08	—	6·8	—	—	—	—	—
2	0·03	—	4·3	—	—	—	—	—
3	0·03	—	4·1	26·0	6·00	4·00	1·70	—
1	0·01	0·20	4·8	—	—	—	—	—
1	<0·01	0·15	1·8	30·0	6·00	3·90	1·80	7·0
2	0·05	0·10	3·1	—	—	—	—	—
3	0·04	0·12	1·1	—	—	—	—	—
2	0·04	0·07	2·0	22·0	6·20	4·00	2·20	—
	—	—	—	—	—	—	—	—
2	0·04	0·11	2·4	—	—	—	—	5·0
2	0·02	0·10	<1·0	—	—	—	—	—
2	0·04	0·06	<1·0	—	—	—	—	—
	—	—	—	—	—	—	—	—
4	—	0·07	<1·0	25·0	6·40	3·60	2·40	3·0
2	—	0·07	1·0	27·0	6·30	3·60	2·40	4·0
3	—	0·06	1·5	—	—	—	—	—
	—	0·10	—	—	—	—	—	—
6	—	0·06	1·6	—	—	—	—	—
2	—	0·06	1·2	20·0	6·60	3·75	2·60	5·0
5	—	0·07	1·6	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—

Williams, D.											
Normal	1.10.44	—	0·30	—	—	—	—	—	—	—	
diet	15.10.44	—	0·33	—	—	—	—	—	—	—	
	22.10.44	0·70	0·50	—	—	27·0	7·40	4·70	2·40	13·0	
	5.11.44	0.64	0·59	—	15·0	—	—	—	—	—	
0	13.11.44	—	—	—	—	—	—	—	—	—	
	19.11.44	0·49	0·32	—	23·0	—	—	—	—	—	
	26.11.44	—	0·13	—	—	—	—	—	—	—	
	3.12.44	0·40	0·13	—	14·0	26·0	7·20	3·90	2·80	9·0	
	17.12.44	0·02	0·01	—	9·6	—	—	—	—	—	
	21. 1.45	0·02	0·04	—	3·6	27·0	6·80	3·55	3·00	5·0	
	4. 2.45	0·02	0·01	—	3·2	—	—	—	—	—	
	18. 2.45	<0·01	0·01	0·07	1·3	—	—	—	—	—	
	4. 3.45	<0·01	0·01	0·04	1·0	31·0	6·40	3·70	2·20	8·0	
	25. 3.45	<0·01	0·05	0·03	1·0	—	—	—	—	—	
	15. 4.45	<0·01	0·03	0·05	1·0	31·0	7·10	3·50	3·20		
	13. 5.45	<0·01	0·03	0·06	1·0	24·0	8·10	3·50	4·20	8·0	
Normal diet + 500	17. 5.45	—	—	—	—	—	—	—	—	—	
	10. 6.45	0·75	—	0·90	—	—	—	—	—	—	
	24. 6.45	0·72	0·79	0·81	12·0	—	—	—	—	—	

TABLE 38 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of: plasma (dye) O (mg./100 ml.)	plasma (dye) S (mg./100 ml.)	whole blood (DNPH) O (mg./100 ml.)	white cells (dye) O (mg./100 g.)	Urea content of plasma (mg./100 ml.)	Protein content of plasma: Total proteins (g./100 ml.)	Albumin (g./100 ml.)	Globulin (g./100 ml.)	Phosphatase content of plasma (units/100 ml.)
Williams, H.										
Normal	15.10.44	—	0·32	—	—	—	—	—	—	—
diet	22.10.44	0·28	0·37	—	—	23·0	6·40	4·40	1·70	10·0
	5.11.44	0·48	0·65	—	17·0	—	—	—	—	—
0	13.11.44	—	—	—	—	—	—	—	—	—
	19.11.44	0·62	0·68	—	—	—	—	—	—	—
	26.11.44	—	0·27	—	—	—	—	—	—	—
	3.12.44	0·15	0·20	—	12·5	24·0	6·00	4·20	1·40	9·0
	17.12.44	0·02	0·06	—	12·3	—	—	—	—	—
	7. 1.45	0·04	0·04	—	3·6	—	—	—	—	—
	21. 1.45	0·02	0·01	—	3·3	23·0	6·30	4·10	2·00	6·0
	4. 2.45	<0·01	0·01	—	1·6	—	—	—	—	—
	18. 2.45	<0·01	0·01	0·02	2·0	—	—	—	—	—
	4. 3.45	<0·01	0·02	0·05	1·0	26·0	6·35	4·00	1·90	10·0
	25. 3.45	<0·01	<0·01	0·10	2·0	—	—	—	—	—
	15. 4.45	0·01	0·04	0·09	1·0	28·0	6·50	3·90	2·30	—
	13. 5.45	<0·01	0·03	0·09	1·0	23·0	6·60	3·70	—	7·0
	3. 6.45	<0·01	—	0·06	3·0	—	—	—	—	6·0
20	5. 6.45	—	—	—	—	—	—	—	—	—
	10. 6.45	0·07	—	0·12	—	—	—	—	—	—
	24. 6.45	0·08	0·05	0·08	2·0	—	—	—	—	—
	15. 7.45	0·01	0·03	0·12	2·8	22·0	5·80	4·00	1·50	8·0
Normal diet + 100	27. 7.45	—	—	—	—	—	—	—	—	—
	12. 8.45	0·78	—	1·15	9·1	—	—	—	—	—

Wodeman										
Normal	15.10.44	—	0·52	—	—	—	—	—	—	—
diet	22.10.44	0·46	0·35	—	—	27·0	6·90	4·40	2·20	13·0
	5.11.44	0·42	0·43	—	20·0	—	—	—	—	—
0	13.11.44	—	—	—	—	—	—	—	—	—
	19.11.44	0·39	0·21	—	19·3	—	—	—	—	—
	26.11.44	—	0·04	—	—	—	—	—	—	—
	3.12.44	0·02	0·08	—	5·5	28·0	6·20	3·70	2·20	4·0
	17.12.44	0·02	0·04	—	6·5	—	—	—	—	—
	7. 1.45	0·02	0·04	—	4·7	—	—	—	—	—
	21. 1.45	0·02	0·04	—	2·2	27·0	6·20	4·20	1·70	—
	4. 2.45	<0·01	0·03	—	1·2	—	—	—	—	5·0
	18. 2.45	<0·01	0·03	0·05	1·8	—	—	—	—	—
	4. 3.45	0·02	0·06	0·09	1·0	30·0	6·30	4·30	1·60	8·0
	28. 3.45	<0·01	0·02	0·10	1·7	—	—	—	—	—
	15. 4.45	<0·01	0·03	0·05	<1·0	—	—	—	—	—
	13. 5.45	<0·01	<0·01	0·06	<1·0	27·0	6·20	—	—	—
	3. 6.45	<0·01	0·03	0·03	<1·0	—	—	—	—	3·0
	24. 6.45	<0·01	0·02	0·03	<1·0	—	—	—	—	—
10	25. 6.45	—	—	—	—	—	—	—	—	—
	15. 7.45	<0·01	0·01	0·04	<1·0	—	—	—	—	—
	12. 8.45	0·01	—	0·07	1·4	30·0	6·30	3·60	2·20	3·0
	9. 9.45	0·02	—	0·07	2·2	28·0	6·90	3·50	3·00	4·0
	30. 9.45	0·02	—	0·07	1·9	—	—	—	—	—
20	4.10.45	—	—	—	—	—	—	—	—	—
	21.10.45	0·10	—	0·10	—	—	—	—	—	—
	28.10.45	0·06	—	0·08	3·0	—	—	—	—	—
	11.11.45	0·06	—	0·09	3·6	20·0	6·90	4·10	2·50	4·0
	25.11.45	0·10	—	0·12	3·3	—	—	—	—	—
	16.12.45	0·06	—	0·07	3·5	—	—	—	—	—
830 (Saturation test, see Table 42)	4. 1.46	—	—	—	—	—	—	—	—	—
Normal diet + 50	18. 1.46	—	—	—	—	—	—	—	—	—
	10. 2.46	0·61	—	1·10	17·7	30·0	7·25	4·80	2·20	4·0

TABLE 38 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of: plasma (dye) O (mg./100 ml.)	plasma (dye) S (mg./100 ml.)	whole blood (DPNH) O (mg./100 ml.)	white cells (dye) O (mg./100 g.)	Urea content of plasma (mg./100 ml.)	Protein content of plasma: Total proteins (g./100 ml.)	Albumin (g./100 ml.)	Globulin (g./100 ml.)	Phosphatase content of plasma (units/100 ml.)
Woodhouse										
Normal	8.10.44	—	0·70	—	—	—	—	—	—	—
diet	15.10.44	—	0·60	—	—	—	—	—	—	—
	22.10.44	0·68	0·56	—	—	28·0	6·20	4·40	1·60	11·0
	5.11.44	0·62	0·63	—	—	—	—	—	—	—
10	13.11.44	—	—	—	—	—	—	—	—	—
	19.11.44	0·39	0·36	—	19·3	—	—	—	—	—
	26.11.44	—	0·23	—	—	—	—	—	—	—
	3.12.44	0·16	0·19	—	14·0	40·0?	6·00	3·90	1·80	—
	7. 1.45	0·02	0·06	—	4·2	—	—	—	—	—
	21. 1.45	0·04	0·01	—	3·9	25·0	6·20	4·00	1·90	—
	4. 2.45	0·03	0·01	—	2·2	—	—	—	—	—
	18. 2.45	<0·01	0·04	0·11	2·7	—	—	—	—	—
	4. 3.45	0·03	0·05	0·06	1·1	26·0	5·80	3·90	1·70	9·0
	25. 3.44	0·02	0·01	0·08	1·0	—	—	—	—	—
	15. 4.45	0·02	0·04	0·12	1·0	—	—	—	—	—
	13. 5.45	<0·01	<0·01	0·10	1·0	24·0	5·90	4·10	—	—
	24. 6.45	0·08	0·03	0·09	2·0	—	—	—	—	—
	15. 7.45	0·01	0·06	0·12	1·7	24·0	6·65	3·70	2·60	9·0
	12. 8.45	—	—	0·08	2·5	26·0	—	—	—	—
	9. 9.45	0·02	—	0·09	1·0	32·0	6·00	3·60	2·20	7·0
	30. 9.45	0·05	—	0·10	2·7	—	—	—	—	—
	21.10.44	0·04	—	0·15	—	—	—	—	—	—
	28.10.45	0·03	—	0·09	2·0	—	—	—	—	—
	11.11.45	0·12	—	0·10	3·0	22·0	6·30	3·60	2·50	6·0
	25.11.45	0·09	—	0·10	3·7	—	—	—	—	—
	16.12.45	0·04	—	0·10	3·7	—	—	—	—	—
640 (Saturation test, see Table 42) Normal diet + 50	9. 1.46	—	—	—	—	—	—	—	—	—
	10. 2.46	0·66	—	1·12	13·8	28·0	6·20	4·30	1·70	6·0

Another Normal diet	1.10.44	—	—	—	—	—	—	—	—	—	—
	15.10.44	—	0·80	—	—	—	—	—	—	—	—
	22.10.44	0·82	0·60	—	—	29·0	6·00	4·00	1·80	9·0	
	5.11.44	0·62	0·63	—	18·0	—	—	—	—	—	
0	13.11.44	—	—	—	—	—	—	—	—	—	
	19.11.44	0·34	0·26	—	26·0	—	—	—	—	—	
	26.11.44	—	0·11	—	—	33·0	6·10	3·60	2·30	5·0	
	3.12.44	0·06	0·13	—	11·6	—	—	—	—	—	
	17.12.44	0·02	0·08	—	9·4	—	—	—	—	—	
	7. 1.45	0·03	0·09	—	9·0	—	—	—	—	—	
	21. 1.45	0·02	0·01	—	3·6	27·0	6·20	3·20	—	2·0	
	4. 2.45	<0·01	0·01	—	2·0	—	—	—	—	—	
	18. 2.45	<0·01	<0·01	0·05	2·3	—	—	—	—	—	
	4. 3.45	<0·01	0·01	0·10	1·8	30·0	5·80	3·80	1·75	6·0	
	25. 3.45	<0·01	<0·01	0·09	1·0	—	—	—	—	—	
	15. 4.45	0·03	<0·01	0·01	<1·0	—	—	—	—	—	
	13. 5.45	<0·01	0·04	0·04	<1·0	22·0	5·80	3·50	—	—	
	3. 6.45	<0·01	0·03	0·02	—	—	—	—	—	7·0	
	24. 6.45	<0·01	0·05	0·08	2·0	—	—	—	—	—	
	15. 7.45	<0·01	0·01	0·06	<1·0	33·0	5·80	3·20	2·30	4·0	
3600	7. 8.45	—	—	—	—	—	—	—	—	—	
	12. 8.45	1·64	—	3·05	6·7	—	—	—	—	—	
Normal diet	25. 8.45	—	—	—	—	—	—	—	—	—	

TABLE 39

Content of vitamin C in the total amount of urine excreted in the 24 hours by all the volunteers throughout the experiment

(Vitamin C was estimated in Cambridge (C), and in Oxford (O), by titration with dichlorophenolindophenol (dye) and by the dinitrophenylhydrazine method (DNPH) and in Cambridge only, by titration with dichlorophenolindophenol in the presence of formaldehyde (formaldehyde dye).)

Dose of vitamin C (mg./day)	Date	Vitamin C content of urine in 24 hours				
		Dye		Formal-dehyde dye	DNPH	
		C (mg.)	O (mg.)	C (mg.)	C (mg.)	O (mg.)
Bartley						
Normal diet	13.10.44	—	95·5	—	—	—
	17.10.44	50·0	95·5	—	—	—
	21.10.44	62·5	60·0	—	—	—
70	13.11.44	—	—	—	—	—
	23.11.44	26·0	—	—	—	—
	28.11.44	24·2	—	—	—	—
	14.12.44	45·0	—	—	—	—
	15. 1.45	16·0	29·4	8·3	16·7	16·0
	23. 1.45	—	—	6·0	17·9	—
	28. 1.45	28·0	23·6	11·0	14·1	—
	30. 1.45	—	23·6	7·0	14·1	10·0
	7. 5.45	34·4	—	9·7	9·8	—
	3. 6.45	26·6	—	10·0	—	—
Normal diet	8. 9.45	—	—	—	—	—
	7.11.45	—	35·4	—	—	16·4
	14. 1.46	—	31·0	—	—	3·5
	10. 2.46	—	27·3	—	—	—
	12. 2.46	—	28·0	—	—	12·6
Drake						
Normal diet	16.10.44	—	38·5	—	—	—
	18.10.44	22·5	30·0	—	—	—
	21.10.44	24·0	21·6	—	—	—
	5.11.44	—	20·0	—	—	—
0	13.11.44	—	—	—	—	—
	23.11.44	17·8	—	—	—	—
	28.11.44	23·8	—	—	—	—
	14.12.44	31·0	—	—	—	—
	17.12.44	27·5	—	—	—	—
	16. 1.45	—	19·3	~0	1·7	5·8
	23. 1.45	—	—	~0	1·8	—
	26. 2.45	17·0	—	~0	—	—
	26. 3.45	17·2	—	1·2	—	—
	6. 5.45	16·0	—	4·4	3·2	—
	12. 5.45	23·0	—	5·2	5·8	—
	13. 5.45	20·0	—	4·9	4·4	—
	23. 5.45	19·4	—	3·2	—	—
10	4. 7.45	—	—	—	—	—
20	5.11.45	—	—	—	—	—
	9.11.45	—	25·2	—	—	5·0
	13.12.45	26·0	—	3·0	6·8	—
510 (Saturation test, see Table 42)	4. 1.46	—	—	—	—	—
Normal diet	15. 1.46	—	—	—	—	—
	10. 2.46	—	19·8	—	—	7·0
	12. 2.46	—	24·5	—	—	4·4

TABLE 39 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of urine in 24 hours				
		Dye		Formal-dehyde dye	DNPH	
		C (mg.)	O (mg.)	C (mg.)	C (mg.)	O (mg.)
Garling						
Normal diet	11.10.44	—	39·2	—	—	—
	17.10.44	24·7	32·6	—	—	—
	20.10.44	20·5	21·6	—	—	—
70	13.11.44	—	—	—	—	—
	23.11.44	22·2	—	—	—	—
	28.11.44	28·5	—	—	—	—
	15.12.44	37·0	—	—	—	—
	19.12.44	45·5	—	—	—	—
	15. 1.45	—	26·4	7·5	8·0	9·7
	24. 1.44	—	—	13·2	9·1	—
	28. 1.45	32·0	28·6	10·0	13·5	10·0
	30. 1.45	—	28·6	9·0	13·5	10·0
	8. 5.45	34·6	—	11·0	10·0	—
	3. 6.45	24·0	—	9·8	—	—
	3. 9.45	21·0	—	8·5	—	—
50	4.10.45	—	—	—	—	—
	8.11.45	—	22·2	—	—	4·5
	26.11.45	31·0	—	5·7	—	—
630 (Saturation test, see Table 42)	11.12.45	—	—	—	—	—
Normal diet	21.12.45	—	—	—	—	—
	7. 1.46	—	20·7	—	—	7·0
	10. 2.46		23·7	—	—	8·0
	12. 2.46	—	23·7	—	—	—
Golding						
Normal diet	21.10.44	19·0	24·9	—	—	—
	22.10.44	20·8	20·6	—	—	—
10	13.11.44	—	—	—	—	—
	23.11.44	17·1	—	—	—	—
	28.11.44	19·3	—	—	—	—
	17.12.44	30·0	—	—	—	—
	19.12.44	30·5	—	—	—	—
	17. 1.45	—	—	~0	4·2	—
	24. 1.45	—	—	~0	4·8	—
	26. 1.45	23·0	—	1·9	4·9	—
	13. 5.45	18·6	—	4·7	4·2	—
	9.11.45	—	13·8	—	—	3·1
570 (Saturation test, see Table 42)	9. 1.46	—	—	—	—	—
Normal diet + 50	19. 1.46	—	—	—	—	—
Normal diet	10. 2.46	—	24·4	—	—	9·3
	12. 2.46	—	19·8	—	—	7·3

TABLE 39 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of urine in 24 hours				
		Dye		Formal-dehyde dye	DNPH	
		C (mg.)	O (mg.)	C (mg.)	C (mg.)	O (mg.)
Hill						
Normal diet	21.10.44	24·5	25·2	—	—	—
	22.10.44	20·0	22·8	—	—	—
70	13.11.44	—	—	—	—	—
	23.11.44	18·6	—	—	—	—
	28.11.44	24·0	—	—	—	—
	17.12.44	37·0	—	—	—	—
	18.12.44	41·0	—	—	—	—
	16. 1.45	—	19·8	4·5	6·5	6·5
	23. 1.45	—	—	14·3	16·4	—
	28. 1.45	30·0	31·0	8·0	6·4	9·0
	4. 2.45	—	31·0	—	—	9·5
	9. 5.45	31·2	—	10·5	11·5	—
	3. 6.45	23·0	—	7·0	—	—
50	8.10.45	—	—	—	—	—
	9.11.45	—	23·7	—	—	9·9
	26.11.45	27·0	—	3·2	—	—
630 (Saturation test, see Table 42)	11.12.45	—	—	—	—	—
Normal diet	21.12.45	—	—	—	—	—
Normal diet + 50	11. 1.46	—	—	—	—	—
	13. 1.46	—	18·4	—	—	5·1
	20. 1.46	—	18·2	—	—	7·3
Normal diet	29. 1.46	—	—	—	—	—
	11. 2.46	—	15·9	—	—	5·4
	12. 2.46	—	21·0	—	—	4·4
Hudson						
Normal diet	18.10.44	25·0	—	—	—	—
	21.10.44	37·5	—	—	—	—
0	13.11.44	—	—	—	—	—
	23.11.44	28·5	—	—	—	—
	28.11.44	26·5	—	—	—	—
	18.12.44	36.5	—	—	—	—
	19.12.44	35·5	—	—	—	—
	17. 1.45	27·0	27·0	~0	26·0	2·5
	22. 1.45	—	—	~0	1·7	—
	8. 3.45	16·4	—	~0	—	—
	8. 5.45	31·4	—	0·6	5·5	—
	14. 5.45	24·8	—	0·8	2·2	—
	27. 5.45	21·0	—	3·6	—	—
10	31. 7.45	—	—	—	—	—
	4. 9.45	23·0	—	1·0	—	—
	9.11.45	—	31·8	—	—	8·6
20	19.11.45	—	—	—	—	—
670 (Saturation test, see Table 42)	4. 1.46	—	—	—	—	—
Normal diet + 50	16. 1.46	—	—	—	—	—
Normal diet	5. 2.46	—	—	—	—	—
	10. 2.46	—	21·1	—	—	8·7
	11. 2.46	—	23·0	—	—	14·1

TABLE 39 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of urine in 24 hours				
		Dye		Formal-dehyde dye	DNPH	
		C (mg.)	O (mg.)	C (mg.)	C (mg.)	O (mg.)
Jackson	11.12.44	50·0	—	—	—	—
0	12.12.44	—	—	—	—	—
	17.12.44	38·5	—	—	—	—
10	18.12.44	—	—	—	—	—
	15. 1.45	—	20·7	~0	3·0	2·3
	23. 1.45	—	—	~0	0·5	—
	8. 5.45	26·4	—	4·3	6·3	—
0	28. 5.45	—	—	—	—	—
5	8. 8.45	—	—	—	—	—
	10. 9.45	26·0	—	2·5	—	—
	9.11.45	—	22·8	—	—	10·0
630 (Saturation test, see Table 42)	11.12.45	—	—	—	—	—
Normal diet	20.12.45	—	—	—	—	—
Normal diet + 50	5. 1.46	—	—	—	—	—
	6. 1.46	—	38·6	—	—	23·8
	13. 1.46	—	52·0	—	—	11·0
Normal diet	27. 1.46	—	—	—	—	—
	10. 2.46	—	21·1	—	—	3·8
	11. 2.46	—	23·0	—	—	9·5
Milburn						
Normal diet	13.10.44	—	46·0	—	—	—
	18.10.44	32·0	30·0	—	—	—
	21.10.44	32·6	30·0	—	—	—
0	13.11.44	—	—	—	—	—
	23.11.44	24·0	—	—	—	—
	28.11.44	18·3	—	—	—	—
	18.12.44	38·2	—	—	—	—
	19.12.44	48·0	—	—	—	—
	17. 1.45	—	15·8	~0	4·9	3·8
	22. 1.45	—	—	~0	1·4	—
	28. 1.45	26·0	23·6	1·5	3·3	5·9
	26. 3.45	23·0	—	~0	—	—
	11. 5.45	38·6	—	5·0	5·2	—
	14. 5.45	25·4	—	5·8	6·2	—
	23. 5.45	25·0	—	1·9	—	—
	27. 5.45	7·0	—	~0	—	—
	12. 7.45	32·8	—	1·8	—	—
6,000	21. 7.45	—	—	—	—	—
Normal diet + 100	22. 7.45	—	—	—	—	—
Normal diet	1. 9.45	—	—	—	—	—
20	4.10.45	—	—	—	—	—
	4.11.45	—	31·6	—	—	12·6
670 (Saturation test, see Table 42)	4. 1.46	—	—	—	—	—
Normal diet + 50	16. 1.46	—	—	—	—	—
Normal diet	3. 2.46	—	—	—	—	—
	12. 2.46	—	17·3	—	—	7·3
	13. 2.46	—	23·2	—	—	7·5

TABLE 39 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of urine in 24 hours				
		Dye		Formal-dehyde dye	DNPH	
		C (mg.)	O (mg.)	C (mg.)	C (mg.)	O (mg.)
Parry						
70	16.12.44	25·0	—	—	—	—
10	18.12.44	—	—	—	—	—
	17.12.44	25·0	—	—	—	—
	17. 1.45	—	14·6	~0	5·4	4·1
	10. 5.45	18·4	—	3·6	2·1	—
	12. 7.45	23·0	—	1·0	—	—
	22. 1.46	—	—	3·0	2·4	—
Proctor						
Normal diet	11.10.44	11·5	11·5	—	—	—
	17.10.44	42·0	35·0	—	—	—
	20.10.44	44·5	38·1	—	—	—
10	13.11.44	—	—	—	—	—
	23.11.44	26·3	—	—	—	—
	28.11.44	27·7	—	—	—	—
	15.12.44	41·3	—	~0	—	—
	16.12.44	40·0	—	2·5	—	—
	24. 1.45	—	—	~0	5·2	—
	8. 5.45	31·8	—	3·6	6·6	—
	4. 9.45	23·0	—	4·2	—	—
	8.11.45	—	25·5	—	—	3·7
560 (Saturation test, see Table 42)	9. 1.46	—	—	—	—	—
Normal diet + 50	19. 1.46	—	—	—	—	—
	10. 2.46	—	51·0	—	—	15·0
	12. 2.46	—	39·2	—	—	17·0
Robinson						
0	13.11.44	—	—	—	—	—
	23.11.44	17·2	—	—	—	—
	28.11.44	20·0	—	—	—	—
	16.12.44	27·6	—	—	—	—
	19.12.44	35·5	—	—	—	—
	15. 1.45	—	23·8	~0	7·0	~0
	23. 1.45	—	—	~0	1·4	—
	11. 5.45	25·4	—	4·6	7·8	—
	13. 5.45	—	—	—	5·5	—
	14. 5.45	20·0	—	0·6	2·8	—
	23. 5.45	19·2	—	1·6	—	—
10	27. 7.45	—	—	—	—	—
	5.11.45	25·0	20·0	0·6	—	6·5
	7.11.45	—	23·9	—	—	6·5
20	19.11.45	—	—	—	—	—
	13.12.45	20·0	—	4·4	6·8	—
	14.12.45	19·0	—	4·0	4·4	—
420 (Saturation test, see Table 42)	4. 1.46	—	—	—	—	—
Normal diet + 50	17. 1.46	—	—	—	—	—
Normal diet	8. 2.46	—	—	—	—	—
	11. 2.46	—	44·0	—	—	23·6
	12. 2.46	—	24·7	—	—	13·3

TABLE 39 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of urine in 24 hours				
		Dye		Formal-dehyde dye	DNPH	
		C (mg.)	O (mg.)	C (mg.)	C (mg.)	O (mg.)
Sanderson						
Normal diet	16.10.44	—	33·0	—	—	—
	18.10.44	22·0	33·0	—	—	—
	20.10.44	20·5	27·0	—	—	—
0	13.11.44	—	—	—	—	—
	23.11.44	23·0	—	—	—	—
	28.11.44	21·3	—	—	—	—
	15.12.44	40·0	—	—	—	—
	17.12.44	31·0	—	—	—	—
	16. 1.45	—	27·8	~0	2·2	5·6
	28. 1.45	21·0	15·0	1·7	2·1	1·9
	10. 5.45	24·8	—	0·6	2·5	—
	13. 5.45	24·4	—	4·1	3·2	—
	23. 5.45	24·0	—	1·8	—	—
10	27. 7.45	—	—	—	—	—
	5.11.45	28·0	23·9	1·7	—	5·2
	7.11.45	—	23·8	—	—	5·2
20	19.11.45	—	—	—	—	—
	13.12.45	28·0	—	3·4	5·3	—
	14.12.45	31·0	—	6·4	8·3	—
660 (Saturation test, see Table 42)	4. 1.46	—	—	—	—	—
Normal diet + 50	17. 1.45	—	—	—	—	—
Normal diet	10. 2.46	—	30·2	—	—	9·6
	12. 2.46	—	24·2	—	—	8·4
Tridgell						
Normal diet	11.10.44	—	86·0	—	—	—
	17.10.44	34·5	32·0	—	—	—
	19.10.44	55·5	—	—	—	—
0	13.11.44	—	—	—	—	—
	23.11.44	18·5	—	—	—	—
	28.11.44	23·0	—	—	—	—
	14.12.44	30·5	—	—	—	—
	20.12.44	48·5	—	—	—	—
	17. 1.45	—	14·9	0·7	2·4	5·1
	22. 1.45	—	—	1·5	1·5	—
	6. 5.45	33·0	—	5·6	5·9	—
	11. 5.45	22·0	—	2·2	4·0	—
	13. 5.45	19·8	—	1·9	0·6	—
	27. 5.45	19·0	—	1·0	—	—
10	5. 8.45	—	—	—	—	—
	6.11.45	—	21·2	—	—	7·1
620 (Saturation test, see Table 42)	9. 1.46	—	—	—	—	—
Normal diet + 50	21. 1.46	—	—	—	—	—
Normal diet	10. 2.46	—	44·3	—	—	30·3
	11. 2.46	—	28·0	—	—	9·0

TABLE 39 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of urine in 24 hours				
		Dye		Formal-dehyde dye	DNPH	
		C (mg.)	O (mg.)	C (mg.)	C (mg.)	O (mg.)
Way						
70	14.12.44	37·5	—	—	—	—
	16.12.44	37·0	—	—	—	—
10	18.12.44	—	—	—	—	—
	14. 1.45	—	25·3	~0	8·9	11·9
	23. 1.45	—	—	~0	5·8	—
	28. 1.45	18·0	21·0	2·5	4·9	6·3
	4. 2.45	—	21·0	~0	5·7	6·3
	18. 2.45	—	—	~0	4·8	—
	10. 5.45	20·6	—	3·6	1·8	—
0	28. 5.45	—	—	—	—	—
10	5. 6.45	—	—	—	—	—
	2. 7.45	—	—	—	—	—
	10. 7.45	15·5	—	0·6	—	—
5	8. 8.45	—	—	—	—	—
	3. 9.45	19·0	—	~0	—	—
	6.11.45	—	18·5	—	—	3·9
670 (Saturation test, see Table 42)	11.12.45	—	—	—	—	—
Normal diet	21.12.45	—	—	—	—	—
Normal diet + 50	5. 1.46	—	—	—	—	—
	6. 1.46	—	30·2	—	—	8·6
	13. 1.46	—	26·2	—	—	4·8
Normal diet	27. 1.46	—	—	—	—	—
	10. 2.46	—	20·4	—	—	5·6
	12. 2.46	—	22·3	—	—	10·8
Whinfield						
70	23.11.44	20·7	—	—	—	—
	28.11.44	14·1	—	—	—	—
	15.12.44	32·2	—	—	—	—
0	11.12.44	—	—	—	—	—
10	19.12.44	35·5	—	—	5·5	—
	17. 1.45	—	17·5	0·8	1·4	0
	23. 1.45	—	18·1	2·0	1·4	3·6
	30. 1.45	19·0	18·1	2·5	2·8	3·6
	7. 5.45	30·6	—	1·9	4·9	—
0	28. 5.45	—	—	—	—	—
5	8. 8.45	—	—	—	—	—
	6. 9.45	21·0	—	3·3	—	—
	8.11.45	—	17·4	—	—	3·9
670 (Saturation test, see Table 42)	11.12.45	—	—	—	—	—
Normal diet	21.12.45	—	—	—	—	—
Normal diet + 50	5. 1.46	—	—	—	—	—
	6. 1.46	—	51·0	—	—	39·0
	13. 1.46	—	45·0	—	—	26·7
Normal diet	27. 1.46	—	—	—	—	—
	11. 2.46	—	18·3	—	—	5·8
	12. 2.46	—	31·4	—	—	10·0

TABLE 39 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of urine in 24 hours				
		Dye		Formal-dehyde dye	DNPH	
		C (mg.)	O (mg.)	C (mg.)	C (mg.)	O (mg.)
Williams, D.						
Normal diet	13.10.44	33·0	23·3	—	—	—
	20.10.44	33·0	29·7	—	—	—
0	13.11.44	—	—	—	—	—
	23.11.44	18·2	—	—	—	—
	28.11.44	22·8	—	—	—	—
	16.12.44	27·5	—	—	—	—
	18.12.44	—	24·4	~0	2·1	4·6
	16. 1.45	—	—	~0	2·1	—
	24. 1.45	—	—	1·8	2·6	—
	9. 5.45	22·8	—	2·5	3·8	—
	14. 5.45	16·6	—	1·0	4·2	—
Williams, H.						
Normal diet	19.10.44	12·5	29·0	—	—	—
	20.10.44	16·0	17·0	—	—	—
0	13.11.44	—	—	—	—	—
	28.11.44	13·3	17·0	—	—	—
	20.12.44	27·0	—	—	—	—
	16. 1.45	—	16·7	0·3	2·2	2·7
	24. 1.45	—	—	2·4	5·5	—
	9. 5.45	27·4	—	1·3	4·1	—
Wodeman						
Normal diet	11.10.44	23·5	47·0	—	—	—
	18.10.44	23·5	—	—	—	—
	22.10.44	24·5	29·0	—	—	—
0	13.11.44	—	—	—	—	—
	23.11.44	22·0	—	—	—	—
	28.11.44	23·0	—	—	—	—
	16.12.44	31·5	—	—	—	—
	18.12.44	42·0	—	—	—	—
	19.12.44	38·0	—	—	—	—
	15. 1.45	—	22·6	1·6	13·0	10·5
	24. 1.45	—	—	~0	5·6	—
	26. 1.45	17·0	—	~0	3·0	—
	26. 4.45	17·0	—	1·2	—	—
	12. 5.45	18·0	—	3·4	3·8	—
	14. 5.45	19·0	—	2·9	6·8	—
	23. 5.45	20·0	—	3·4	—	—
10	25. 6.45	—	—	—	—	—
20	4.10.45	—	—	—	—	—
	4.11.45	—	19·7	—	—	5·7
	5.11.45	25·0	—	2·0	—	—
830 (Saturation test, see Table 42)	4. 1.46	—	—	—	—	—
Normal diet + 50	18. 1.46	—	—	—	—	—
	10. 2.46	—	25·2	—	—	—
Normal diet	13. 2.46	—	—	—	—	—
	12. 2.46	—	48·2	—	—	38·8

TABLE 39 (*continued*)

Dose of vitamin C (mg./day)	Date	Vitamin C content of urine in 24 hours: Dye C (mg.)	Dye O (mg.)	Formaldehyde dye C (mg.)	DNPH C (mg.)	DNPH O (mg.)
Woodhouse						
Normal diet	21.10.44	27·5	30·0	—	—	—
	22.10.44	21·5	24·0	—	—	—
10	13.11.44	—	—	—	—	—
	23.11.44	19·1	—	—	—	—
	28.11.44	16·2	—	—	—	—
	16. 1.45	—	21·3	~0	1·6	2·7
	9.11.45	—	21·5	—	—	6·3
	17.12.45	33·5	—	—	—	—
640 (Saturation test, see Table 42)	19. 1.46	—	—	—	—	—
	22. 1.46	—	—	0·5	1·6	—
Normal diet + 50	11. 2.46	—	27·8	—	—	15·0
	12. 2.46	—	24·4	—	—	10·4
	11. 5.46	24·6	—	1·7	4·4	—
Another						
Normal diet	13.10.44	34·5	48·0	—	—	—
	18.10.44	34·5	37·0	—	—	—
	19.10.44	31·5	37·0	—	—	—
0	13.11.44	—	—	—	—	—
	23.11.44	15·4	—	—	—	—
	14.12.44	31·0	—	—	—	—
	18.12.44	39·0	—	—	—	—
	14. 1.45	—	—	~0	1·7	—
	17. 1.45	—	19·8	~0	1·7	3·7
	22. 1.45	—	—	~0	2·5	—
	26. 2.45	24·0	—	~0	—	—
	26. 4.45	21·0	—	~0	—	—
	7. 5.45	34·4	—	2·6	2·8	—
	23. 5.45	18·0	—	0·8	—	—
	10. 7.45	15·0	—	~0	—	—

TABLE 40

Dates on which bleeding of the gums, gingivitis, aphthous ulcers, pain or haemorrhage were recorded in the groups receiving supplements

Name	Vitamin C supplement (mg. daily)	Dates when the following signs were noted: bleeding of the gums	gingivitis	aphthous ulcers	tenderness or pain	non-progressive haemorrhages into the gums
Bartley	70		12.11.44	8. 8.45		22. 3.45
			1.12.44			16. 5.45
			13. 2.45			
			22. 9.45			
			15. 1.46			
Garling	70	4. 9.45				
Hill	70	15. 1.45	13.11.44	3. 1.45		
		25. 4.45	7. 2.45	20. 1.45		
		1. 5.45	20. 6.45			
		30. 5.45	17. 7.45			
		12. 6.45	6. 9.45			
		26. 7.45	23.10.45			
			16.11.45			
Golding	10				24. 2.45	14. 5.45
Jackson	10			22.12.44		
				15. 5.45	7. 5.45	20. 7.45
				22. 7.45		
				14. 8.45		
Parry	10			23. 5.45	6. 2.45	
Proctor	10	16. 3.45	13.11.44	13.11.44	21. 3.45	14. 5.45
		3. 7.45	28.11.44			12.10.45
		8. 8.45	2. 1.45			
		14. 9.45	6. 3.45			
		29. 9.45	10. 4.45			
		16.10.45	15. 5.45			
			8. 8.45			
			29. 9.45			
			16.10.45			
			13.11.45			
			15. 1.46			
Way	10	12. 4.45		2. 3.45		
		15. 5.45		24.11.45		
		10. 8.45	27. 1.46			
		5. 9.45				
		6. 9.45				
Whinfield	10	10. 4.44			8. 8.45	
		15. 1.46				
Woodhouse ..	10	20. 6.45	13.11.44	13.11.44	31.12.44	
		14. 8.45	9.12.44		23. 5.45	
		6. 9.45	20. 1.45			
		10.10.45	7. 2.45			
			29. 3.45			
			30. 4.45			
			23. 5.45			
			20. 6.45			
			17. 7.45			
			14. 8.45			
			6. 9.45			
			10.10.45			
			24.11.45			
			20.12.45			
			21. 2.46			

TABLE 41

Dates on which bleeding of the gums, gingivitis, aphthous ulcers, pain or haemorrhage were recorded in the deprived group

(Full details of the scorbutic lesions will be found in the case histories.)

Name	Vitamin C supplement (mg. daily)	Dates when the following signs were noted: bleeding of the gums	gingivitis	aphthous ulcers	tenderness or pain	non-progressive haemorrhages into the gums	Remarks
Drake ..	0	13. 2.45 13. 3.45 14. 6.45 15. 6.45 3. 7.45 17. 7.45	30. 4.45 11.12.45				Scorbutic lesion 29.5.45
Hudson ..	0	13. 7.45 31. 7.45	22.12.44 24. 4.45 27. 7.45			20. 7.45 31. 7.45 3. 8.45	No typical scorbutic lesion
Milburn ..	0	6. 3.45 23. 3.45 10. 4.45 26. 6.45 17. 7.45	14. 5.45 11. 6.45	23. 2.45	22.12.44 30. 4.45	28. 6.45	Small scorbutic lesion 20.7.45
Robinson	0	6. 2.45 6, 3.45 10. 4.45 15. 5.45 12. 6.45 17. 7.45 31. 7.45 8. 8.45 15. 8.45 4. 9.45 29. 9.45 23.10.45 13.11.45 11.12.45 15. 1.46	13.11.44 9. 1.44 31. 1.45 6. 2.45 6. 3.45 10. 4.45 15. 5.45 12. 6.45 17. 7.45 27. 7.45 8. 8.45 4. 9.45 29. 9.45 23.10.45 13.11.45 11.12.45 15. 1.46	12. 6.45 29. 9.45	13.11.44 9. 1.44 6. 2.45 6. 3.45 10. 4.45 15. 5.45 12. 6.45 17. 7.45 27. 7.45 31. 7.45 8. 8.45 4. 9.45 29. 9.45 23.10.45 13.11.45 11.12.45 15. 1.46		Typical lesion 11.6.45
Sanderson	0	21. 6.45 28. 6.45 29. 6.45 3. 7.45 17. 7.45	16. 4.45	12. 6.45	12. 6.45 31. 7.45		Typical lesion 9.7.45

TABLE 41 (*continued*)

Name	Vitamin C supplement (mg. daily)	Dates when the following signs were noted: bleeding of the gums	gingivitis	aphthous ulcers	tenderness or pain	non-progressive haemorrhages into the gums	Remarks
Tridgell ..	0	31. 7.45	17. 7.45	15. 5.45	13. 3.45	17. 7.45	No typical lesion
Williams, D.	0	5. 4.45 8. 4.45 9. 4.45 10. 4.45 11. 4.45 13. 4.45 22. 4.45 27. 4.45	17. 1.45				Typical lesion 24.4.45
Williams, H.	0	30. 3.45 3. 4.45 11. 4.45 21. 4.45 2. 5.45 29. 5.45 29. 6.45 23. 7.45					Typical lesion 28.5.45
Wodeman	0	29. 6.45 30. 6.45	13.11.44	6. 3.45	13.11.44 16. 1.45 6. 2.45 15. 5.45 12. 6.45		Typical lesion 15.5.45
Another ..	0		13.11.44 2.12.44 11. 6.45 25. 6.45	13.12.44 22.12.44 2. 1.45 10. 4.45 19. 6.45		9. 7.45 3. 8.45	No typical lesion

TABLE 42

Vitamin C content of the whole blood, plasma, white blood cells and urine of the volunteers during saturation tests with series of daily doses of 10 *mg. vitamin C per kg. body weight*

(*Vitamin C was estimated by the Cambridge and Oxford teams, in the home laboratory* (*CC, OO*) *and in the Sheffield laboratory* (*CS, OS*). *Methods of estimation used were: the dinitrophenylhydrazine method* (*DNPH*), *titration with* 2:6-*dichlorophenolindophenol* (*dye*), *and titration with dye in conjunction with formol treatment* (*formol dye*). **The beginning of the saturation test is indicated by the use of bold figures in column* 2.)

Date	Dose of vitamin C* (mg./day)	Vitamin C content of various fractions of the blood: Whole blood DNPH OO (mg./100 ml.)	Plasma DNPH OO (mg./100 ml.)	Plasma Dye OS (mg./100 ml.)	White cells Dye OS (mg./100 g.)	Urine: Volume (ml./24 hr.)	Vitamin C content: Dye OO (mg./24 hr).	Dye OS (mg./24 hr.)	Dye CS (mg./24 hr.)	DNPH OO (mg./24 hr.)	Formol dye CC (mg./24 hr.)
Drake											
3. 1.46	20	—	—	—	—	1,070	—	16·4	15·5	—	1·4
4. 1.46	**510**	0·13	—	0·03	3·2	2,270	17·6	25·2	25·3	6·8	13·3
5. 1.46	510	0·21	0·13	0·07	5·6	1,445	13·7	24·0	24·0	8·6	12·4
6. 1.46	510	0·55	0·10	0·15	9·0	1,620	25·0	27·0	27·4	12·2	14·5
7. 1.46	510	0·55	0·28	0·17	9·2	1,455	32·6	36·4	40·7	29·1	28·7
8. 1.46	510	0·75	0·43	0·50	9·1	1,810	88·0	87·0	93·0	67·0	79·0
9. 1.46	510	1·00	0·46	0·60	14·9	1,810	128	136	152	121	126
10. 1.46	510	1·00	0·62	0·81	16·0	1,550	198	201	200	193	199
11. 1.46	510	—	—	—	—	1,700	139	145	142	170	—
12. 1.46	510	0·95	0·81	0·98	17·0	1,565	305	313	321	235	—
13. 1.46	510	—	—	—	—	1,290	472	436	415	243	—
14. 1.46	510	0·96	0·57	0·92	18·0	1,820	295	340	314	200	—
15. 1.46	Uncontrolled diet + 510	—	—	—	—	—	—	—	—	—	—
16. 1.46	Uncontrolled diet	1·05	0·94	0·70	21·8	—	—	—	—	—	—
12. 2.46	Uncontrolled diet	0·76	0·50	0·42	17·2	2,330	20·0	24·5	—	4·4	15·4
14. 2.46	Uncontrolled diet	—	—	—	—	1,850	16·9	19·8	—	7·0	12·5

Garling											
9.12.45	50	—	—	—	—	1,090	—	—	10·9	—	—
10.12.45	50	—	—	—	—	1,610	13·4	18·6	17·0	12·2	7·3
11.12.45	**630**	0·63	0·30	0·29	10·0	1,795	81·5	97·0	88·6	90·0	86·0
12.12.45	630	1·00	0·45	0·69	9·6	1,590	107	125	130	123	125
13.12.45	630	1·15	0·64	0·89	11·3	1,480	160	188	196	192	—
14.12.45	630	1·26	0·92	0·90	14·2	1,570	285	342	299	336	—
15.12.45	630	1·34	0·90	0·92	16·1	995	—	317	353	—	—
16.12.45	630	1·15	—	0·98	18·0	850	—	170	178	—	—
17.12.45	630	1·23	1·10	0·99	19·0	1,600	—	317	319	—	—
18.12.45	630	—	—	—	—	1,510	—	252	274	—	—
19.12.45	630	1·15	—	0·98	18·0	2,605	—	250	258	—	—
11. 1.46	Uncontrolled diet + 50	0·78	0·53	0·50	13·6	—	—	—	—	—	—
16. 1.46	Uncontrolled diet + 50	—	—	—	—	lost	—	—	—	—	—
17. 1.46	Uncontrolled diet + 50	—	—	—	—	2,350	18·3	20·7	—	7·0	7·0
10. 2.46	Uncontrolled diet	—	—	—	—	1,565	19·3	23·7	—	7·1	10·4
12. 2.46	Uncontrolled diet	0·80	0·45	0·42	15·5	2,135	19·8	23·7	—	8·0	13·0
Golding											
5. 1.46	10	—	—	—	—	1,315	10·6	15·4	14·4	0·0	7·7
9. 1.46	**570**	0·07	0·0	0·02	2·2	1,550	14·3	17·3	17·0	4·7	10·5
10. 1.46	570	0·17	0·08	0·06	2·9	1,960	17·8	21·6	20·3	5·9	12·9
11. 1.46	570	0·26	0·15	0·13	4·2	1,730	17·2	23·5	22·3	6·5	13·5
12. 1.46	570	0·55	0·41	0·28	10·0	2,500	63·0	67·5	68·0	47·5	55·2
13. 1.46	570	0·81	0·75	0·75	11·5	1,600	75·5	92·0	86·0	62·5	86·0
14. 1.46	570	0·87	0·79	0·78	14·1	1,760	285	300	275	188	296
15. 1.46	570	1·15	0·92	0·98	14·2	1,796	324	328	356	254	325
16. 1.46	570	—	—	—	—	1,890	183	195	206	172	195
17. 1.46	570	1·15	1·01	0·98	13·3	1,135	256	341	335	282	341
18. 1.46	570	—	—	—	—	1,945	326	332	303	202	332
19. 1.46	Uncontrolled diet + 50	1·25	0·93	0·88	16·7	—	—	—	—	—	—
12. 2.46	Uncontrolled diet	—	—	—	—	2,075	23·6	24·4	—	9·3	12·9
13. 2.46	Uncontrolled diet	0·93	0·83	0·64	15·3	1,790	19·2	19·8	—	7·3	12·5

TABLE 42 (*continued*)

Date	Dose of vitamin C* (mg./day)	Vitamin C content of various fractions of the blood				Urine					
		Whole blood DNPH OO (mg./100 ml.)	Plasma DNPH OO (mg./100 ml.)	Plasma Dye OS (mg./100 ml.)	White cells Dye OS (mg./100 g.)	Volume (ml./24 hr.)	Vitamin C content: Dye OO (mg./24 hr).	Vitamin C content: Dye OS (mg./24 hr.)	Vitamin C content: Dye CS (mg./24 hr.)	Vitamin C content: DNPH OO (mg./24 hr.)	Vitamin C content: Formol dye CC (mg./24 hr.)
Hill											
9.12.45	50	—	—	—	—	980	—	—	17·8	—	—
10.12.45	50	—	—	—	—	1,820	19·6	21·7	17·8	11·0	11·0
11.12.45	630	0·61	0·18	0·30	9·8	2,615	210	240	204	216	230
12.12.45	630	0·99	0·51	0·69	13·0	1,525	285	351	360	384	350
13.12.45	630	1·20	0·68	1·00	13·2	2,090	352	439	463	500	—
14.12.45	630	1·23	0·57	0·93	14·0	1,400	420	478	480	368	—
15.12.45	630	1·28	0·84	0·94	14·0	1,045	—	512	538	—	—
16.12.45	630	—	—	—	—	680	—	412	432	—	—
17.12.45	630	1·00	0·93	0·85	21·4	1,000	—	360	385	—	—
18.12.45	630	—	—	—	—	915	—	449	434	—	—
19.12.45	630	0·96	—	0·86	21·6	2,360	—	460	500	—	—
11. 1.46	Uncontrolled diet + 50	0·67	0·43	0·46	11·8	—	—	—	—	—	—
16. 1.46	Uncontrolled diet + 50	—	—	—	—	1,700	9·5	18·4	—	5·1	9·2
17. 1.46	Uncontrolled diet + 50	—	—	—	—	1,945	16·3	18·2	—	7·3	6·2
11. 2.46	Uncontrolled diet	—	—	—	—	965	13·3	15·9	—	5·4	7·8
12. 2.46	Uncontrolled diet	0·60	0·40	0·28	15·3	1,480	16·1	21·0	—	4·5	11·2

Hudson											
3. 1.46	20	—	—	—	—	1,225	17·1	21·2	16·3	5·5	3·6
4. 1.46	**670**	0·13	0·03	0·02	4·1	1,640	21·1	31·4	29·0	5·8	11·9
5. 1.46	670	0·17	0·06	0·02	5·0	1,630	23·6	30·6	30·8	(48 ?)	12·6
6. 1.46	670	0·49	0·19	0·18	6·6	895	34·6	35·4	35·5	28·2	27·0
7. 1.46	670	0·63	0·34	0·27	7·8	1,350	85·8	90·0	95·5	81·0	75·5
8. 1.46	670	0·75	0·38	0·43	10·0	2,100	84·0	105	106	82·0	97·0
9. 1.46	670	0·84	0·47	0·48	16·0	1,770	213	221	229	262	210
10. 1.46	670	0·86	0·53	0·62	17·4	1,695	264	260	247	249	257
11. 1.46	670	—	—	—	—	1,725	328	416	430	353	416
12. 1.46	670	0·77	0·56	0·60	21·6	1,610	340	304	298	340	304
13. 1.46	670	—	—	—	—	1,380	332	343	323	390	343
14. 1.46	Uncontrolled diet + 670	0·75	0·58	0·72	21·0	—	—	—	—	—	—
16. 1.46	Uncontrolled diet + 50	0·81	0·62	0·50	22·5	—	—	—	—	—	—
10. 2.46	Uncontrolled diet	—	—	—	—	1,090	19·4	28·2	—	8·7	12·7
11. 2.46	Uncontrolled diet	0·78	0·56	0·33	18·0	1,655	30·3	34·2	—	14·1	18·1
Jackson											
9.12.45	5	—	—	—	—	1,925	—	—	12·8	—	—
10.12.45	5	—	—	—	—	1,460	18·5	20·4	19·2	6·6	5·9
11.12.45	**630**	0·09	0·04	0·05	1·9	2,075	23·8	24·0	21·1	6·2	11·2
12.12.45	630	0·15	0·05	0·12	2·0	1,930	22·4	23·0	20·5	4·6	10·8
13.12.45	630	0·26	0·08	0·12	4·8	2,015	18·4	27·6	27·8	6·0	15·2
14.12.45	630	0·79	0·14	0·27	5·0	2,010	28·5	38·0	29·8	23·2	32·0
15.12.45	630	0·68	0·64	0·83	8·0	1,290	93·0	76·6	81·8	77·5	75·0
16.12.45	630	—	—	—	—	1,130	368	329	302	248	—
17.12.45	630	1·00	0·96	0·89	13·9	1,760	—	250	247	—	—
18.12.45	630	1·10	0·97	0·98	14·3	2,220	—	326	355	—	—
19.12.45	630	1·04	—	0·89	13·4	3,190	—	252	283	—	—
11. 1.46	Uncontrolled diet + 50	1·05	0·78	0·80	13·0	—	—	—	—	—	—
15. 1.46	Uncontrolled diet + 50	—	—	—	—	2,380	43·2	38·6	—	23·8	26·2
16. 1.46	Uncontrolled diet + 50	—	—	—	—	2,450	44·5	52·5	—	11·0	44·0
10. 2.46	Uncontrolled diet	0·92	0·34	0·30	12·9	2,550	20·9	21·1	—	3·8	10·1
11. 2.46	Uncontrolled diet	—	—	—	—	1,785	24·6	23·0	—	10·6	9·5

TABLE 42 (*continued*)

Date	Dose of vitamin C* (mg./day)	Vitamin C content of various fractions of the blood				Urine					
		Whole blood DNPH OO (mg./100 ml.)	Plasma DNPH OO (mg./100 ml.)	Plasma Dye OS (mg./100 ml.)	White cells Dye OS (mg./110 g.)	Volume (ml./24 hr.)	Vitamin C content: Dye OO (mg./24 hr).	Vitamin C content: Dye OS (mg./24 hr.)	Vitamin C content: Dye CS (mg./24 hr.)	Vitamin C content: DNPH OO (mg./24 hr.)	Vitamin C content: Formol dye CC (mg./24 hr.)
Milburn											
3. 1.46	20	—	—	—	—	735	16·2	23·3	19·8	7·4	1·4
4. 1.46	**670**	0·15	0·16	0·06	5·6	1,465	21·6	29·3	27·9	9·9	7·3
5. 1.46	670	0·31	0·16	0·09	4·5	2,250	24·0	31·0	33·7	15·2	12·1
6. 1.46	670	0·51	0·16	0·39	7·7	1,605	43·5	43·5	45·0	28·0	26·1
7. 1.46	670	1·00	0·82	0·71	8·7	1,465	254	300	325	312	279
8. 1.46	670	1·32	1·12	1·04	7·6	1,075	508	440	437	455	430
9. 1.46	670	1·25	1·13	1·09	12·0	1,080	280	332	312	318	329
10. 1.46	670	1·27	1·07	1·10	16·6	1,350	481	444	425	461	—
11. 1.46	670	—	—	—	—	1,010	470	512	500	400	—
12. 1.46	670	1·23	1·15	1·46	17·8	—	—	—	—	—	—
13. 1.46	670	—	—	—	—	—	—	—	—	—	—
14. 1.46	Uncontrolled diet + 670	0·92	0·90	1·00	20·0	—	—	—	—	—	—
15. 1.46	Uncontrolled diet + 670	—	—	—	—	—	—	—	—	—	—
16. 1.46	Uncontrolled diet + 670	1·17	1·13	0·99	21·3	—	—	—	—	—	—
12. 2.46	Uncontrolled diet	0·78	0·68	0·51	12·3	1,490	17·0	17·3	—	7·3	10·2
13. 2.46	Uncontrolled diet	—	—	—	—	655	16·7	23·2	—	7·5	11·3

Proctor											
8. 1.46	10	—	—	—	—	2,915	20·1	25·6	24·0	15·5	7·9
9. 1.46	**560**	1·07	0·04	0·0	2·0	2,780	21·6	24·8	25·0	0·0	12·4
10. 1.46	560	0·12	0·06	0·04	4·0	2,510	22·1	24·3	23·5	8·5	14·3
11. 1.46	560	0·25	0·12	0·14	6·1	2,410	19·0	32·0	29·4	6·4	20·2
12. 1.46	560	0·37	0·46	0·36	8·4	3,350	32·2	40·0	43·0	13·7	24·5
13. 1.46	560	0·75	0·57	0·61	13·0	3,225	72·0	90·2	88·0	47·0	75·0
14. 1.46	560	0·96	0·88	0·97	13·0	3,500	206	220	203	138	202
15. 1.46	560	1·10	1·09	1·20	17·0	3,200	323	266	277	189	223
16. 1.46	560	—	—	—	—	3,710	292	308	328	242	300
17. 1.46	560	1·21	1·32	1·05	18·1	3,075	—	323	350	—	303
18. 1.46	560	—	—	—	—	4,270	324	294	312	184	—
19. 1.46	Uncontrolled diet + 50	1·35	1·05	1·09	20·2	—	—	—	—	—	—
10. 2.46	Uncontrolled diet	—	—	—	—	3,900	43·0	51·0	—	1·5	30·5
12. 2.46	Uncontrolled diet	1·30	1·22	1·00	lost	4,150	36·2	39·2	—	17·0	26·4
Robinson											
3. 1.46	20	—	—	—	—	2,245	27·5	19·5	17·2	5·1	8·2
4. 1.46	**420**	0·13	0·02	0·05	3·7	1,565	15·8	17·8	18·7	2·4	8·7
5. 1.46	420	0·23	0·05	0·09	3·6	1,510	17·3	19·2	27·0	21·5	9·2
6. 1.46	420	0·35	0·17	0·17	7·2	590	12·4	14·6	15·2	8·6	7·6
7. 1.46	420	0·59	0·21	0·32	10·0	1,440	25·8	28·8	31·0	32·5	22·5
8. 1.46	420	0·85	0·61	0·60	13·3	1,330	71·0	72·5	71·5	58·5	62·2
9. 1.46	420	0·92	0·79	0·75	17·5	1,710	104	111	109	102	97·0
10. 1.46	420	1·05	0·82	0·93	17·1	2,250	208	186	184	178	186
11. 1.46	420	—	—	—	—	1,880	168	172	176	140	—
12. 1.46	420	1·05	0·76	1·30	18·4	2,250	212	175	175	114	—
13. 1.46	420	—	—	—	—	1,760	265	224	214	146	—
14. 1.46	420	0·91	0·80	0·92	19·0	2,460	285	304	287	184	—
15. 1.46	420	—	—	—	—	2,080	254	186	194	150	—
16. 1.46	Uncontrolled diet + 420	1·18	0·96	0·82	18·0	2,180	242	250	256	214	—
11. 2.46	Uncontrolled diet	1·35	1·01	0·78	19·6	2,255	36·6	44·0	—	23·6	330
12. 2.46	Uncontrolled diet	—	—	—	—	2,530	24·8	24·7	—	13·3	16·5

TABLE 42 (*continued*)

Date	Dose of vitamin C* (mg./day)	Vitamin C content of various fractions of the blood				Urine					
		Whole blood DNPH	Plasma		White cells Dye	Volume	Vitamin C content				
							Dye			DNPH	Formol dye
		OO (mg./100 ml.)	DNPH OO (mg./100 ml.)	Dye OS (mg./100 ml.)	OS (mg./100 g.)	(ml./24 hr.)	OO (mg./24 hr).	OS (mg./24 hr.)	CS (mg./24 hr.)	OO (mg./24 hr.)	CC (mg./24 hr.)
Sanderson											
3. 1.46	20	—	—	—	—	1,950	17·1	21·0	19·3	7·3	1·0
4. 1.46	660	0·09	0·10	0·02	4·0	1,915	18·4	24·5	24·3	8·6	5·9
5. 1.46	660	0·15	0·11	0·06	4·2	2,060	19·2	25·6	19·0	7·7	12·0
6. 1.46	660	0·39	0·06	0·16	5·5	1,480	21·2	25·0	28·5	11·1	9·3
7. 1.46	660	0·47	0·30	0·29	8·6	1,355	63·0	81·5	89·0	74·2	63·7
8. 1.46	660	0·80	0·56	0·55	10·0	1,520	226	256	249	219	244
9. 1.46	660	1·03	0·73	0·82	15·4	2,155	320	350	330	293	343
10. 1.46	660	1·05	0·71	0·92	16·0	1,315	509	475	463	505	—
11. 1.46	660	—	—	—	—	1,965	401	465	459	412	—
12. 1.46	660	1·05	0·89	1·50	18·9	2,080	225	386	400	283	—
13. 1.46	660	—	—	—	—	1,300	351	380	361	201	—
14. 1.46	Uncontrolled diet + 660	0·95	0·84	0·91	18·6	—	—	—	—	—	—
15. 1.46	Uncontrolled diet + 660	—	—	—	—	—	—	—	—	—	—
16. 1.46	Uncontrolled diet	1·10	0·87	0·94	18·0	—	—	—	—	—	—
11. 2.46	Uncontrolled diet	1·15	(1·82 ?)	0·61	16·5	1,600	26·6	30·2	—	9·6	13·6
12. 2.46	Uncontrolled diet	—	—	—	—	1,405	22·2	24·2	—	8·4	11·6

Trigdell											
8. 1.46	10	—	—	—	—	2,165	lost	25·2	24·6	lost	9·0
9. 1.46	**620**	0·01	0·00	0·03	2·6	1,770	12·6	16·3	16·2	2·7	7·0
10. 1.46	620	0·15	0·01	0·04	3·3	2,230	18·2	22·9	19·8	8·9	14·2
11. 1.46	620	0·24	(0·24 ?)	0·06	6·4	1,860	19·2	23·8	23·4	5·6	15·4
12. 1.46	620	0·35	0·16	0·16	8·6	1,610	16·6	24·2	23·0	9·7	10·7
13. 1.46	620	0·40	0·23	0·18	11·2	2,200	25·5	29·2	31·0	9·9	16·6
14. 1.46	620	0·59	0·25	0·24	11·6	1,630	71·0	84·0	78·3	36·0	74·0
15. 1.46	620	0·77	—	0·50	16·1	2,040	132	110	120	80·0	92·0
16. 1.46	620	—	—	—	—	1,930	184	200	220	203	174
17. 1.46	620	0·99	0·60	0·58	17·0	1,965	130	143	168	133	124
18. 1.46	620	—	—	—	—	1,940	174	232	312	240	—
19. 1.46	620	0·95	0·65	0·59	17·8	2,050	470	470	440	476	—
20. 1.46	620	—	—	—	—	1,275	294	294	—	306	—
21. 1.46	Uncontrolled diet + 50	1·04	0·55	—	—	—	—	—	—	—	—
10. 2.46	Uncontrolled diet	—	—	—	—	1,890	42·4	44·3	—	30·3	20·9
11. 2.46	Uncontrolled diet	1·05	0·71	0·51	22·0	2,645	26·0	28·2	—	9·0	16·5
Way											
9.12.45	5	—	—	—	—	2,425	—	—	17·2	—	—
10.12.45	5	—	—	—	—	1,930	16·0	15·6	15·8	5·0	6·0
11.12.45	**670**	0·07	0·10	0·06	2·1	2,155	22·6	24·8	20·5	9·7	10·0
12.12.45	670	0·10	0·07	0·08	2·9	1,104	16·5	21·0	15·4	6·4	11·0
13.12.45	670	0·20	0·08	0·14	4·3	945	10·6	18·0	16·0	8·0	9·0
14.12.45	670	0·26	0·06	0·20	5·0	2,290	19·2	27·7	22·4	12·6	12·5
15.12.45	670	0·47	0·21	0·24	9·4	1,120	28·3	22·0	24·2	6·0	13·0
16.12.45	670	—	—	—	—	1,150	48·5	48·3	45·7	41·0	34·0
17.12.45	670	1·10	0·59	0·55	10·6	930	97·0	77·6	76·0	76·0	75·0
18.12.45	670	1·37	0·70	0·78	15·2	1,570	80·0	82·0	90·0	67·5	78·0
19.12.45	670	0·91	—	0·75	16·7	1,740	142	142	149	131	140
11. 1.46	Uncontrolled diet + 50	0·64	0·39	0·47	16·0	—	—	—	—	—	—
14. 1.46	Uncontrolled diet + 50	—	—	—	—	1,570	28·5	30·2	—	8·6	17·5
15. 1.46	Uncontrolled diet + 50	—	—	—	—	2,530	27·8	26·2	—	4·8	13·6
10. 2.46	Uncontrolled diet	0·69	0·41	0·25	11·2	1,875	18·9	20·4	—	5·6	9·8
12. 2.46	Uncontrolled diet	—	—	—	—	2,625	18·9	22·3	—	10·8	12·9

TABLE 42 (*continued*)

Date	Dose of vitamin C* (mg./day)	Vitamin C content of various fractions of the blood				Urine					
		Whole blood DNPH	Plasma		White cells Dye	Volume	Vitamin C content				
			DNPH	Dye			Dye			DNPH	Formol dye
		OO (mg./ 100 ml.)	OO (mg./ 100 ml.)	OS (mg./ 100 ml.)	OS (mg./ 100 g.)	(ml./ 24 hr.)	OO (mg./ 24 hr).	OS (mg./ 24 hr.)	CS (mg./ 24 hr.)	OO (mg./ 24 hr.)	CC (mg./ 24 hr.)
Whinfield											
9.12.45	5	—	—	—	—	—	—	—	—	—	—
10.12.45	5	—	—	—	—	2,030	18·0	17·0	16·8	4·6	7·0
11.12.45	**670**	0·06	0·03	0·05	2·1	1,785	20·8	23·2	17·6	6·7	9·3
12.12.45	670	0·13	0·05	0·07	3·5	1,267	16·9	24·0	17·6	5·2	11·0
13.12.45	670	0·19	0·04	0·09	3·0	1,480	17·3	24·0	22·6	8·9	11·0
14.12.45	670	0·33	0·05	0·20	5·6	1,650	16·0	21·6	16·7	9·0	9·6
15.12.45	670	0·47	0·21	0·25	7·8	1,925	21·7	26·9	25·3	14·4	12·3
16.12.45	670	—	—	—	—	1,345	22·3	22·6	20·6	8·2	11·9
17.12.45	670	0·93	0·79	0·64	15·0	1,930	172	135	132	129	132
18.12.45	670	1·13	0·85	0·87	15·0	1,680	301	296	325	304	296
19.12.45	670	1·25	—	0·91	14·6	1,780	—	210	217	—	—
11. 1.46	Uncontrolled diet + 50	1·15	0·81	0·82	22·9	—	—	—	—	—	—
16. 1.46	Uncontrolled diet + 50	—	—	—	—	1,040	48·0	51·0	—	39·0	40·4
17. 1.46	Uncontrolled diet + 50	—	—	—	—	1,355	38·2	45·0	—	26·7	25·2
11. 2.46	Uncontrolled diet	—	—	—	—	1,100	19·2	18·3	—	5·8	10·0
12. 2.46	Uncontrolled diet	0·97	0·79	0·53	17·0	2,780	26·5	31·4	—	14·6	18·8

Wodeman											
3. 1.46	20	—	—	—	—	1,745	7·1	17·6	13·0	2·2	2·9
4. 1.46	**830**	0·12	0·10	0·02	3·9	2,115	17·0	21·2	22·0	6·3	5·1
5. 1.46	830	0·13	0·05	0·14	5·0	1,500	13·8	21·0	22·0	9·0	6·5
6. 1.45	830	0·27	0·15	0·11	6·3	2,085	21·2	21·2	22·0	15·6	8·7
7. 1.46	830	0·45	0·13	0·19	7·3	2,090	18·7	28·7	28·1	15·7	14·8
8. 1.46	830	0·52	0·32	0·26	(6·6 ?)	2,420	32·4	36·4	35·0	29·0	24·0
9. 1.46	830	0·68	0·53	0·46	15·2	2,155	176	191	194	196	180
10. 1.46	830	0·87	0·64	0·70	15·8	2,270	355	265	268	329	265
11. 1.46	830	—	—	—	—	1,720	237	253	268	220	—
12. 1.46	830	0·79	(0·36 ?)	0·77	16·4	1,755	360	290	302	210	—
13. 1.46	830	—	—	—	—	2,420	350	380	357	225	—
14. 1.46	830	0·82	0·55	0·75	18·3	2,230	390	490	478	290	—
15. 1.46	830	—	—	—	—	3,025	327	273	289	191	—
16. 1.46	830	0·80	0·59	0·69	21·0	1,930	334	310	338	250	—
17. 1.46	830	—	—	—	—	2,460	197	214	208	175	—
11. 2.46	Uncontrolled diet + 50	1·10	0·83	0·61	17·7	1,110	lost	25·2	—	lost	13·4
12. 2.46	Uncontrolled diet + 50	—	—	—	—	2,040	40·0	48·2	—	38·8	37·0
Woodhouse											
8. 1.46	10	—	—	—	—	1,065	14·3	15·1	14·4	3·1	5·9
9. 1.46	**640**	0·06	0·03	0·04	4·2	1,610	18·8	23·0	21·5	3·7	12·1
10. 1.46	640	0·27	0·12	0·10	6·2	1,400	16·0	21·6	18·7	6·0	9·9
11. 1.46	640	0·38	0·29	0·19	6·0	1,680	15·2	21·6	22·0	12·6	13·5
12. 1.46	640	0·55	0·56	0·50	11·2	1,380	176	211	203	122	198
13. 1.46	640	0·64	0·90	0·65	13·0	1,820	205	284	248	188	279
14. 1.46	640	0·92	0·75	0·90	lost	1,340	295	310	283	205	—
15. 1.46	640	1·13	0·90	0·95	13·8	1,060	246	213	228	176	—
16. 1.46	640	—	—	—	—	1,865	440	460	485	400	—
17. 1.46	640	1·10	0·94	0·76	16·0	1,550	306	400	405	382	—
18. 1.46	640	—	—	—	—	—	—	—	—	—	—
19. 1.46	Uncontrolled diet + 50	0·84	0·77	0·70	17·0	—	—	—	—	—	—
11. 2.46	Uncontrolled diet	1·12	0·90	1·66	13·8	1,110	25·8	27·8	—	15·0	15·3
12. 2.46	Uncontrolled diet	—	—	—	—	1,300	18·0	24·4	—	10·4	12·9

TABLE 43

*Number of days on which a body temperature above 98·4° F. was recorded for all the volunteers except D. Williams**

Name	Vitamin C supplement (mg. daily)	Raised temperature		Dates of attacks	Remarks
		No. of days	No. of attacks		
Bartley	70	6	1	1.9.45	Attack diagnosed as influenza
Garling	70	0	0	—	
Hill	70	1	1	12.11.45	
Group total ..		7	2		
Group average ..		2·3	0·6		
Golding	10	2	2	26.8.45; 9.10.45	
Jackson	10	3	3	24.2.45; 10.11.45; 22.12.45	
Parry	10	7	5	11.2.45; 2.6.45; 14.6.45; 5.7.45; 20.8.45	
Proctor	10	Records lost			
Way	10	1	1	6.12.45	
Whinfield	10	2	2	15.12.44; 31.1.45	No record after February, 1945
Woodhouse	10	0	0	—	
Group total ..		15	13		
Group average ..		2·5	2·2		

TABLE 43 (*continued*)

Name	Vitamin C supplement (mg. daily)	Raised temperature		Dates of attacks	Remarks
		No. of days	No. of attacks		
Drake	0	2	2	2 while deprived, 26.6.45 1 after dosing with vitamin C, 18.9.45	
Hudson	0	7	7	3 while deprived, 16.12.44; 22.12.44; 28.2.45 4 after dosing with vitamin C, 28.10.45; 12.11.45; 14.11.45; 29.11.45	All but three of the temperature increases were slight, up to 98·5° or 98·6°
Milburn	0	2	2	2 while deprived, 30.1.45; 23.6.45	The temperature increase was associated with cardiac emergency
Robinson	0	1	1	After dosing with vitamin C, 26.10.45	
Sanderson	0	3	3	2 while deprived, 30.1.45; 5.2.45 1 after dosing with vitamin C; 10.9.45	
Tridgell	0	4	3	1 while deprived, 4.4.45 2 after dosing with vitamin C, 12.10.45; 9.1.46	
Williams, H.	0	17	10	All during the depletion period, 14.11.44; 30.12.44; 28.1.45; 20.2.45; 3.3.45; 14.3.45; 20.3.45; 11.4.45; 14.4.45; 8.5.45	
Wodeman	0	1	1	After dosing with vitamin C, 10.2.46	
Another	0	1	1	While deprived, 7.8.45	This attack was associated with chest pains and the subject was given large doses of vitamin C
Group total ..		38	30		
Group average ..		4·2	3·3		

* D. Williams was found to be infected with benign tertian malaria.

TABLE 44

Number and duration of colds as recorded by certain of the volunteers in their own notebooks

Name	Vitamin C (mg. daily)	Date	Number of days	Number of colds	Remarks
Bartley ..	70	9. 1.45	2	1	
		11. 3.45	2	1	
		18. 5.45	5	1	
		27. 9.45	4	1	
		11.10.45	1	1	
		13.12.45	6	1	
		Total	20	6	
Garling ..	70	7. 1.45	15	1	
		26. 2.45	5	1	
		7. 5.45	3	1	
		Total	23	3	
Hill ..	70	12.11.44	1	1	
		13. 1.45	6	1	
		29. 6.45	1	1	
		22. 8.45	1	1	
		29.12.45	7	1	
		Total	16	5	
Golding ..	10	5.12.44	14	1	
		28.12.44	13	1	
		23. 2.45	8	1	
		1. 5.45	11	1	
		1. 6.45	1	1	
		23. 6.45	11	1	
		20.10.45	7	1	
		28.11.45	6	1	
		Total	71	8	
Jackson ..	10	11. 1.45	1	1	
		5. 3.45	1	1	
		1.11.45	3	1	
		10.11.45	7	1	
		Total	12	4	
Way ..	10	3. 1.46	4	1	
		Total	4	1	
Woodhouse	10	23.11.44	3	1	
		29.12.44	1	1	
		9. 4.45	1	1	
		2. 6.45	5	1	
		17. 8.45	1	1	
		Total	11	5	

TABLE 44 (*continued*)

Name	Vitamin C (mg. daily)	Date	Number of days	Number of colds	Remarks
Drake ..	0	8.12.44	2	1	
		27.12.44	16	1	
		Total	18	2	Dosed 3.7.45
Hudson ..	0	7.12.44	16	1	
		21. 3.45	9	1	
		14. 7.45	11	1	Dosed 30.7.45
		2.10.45	13	1	
		11.11.45	16	1	
		9. 1.46	9	1	
		Total	74	6	
Robinson	0	31.12.44	2	1	
		26. 6.45	3	1	Dosed 27.7.45
		26.10.45	1	1	
		Total	6	3	
Sanderson	0	21. 1.45	24	1	
		27. 4.45	17	1	
		18. 6.45	3	1	Dosed 27.7.45
		22. 9.45	7	1	
		Total	51	4	
Tridgell ..	0	13.11.44	8	1	
		28.12.44	4	1	
		15. 1.45	14	1	
		2. 4.45	15	1	
		27. 4.45	3	1	
		2. 5.45	9	1	
		29. 6.45	8	1	Dosed 5.8.45
		9. 8.45	1	1	
		22. 9.45	5	1	
		12.10.45	7	1	
		23.11.45	6	1	
		26.12.45	21	1	
		30. 1.46	5	1	
		Total	106	13	
Wodeman	0	23. 3.45	3	1	Dosed 25.6.45
		9. 1.46	3	1	
		Total	6	2	

TABLE 45

Oral condition of the volunteers at the start of the experiment

Name	Vitamin C supplement (mg. daily)	Dentition	Condition of the gums	General remarks
Bartley ..	70	87 4321 12345 78 87 54321 1234 78	Slight marginal gingivitis	
Garling ..	70	8 321 1234 78 8 54321 123 5	Normal and healthy	
Hill ..	70	87 5 321 123 67 7 54321 1234 78	Slight swelling of interdental papillae and slight gingivitis	
Golding ..	10	5432 3 7 54321 123456	Normal and healthy	
Jackson ..	10	Full dentition	Normal and healthy	
Parry ..	10	7 54321 1234567 7654321 12345 7	Normal and healthy	
Proctor ..	10	87 543 1 1234 8 54321 12345 8	Slight marginal gingivitis front of lower jaw; annular hypoplasia under central incisors	Aphthous ulcer under lower lip; gums occasionally bled on brushing
Way ..	10	7 321 1 3 7 7 4321 12345 7	Normal and healthy	
Whinfield ..	10	76 4321 1234 78 8 65 321 123 56 8	Slight retraction of the gums round the lower incisors	
Woodhouse	10	87 54321 1234567 87 4321 1234 78	Some gingivitis round right upper incisors and canines, with slight amount of periodontal pus	Aphthous ulcer under lower lip
Drake ..	0	54321 123456 8 8 54321 1234 8	Slight gingivitis	
Hudson ..	0	87 54321 123 5 78 87 54321 12345 78	Normal and healthy	
Milburn ..	0	8765 21 12345678 87 54321 12345 78	Normal and healthy	Recurring aphthous ulcers in the mouth

TABLE 45 (*continued*)

Name	Vitamin C supplement (mg. daily)	Dentition	Condition of the gums	General remarks
Robinson ..	0	321 123	Tenderness and retraction round lower incisors, some swelling	
Sanderson	0	7654321 12345678 7654321 1234567	Normal and healthy	
Tridgell ..	0	7 54321 12345 78 87 54321 12345678	Normal and healthy	
Williams, D.	0	Full dentition	Normal and healthy	
Williams, H.	0	87654321 12345678 87 54321 12345 78	Normal and healthy	
Wodeman	0	87 4321 123 78 87 54321 12345678	Localized gingivitis upper alveolar margin; marked recession of upper gums	
Another ..	0	3 3	Marginal gingivitis round lower canines; gums tender	

Note: The mouths of Robinson, Wodeman and Another were considered to be in poorer condition than those of the other volunteers.

TABLE 46

Measurements of capillary resistance by a positive-pressure method, expressed as numbers of petechiae (see p. 48), for all the volunteers individually

(Measurements by J. Pemberton)

Name	Date	Vitamin C supplement (mg. daily)	No. of petechiae
Bartley ..	31.10.44	Normal diet	0
	13.11.44	70	–
	1.12.44		2
	2. 1.45		3
	13. 2.45		0
	13. 3.45		2
	17. 4.45		9
	23. 5.45		1
	12. 6.45		0
	3. 7.45		0
	17. 7.45		1
	8. 8.45		2
	8. 9.45	Normal diet	–
	29. 9.45		1
	16.10.45		0
	11.12.45		1
Drake ..	31.10.44	Normal diet	9*
	13.11.44	0	–
	28.11.44		2
	9. 1.45		6
	13. 2.45		2
	13. 3.45		22*
	17. 4.45		9*
	15. 5.45		1
	12. 6.45		9*
	3. 7.45		1*
	3. 7.45	10	–
	17. 7.45		8
	8. 8.45		4
	4. 9.45		1
	29. 9.45		2
	23.10.45		13
	5.11.45	20	–
	24.11.45		4
	11.12.45		9
	4. 1.46	510	–
	15. 1.46		4
Garling ..	24.10.44	Normal diet	0
	13.11.44	70	–
	28.11.44		3
	2. 1.45		1
	6. 2.45		8
	6. 3.45		0
	17. 4.45		20*
	23. 5.45		10*
	19. 6.45		18
	17. 7.45		0
	14. 8.45		3
	4. 9.45		0
	29. 9.45		1*
	4.10.45	50	–
	16.10.45		3
	13.11.45		2
	11.12.45	630	3
	20.12.45	Normal diet	–
	5. 1.46	Normal diet + 50	–
	15. 4.46		5*
Golding ..	13.11.44	10	–
	28.11.44		0
	17. 1.45		1
	24. 2.45		1
	13. 3.45		0
	21. 4.45		0
	17. 5.45		2
	27. 6.45		1
	17. 7.45		0
	6. 9.45		1
	2.10.45		3
	23.10.45		0
	11.12.45		1
Hill ..	24.10.44	Normal diet	0
	13.11.44	70	–

* On these occasions a crop of petechiae was found under the cuff when the test was ended. The phenomenon showed no correlation with the vitamin C intake.

TABLE 46 (*continued*)

Name	Date	Vitamin C supplement (mg. daily)	No. of petechiae
Hill (*contd.*)	6.12.44		3
	3. 1.45		0
	7. 2.45		2
	12. 4.45		7
	23. 5.45		0
	20. 6.45		0*
	17. 7.45		0
	6. 9.45		0
	8.10.45	50	–
	23.10.45		0
	10.11.45		1
	11.12.45	630	0
Hudson ..	31.10.44	Normal diet	1
	13.11.44	0	–
	28.11.44		3
	2. 1.45		0
	6. 2.45		7
	6. 3.45		0
	10. 4.45		7
	15. 5.45		4
	19. 6.45		30
	3. 7.45		0
	17. 7.45		25
	31. 7.45		100
	31. 7.45	10	–
	8. 8.45		5
	14. 8.45		0
	4. 9.45		9*
	29. 9.45		1
	16.10.45		1
	13.11.45		7
	19.11.45	20	–
	11.12.45		14
	4. 1.46	670	–
	15. 1.46		13
Jackson ..	16. 1.45	10	0
	13. 2.45		0
	13. 3.45		0
	10. 4.45		0
	15. 5.45		0
	28. 5.45	0	–
	19. 6.45		3
	3. 7.45		0
	17. 7.45		4
Jackson (*contd.*)	8. 8.45	5	–
	14. 8.45		0
	4. 9.45		1
	16.10.45		0
	13.11.45		0
	10.12.45		0
	11.12.45	630	–
	20.12.45	Normal diet	–
	5. 1.46	Normal diet + 50	–
	15. 1.46		0
Milburn	7.11.44	Normal diet	1
	13.11.44	0	–
	28.11.44		2
	2. 1.45		0
	6. 2.45		1
	6. 3.45		0
	10. 4.45		1*
	15. 5.45		4
	19. 6.45		12*
	3. 7.45		5
	17. 7.45		5
	21. 7.45	6,000	–
	22. 7.45	Normal diet + 100	–
	1. 9.45	Normal diet	–
	4.10.45	20	–
	31.10.45		0
	24.11.45		4*
	20.12.45		35
Parry ..	16. 1.45	10	0
	6. 2.45		30
	7. 3.45		0
	17. 4.45		1
	23. 5.45		0
	12. 6.45		0
	23. 7.45		0
	27. 8.45	Normal diet	–
	12. 9.45		0

* On these occasions a crop of petechiae was found under the cuff when the test was ended. The phenomenon showed no correlation with the vitamin C intake.

TABLE 46 (*continued*)

Name	Date	Vitamin C supplement (mg. daily)	No. of petechiae
Proctor ..	21.10.44	Normal diet	0
	13.11.44	10	–
	28.11.44		2
	2. 1.45		2
	6. 2.45		3
	16. 3.45		1
	10. 4.45		13
	15. 5.45		1
	12. 6.45		7
	3. 7.45		2
	17. 7.45		2
	8. 8.45		5
	4. 9.45		4
	29. 9.45		1
	16.10.45		1
	13.11.45		2
	11.12.45		10
	9. 1.46	560	–
	15. 1.46		1
Robinson	31.10.44	Normal diet	50
	13.11.44	0	–
	28.11.44		80
	1. 1.45		37
	6. 2.45		24
	6. 3.45		30
	10. 4.45		30
	15. 5.45		7*
	12. 6.45		100
	17. 7.45		2
	27. 7.45	10	–
	31. 7.45		1
	8. 8.45		50
	15. 8.45		5
	4. 9.45		26*
	29. 9.45		33*
	23.10.45		13
	13.11.45		26*
	19.11.45	20	–
	11.12.45		10
	4. 1.46	420	–
	15. 1.46		15
Sanderson	31.10.44	Normal diet	0
	13.11.44	0	–
	28.11.44		0
	9. 1.45		0
	6. 2.45		1
	6. 3.45		0
	10. 4.45		0
	15. 5.45		2
	12. 6.45		4
	3. 7.45		0
	17. 7.45		0
	27. 7.45	10	–
	31. 7.45		0
	8. 8.45		0
	15. 8.45		1
	4. 9.45		0
	2.10.45		2
	23.10.45		50
	13.11.45		3
	19.11.45	20	–
	11.12.45		7
	4. 1.46	660	–
	15. 1.46		3
Tridgell ..	31.10.44	Normal diet	2
	13.11.44	0	–
	28.11.44		0
	2. 1.45		1
	13. 2.45		0
	13. 3.45		1
	10. 4.45		2
	15. 5.45		0
	12. 6.45		0
	3. 7.45		0
	17. 7.45		0
	31. 7.45		7*
	5. 8.45	10	–
	8. 8.45		0
	15. 8.45		1
	5. 9.45		0
	29. 9.45		0
	23.10.45		1
	13.11.45		2

* On these occasions a crop of petechiae was found under the cuff when the test was ended. The phenomenon showed no correlation with the vitamin C intake.

TABLE 46 (*continued*)

Name	Date	Vitamin C supplement (mg. daily)	No. of petechiae
Way ..	2.12.44	70	1
	18.12.44	10	–
	9. 1.45		4
	6. 2.45		4
	6. 3.45		0
	10. 4.45		0
	15. 5.45		0
	28. 5.45	0	–
	5. 6.45	10	–
	19. 6.45		7
	2. 7.45	0	–
	3. 7.45		0
	17. 7.45		2
	8. 8.45	5	0
	4. 9.45		3
	2.10.45		3
	16.10.45		0
	13.11.45		1
	24.11.45		1
	10.12.45		0
	11.12.45	670	–
	21.12.45	Normal diet	–
	5. 1.46	Normal diet + 50	–
	15. 1.46		0
Whinfield	24.11.44	70	0
	18.12.44	10	–
	9. 1.45		1
	6. 2.45		0
	6. 3.45		0
	10. 4.45		0
	15. 5.45		0
	28. 5.45	0	–
	12. 6.45		1
	8. 8.45	5	0
	4. 9.45		1
	2.10.45		1
	16.10.45		0
	13.11.45		1
	10.12.45		0
	11.12.45	670	–
	21.12.45	Normal diet	–

Name	Date	Vitamin C supplement (mg. daily)	No. of petechiae
Whinfield (*contd.*)	5. 1.46	Normal diet + 50	–
	15. 1.46		1
Williams, D.	2.12.44	0	0
	16. 1.45		0
	7. 2.45		3
	7. 3.45		1
	24. 4.45		2
Williams, H.	31.10.44	Normal diet	8
	13.11.44	0	–
	1.12.44		3
	16. 1.45		1
	7. 2.45		12
	24. 3.45		2
	21. 4.45		4
	29. 5.45		11*
	5. 6.45	20	–
	23. 7.45		12*
Wodeman	31.10.44	Normal diet	1
	13.11.44	0	–
	28.11.44		2
	16. 1.45		3
	6. 2.45		8
	6. 3.45		0
	10. 4.45		0
	15. 5.45		3
	12. 6.45		2
	25. 6.45	10	–
	3. 7.45		0
	17. 7.45		3
	8. 8.45		0
	4. 9.45		2
	29. 9.45		0
	4.10.45	20	–
	16.10.45		0
	13.11.45		0
	11.12.45		0
	4. 1.46	830	–
	15. 1.46		1

* On these occasions a crop of petechiae was found under the cuff when the test was ended. The phenomenon showed no correlation with the vitamin C intake.

TABLE 46 (*continued*)

Name	Date	Vitamin C supplement (mg. daily)	No. of petechiae	Name	Date	Vitamin C supplement (mg. daily)	No. of petechiae
Woodhouse	27.10.44	Normal diet	1	*Another* ..	31.10.44	Normal diet	1
	13.11.44	10	–		13.11.44	0	–
	9.12.44		6		2.12.44		1
	20. 1.45		1		2. 1.45		1
	7. 2.45		3		13. 2.45		1
	29. 3.45		4		13. 3.45		0
	23. 5.45		5		10. 4.45		0
	20. 6.45		10		15. 5.45		0
	17. 7.45		0		19. 6.45		2
	14. 8.45		2		3. 7.45		0
	6. 9.45		0		17. 7.45		2
	10.10.45		2		7. 8.45	3,600	
	24.11.45		2		25. 8.45	Normal diet	–
	20.12.45		2		23.10.45		2

* On these occasions a crop of petechiae was found under the cuff when the test was ended. The phenomenon showed no correlation with the vitamin C intake.

SUMMARY

1. Twenty volunteers were given a diet containing less than 1 mg. vitamin C daily. Three received a vitamin C supplement of 70 mg. daily, seven received 10 mg. and ten had no supplement. No signs of deficiency were observed in those receiving supplements during a period of observation of up to 14 months.

2. All ten volunteers receiving no supplement developed clinical signs of scurvy, though in varying degree. The first changes were enlargement and keratosis of the hair follicles, beginning after 17 weeks of deprivation. Later the enlarged hair follicles became haemorrhagic and formed the characteristic scorbutic spots. Scorbutic gum changes began to appear after 26 weeks of deprivation.

3. Five of the ten deprived volunteers showed a very pronounced exacerbation of the acne present in a mild form at the start of the trial. The exacerbation began after about 22 weeks of deprivation.

4. In one case effusion into both knee joints and ecchymoses of the leg occurred, and two volunteers developed cardiac complications which are described and discussed.

5. A dose of 10 mg. of vitamin C daily given to six of the scorbutic volunteers removed the clinical signs of scurvy in all cases. Within 1 or 2 weeks the scorbutic spots began to fade and within from 7 to 9 weeks the appearance of the skin became normal. The gum lesions responded more slowly, restoration being complete within from 10 to 14 weeks.

6. The volunteers receiving a 70 mg. supplement maintained the initial vitamin C level of the plasma which on an average was 0·55 mg. per 100 ml. In the totally deprived volunteers the vitamin C level of the plasma fell rapidly, reaching about 0·03 mg. per 100 ml. after 37 days and remaining below this value for the rest of the deprivation period. In the volunteers receiving a supplement of 10 mg. the plasma level of vitamin C was of the same order throughout as in the totally deprived group. Thus a difference in the vitamin C intake of 10 mg. daily (enough to prevent and cure clinical scurvy) was not reflected in differences in the plasma level of vitamin C.

7. The volunteers receiving a 70 mg. supplement maintained the initial vitamin C level of the white blood cells which on an average was 16.6 mg. per 100 g. In the totally deprived volunteers the vitamin C level of the white blood cells fell to 1 mg. per 100 g. in 113 days, remaining below this value for the rest of the deprivation period. In the volunteers receiving a supplement of 10 mg. the vitamin C level in the white cells also fell but remained roughly 1 mg. per 100 g. above the value for the deficient group.

8. In the deprived group there was no change in body weight and no increased incidence of infection although colds seemed to last longer. Dark adaptation measurements and audiometry gave no abnormal results. The haemoglobin concentration, red cell count, white cell count and bleeding time showed no significant changes.

9. Conventional tests of so-called capillary strength failed to show correlation with the state of vitamin C depletion.

10. Pains in the back, joints and limbs were reported with increasing frequency by the depleted volunteers as the signs of scurvy developed.

11. No significant variations from normal were observed in the plasma values for protein, urea or phosphatase, or in the albumin-globulin ratio.

12. Experimental wounds were made with the object of studying the process of wound healing. The scars left after such wounds had been excised became

haemorrhagic at the height of scurvy. Examination of scar tissue by histological methods and breaking-strain tests gave abnormal findings in the deprived group but not in the two groups receiving a supplement.

13. Saturation tests carried out towards the end of the experiment differentiated between vitamin C intakes above 20 mg. but not between the levels so important in practice of 20 mg. and below. An intake of 10 or 20 mg. (sufficient to cure and prevent scurvy) gave about the same result as an intake of 5 mg. (which is below the safety level).

14. Administration to scorbutic volunteers of a tyrosine supplement, which increased the normal daily tyrosine intake from about 5·6 g. to 25 g., did not lead to an increase in urinary excretion of tyrosine and its derivatives as measured by the phenol test of Folin and Ciocalteu.

15. The changes in the capillaries round the hair follicles were examined by capillaroscopy and are described in detail.

16. The urine of deprived volunteers when the true vitamin C content must have been near zero was used for testing the specificity of vitamin C estimations. Direct titration with 2:6-dichlorophenolindophenol gave values between 13 and 48 mg. It is pointed out that the quantity of material behaving like vitamin C in this method is likely to vary with the diet. The dinitrophenylhydrazine method of Roe and Kuether and the dichlorophenolindophenol-formaldehyde method are more specific, but interfering substances simulating vitamin C were still not completely eliminated.

17. The vitamin C requirements of human adults are considered in the light of the results of the present trial. The fact that a supplement of 10 mg. daily cured clinical scurvy in all six cases examined, together with the observation that 10 mg. daily protected seven volunteers for periods of up to 424 days, are taken to indicate that in the group under test the minimum protective dose of vitamin C, measured by the presence or absence of the signs of scurvy, was in the region of 10 mg. daily. In order to arrive at a figure for a daily allowance which covers individual variations and includes a safety margin, it is suggested that the minimum protective dose of 10 mg. be trebled. An allowance of 30 mg. daily is in accordance with the recommendation by the League of Nations Health Organisation Technical Commission on Nutrition made in 1938.

APPENDIX A

Estimation of Ascorbic Acid in Urine

by L. W. Mapson*

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INTRODUCTION

SEVERAL methods for estimating ascorbic acid in urine have been published, but comparison shows that there are wide discrepancies among the results. It is difficult to test the validity of any method because the various substances in normal urine that interfere with the test have not been characterized, so that it is impossible to make synthetic mixtures of them and thus determine exactly the limits of error. Recovery tests, while valuable as checks, simply indicate that the test will estimate added ascorbic acid, and not whether it includes other substances. The approach has to be a less direct one and consequently the evidence of validity is less conclusive. In the present research, advantage has been taken of the opportunity to examine urine from human subjects with well-defined signs of scurvy. On the assumption that the vitamin C in such urine should be none or very little, while the amounts of other interfering substances should be normal, the specificity of different methods may be assessed.

Four methods were thus tested during the experiment, two involving removal of interfering substances by precipitation with phosphotungstic acid or lead salts, the third that of Roe and Kuether (1943), based on the reaction between the oxidized form of ascorbic acid and 2:4-dinitrophenylhydrazine, and the fourth, a new method based on the use of formaldehyde. Methods in which formaldehyde is used to differentiate ascorbic acid from other reducing substances in foods have been published by Lugg (1942 a, b), by Mapson (1943), and by Snow and Zilva (1944). With suitable modifications the method has been applied to the estimation of ascorbic acid in urine.

METHOD WITH FORMALDEHYDE

The use of formaldehyde to differentiate ascorbic acid from other reducing substances is based on the fact that ascorbic acid condenses with it to give a product without any power to reduce 2:6-dichlorophenolindophenol. The rate of formation of the condensation product is governed primarily by *p*H so that, by changing the *p*H, conditions can be selected to distinguish ascorbic acid from other reducing substances which condense with formaldehyde to give non-reducing compounds, as well as from reducing substances which do not react with formaldehyde at all. The optimum *p*H for the reaction between ascorbic acid and formaldehyde is 3·5; the rate of reaction diminishes rapidly with decreasing *p*H until at *p*H 0·6, even with formaldehyde in relatively high concentration, it becomes very slow. On the other hand, the rate of condensation with formaldehyde of sulphydryl derivatives, sulphides and thiosulphate is rapid at *p*H 0·6. Interference due to sulphur compounds may therefore be eliminated by determining the fall in titre after treatment with formaldehyde at *p*H 0·6, and then determining the further fall in titre when the solution is adjusted to *p*H 3·5; the extent of the last fall represents the reducing titre due to ascorbic acid when the interference has been eliminated of other reducing substances that condense only slowly or not at all with formaldehyde.

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When normal human urine is subjected to such an analysis, there is evidence of the presence of at least three groups of reducing substances, sulphur compounds, ascorbic acid and unknown substances (Fig. A1).

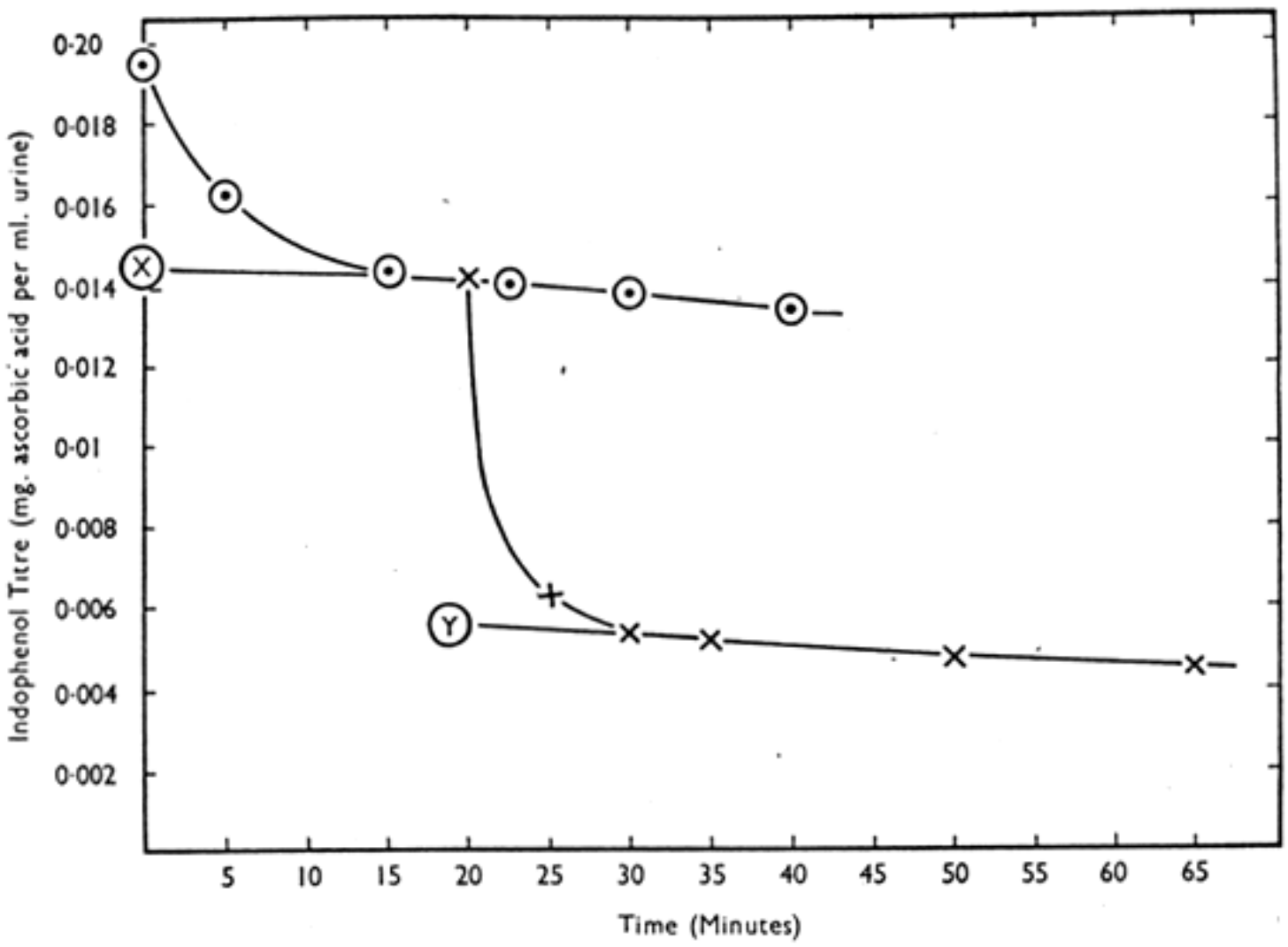

FIG. A1. Estimation of ascorbic acid in normal urine with use of formaldehyde.

—⊙—⊙— urine, *p*H 0·6, treated with formaldehyde (2·5*M*)

—X—X— solution, after 20 min. at *p*H 0·6, adjusted to *p*H 3·5

⊗—Ⓨ reducing titre equivalent to mg. ascorbic acid/ml. urine =0·0091 mg./ml.

In using the method for estimating ascorbic acid in urine, it is assumed that in normal urine ascorbic acid is the only reducing substance that will condense rapidly at *p*H 3·5 with formaldehyde in a concentration of 8 per cent. Of the known reducing substances, which, if present, would interfere at this *p*H, the chief one is hydroxytetronic acid. Interference could be caused also by reductone-like substances such as occur in some processed foods (Mapson, 1943). It would appear, however, from the very low values yielded by this test for the ascorbic acid content of the urine of the scorbutic subjects, that reductone-like substances are not present to any significant extent in otherwise normal urine, but it is essential to bear in mind the possibility of their presence when the results are assessed.

The effect of condensation with formaldehyde on the amounts of reducing substances present in two typical specimens of urine from scorbutic subjects is shown in Fig. A2. Comparison with Fig. A1 shows that only two groups of reducing substances were present since, when the *p*H of the urine was adjusted to 3·5, there was little or no further fall in titre. Experience with many samples of urine has shown that the reaction between formaldehyde and the first group of reducing substances is virtually complete in from 10 to 12 minutes at

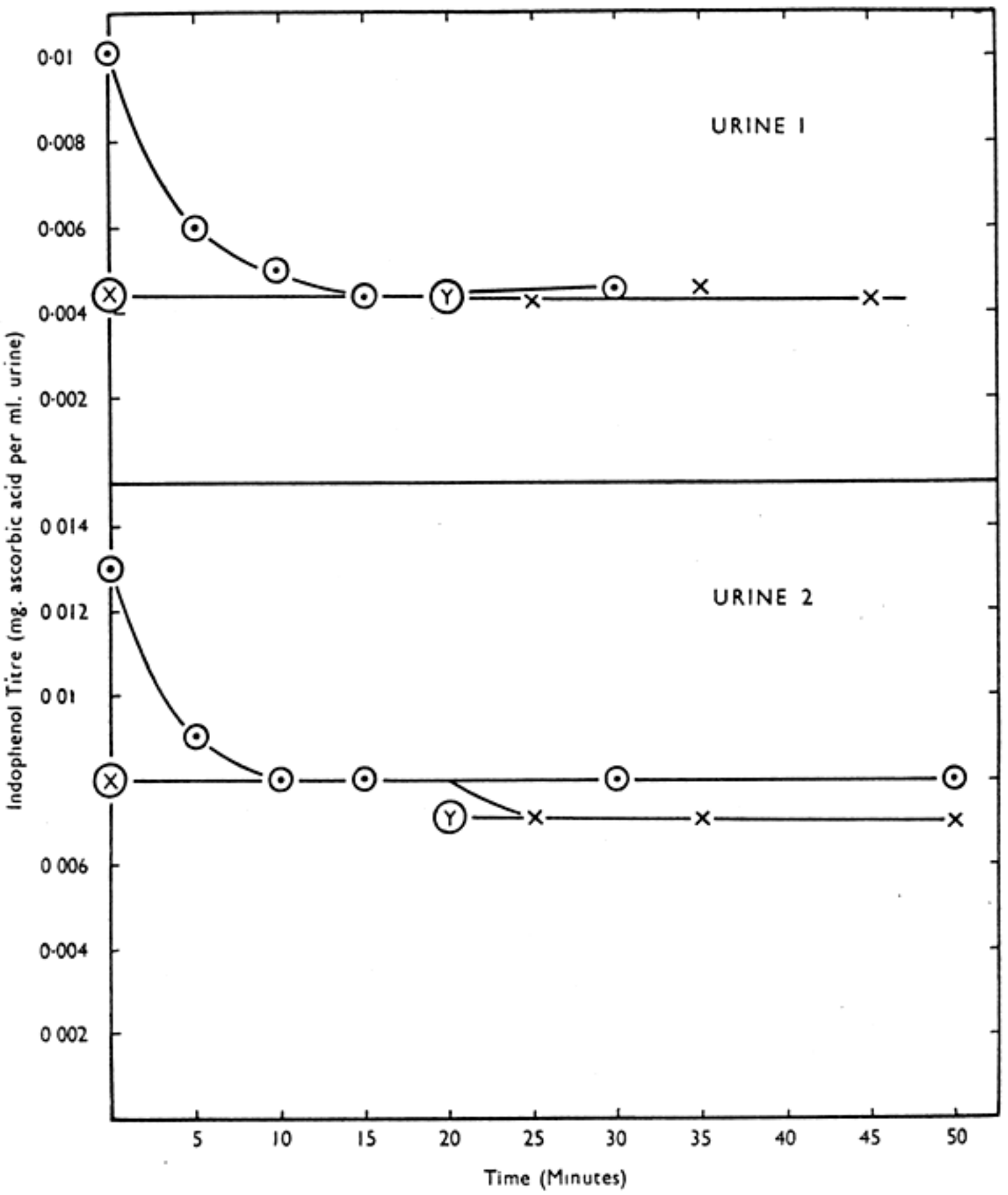

FIG. A2. Estimation of ascorbic acid in scorbutic urine. Procedure as outlined for Fig. A1.

⊗—Ⓨ for urine 1 = 0

⊗—Ⓨ for urine 2 = 0·0008 mg./ml.

20° C. in the conditions of the test. Fifteen minutes has, therefore, been selected as the time to be allowed for their removal by condensation.

To allow for any loss of ascorbic acid, if present, by condensation with the reagent during the period of 15 minutes, the reaction is allowed to proceed for a further 15 minutes; the fall in titre in the second period can be used to evaluate any such losses by extrapolation or by calculation.

Similarly, after adjustment to *p*H 3·5 of a further portion of the urine a period of 15 minutes is allowed for the reaction between formaldehyde and ascorbic acid to be completed, and the reducing titre is determined again at the end of

another 15 minutes, so that interference due to condensation of any of the reducing substances that condense only very slowly at *p*H 3·5 may be allowed for.

At the outset of the work difficulty was encountered because of the formation of heavy precipitates when formaldehyde was added to urine; the precipitation occurred at *p*H values below 2·0 and was found to be caused by a reaction between the formaldehyde and urea. The formation of such precipitates made accurate estimation of the reducing titre very difficult. Attempts to remove urea with specific reagents before the addition of formaldehyde were not successful, but the problem was solved by diluting the day's output of urine to 2,000 ml., if necessary, and by using the formaldehyde in a concentration as high as 2·5*M*.

Detailed Procedure

The details of the method finally adopted were as follows. Urine was collected for 24 hours in containers to which 40 g. of powdered metaphosphoric acid had been added. Precautions were taken to prevent access of light during the collection. The volume of urine excreted in the 24 hours was recorded and, when it was less than 2,000 ml., was made up to that volume with distilled water; a portion was filtered before testing.

The amount of 50 per cent v/v H_2SO_4 solution needed to bring the acidity of 10 ml. of the urine to *p*H 0·6 was determined, and then the amount of 30 per cent w/v sodium citrate solution required to readjust the *p*H to 3·5. The required amount of 50 per cent H_2SO_4 solution was then added to 50 ml. of urine, and the reducing titre of 2 ml. of this solution was determined by the photo-electric procedure described below. Ten ml. of a 40 per cent solution of formaldehyde, adjusted to *p*H 0·6, was then added to 40 ml. of this solution (solution A). The reducing titre of a 2 ml. portion of solution A was determined after the solution had stood at 20° C. for 15 minutes and for 30 minutes. At the end of 20 minutes a 20 ml. sample was removed and pipetted into a flask containing sufficient sodium citrate solution to re-adjust the *p*H to 3·5 and sufficient formaldehyde, adjusted to *p*H 3·5, to maintain a concentration of 8 per cent (solution B). The reducing titre of solution B also was estimated after standing for periods of 15 and 30 minutes.

The value of the reducing titre was corrected in all cases for any dilution with formaldehyde or sodium citrate solution or both, and from these values the concentration of ascorbic acid could be estimated by graphical extrapolation, or by calculation according to a formula similar to that proposed by Snow and Zilva (1944).

If V_1 = reducing titre of the urine after 15 minutes at *p*H 0·6
V_2 = ,, ,, ,, ,, ,, ,, 30 ,, ,, ,, 0·6
V_3 = ,, ,, ,, ,, ,, ,, 15 ,, ,, ,, 3·5
V_4 = ,, ,, ,, ,, ,, ,, 30 ,, ,, ,, 3·5
X = percentage of ascorbic acid condensed at equilibrium (98 per cent),

then the ascorbic acid concentration for 2 ml. of urine

$$= \frac{100}{X} (2V_1 - 2V_3 - V_2 + V_4)$$

By this means allowance is made for any condensation of ascorbic acid at *p*H 0·6 and for condensation of reducing substances other than ascorbic acid at *p*H 3·5.

Assessment of the End Point in Determining the Reducing Titre

The mode of estimating the reducing titre of urine with 2:6-dichlorophenol-indophenol calls for some comment. The accuracy of the method depends ultimately on the values for the reducing titre obtained during treatment with formaldehyde, and several methods of determining them were examined. The concentration of ascorbic acid in the urine was often only about 0·5 μg. per ml. or less, representing about 1 mg. in a daily output of 2,000 ml., so that the need for accuracy requires no emphasis. Some visual methods of titration were tried, and of these, that in which an excess of dye was added to the urine was the best, the excess being titrated with ascorbic acid; this kind of method, however, proved to be insufficiently accurate to yield consistent results, owing to the subjective difficulty of determining the exact end point, and was, therefore, abandoned. More consistent results were obtained by the use of a photo-electric colorimeter, and all the results given in this Report have been obtained by using this procedure.

The photo-electric colorimeter contained a Contrast Green Wratten filter 61N, with an absorption range of 490 to 600 mμ., with maximum transmission at 520 mμ. Glass cells of 10 ml. capacity were used with the instrument, which was calibrated with solutions of pure ascorbic acid and of indophenol dye. The reducing titre of the urine was determined as follows: 2 ml. of the urine were added to a total volume of 8 ml. of solution in the cell. The 8 ml. of solution contained

TABLE 1A

Content of ascorbic acid determined by the indophenol dye method and without formaldehyde condensation in samples of urine from scorbutic subjects, together with the content after formaldehyde condensation, of ascorbic acid added to the same samples of urine

Sample no.	Scorbutic urine		Amount of ascorbic acid added to scorbutic urine (mg./l.)	Total amount of ascorbic acid present in urine (mg./l.)	Total amount of ascorbic acid found by formaldehyde method in urine (mg./l.)
	Amount of ascorbic acid found with formaldehyde (mg./l.)	Total reducing titre estimated by dye method without formaldehyde and expressed as ascorbic acid (mg./l.)			
1	0·0	10·0	20·6	20·6	20·3
1	0·0	10·0	8·0	8·0	7·0
1	0·0	10·0	10·0	10·0	10·0
1	0·0	10·0	1·9	1·9	1·95
2	1·0	12·5	2·2	3·2	2·85
3	0·6	9·5	10·5	11·1	9·5
3	0·6	9·5	5·2	5·8	5·2
4	0·0	14·6	2·3	2·3	1·9
5	0·0	12·1	3·9	3·9	4·1
5	0·0	12·1	1·6	1·6	2·0
5	0·0	12·1	20·0	20·0	17·0

The standard deviation of the difference between the amount of ascorbic acid present and added and that found is ± 1·095.

5 ml. of a solution of 0.5*M* Na_2HPO_4 buffered with citric acid to *p*H 3·5, 2 ml. of a standard indophenol solution (≡ 0·040 mg. per ml. ascorbic acid) and 1 ml. of water. When the urine sample was at *p*H 0·6, 1 ml. of water was replaced by 1 ml. of a solution containing sufficient sodium citrate to adjust the *p*H to 3·5, so that the reaction between the reducing substances in the urine and the dye was always carried out at *p*H 3·5. Exactly one minute was allowed for the reaction between the reducing substances in the urine and the dye, the reading on the galvanometer being recorded at the end of that time. Excess dye was then immediately decolorized by the addition of one drop of a strong solution of ascorbic acid, and the reading (blank) was made. The blank reading measured the amount of natural colour in the urine. The difference between the values for these readings in terms of indophenol dye, derived from the calibration chart, represented the amount of dye in excess at the end of the reactions. From it the reducing titre of the urine could be obtained.

Recovery Experiments

Table 1A gives the results of recovery experiments in which known amounts of ascorbic acid were added to urine from scorbutic subjects. The results show that with the use of formaldehyde added ascorbic acid could be estimated to within 1 to 2 mg. of the correct value, and often the agreement was closer than this.

COMPARISON OF THE RESULTS FOUND BY THE FORMALDEHYDE METHOD WITH THOSE FOUND BY OTHER METHODS

A comparison has been made of the results of estimating ascorbic acid in the urine of scorbutic subjects by four different methods. In the published accounts of these methods, recovery experiments were reported, indicating that ascorbic acid added to urine could be estimated with a fair degree of accuracy. For the purposes of the present research, however, the important point was not whether added ascorbic acid was estimated, but whether other substances likewise were included. It was for this reason that the urine of scorbutic subjects was examined, since errors of the type under consideration would be more apparent when the excretion of ascorbic acid was likely to be low.

The four methods examined were (1) that of Nagayama, Tomoi and Sagara (1939–40) in which interfering substances are removed by precipitation with phosphotungstic acid; (2) that of Richter and Croft (1943) in which lead acetate is used to remove other reducing substances; (3) that of Roe and Kuether (1943) in which ascorbic acid is oxidized by activated charcoal and the oxidation product is coupled with 2:4-dinitrophenylhydrazine, and (4) that with formaldehyde which has just been described. In addition some of the samples of urine were analysed by Dr. Scarborough by an enzyme method similar to that described by Meiklejohn and Stewart (1941), and by his kind permission his results are quoted in this Report.

The results obtained for scorbutic urine by the precipitation methods with phosphotungstic acid or lead acetate are considerably higher than those obtained by the formaldehyde method (Table 2A). Indeed, in many cases as much as from 6 to 8 mg. of apparent ascorbic acid were estimated to have been excreted in 24 hours. The subjects of the test had at this time been some 5 to 6 months on a scorbutogenic diet and many were already showing signs of scurvy, so that the probability was that substances which were not ascorbic acid were being estimated by the two methods. The results obtained with formaldehyde were

much lower although, even with this method, the three values of 3·4, 3·2 and 3·6 mg. for ascorbic acid excreted in the 24 hours may have included a small amount of some other interfering substance.

TABLE 2A

Comparison of the ascorbic acid content of 24-hour samples of scorbutic urine estimated by the formaldehyde method, the phosphotungstic acid method (Nagayama, Tomoi and Sagara, 1939–40) and the lead acetate method (Richter and Croft, 1943)

Sample no.	Total reducing titre by the dye method expressed as ascorbic acid (mg. daily)	Ascorbic acid content as estimated with:		
		formaldehyde (mg. daily)	phosphotungstic acid (mg. daily)	lead acetate (mg. daily)
1	25·0	1·96	8·5	7·0
2	24·0	1·85	6·3	8·6
3	18·0	0·8	6·8	5·5
4	20·0	3·4	6·0	5·2
5	19·4	3·2	5·2	7·0
6	19·2	1·6	7·2	7·25
7	19·0	1·0	5·0	5·4
8	21·0	3·6	7·4	7·0
9	7·0	0·3	1·5	1·4

The agreement obtained between the results of the method of Roe and Kuether and of the formaldehyde method was better; a considerable number of direct comparisons are shown in Table 3A. Agreement was often very close, and the results did not generally differ by more than 2 or 3 mg. for the total daily excretion of ascorbic acid. For the majority of samples the formaldehyde method yielded lower results than the method of Roe and Kuether. It is not possible from the data available to say which of these two methods gives a more correct answer but the following points should not be forgotten. The formaldehyde method, as used here, fails to estimate any dehydroascorbic acid that might be present, but the method of Roe and Kuether would estimate it. The method of Roe and Kuether would estimate also 2:3-diketogulonic acid, the inactive mutarotated product of dehydroascorbic acid, which Penney and Zilva (1943) have found to be present in urine after the administration of dehydroascorbic acid. It is perhaps for such reasons that the results with formaldehyde were sometimes lower than those given by the method of Roe and Kuether. The formaldehyde method can be adapted to estimate dehydroascorbic acid by first subjecting the urine to treatment with sulphuretted hydrogen at *p*H 3·5 for 2 hours, and then, after removal of the sulphuretted hydrogen, proceeding in the manner described above. A few tests have been made on samples of normal and other urine, and the results set out in Table 4A show that little, if any, dehydroascorbic acid was present. In view of the time needed for such a test, and of the smallness of the amounts of dehydroascorbic acid found, which in many cases were within the experimental error of the method, there would be little advantage in including the added complication of this procedure.

TABLE 3A

Comparison of the ascorbic acid content of scorbutic and other urine, estimated by the formaldehyde method and by the method of Roe and Kuether (1943)

Date	Sample no.	Supplement of ascorbic acid (mg. daily)	Total reducing titre of the urine, determined by the dye method and expressed as ascorbic acid (mg. daily)	Content of ascorbic acid in the urine estimated by: formaldehyde method (mg. daily)	Content of ascorbic acid in the urine estimated by: method of Roe and Kuether (mg. daily)
11. 5.45	19	0	22·0	2·20	4·0
13. 5.45	3	0	19·8	1·94	0·6
6. 5.45	2	0	16·0	4·40	3·2
12. 5.45	22	0	23·0	5·20	5·8
14. 5.45	2	0	20·0	4·90	4·4
11. 5.45	18	0	25·5	4·60	7·8
14. 5.45	27	0	19·5	0·90	3·2
12. 5.45	23	0	18·0	3·45	3·8
14. 5.45	29	0	19·0	2·95	6·8
8. 5.45	9	0	31·4	0·60	5·5
14. 5.45	25	0	24·8	0·80	2·2
9. 5.45	3	0	22·8	2·50	3·8
14. 5.45	28	0	16·6	0·95	4·2
10. 5.45	15	0	24·8	0·60	2·5
13. 5.45	4	0	24·4	4·10	3·2
11. 5.45	21	0	38·6	5·00	5·2
14. 5.45	26	0	25·4	5·80	6·2
7. 5.45	6	0	34·0	2·57	2·8
9. 5.45	4	0	27·4	1·28	4·1
7. 5.45	13	10	30·6	1·94	4·9
8. 5.45	5	10	31·8	3·60	6·6
8. 5.45	10	10	26·4	4·30	6·3
10. 5.45	16	10	18·4	3·60	2·1
10. 5.45	17	10	20·6	3·60	1·8
11. 5.45	20	10	24·6	1·65	4·4
13. 5.45	5	10	18·6	4·70	4·2
7. 5.45	8	70	34·4	9·70	9·8
8. 5.45	7	70	34·6	11·00	10·0
9. 5.45	2	70	31·2	10·50	11·5

Enzyme Method

The results obtained by Scarborough with ascorbic acid oxidase in a method similar to that described by Meiklejohn and Stewart (1941) are shown in Table 5A, together with some results of the formaldehyde method.

Like the two precipitation methods, the enzyme method gave results for the true ascorbic values which were higher than those obtained by the formaldehyde method, the amounts of apparent ascorbic acid excreted by persons on a scorbutogenic diet being often as high as by those receiving 70 mg. ascorbic acid daily. The results are few in number, but they suggest that the method is not as reliable as the formaldehyde method or that of Roe and Kuether.

TABLE 4A

Content of ascorbic acid in urine determined by the formaldehyde method before and after treatment with sulphuretted hydrogen to reduce dehydroascorbic acid

Dietary regime of subject	Ascorbic acid content: before H_2S (mg./l.)	Ascorbic acid content: after H_2S (mg./l.)
1. Scorbutogenic diet	2·8	2·6
2. „ „	1·5	1·9
3. „ „	4·4	4·4
4. „ „	0·9	1·2
5. „ „	2·5	3·9
6. Supplement of 70 mg. ascorbic acid daily ..	9·7	10·8
7. Single dose of 300 mg. ascorbic acid previous to collection	35·0	37·0

TABLE 5A

Content of ascorbic acid in various samples of urine, determined by the formaldehyde method and by an enzyme method

Subject	Date of collection	Daily supplement of ascorbic acid (mg.)	Content of ascorbic acid determined by: enzyme method (mg. daily)	Content of ascorbic acid determined by: formaldehyde method (mg. daily)
Another ..	9.7.45	0	12·8	0·0
Way	9.7.45	0	17·4	0·6
Drake	10.7.45	10	10·3	—
Bartley ..	10.7.45	70	15·6	—
Milburn ..	11.7.45	0	2·4	1·8
Parry	11.7.45	10	0·0	1·0
Whinfield ..	14.8.45	5	7·3	—
Tridgell ..	15.8.45	10	1·6	—
Wodeman ..	15.8.45	10	6·9	—
Jackson ..	15.8.45	5	11·4	—
Garling ..	4.9.45	70	12·1	7·0
Way	4.9.45	5	0·0	0·0
Jackson ..	5.9.45	5	9·5	—
Hudson ..	5.9.45	10	8·4	1·0
Proctor ..	6.9.45	10	15·2*	4·2*
Whinfield ..	6.9.45	5	0·9	3·3

* Volume excreted by this subject on this day exceptionally large (3,460 ml.).

APPENDIX B

Capillary Resistance Measurements at the Sorby Research Institute, Sheffield, November 1944 to February 1946

by H. Scarborough*

THE methods at present available for the determination of capillary resistance are of two kinds. The procedure most commonly used employs the positive-pressure principle, in which an increased intracapillary pressure is maintained for a standard time by inflating to a standard pressure a sphygmomanometer cuff applied to the upper arm. The capillary resistance is assessed in terms of the number of petechiae in a standard area of skin below the cuff. Broadly speaking, there are two varieties of this type of test (see Hess, 1920; Hess and Fish, 1914; Göthlin, 1931; Bell, Lazarus and Munro, 1940), One uses a relatively high collecting pressure applied for a relatively short time (Hess) and the other a lower collecting pressure for a longer time (Göthlin). Both have been used in this study.

The second method of measuring capillary resistance employs the negative-pressure principle in which suction is applied to the capillaries through the skin. There are various ways in which such tests may be made, but in this investigation the capillary resistance was measured in terms of the smallest negative pressure required to produce a single petechial haemorrhage in each of three standard areas on the front of the forearm, the suction being allowed to act for 30 seconds in each case. Later the same technique was applied to areas inside the mouth.

There are three points to which the attention should be directed before the methods used are described in detail. Firstly, serial determination of capillary resistance, by whatever method, is frequently punctuated by sudden temporary fluctuations which cannot at present be explained. This is particularly true of the positive-pressure tests. In the present study an attempt was made to elucidate and so to control, such variations by measuring the temperature of the room and surface of the skin at the times when the tests were made, and by recording the activities of the subjects immediately beforehand. However, even when care was taken to perform the tests under relatively standard conditions, variations were still encountered from time to time. Secondly, examination of data obtained in other studies has shown that the correlations between the results of positive- and negative-pressure tests, though invariably significant, are not of a high order (r lies between $-0{\cdot}2$ and $-0{\cdot}5$). Therefore agreement between the results of the tests is not to be expected at all times. Lastly, all the procedures used in this investigation, except that for determining the capillary resistance in the mouth, have been used repeatedly in other studies.

METHODS

All capillary resistance determinations were made with the subject lying on a couch in a warm room, the temperature of which was always over 19° C. After the first few months no attempt was made to control the temperature of the skin provided that it felt comfortably warm to the observer. The negative-pressure test was applied always to the right arm, and the positive-pressure test to the

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left. The Göthlin and the negative-pressure tests were performed simultaneously, and the Hess test immediately afterwards. Care was taken that the left arm had not been previously congested on the day of the test; the congestion during the performance of the Göthlin test may have influenced the results obtained by the Hess test, and these are therefore not strictly comparable with results obtained in other studies. A parallel investigation carried out to test this point showed that the influence of the 15 minutes' congestion at 50 mm. Hg during the Göthlin test cannot have had much effect on the result of the Hess test.

All potential counts were made in strong artificial daylight under a 5 dioptre lens by two independent observers. The mean of the two counts, which never varied by more than 2 in a petechial count of 10, was recorded as the capillary resistance.

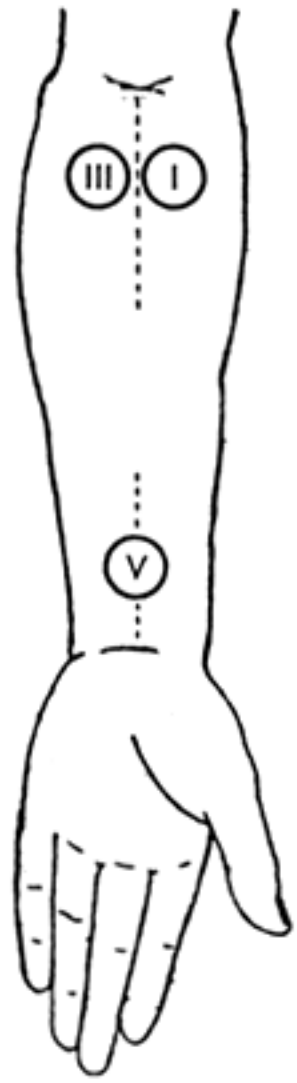
Positive-pressure Tests

The Hess test. The positive-pressure test used by Hess was modified to form a quantitative method for the rapid assessment of capillary resistance. A standard pressure of 100 mm. Hg was applied to a sphygmomanometer cuff on the upper arm and maintained for 3 minutes. After the pressure had been released, the arm was elevated to drain it of blood and the petechiae were counted in a circle 3 cm. in diameter whose centre lay over the insertion of the biceps tendon.

The Göthlin test. Four and a half months from the beginning of the investigation a second positive-pressure test was introduced because the results obtained by the procedure just described were unduly high in a number of subjects. In the Göthlin test a collecting pressure of 50 mm. Hg was maintained for 15 minutes. After release of the pressure the arm was drained of blood and the petechiae were counted in a circle 6 cm. in diameter whose centre lay over the middle of the flexure of the elbow.

Negative-pressure Tests

On the forearm. Suction, measured in mm. Hg, was applied for 30 seconds through a capsule which covered a circular area of skin 2·2 cm. in diameter. Three standard areas of skin were defined on the front of the forearm and each was examined at every test. The two proximal ones lay on either side of the mid-line of the volar surface of the forearm with their proximal edges 4 cm. distal to the elbow flexure; the distal area lay across the mid-line with its distal edge 2 cm. proximal to the flexure of the wrist. These areas, called I, III, V, are shown in the sketch (p. 156). After release of the suction, the skin was examined for petechiae under a standard illumination through a lens system giving a maximum useful magnification of 10, both the source of light and the lens system forming a part of the capsule. The object of the test was to determine the negative pressure just sufficient to produce a single petechial haemorrhage in each area. If the first application of suction for 30 seconds produced no petechia, then the suction was increased by 20 or 25 mm. Hg if the capillary resistance was relatively low (below 250 mm. Hg) and by 50 mm. Hg if it was relatively high (above 250 mm. Hg); the new pressure was applied as before for 30 seconds. The same procedure was repeated until the required pressure was found. If the first application of suction produced more than ten petechiae, the result for that area was discarded. In practice it was rarely necessary to apply suction more than twice to the same area of skin.



In the mouth. Five weeks from the beginning of the investigation a method was devised for measuring the capillary resistance of the looser tissue at the base of the gum between the roots of the teeth and the reflexion of the mucous membrane on to the lip, and of the much firmer gingival tissue investing the roots of the teeth. At first the measurements were made only in the upper jaw immediately to one side of the mid-line. It was hoped in this way to compare alterations in the capillary resistance of a tissue, which is characteristically the seat of pathological lesions early in scurvy, with the condition of a neighbouring area not similarly involved. Determinations of capillary resistance in the marginal areas of gum both in the upper, and later in the lower, jaw had, however, to be abandoned as unreliable. In the 25th week of the study, determinations of capillary resistance in the mouth were extended to include similar (basal) areas in the lower jaw immediately to one side or the other of the mid-line, and also to the inside of the lower lip in order to include an area of mucous membrane not actually part of the gums. Exactly the same method as that previously described for measuring the capillary resistance in the skin was used, but the suction capsule covered a circular area 0·6 cm. in diameter.

The observations on the gums could not have been made without the help of one of the volunteers, Milburn. It is largely due to his continued interest and ingenuity that a satisfactory procedure was devised.

METHODS OF EXPRESSING THE RESULTS

Charts

In order that the measurements of capillary resistance of each subject during the whole study may be rapidly reviewed, a chart for each individual has been prepared (Figs. B 1–20, pp. 166–175).

Statistical Analysis

Negative-pressure results. The results obtained by the negative-pressure method have been submitted to statistical scrutiny.

Although capillary resistance is a normally distributed function, both the standard error of a single observation and the standard error of the mean in many groups of over a hundred subjects are relatively large (coefficient of variation 20 and 30 per cent). In groups of from 5 to 10 subjects and also in larger groups of subjects whose capillary resistance is abnormal, the coefficient of variation may reach 50 per cent. The variance is much reduced if the results are expressed logarithmically.

In other work it has consistently been found that the magnitude of changes in the capillary resistance is related to the initial level before such changes occur. A procedure introduced, whereby an allowance may be made for the effect of the initial level in influencing the final result, involves the use of logarithms. For these reasons it is now customary to measure capillary resistance logarithmically. Measurements obtained in the present investigation have therefore been converted into their logarithms.

Positive-pressure results. In this study the results of the quantitative Hess test and the Göthlin test are not normally distributed and cannot therefore be submitted to a simple statistical procedure. Statistical treatment of these results, even if it were possible, would be complex and it has not, therefore, been attempted.

RESULTS

It may be said at once that determinations of capillary resistance have yielded results of considerable interest. Certain general tendencies suggested by the charts are supported by statistical analysis and it is therefore possible to reach a number of general conclusions. It is convenient first to examine the results in each individual and then to consider differences between the groups in more detail.

Charts

Group Receiving 70 mg. Supplement of Ascorbic Acid daily

(*Bartley, Garling and Hill, Figs.* B 1–3)

Negative pressure. The graphs in this group provide no indication of any progressive alterations in capillary resistance in either the standard areas of the skin or the areas in the mouth.

Positive pressure. In both positive-pressure tests, results which are abnormal by the usually adopted criteria occurred in all three subjects at least once during the period of the investigation. This is a common experience when serial determinations of capillary resistance are made on a single individual but its meaning is doubtful. The variations shown in the charts in both negative- and positive-pressure tests are not greater or more frequent than those commonly found in such investigations.

Group Receiving 10 mg. Supplement of Ascorbic Acid daily

(*Golding, Jackson, Parry, Proctor, Way, Whinfield and Woodhouse, Figs.* B 4–10)

Negative pressure. In three subjects of this group, Jackson, Parry and Whinfield, no progressive alterations in capillary resistance occurred. A fall (standard areas) followed by a recovery is suggested for Jackson. In Proctor and Way (standard

areas) and in Golding and Woodhouse (mouth areas), the graphs suggest some progressive fall in capillary resistance. In some areas this was not very definite and it was not maintained throughout the whole period of dosing with 10 mg. In the group (Jackson, Way and Whinfield), in which the dosage was further reduced, no further fall in capillary resistance occurred, but there is a suggestion of a progressive increase in capillary resistance on a daily dose of 5 mg. given after a period of complete deprivation.

Positive pressure. 'Abnormal' results occurred at various times in four out of the seven subjects.

Group Receiving no Supplement

(*Drake, Hudson, Milburn, Robinson, Sanderson, Tridgell, D. Williams, H. Williams, Wodeman and Another, Figs.* B11–20)

Negative pressure. With the exception of Another, all the subjects showed a progressive fall in capillary resistance quite unlike any results found in either of the other two groups. This gradual fall became evident in the various individuals between 4 and 6 months from the start of the investigation, by which time all the wholly deprived subjects, with the exception of Another and perhaps H. Williams, could be distinguished from the groups having 10 or 70 mg. daily. It is known that females very rarely develop scurvy—a series of 74 cases examined within the last 10 years contained only one female—and it is possible that they may behave differently towards a deficiency of ascorbic acid. However, even H. Williams showed a striking fall in capillary resistance in the gums. As regards Another, the conditions under which the tests were made varied from time to time, and it was thought that the results in his case were not reliable. This volunteer has therefore been excluded entirely from subsequent consideration and his results are not included in any of the statistical analyses. Robinson had a low capillary resistance to begin with and the subsequent fall, though definite, was not conspicuous.

All these changes in the capillary resistance were reversed by dosing. It seems clear that a daily dose of 10 mg. of ascorbic acid was associated with a return of the capillary resistance to values corresponding roughly with those at the beginning of the study; the increase in capillary resistance was relatively rapid (within 5 weeks) in Drake, Hudson and Tridgell, but much slower in Sanderson and Wodeman. An increase in the daily dose of ascorbic acid to 20 mg. had little further effect on the capillary resistance in the standard areas but may have been associated with further improvement in the mouth areas, in which, in any case, the rise in capillary resistance occurred more gradually.

Positive pressure. There is a rough parallelism between the results of the positive-pressure tests and of the negative-pressure ones. This is most clearly seen with Drake, Sanderson, D. Williams and H. Williams. Some of the other records are marred by the unusually high figures for the Hess test in the first 3 months, to which reference has already been made, but which cannot at present be accounted for. Even so, all the subjects, with the exception of Another showed abnormal results in both tests later in the investigation at times when the capillary resistance, measured by the negative-pressure technique, had fallen perceptibly from its initial level.

It is quite clear that in the present study the Göthlin test has proved to be more reliable than the Hess test. The results are not only more regular but follow more closely the negative-pressure values.

Statistical Analysis of the Results of the Negative-pressure Method

Statistical analysis of the available data has yielded a number of conclusions which are stated briefly below. A difference has been taken to be significant when the "t" test gives $P<0{\cdot}05$.

Results at the Beginning of the Study

The results (actual capillary resistance expressed as mm. Hg suction), obtained from the subjects entering the investigation at the time when the first series of measurements was made on November 8–9, 1944, may be summarized thus:

	N	Mean	Standard deviation	Coefficient of variation (%)
AREA I	16	278·1	96·5	35
AREA III	16	337·5	121·8	36
AREA V	16	433·3	105·7	24

For comparison with these figures, results are given which were obtained in a group of apparently healthy medical students, of ages similar to those of the subjects of the Sheffield investigation, examined in Glasgow in June 1941.

	N	Mean	Standard deviation	Coefficient of variation (%)
AREA I	113	283·8	63·8	22·5
AREA III	103	346·1	78·1	22·6
AREA V	92	454·1	71·9	16·1

The figures in the two groups are thus not significantly different.

This finding may be taken as evidence that the initial figures in the Sheffield subjects lay within the normal range.

Analysis of the log values for capillary resistance of all subjects included in the subsequent studies in Sheffield gives:

	N	Mean	Standard deviation	Coefficient of variation (%)
AREA I	20	2·41	0·35	14·5
AREA III	20	2·49	0·17	7·0
AREA V	20	2·61	0·10	4·0

(Four new subjects entered the investigation at the time when the second series of measurements was made on December 13–14, 1944.)

A satisfactory series of measurements in the mouth (base of upper gum) was first obtained on December 21, 1944, as follows (capillary resistance as log value).

	N	Mean	Standard deviation	Coefficient of variation (%)
Upper base	16	2·32	0·15	6·5

Group Receiving 70 mg. Supplement

Standard areas and gums, upper base. It is clear from the charts, and the conclusion is supported by statistical examination, that the results at the end of the investigation are not different from the findings at the beginning. It is to be

noted particularly that the capillary resistance in this supplemented group had not fallen significantly after from 7 to 9 months measured from the beginning of the study. At this time the volunteers in the unsupplemented group were suffering from clinical scurvy.

Group Receiving 10 *mg. Supplement*

Standard areas. The results after 1 month/1 week have been compared with the results at the end of the period of dosing. The length of this period was 6 months/1 week for Jackson, Whinfield and Way, and over 10 months for Parry, Golding, Woodhouse and Proctor.

	Mean differences	"t"	Degrees of freedom	
AREA I ..	—0·019	0·62	6	$0{\cdot}6>P>0{\cdot}5$
AREA III ..	0·041	0·97	6	$0{\cdot}4>P>0{\cdot}3$
AREA V ..	0·107	2·04	6	$0{\cdot}1>P>0{\cdot}05$

These figures indicate no significant fall in capillary resistance.

Gums, upper base. A similar comparison has been made of the figures obtained in this area:

Mean difference	"t"	Degrees of freedom	
0·313	4·75	5	$P<0{\cdot}01$

Thus there was a significant reduction in capillary resistance in this area of the gums. This finding, together with the gradual fall in the results for this area shown in the charts in five out of the seven subjects suggested that a daily dose of 10 mg., while sufficient to prevent any fall in capillary resistance in the standard areas of skin, was insufficient to prevent it in the gums.

Differences between Groups Receiving 70 *mg. and* 10 *mg. Supplements*

It seemed desirable to find whether there were any differences between the findings in the two groups receiving supplements at the time when the deprived group developed scurvy, namely during the period between 6 months/1 week and 9 months/1 week from the start of the study. For this calculation the findings after 6 months/1 week, 7 months/1 week, 8 months/1 week and 9 months/1 week were taken together in both supplemented groups:

	Mean differences	"t"	Degrees of freedom	
AREA I ..	0·063	1·65	25	$0{\cdot}2>P>0{\cdot}1$
AREA III ..	0·056	1·31	25	$0{\cdot}3>P>0{\cdot}2$
AREA V ..	0·006	0·148	25	$0{\cdot}9>P>0{\cdot}8$
Gums, upper base ..	—0·087	1·98	34	$0{\cdot}05<P<0{\cdot}1$

This analysis indicates no differences in capillary resistance in any of the areas between the two groups given supplements. In subsequent analyses, therefore, the two groups have been taken together. Woodhouse was dropped from this comparison because he developed marginal gingivitis.

Group Receiving no Supplement

The first point to be determined was whether there was a significant difference between the results in this group at the beginning of the investigation and the results at the time when the subjects developed clinical scurvy, a period which varied between 6 and 9 months. (H. Williams was not included in this analysis; although the capillary resistance fell progressively, she never developed scurvy.)

The following table compares the findings after 1 month/1 week with the results obtained at the first appearance of frank scurvy:

	Mean differences	"t"	Degrees of freedom	
AREA I ..	0·379	6·26	7	$P<0{\cdot}01$
AREA III ..	0·365	7·84	7	$P<0{\cdot}01$
AREA V ..	0·396	13·80	7	$P<0{\cdot}01$
Gums, upper base ..	0·717	9·68	6	$P<0{\cdot}01$

At the time when the subjects developed scurvy, then, there had been a significant fall in capillary resistance. It therefore seemed desirable to try and find the first point at which the fall could be detected.

The findings after 1 month/1 week were compared with the results after 3 months/3 weeks:

	Mean differences	"t"	Degrees of freedom	
AREA I ..	0·075	1·76	7	$0{\cdot}2>P>0{\cdot}1$
AREA III ..	0·063	2·28	7	$0{\cdot}1>P>0{\cdot}05$
AREA V ..	0·08	2·12	7	$0{\cdot}1>P>0{\cdot}05$
Gums, upper base ..	0·15	2·91	6	$0{\cdot}05>P>0{\cdot}02$

From this table it is clear that even 3 months/3 weeks from the beginning of the study there was a fall of capillary resistance in the gums in the deprived group. At this time there had been, however, no significant fall in the capillary resistance in the standard areas of skin.

Accordingly, a comparison was made of the findings after 4 months/2 weeks with those after 1 month/1 week, as follows:

	Mean difference	"t"	Degrees of freedom	
AREA I ..	0·129	2·65	7	$0{\cdot}05>P>0{\cdot}02$
AREA III ..	0·128	3.53	7	$P<0{\cdot}01$
AREA V ..	0·131	2·49	7	$0{\cdot}05>P>0{\cdot}02$
Gums, upper base ..	0·263	4·77	6	$P<0{\cdot}01$

Thus the fall in capillary resistance, which occurred in subjects taking a diet free from ascorbic acid, had become significant in all areas 4 months/2 weeks from the time the subjects began taking the diet.

Differences between the Deprived Group and Groups Receiving Supplements

It might be that a part of the fall in capillary resistance found in the deprived group was due to variations in capillary resistance occurring in all groups independently of the intake of ascorbic acid. In order to confirm that the differences found in the previous section are actually related to the absence of ascorbic acid from the diet, the results for the subjects who developed clinical scurvy have been compared with the results for the two groups receiving 70 or 10 mg. daily, taken together, at the same period of the study, namely 6 months/1 week, 7 months/1 week, 8 months/1 week, and 9 months/1 week from the start. Of the group receiving 10 mg., only Parry, Golding, Woodhouse and Proctor were available.

	Mean differences	"t"	Degrees of freedom	
AREA I ..	0·319	7·33	34	$P<0·01$
AREA III ..	0·311	6·59	34	$P<0·01$
AREA V ..	0·294	6·60	34	$P<0·01$
Gums, upper base ..	0·479	9·55	31	$P<0·01$

At the time when the deprived group developed scurvy, therefore, their values for capillary resistance were not only significantly different from their own values at the start of the study, but also from those found at the same time in the groups receiving supplements.

The Behaviour of the Deprived Group during Treatment

Milburn, D. Williams and H. Williams had to be omitted from this analysis because of the different treatment they received.

Differences between the value for the capillary resistance at the time of development of scurvy and the value at the end of a period when 10 mg. of ascorbic acid daily had been given. This period was 12 months/3 weeks for Drake, Hudson, Robinson and Sanderson, 14 months/3 weeks for Tridgell and 11 months/1 week for Wodeman.

	Mean differences	"t"	Degrees of freedom	
AREA I ..	−0·342	3·33	5	$0·05>P>0·02$
AREA III ..	−0·325	4·31	5	$P<0·01$
AREA V ..	−0·362	4·81	5	$P<0·01$
Gums, upper base ..	−0·515	4·31	5	$P<0·01$
Gums, lower base ..	−0·525	4·56	5	$P<0·01$
Lip	−0·372	3·01	5	$0·05>P>0·02$

There was, therefore, a significant increase in the capillary resistance in all areas as the result of dosing with 10 mg. ascorbic acid daily.

The figures were accordingly examined to find at what point, after dosing began, a significant improvement could be detected.

Differences between the value for capillary resistance at the time of development of scurvy and the value at the end of dosing with 10 *mg. daily for from* 4 *to* 5 *weeks.*

	Mean differences	"t"	Degrees of freedom	
AREA I ..	−0·282	4·40	5	$P<0·01$
AREA III ..	−0·278	3·83	5	$0·02>P>0·01$
AREA V ..	−0·303	4·77	5	$P<0·01$
Gums, upper base ..	−0·182	1·94	5	$0·2>P>0·1$
Gums, lower base ..	−0·193	2·28	5	$0·1>P>0·05$
Lips	−0·110	1·92	5	$0·2>P>0·1$

There had, therefore, been a significant increase in the capillary resistance in Areas I, III and V, but not in any of the other areas, at the end of a period of dosing with 10 mg. daily for from 4 to 5 weeks.

Differences between results in the gums at the time when scurvy was present and at the end of a period of between 7 *and* 9 *weeks' dosing with* 10 *mg. daily of ascorbic acid.*

	Mean differences	"t"	Degrees of freedom	
Gums, upper base ..	−0·400	3·08	4	$0·05>P>0·02$
Gums, lower base ..	−0·268	1·77	4	$0·2>P>0·1$

There was, thus, a significant increase in the capillary resistance in the base of the upper gum, but not in the lower, after from 7 to 9 weeks' dosing.

Examination of the figures to determine whether the elevation of the capillary resistance as the result of dosing ever became complete, that is, returned to the initial values.

As far as the standard areas of skin were concerned, the following table shows that the initial values had in fact been reached at the end of from 4 to 5 weeks' dosing with 10 mg. daily, for there were by that time no significant differences between the values at the end of that period and the initial results after 1 month/1 week.

	Mean differences	"t"	Degrees of freedom	
AREA I ..	0·070	0·91	5	$0·5>P>0·4$
AREA III ..	0·065	0·87	5	$0·5>P>0·4$
AREA V ..	0·098	1·38	5	$0·3>P>0·2$

As far as the gums were concerned the only results suitable for analysis were those for the upper base. Analysis of the results showed that the increase in capillary resistance as the result of dosing with 10 mg. daily, though significant at the end of a period of from 7 to 9 weeks of dosing, was at that time not yet complete.

	Mean difference	"t"	Degrees of freedom	
Gums, upper base ..	−0·317	4·40	5	$P<0·01$

In other words, there was still a significant difference between the results at the end of that period of dosing and the initial results after 1 month/1 week.

Accordingly the results in the base of the upper gum, after a further period of dosing for about 4 weeks with 20 mg. daily, were compared with the initial findings at the beginning of the study.

	Mean difference	"t"	Degrees of freedom	
Gums, upper base ..	−0·206	2·02	4	$0·2>P>0·1$

This result shows that the capillary resistance in this area of the gums did finally return to its initial strength.

NOTE ON FOLLICULAR HYPERKERATOSIS

Measurement of capillary resistance by the negative-pressure method on the forearm provides particularly favourable circumstances for detecting mild forms of follicular hyperkeratosis, because the well-illuminated skin is being closely inspected under a lens system giving a maximum useful magnification of 10. The following table gives the times at which the condition was first noticed. The volunteers receiving a supplement of 70 mg. ascorbic acid daily did not show the condition.

Occurrence of follicular hyperkeratosis in volunteers receiving 10 mg. supplements or no supplement

Group	Name	Condition first observed	
		Date	Time after start of experiment (months/weeks)
Supplemented with 10 mg. ..	Golding	9.11.44	0
	Jackson	27.2.45	3/3
	Parry	9.5.45	6/1
	Proctor	27.2.45	3/3
	Way	—	—
	Whinfield	27.2.45	3/3
	Woodhouse	2.8.45	9/1
Unsupplemented	Drake	26.2.45	3/3
	Hudson	26.2.45	3/3
	Milburn	26.2.45	3/3
	Robinson	27.2.45	3/3
	Sanderson	9.5.45	6/1
	Tridgell	21.12.44	1/1
	D. Williams	26.2.45	3/3
	H. Williams	26.2.45	3/1
	Wodeman	27.2.45	3/1
	Another	20.3.45	4/2

GENERAL CONCLUSIONS

(1) The development of clinical scurvy on a diet lacking ascorbic acid, but believed to be adequate in other respects, was associated with, and preceded by, a fall in capillary resistance.

(2) The fall was perceptible in the skin of the forearm and in the gums. It was evident first in the gums within 15 weeks, and could be detected in the skin of the forearm within 18 weeks.

(3) A supplement of 10 mg. ascorbic acid daily, though sufficient to protect from clinical scurvy, was not enough to prevent a progressive reduction in the capillary resistance of the gums, though it was probably enough to prevent it in the skin.

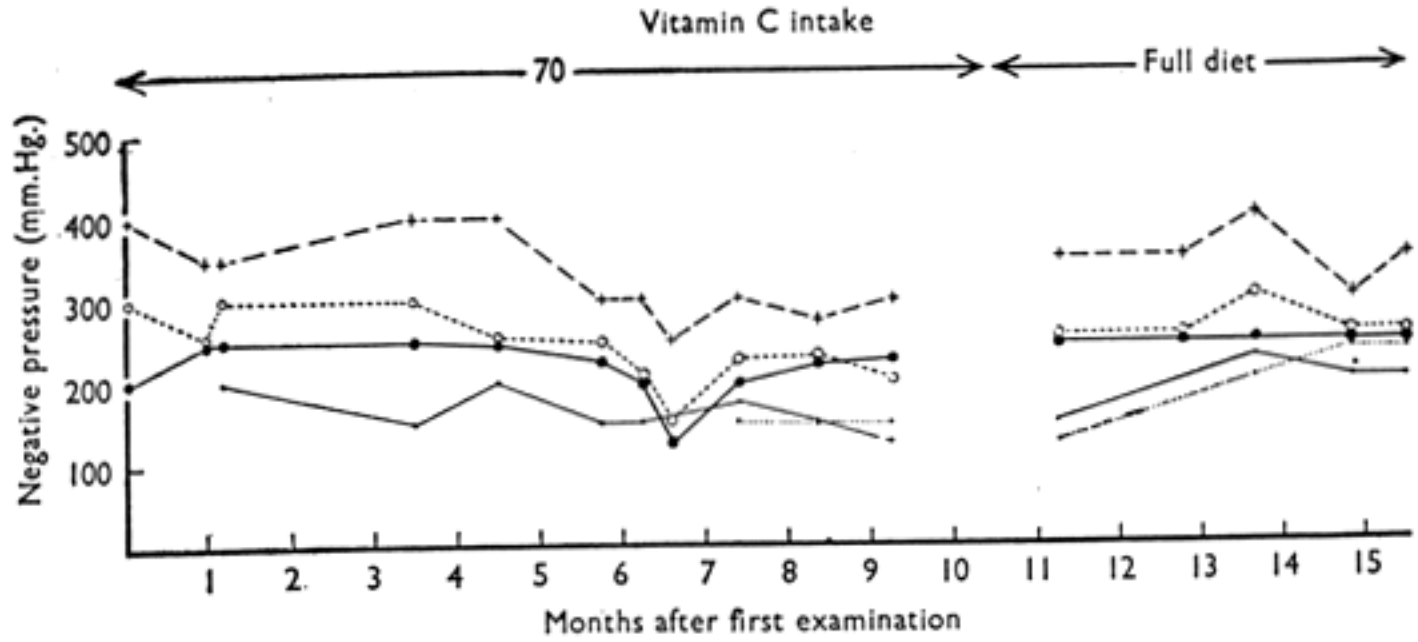
(4) A supplement of 70 mg. ascorbic acid daily was sufficient to prevent any detectable alteration in capillary resistance.

(5) The lowered capillary resistance found in the scorbutic subjects was raised as the result of dosing with 10 mg. ascorbic acid daily. After 4 or 5 weeks' dosing with 10 mg. daily, the capillary resistance of the skin of the forearm had returned to the values found at the beginning of deprivation. The capillary resistance of the gums, however, had not reached the initial values at the end of from 7 to 9 weeks' dosing. After a further period of dosing with 20 mg. ascorbic acid daily for about 4 weeks, the capillary resistance of the gums finally returned to the values found at the beginning of the investigation.

(6) Reduction in capillary resistance as the result of lack of ascorbic acid occurred earlier in the gums than in the skin and was not as easily reversed by dosing. The finding is interesting in view of the early appearance of clinical changes in the gums in the deprived subjects and suggests that the gum tissues may have a greater need for ascorbic acid.

(7) All the volunteers in the deprived group and all save two in the group supplemented with 10 mg. ascorbic acid daily developed follicular hyperkeratosis on the front of the forearm within 3 to 6 months from the beginning of the period of deprivation. These groups therefore differed from the group supplemented with 70 mg. daily in which no lesions were observed.

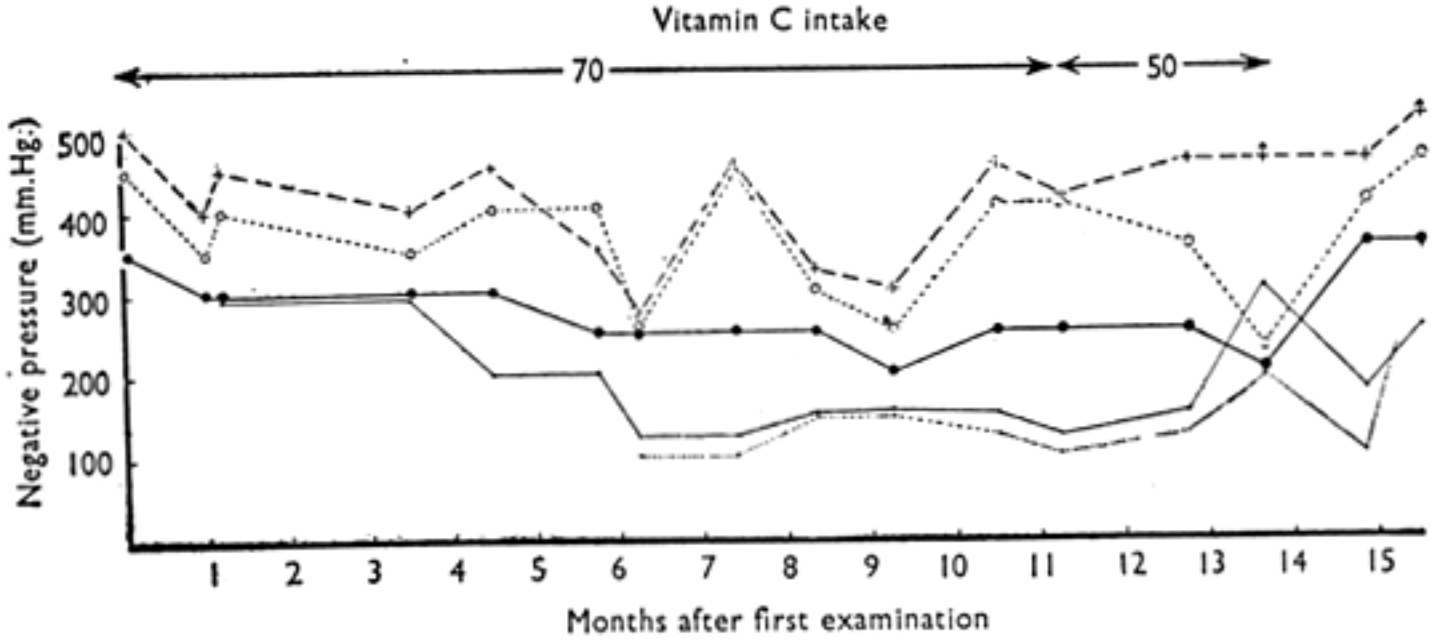
Charts showing results of capillary resistance measurements for each volunteer



Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of petechiae	Hess test	2	5	2	4	10	2	0	*23*	3		3	0	3	5	1
	Göthlin test				2	2	2	3	0	1		1	0	1	2	0

FIG. B1. BARTLEY



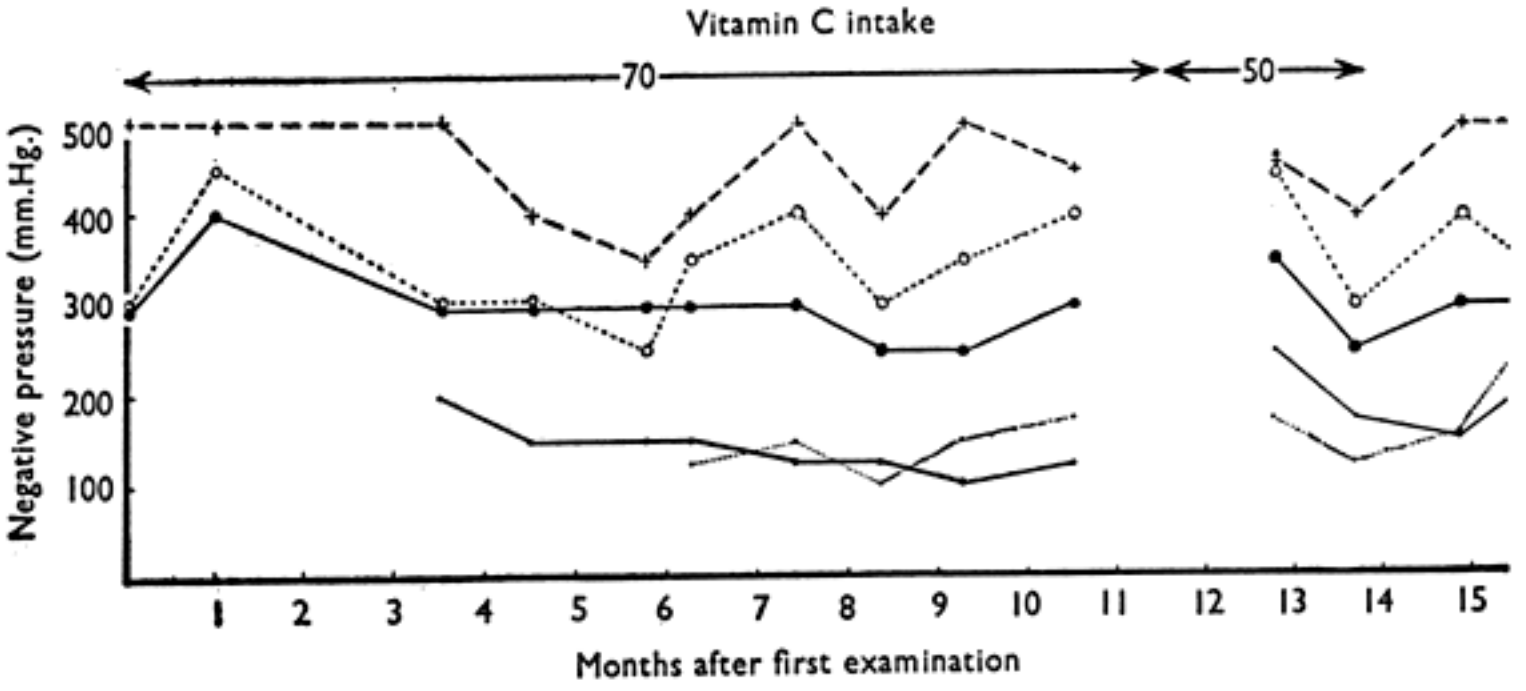
Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of petechiae	Hess test	2	*40*	4	7	6	6	11	*18*	10	9	4	2	6	4	6
	Göthlin test				3	3	*9*	3	5	4	*8*	2	1	2	3	2

FIG. B2. GARLING

KEY: Forearm — Area I —●—●—●—; Area III ···o···o···o···o···; Area V —+—+—+—. Mouth — Upper gum, base —·—·—·—; Lower lip ·····

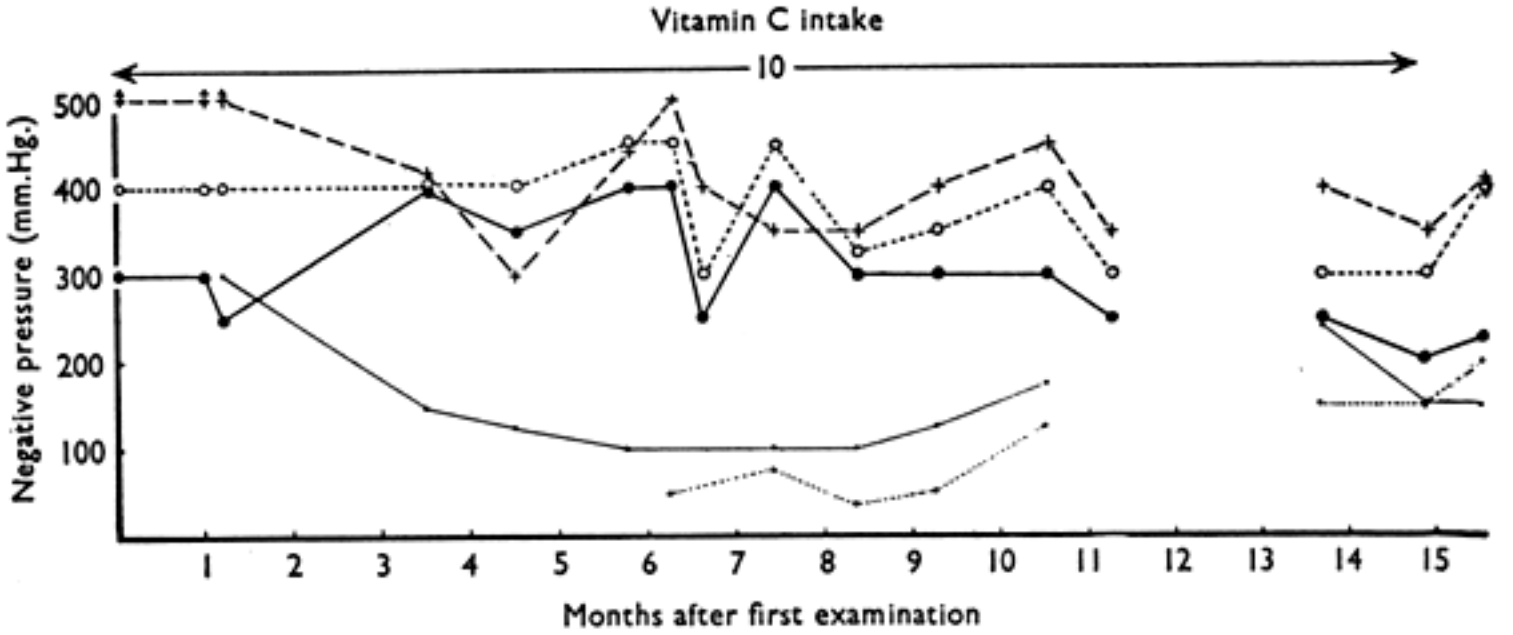
Abnormal positive pressure results are in italics.



Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of pete-chiae	Hess test	5	2	4	8	3	*25*	6	14	1	4		1	1	2	0
	Göthlin test				5	2	*38*	0	2	0	2		3	2	1	0

FIG. B3. HILL



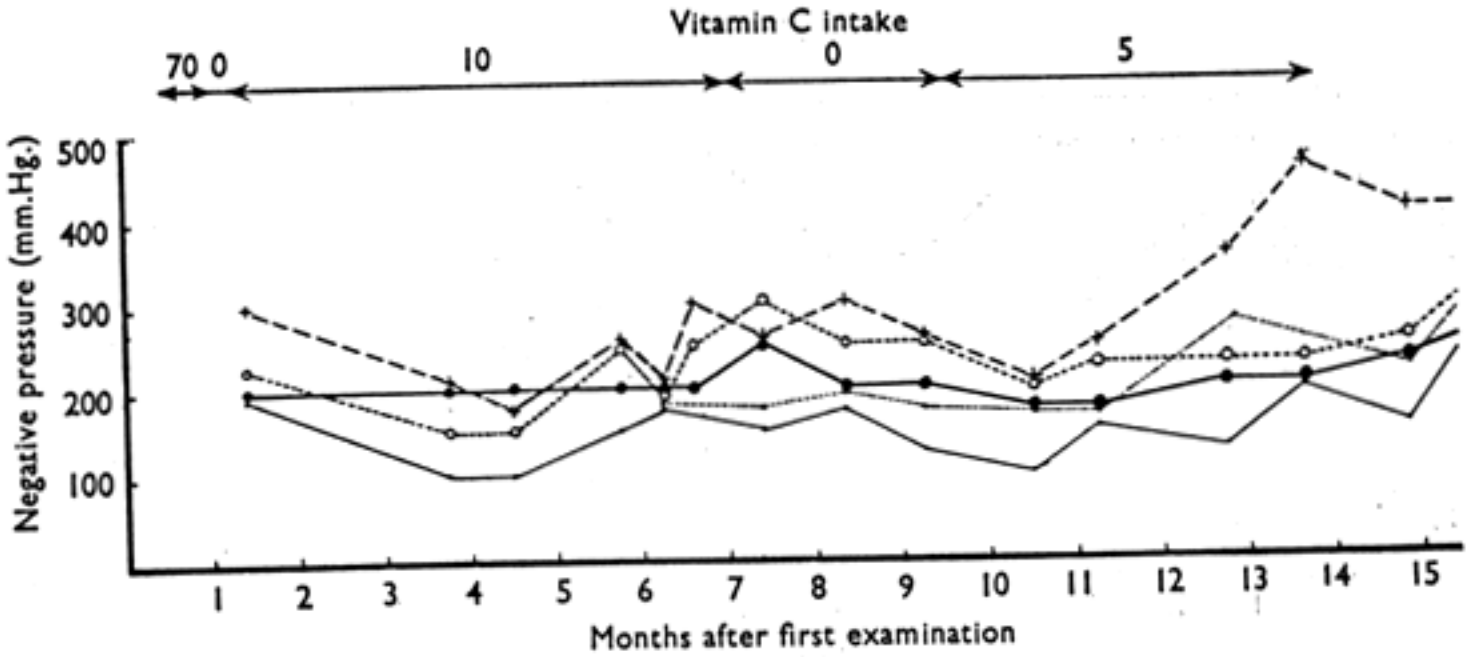
Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of pete-chiae	Hess test	4	0	0	4	0	6	3	4	2	0	3		2	2	0
	Göthlin test				2	1	5	0	3	1	3	2		0	3	0

FIG. B4. GOLDING

KEY: Forearm — Area I —●—●—●—; Area III ···o···o···o···o···; Area V —+—+—+— | Mouth — Upper gum, base —·—·—·—; Lower lip ····•·····•·····•····

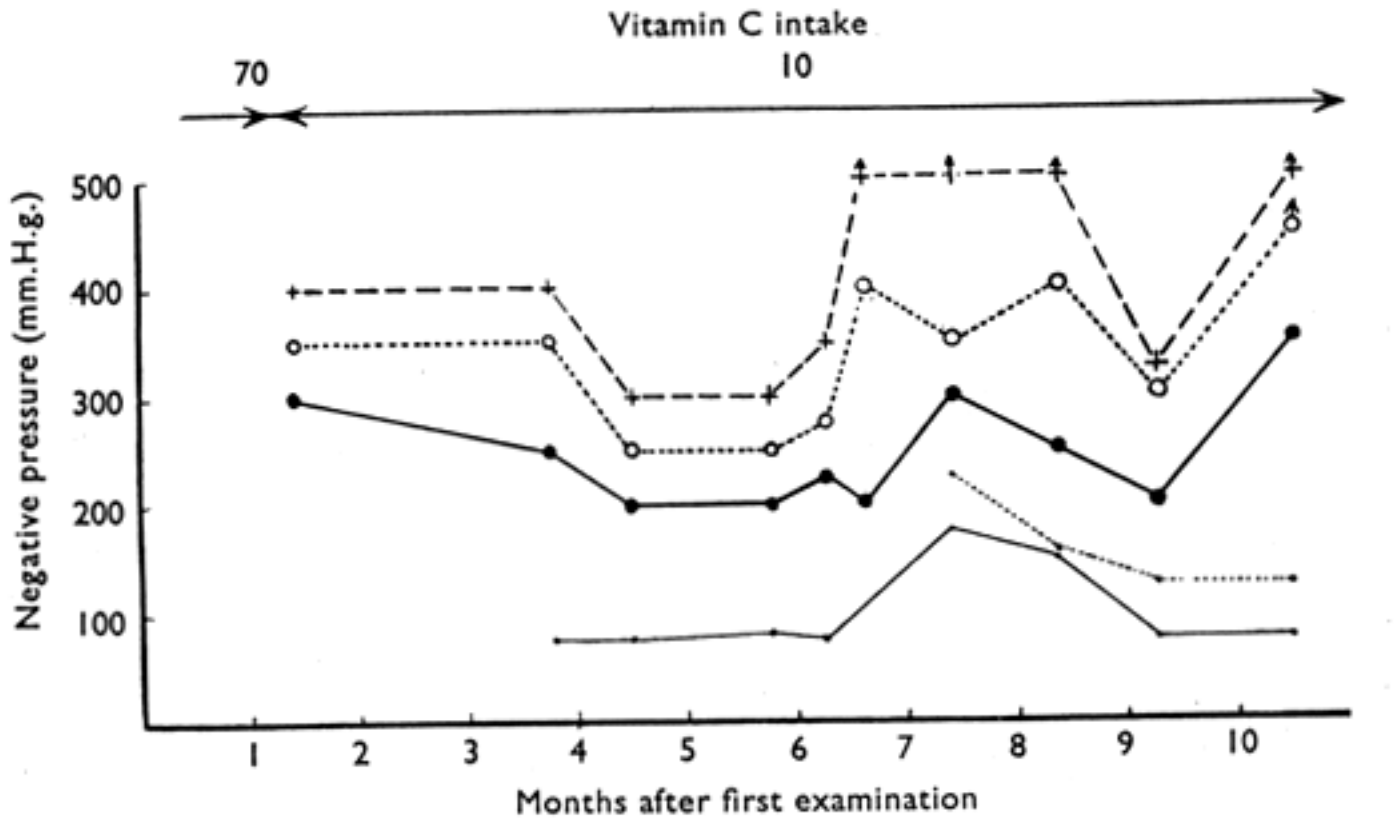
Abnormal positive pressure results are in italics.



Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of petechiae	Hess test		10	3	1	2	4	8	5	6	0	7	5	6	5	2
	Göthlin test				*8*	0	6	6	6	4	*11*	*13*	2	2	5	2

FIG. B5. JACKSON



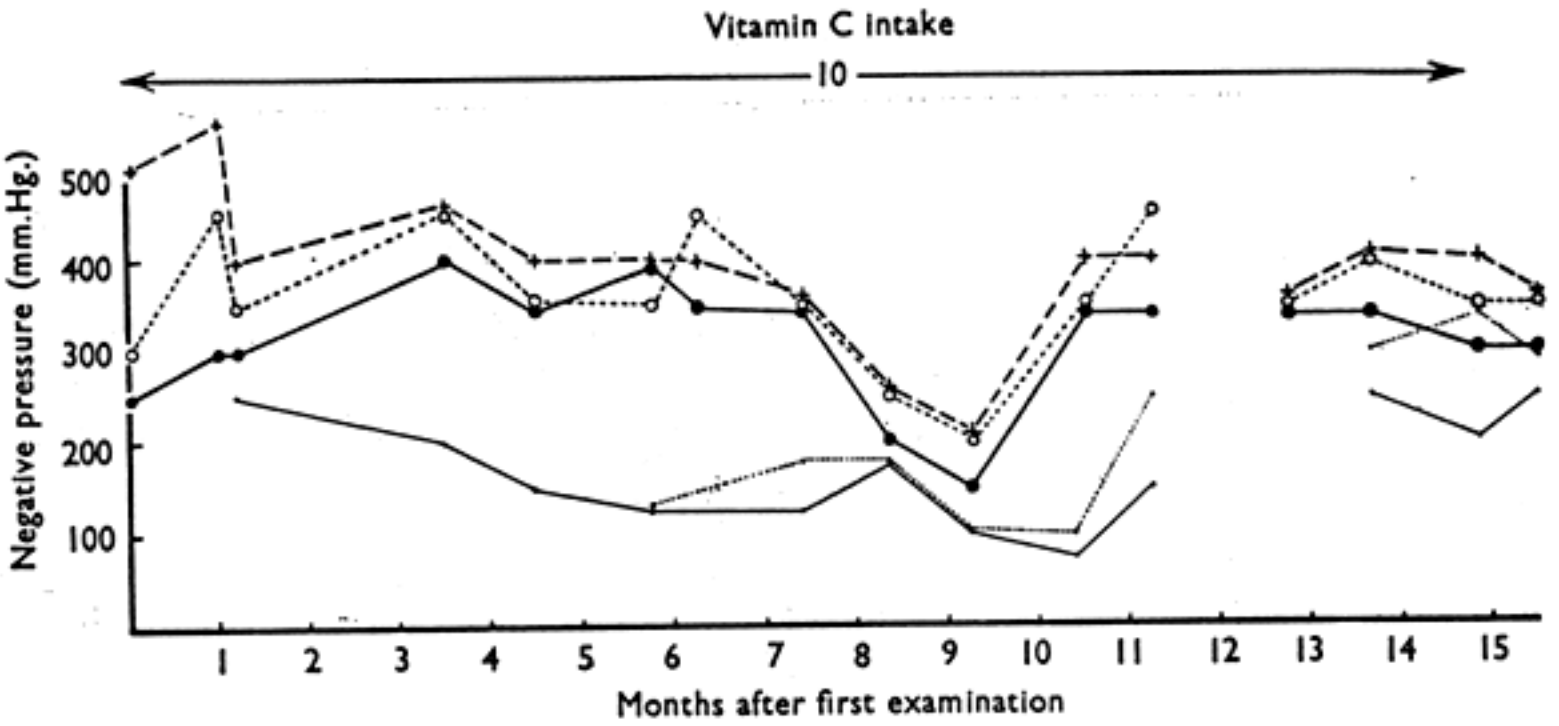
Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·
No. of petechiae	Hess test		*30*	4	8	2	6	1	·8	4	6					
	Göthlin test				4	5	4	0	*8*	1	0					

FIG. B6. PARRY

KEY: Forearm — Area I —●—●—●—; Area III ···o···o···o···o···; Area V —+—+—+—. Mouth — Upper gum, base —·—·—·—; Lower lip ·····

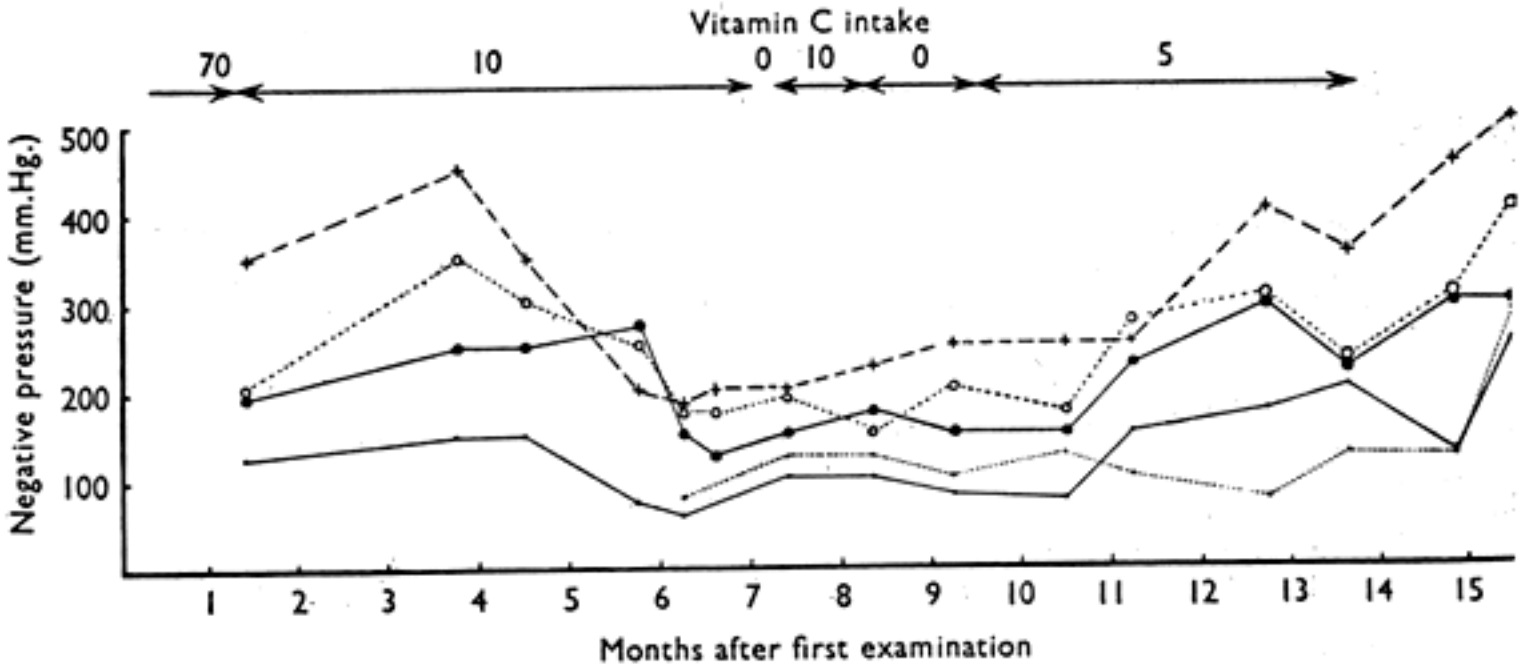
Abnormal positive pressure results are in italics.



Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of petechiae	Hess test	7	1	0	2	3	4	0	7	4	3	2	2	3	2	7
	Göthlin test				2	4	*8*	0	5	3	0	3	3	2	0	1

FIG. B7. PROCTOR



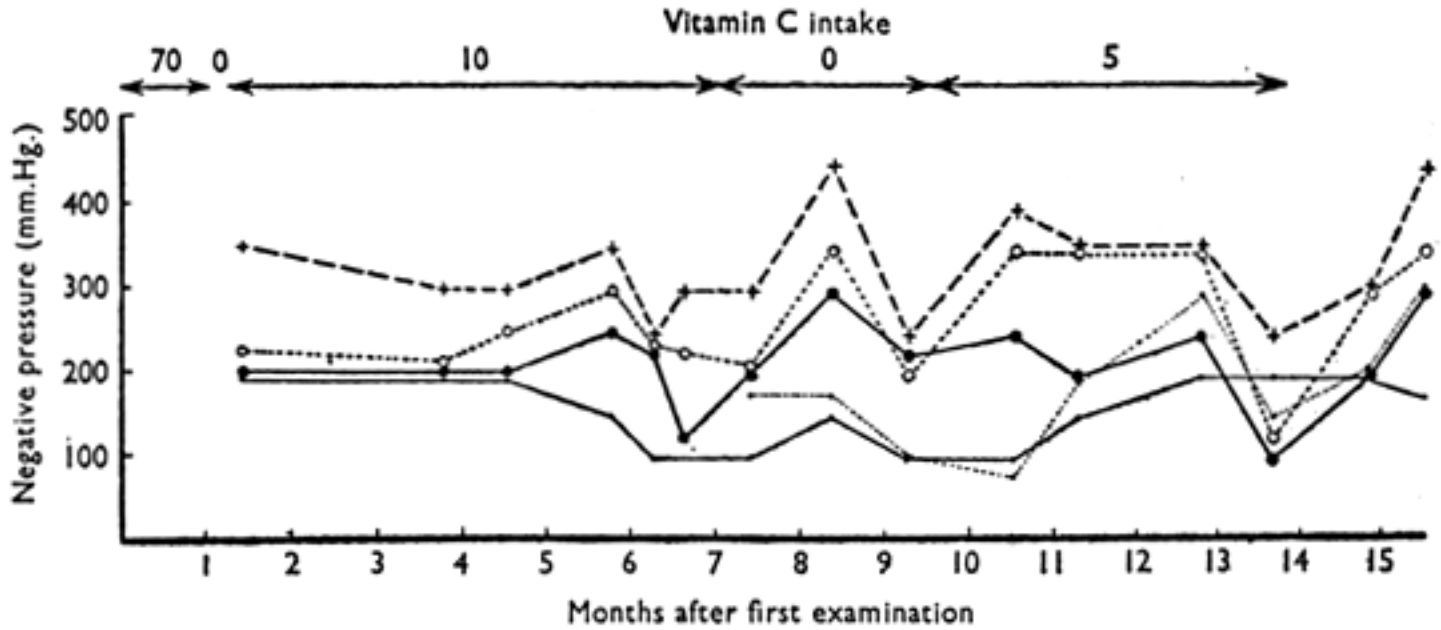
Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of petechiae	Hess test		2	4	4	2	3	5	6	2	5	2	0	0	0	0
	Göthlin test				2	4	2	6	5	0	3	2	2	0	0	1

FIG. B8. WAY

KEY: Forearm — Area I —●—●—●—; Area III ···o···o···o···o···; Area V —+—+—+— . Mouth — Upper gum, base —·—·—·—; Lower lip ·····

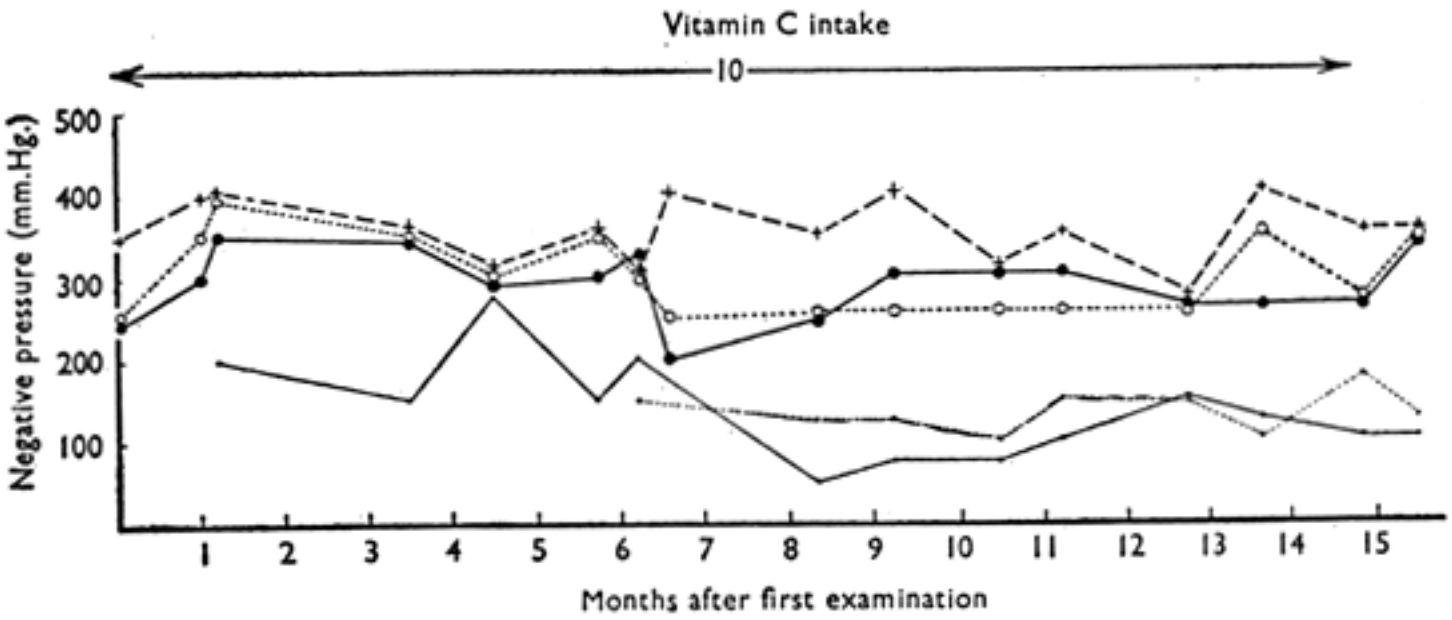
Abnormal positive pressure results are in italics.



Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of petechiae	Hess test		4	1	5	2		5	8	1	2	5	4	12	1	
	Göthlin test				0	1		2	6	1	0	0	0	6	0	1

FIG. B9. WHINFIELD



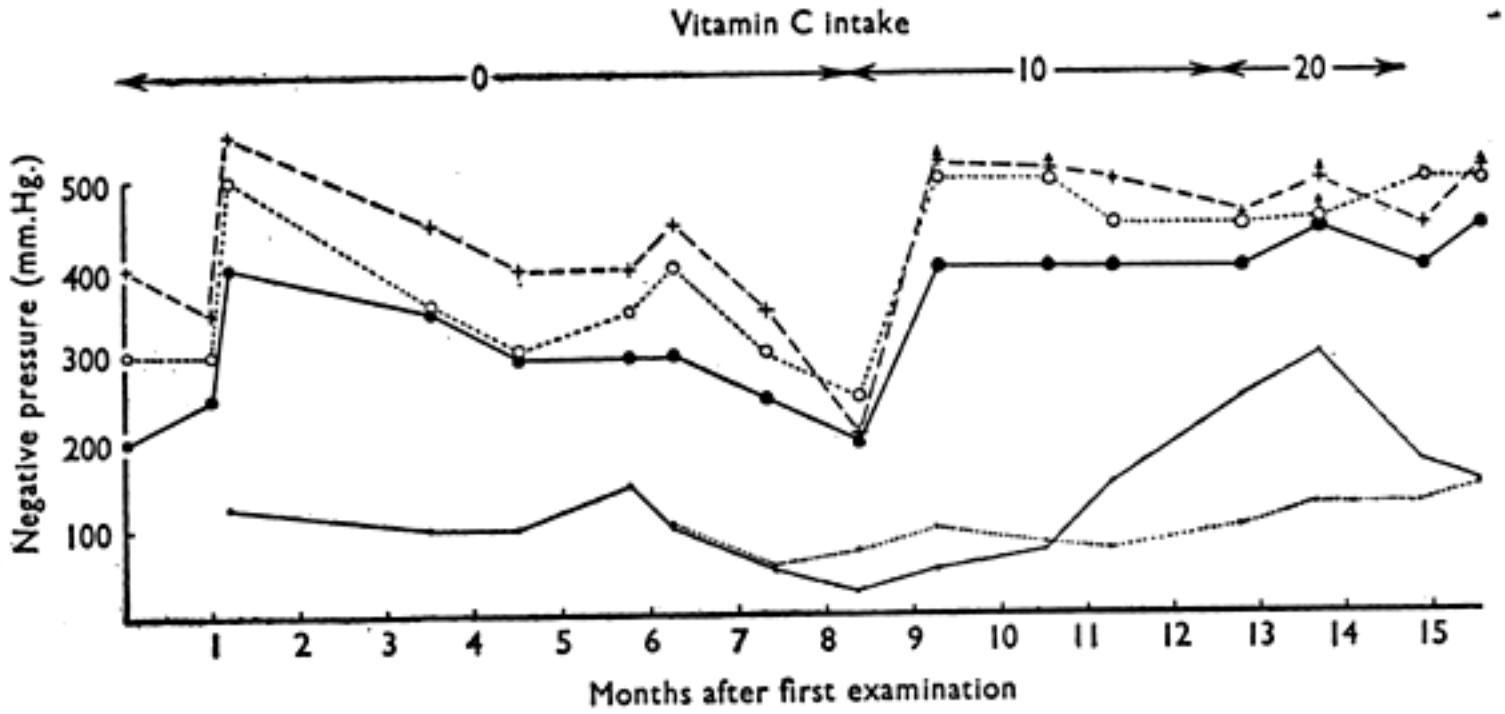
Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of petechiae	Hess test	4	7	2	2	0	*15*		3	4	4	5	4	2	3	6
	Göthlin test				3	0	6		1	1	7	0	0	0	0	0

FIG. B10. WOODHOUSE

KEY: Forearm — Area I —●—●—●—; Area III ···o···o···o···o···; Area V —+—+—+—. Mouth — Upper gum, base —·—·—·—; Lower lip ·····

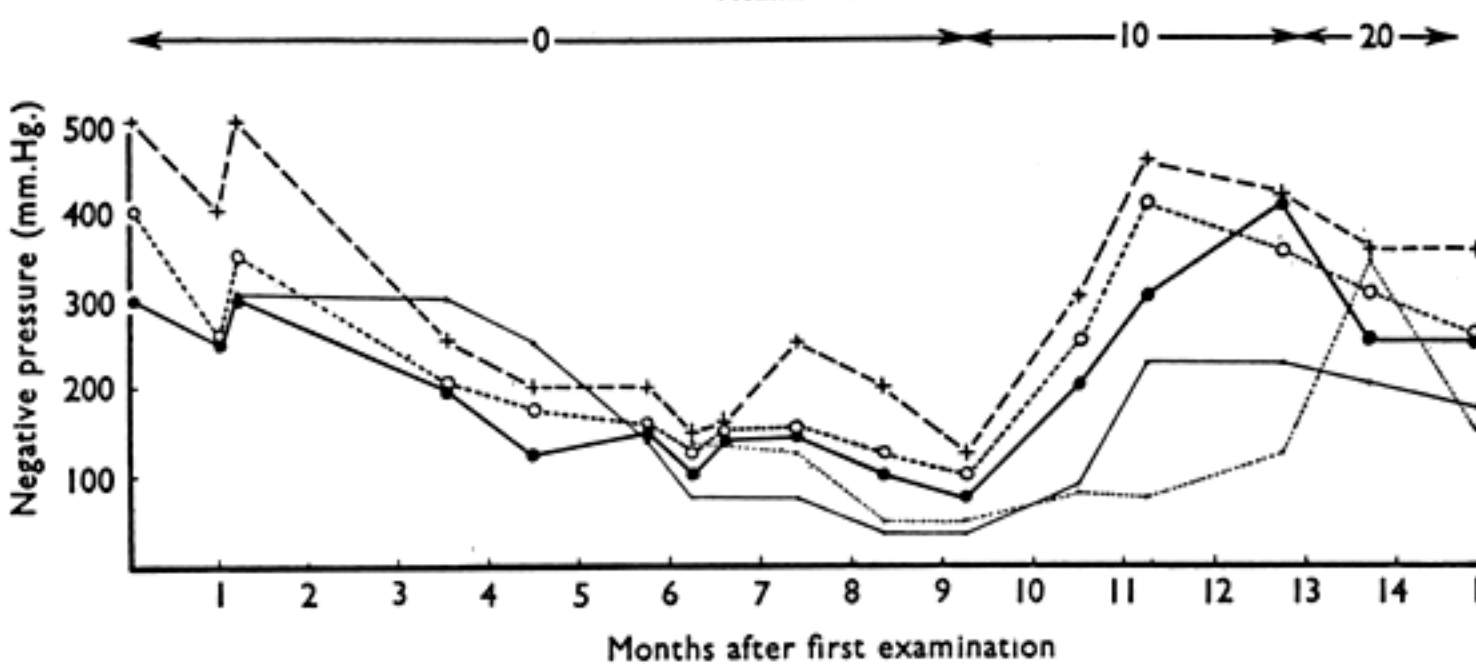
Abnormal positive pressure results are in italics.



Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of petechiae	Hess test	0	20	0	3	7	7	*50+*	*50*	4	4	16	4	10	3	3
	Göthlin test				*8*	*10*	*8*	*17*	*50*	3	*13*	7	6	5	3	2

FIG. B11. DRAKE



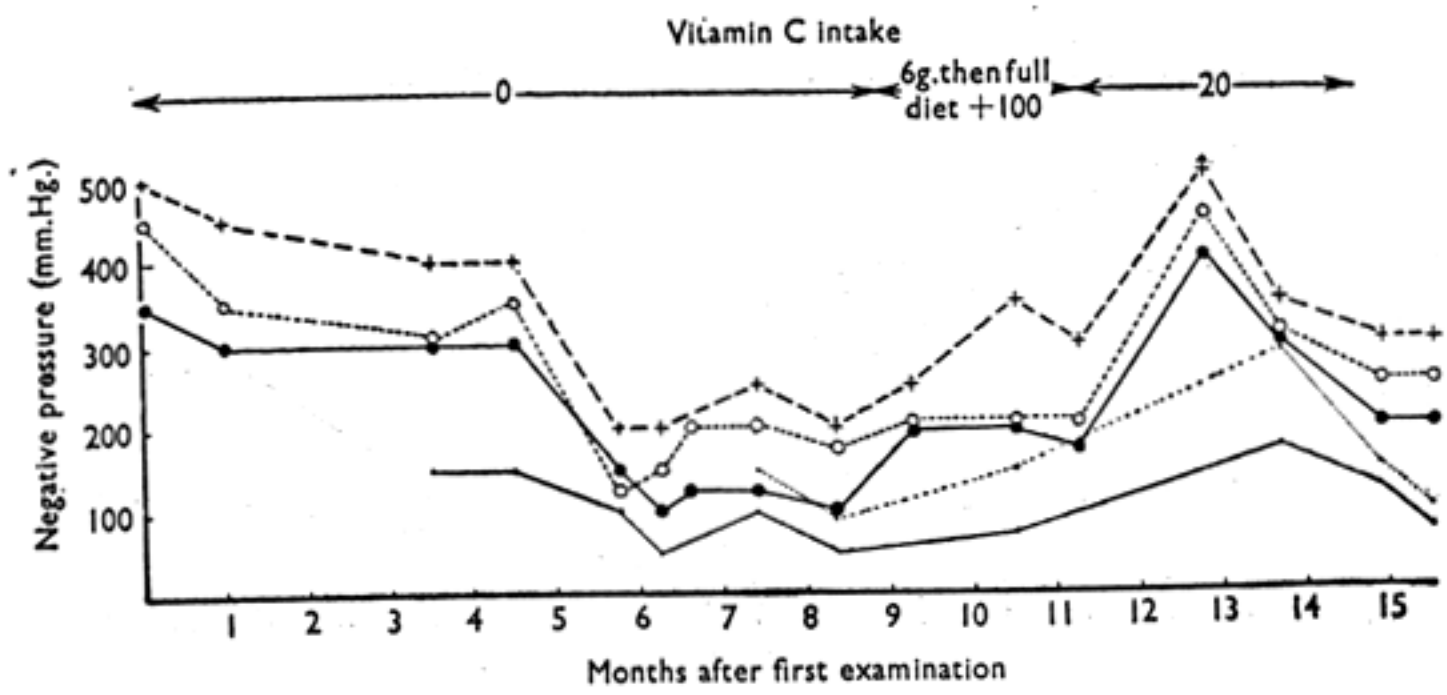
Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of petechiae	Hess test	4	*15*	*15*	*26*	7	*30*	*15*	*60*	*	*35*	3	7	6	5	12
	Göthlin test				2	2	4	*8*	*60*	*25*	*8*	2	1	1	0	1

FIG. B12. HUDSON
*Haemorrhage

KEY:
Forearm — Area I —●—●—●— ; Area III ···o···o···o···o··· ; Area V —+—+—+—
Mouth — Upper gum, base —·—·—·— ; Lower lip ·····

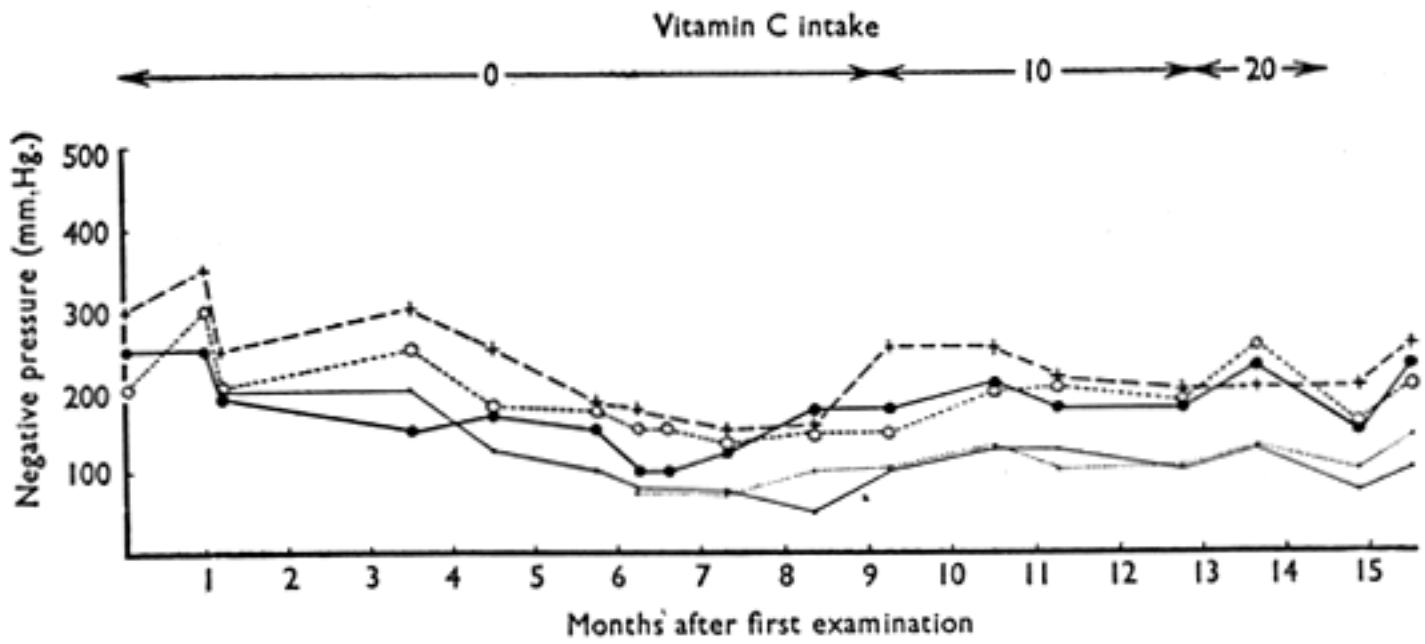
Abnormal positive pressure results are in italics.



Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·
No. of pete-chiae	Hess test	*20*	*20*	0	*35*	5	*18*	*30*	*50*		*60*	11	8	*20*	5	3
	Göthlin test				0	0	0	*10*	*50*		7	0	0	0	1	2

FIG. B13. MILBURN



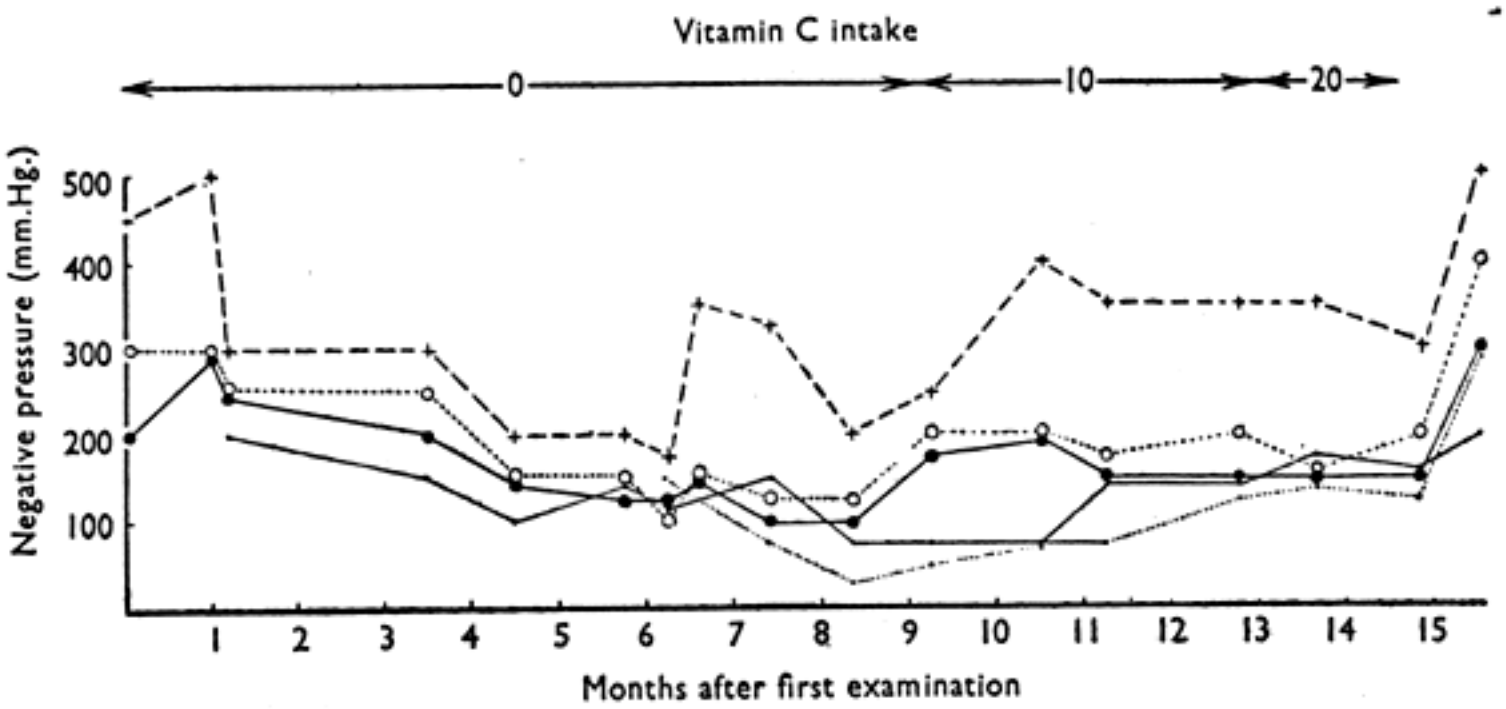
Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	1
No. of pete-chiae	Hess test	*60*	*70*	*25*	*75*	7	7	10	8	10	4	*15*	6	9	3	
	Göthlin test				3	3	6	7	*8*	7	*12*	*18*	*9*	3	1	

FIG. B14. ROBINSON

KEY: Forearm — Area I —●—●—●—; Area III ···o···o···o···o···; Area V —+—+—+—. Mouth — Upper gum, base —·—·—; Lower lip ·····

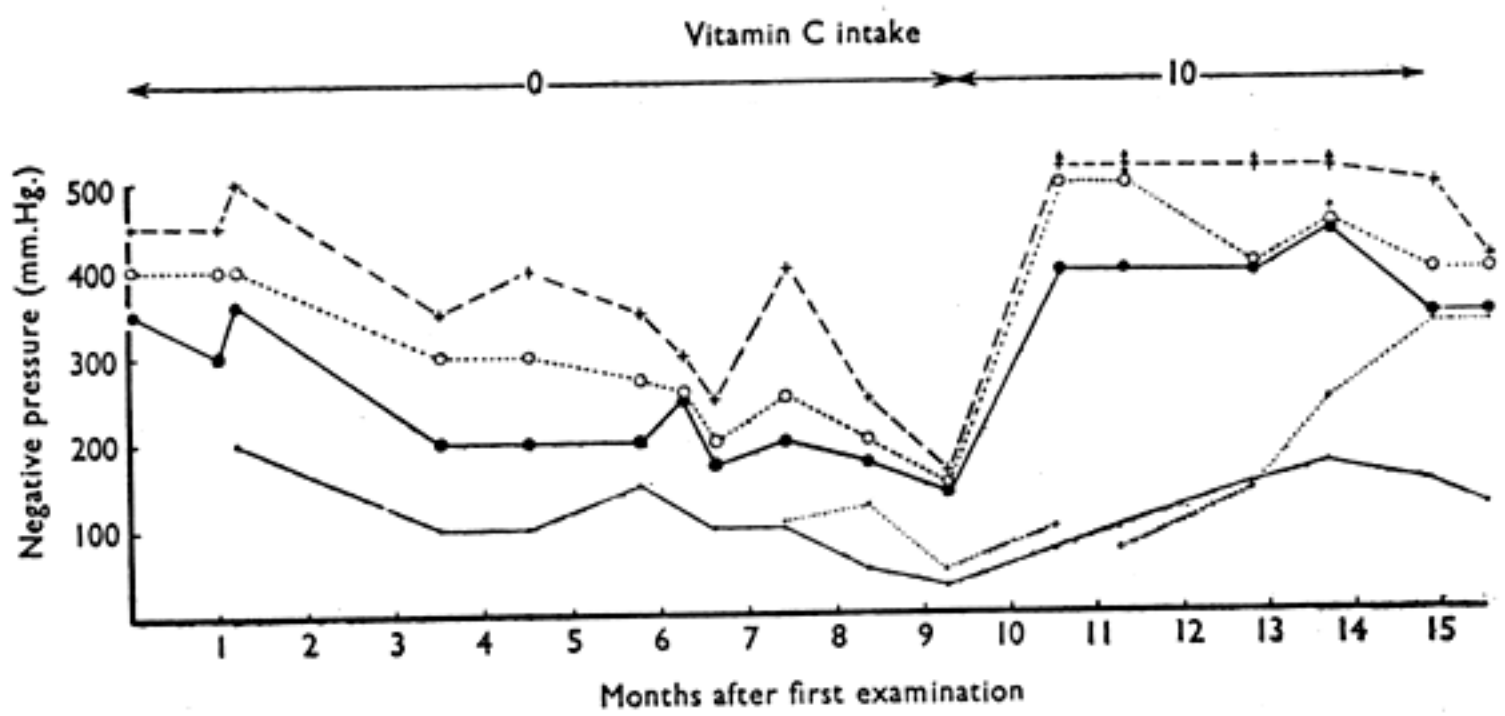
Abnormal positive pressure results are in italics.



Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of petechiae	Hess test	3	2	1	0	6	3	*20*	6	1	5	2	2	8	2	1
	Göthlin test				1	6	3	*12*	*16*	0	*10*	1	1	1	0	1

FIG. B15. SANDERSON



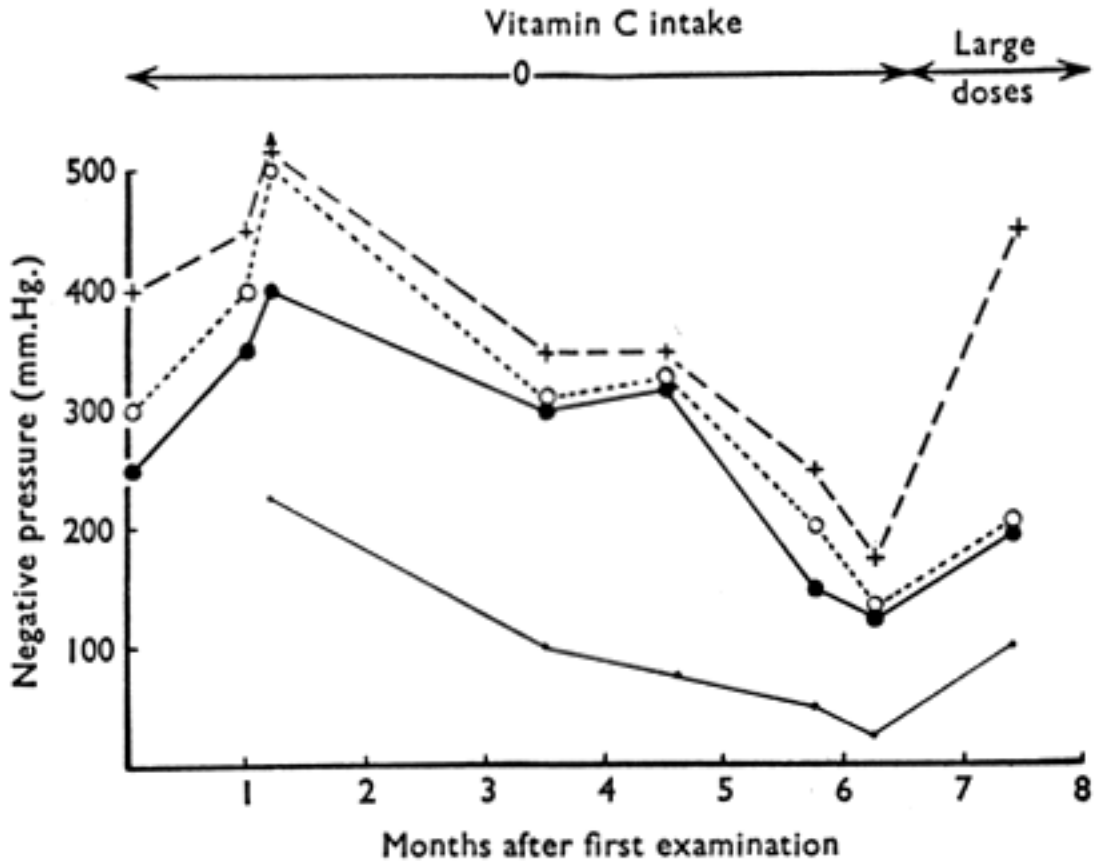
Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of petechiae	Hess test	*20*	3	1	1	1	*15*	3	14	5	1	4	2	1	7	2
	Göthlin test				0	2	*4*	9	*9*	1	1	*8*	0	0	6	0

FIG. B16. TRIDGELL

KEY: Forearm — Area I —●—●—●—; Area III ···o···o···o···o···; Area V —+—+—+— ; Mouth — Upper gum, base —·—·—·—; Lower lip ·····

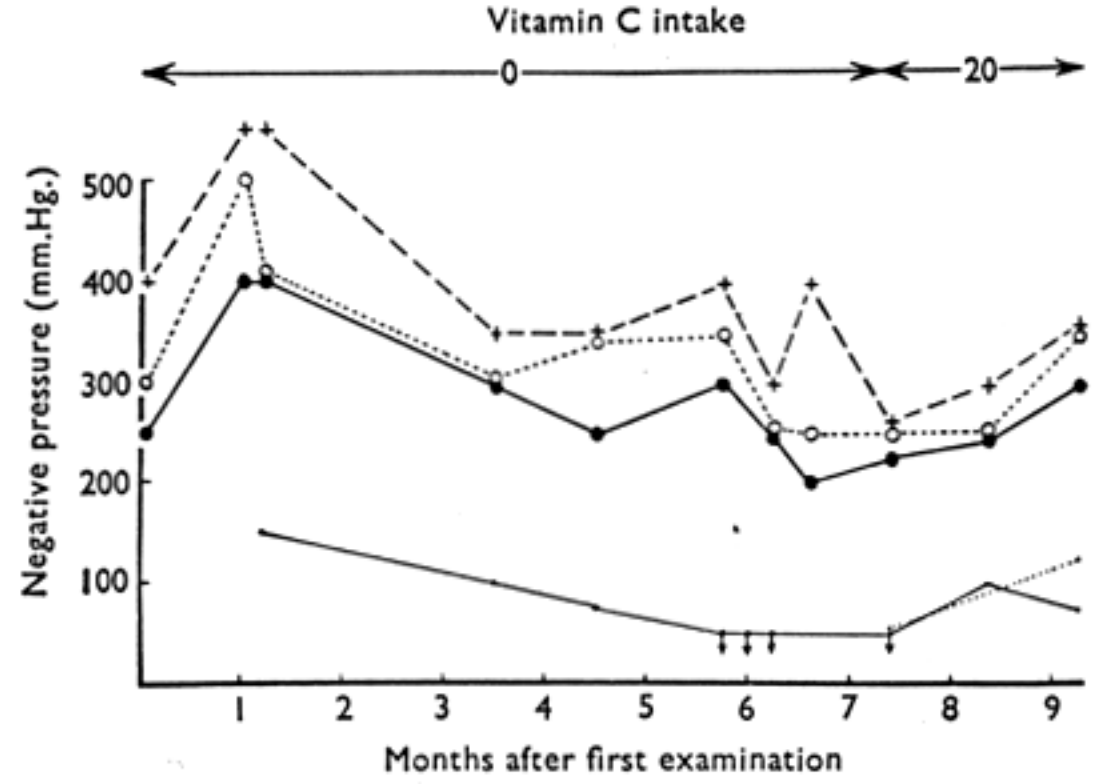
Abnormal positive pressure results are in italics.



Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1
No. of petechiae	Hess test	3	1	0	3	7	*13*	6
	Göthlin test				0	*12*	7	6

FIG. B17. WILLIAMS, D.



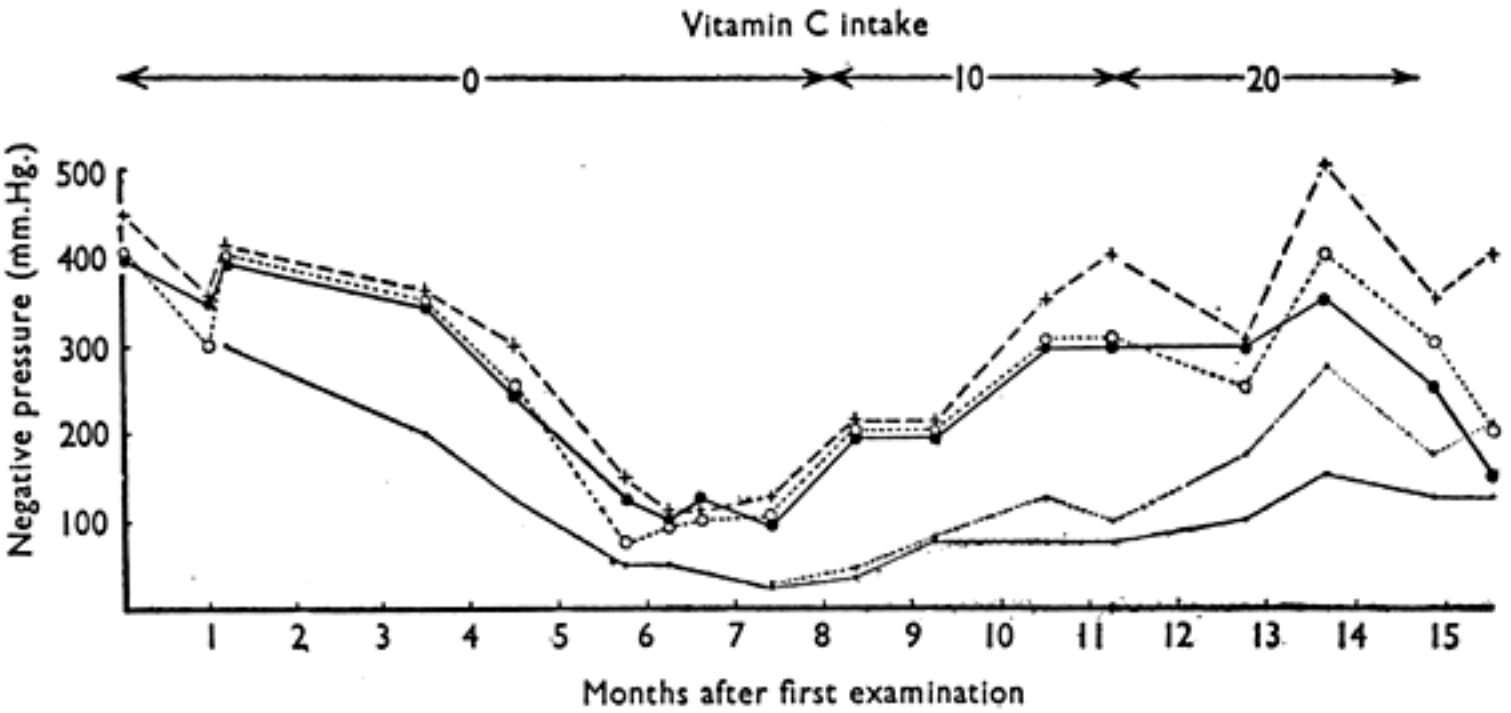
Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of petechiae	Hess test	1	6	2	4	12	4	*40+*		0						
	Göthlin test				3	4	6	*9*		0						

FIG. B18. WILLIAMS, H.

KEY: Forearm — Area I —●—●—●—; Area III ···o···o···o···o···; Area V —+—+—+—. Mouth — Upper gum, base; Lower lip.

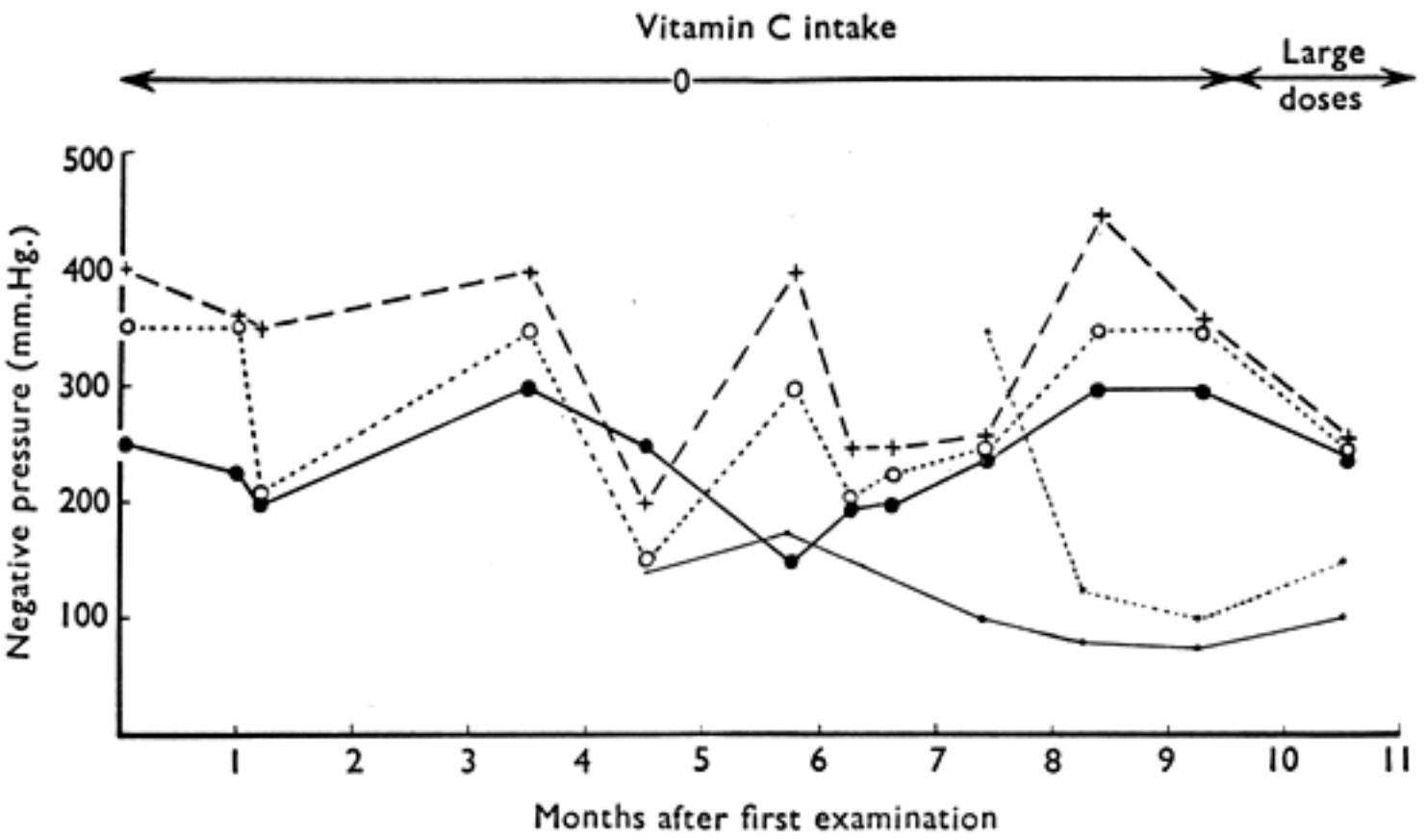
Abnormal positive pressure results are in italics.



Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of petechiae	Hess test	0	3	2	8	12	11	*25*	13	12	13	7	2	2	2	2
	Göthlin test				3	*8*	*9*	*40*	*12*	0	*8*	7	2	1	0	0

FIG. B19. WODEMAN



Positive pressure results

	Months	0	1·1	3·3	4·2	5·3	6·1	7·1	8·1	9·1	10·2	11·1	12·3	13·2	14·3	15·2
No. of petechiae	Hess test	0	0	0	*20*	0	1	2	2	2	3					
	Göthlin test				*8*	2	2	1	3	0	1					

FIG. B20. ANOTHER

KEY:

Forearm	Area I	—●—●—●—	Mouth	Upper gum, base
	Area III	···o···o···o···o···		
	Area V	—+—+—+—		Lower lip

Abnormal positive pressure results are in italics.

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