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EXPERIMENTAL HUMAN SCURVY*

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BOSTON

ALTHOUGH thoroughly investigated in the guinea pig,[1-4] experimental scurvy has not been studied in the human adult. Indeed, until recently there seemed little to warrant such a study. The numerous and widespread clinical reports of scorbutus dating back to Hippocrates[5-7] have drawn a detailed and comprehensive picture of the disease. With the discovery of other vitamins and the isolation of vitamin C, however, the question arises as to what roles such factors as multiple avitaminosis and infection may have played in the syndrome of scurvy and its complications. Obviously the vast majority of cases comprising the literature of this subject involve not only vitamin C deficiency but also multiple subclinical avitaminosis as well; and in many cases infection is an additional complicating factor. Moreover, with the recent development of methods for the determination of ascorbic acid in the blood and urine,[8-12] the necessity has arisen for correlating various blood and urine levels with degrees of pure vitamin C deficiency uncomplicated by other factors.

How long, for example, does it take for an adult on a diet entirely deficient in only vitamin C to become scorbutic? The crews of seventeenth-century and eighteenth-century sailing vessels, having rations undoubtedly poor in many vitamins, frequently developed signs of scurvy after sixty to one hundred and twenty days at sea.[13] Stark,[14] in 1769, produced scorbutus and also some symptoms of vitamin B deficiency in himself by subsisting on a diet of bread and water for ten weeks, an effort which later resulted in his death.

In other communications[15, 16] we have presented partial data concerning an adult male on a diet totally deficient in vitamin C, but supplemented by all the other known vitamins. We now present the completed experiment in detail, representing six months of total vitamin C deficiency. To our knowledge, no controlled vitamin C deficiency has previously been carried longer than twelve days,[17] although Van Eekelen[18] remained on an uncontrolled vitamin C deficient diet for eighty-four days.

EXPERIMENTAL DATA

J. H. C., a male adult weighing one hundred and fifty-eight pounds, with a negative history, physical examination and laboratory findings, placed himself on a diet containing no milk and no fruit or vegetables of any kind.||

Daily plasma determinations were done, using the macromethod of Mindlin and Butler,[9] and frequent plasma determinations, which checked with ours, were also done by Dr. Allan M. Butler and Miss Margaret Cushman of the Children's Hospital. The latter, who have developed a new method for determining the vitamin C in the white-cell-platelet layer of centrifuged blood,[10] also did such determinations at frequent intervals.

In Figure 1 it will be seen that the plasma vitamin C fell irregularly but rapidly, reaching zero after forty-one days of the diet. It remained at zero constantly thereafter. Between the one hundred and sixty-fourth and the one hundred and seventy-first day of the diet, during which time the subject visited Chicago, daily plasma determinations were done by the Farmer and Abt titration method[8] at the Illinois Research Hospital, through the courtesy of Dr. Henry G. Poncher. A microdetermination was also done by Dr. Chester J. Farmer, using his own method, at Northwestern University. All these determinations gave zero readings.

The white-cell-platelet ascorbic acid, as determined by Butler and Cushman by their own method, fell gradually from a relatively normal level of 28 mg. per 100 cc. on the seventeenth day of the diet to 4 mg. per 100 cc. on the eighty-second day, after which the level remained at zero.

*From the Fifth Surgical Service and the Surgical Research Laboratory, Boston City Hospital, the Department of Surgery, Harvard Medical School, and the Fatigue Laboratory, Harvard School of Business Administration. This work was aided by anonymous donors and Hoffman–La Roche, Inc., Nutley, New Jersey. Assistance in securing the data was in part furnished by the personnel of the Work Projects Administration (Project 17580).

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||For the first two months well-cooked meat (not over 200 gm. per day) and small amounts of cream were allowed, but thereafter all meat and cream were also omitted. For the last four months the diet consisted of cheese (Swiss and American), bread, crackers, eggs, beer (not over 1500 cc. per week), black coffee and a standard brand of pure chocolate candy, consisting only of chocolate bean and sugar, roasted and processed at 215°F. for seventy-two hours. During the entire dietary period the subject received daily 30,000 international units of vitamin A, 132 international units of vitamin B_1, and 80 microgm. of riboflavin, fortified by 10 Harris yeast tablets, 50 mg. nicotinic acid, 5280 international units of vitamin D and, after the second month, 4 cc. of wheat-germ oil.

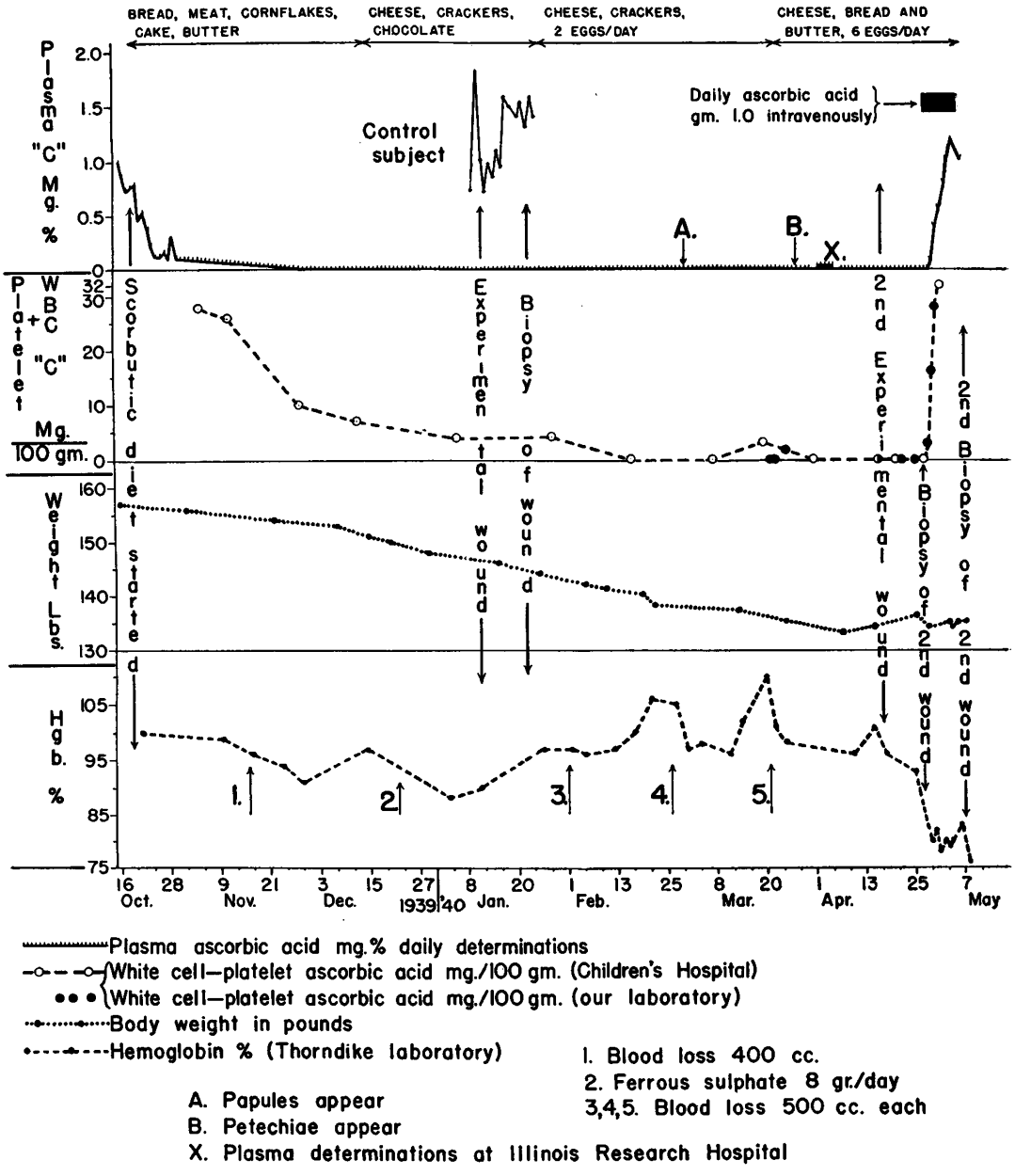

FIGURE 1. *Graph of Pertinent Data in a Case of Experimental Human Scurvy.*

ADDITIONAL DATA

Vitamin C Content of Plasma (mg. per 100 cc.): (17)† 0.2; (23) 0.1; (41) 0.0; (55) 0.0; (79) 0.0; (87) 0.0; (100) 0.0; (121) 0.0; (135) 0.0; (153) 0.0; (181) 0.0; (185) 0.0; (192) 0.0.*

Vitamin C Content of Whole Blood (mg. per 100 cc.): (17) 0.2; (23) 0.1; (41) 0.1; (79) 0.1; (87) 0.0; 0.0 thereafter, as plasma above.*

Basal Metabolic Rate (per cent): (1) +11.5; (7) +5.1; (10) +0.9; (13) –12.5; (17) –12.6; (23) –10.7; (30) –5.0; (36) –8.7; (44) –6.2; (48) –14.1; (51) –16.6; (59) –6.4; (70) –20.0; (77) –21.0; (82) –19.6; (89) –9.2; (93) –13.6; (105) –10.3; (110) –10.0; (114) –15.4; (118) –11.0; (133) –8.3; (139) –17.0; (150) –15.9; (156) –15.9; (161) –22.0; (175) –21.8; (181) –17.4; (187) –6.4; thereafter see Table 3.

Red-Cell Count (millions): (0) 5.0; (5) 5.0; (12) 5.0; (23) 5.0; (29) donor for transfusion, 400 cc.; (30) 4.6; (37) 4.2; (42) 4.5; (57) 4.8; (77) 4.3; (85) 4.5; (102) 5.0; (106) donor for transfusion, 500 cc.; (110) 4.9; (122) 5.5; (124) 5.6; (131) donor for transfusion, 500 cc.; (132) 5.3; (138) 5.1; (148) 5.4; (156) 5.2; (158) acute blood loss, 500 cc.; (159) 5.2; (175) 5.2; (185) 5.2; (190) 4.8; ‡; (194) 4.2; (195) 4.3; (196) 4.2; (197) 4.3; (198) 4.3; (201) 4.2; (203) 3.8; (205) 4.3; (209) 4.5.

White-Cell Count (thousands): (0) 4.7; (57) 4.8; (85) 4.5; (102) 4.2; (124) 4.3; (132) 3.5; (138) 4.4; (156) 3.2; (175) 3.4; (180) 3.6; ‡; (193) 5.0; (195) 4.7; (197) 3.7; (198) 5.4; (201) 9.0; (205) 6.0.

During the first four months of the deficient diet all physical findings were negative. There was a slight fall in weight and basal metabolic rate and a feeling of easy fatigability and slight weakness (see below).

tion of hairs. These lesions, which progressed in severity during the ensuing three weeks, resembled a mild form of the lesion described as typical of vitamin A deficiency.[19] Each papule contained an ingrown hair, which could be

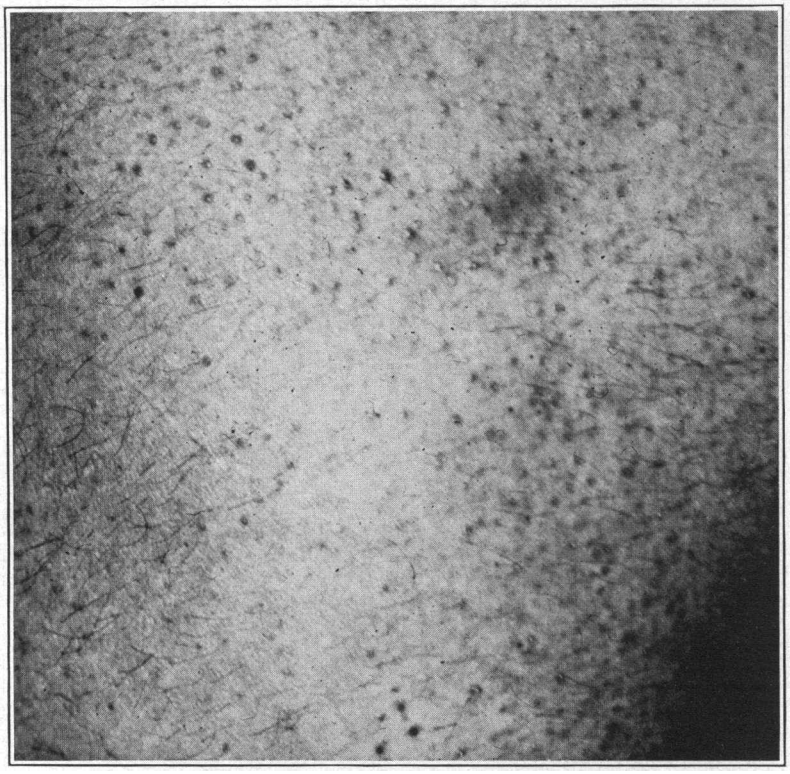

FIGURE 2. *Lateral View of the Thigh after Five and a Half Months of Vitamin C-Free Diet.*
Note the hyperkeratotic papules surrounding the hair follicles.

After one hundred and thirty-four days had elapsed, small perifollicular hyperkeratotic papules began to develop over the buttocks and the posterior aspects of the calves (Figs. 2 and 3). There was noticeable fragmentation of hairs. These lesions, which progressed in severity during the ensuing three weeks, resembled a mild form of the lesion described as typical of vitamin A deficiency.[19] Each papule contained an ingrown hair, which could be seen if the hyperkeratotic plug was picked or scraped off, leaving a small, slightly bleeding crater. Associated with these papules was a marked dryness of the skin, particularly over the extensor surfaces, and the backs

ADDITIONAL DATA (*continued*)

Differential White-Cell Count (per cent): (76) 70 P, 26 L, 2 M, 1 E, 1 B; (132) 55 P, 38 L, 4 M, 1 E, 1 B; (180) 60 P, 24 L, 13 M, 2 E, 1 B; ‡; (204) 66 P, 23 L, 8 M, 2 E, 1 B.
Hematocrit: (0) 0.48; (77) 0.50; (87) 0.49; (107) 0.45; (122) 0.45; (122) 0.47; (154) 0.48; (180) 0.46; ‡; (194) 0.39; (198) 0.38; (203) 0.36; (205) 0.41.
Sedimentation Rate (mm. per minute): (28) 0.4; (75) 0.01; (93) 0.03; (111) 0.05; (145) 0.2; (178) 0.1; (191) 0.2.
Blood Sodium (milliequiv. per liter): (33) 142; (43) 136; (78) 143; (89) 147; (122) 149; (146) 137; (187) 136; ‡; (198) 143.
Blood Complement: normal titer throughout (78, 121, 152, 190).
Göthlin Test: negative throughout (36, 53, 67, 88, 105, 110, 120, 129, 134, 145, 160, 170, 192).
Bleeding Time (Ivy, seconds): (71) 124; (102) 290; (122) 190; (149) 182; (178) 110; (187) 125.
Clotting Time (Ivy, minutes): (71) 9; (102) 8; (122) 12; (149) 10; (187) 6.
Guaiac Test on Stool: negative throughout (54, 95, 120, 139, 145, 192).
Urine (sugar, albumin and sediment): normal throughout (0, 55, 120, 130, 188, 192).
Serum Protein (gm. per 100 cc.): (93) 6.5, A:G as 4.3:2.2; (121) 5.0; (154) 6.5; (187) 7.1.
Chest X-Ray Film: normal throughout (0, 46, 85, 112, 146, 177).
Electrocardiogram: normal throughout (35, 85, 112, 146, 177).
Red-Cell Fragility Test: (156) normal.

*Determinations at the Children's Hospital (Boston) Laboratory.
†Numbers in parentheses refer to days of diet.
‡Vitamin C therapy started on the one hundred and ninety-second day of the diet.

of the hands became markedly roughened, the pores standing out in exaggerated fashion. The similarity between the lesions of vitamin A deficiency and those of vitamin C deficiency has been previously point out.[20, 21] In order to determine the presence or absence of vitamin A deficiency a biophotometric test [22] was done by Dr. Harold J. Jeghers, of the Boston City Hospital. This showed perfectly normal visual adaptation in the dark. At the same time vitamin A determinations done on the plasma were within normal limits, both in our laboratory and that at the Children's Hospital. These findings, coupled with the fact that at least 30,000 international units of vitamin A had been taken daily, — the normal requirement being only 5000 units,[22] — seem to us to rule out vitamin A deficiency as a possible cause for these lesions.

After one hundred and sixty-one days of the diet, the plasma ascorbic acid having been zero for one hundred and twenty days, there appeared for the first time small perifollicular hemorrhages or petechiae over the lower legs (Fig. 4). These lesions did not fade on pressure and were not elevated. They seemed to occur in greatest numbers after the subject had been standing for some time (five hours), appearing for the first time, in fact, following a long period of operating. First seen over the inner and extensor aspects of the lower legs, the petechiae crept upward as the experiment progressed, until at the end of six months they were abundant over the lower thighs, where they seemed to take the place of the hyperkeratotic lesions already described.

Following the administration of vitamin C all skin lesions rapidly cleared (Fig. 5).

Wound Healing

At the end of three months, after the plasma ascorbic acid had been zero for forty-four days, a sizable wound was made in the right mid-back of the subject. Ten days later biopsy of this wound showed good healing, as compared with a normal control[15]; histological study showed ample intercellular substance and capillary formation (Fig. 6). At the end of one hundred and eighty-two days, after the plasma ascorbic acid had been at zero for one hundred and forty-one days, the white-cell-platelet ascorbic acid at zero for sixty-one days, and the petechiae apparent over the lower limbs for twenty-one days, a similar wound was made in the left mid-back.*

The skin sutures were removed on the sixth postoperative day, at which time the wound seemed to be progressing normally.

On the tenth postoperative day, under Pentothal anesthesia, a biopsy was made of the wound through an incision transverse to it and again extending down to the sacrospinalis muscle. Beneath the skin, which appeared well healed, there was no healing of the wound, which was filled with unorganized blood clot (Fig. 7). This was in marked contradistinction to the findings at three months, when there was perfect wound healing. So little healing had occurred that it was necessary to insert a small rubber drain, and the wound was brought together only with silk sutures through skin and subcutaneous tissue.

Sections of this wound, examined by Dr. Frederick Parker, Jr., of the Boston City Hospital, and Dr. S. Burt

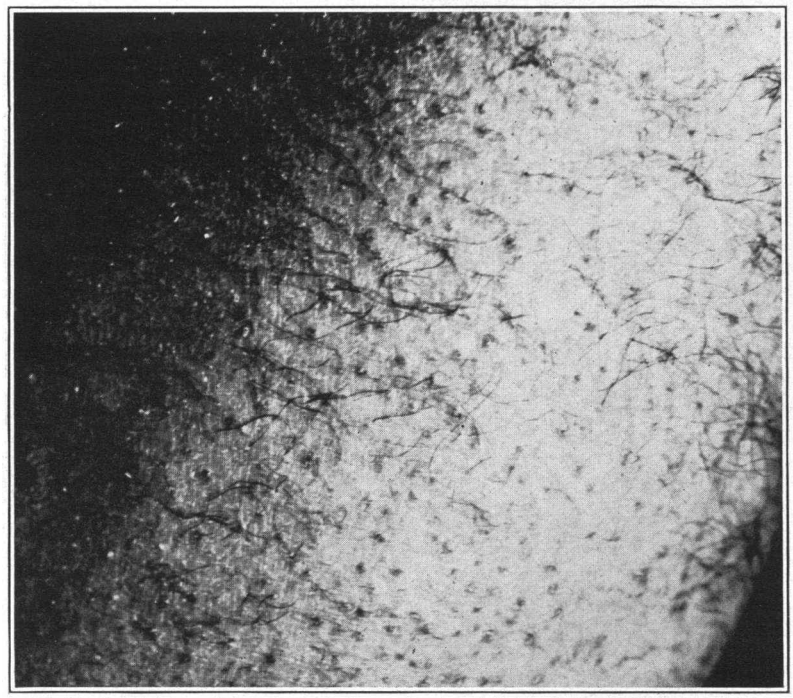

FIGURE 3. *Posterior View of the Thigh after Five and a Half Months of Vitamin C-Free Diet.*
Note the fragmentation of the hairs.

*A 6-cm. transverse incision was made in the left mid-back under local anesthesia (1 per cent novocain). The latissimus dorsi aponeurosis was divided in the plane of its fibers and the anterior sacrospinalis fascia divided, revealing the sacrospinalis muscle, 1 to 2 gm. of which was removed. The sacrospinalis fascia was then closed with interrupted mattress sutures of plain No. 00 catgut. Considerable difficulty was experienced in approximating the edges of this fascia, the sutures tearing out with great ease. (No such difficulty had been encountered at the previous operation.) The latissimus dorsi aponeurosis was then approximated with a running suture of plain No. 00 catgut and silk to the skin.

Postoperatively the movements of the subject were unrestricted as after the previous operation, but on the second postoperative day there was an elevation of temperature to 101°F. On examination at this time the wound appeared perfectly normal and it was left alone, the subject being admitted to the hospital for forty-eight hours. Thereafter he again continued his activities, unrestricted as before.

Wolbach, of the Harvard Medical School, showed lack of intercellular substance and capillary formation (Fig. 8), as had been found by Wolbach[3] in the wounds of scorbutic guinea pigs. This finding was in marked contrast to the section from the wound biopsy at the end of three months (Fig. 6).

Immediately after the biopsy the subject began daily to receive 1000 mg. of ascorbic acid intravenously, continuing as before on the same vitamin C-free diet (see below). After forty-eight hours the activities were again unrestricted, the drain being removed at this time. On the tenth postoperative day, another biopsy specimen was taken from this wound. There was good healing, the sections showing ample intercellular substance and capillary formation, as seen in Figures 9 and 10.

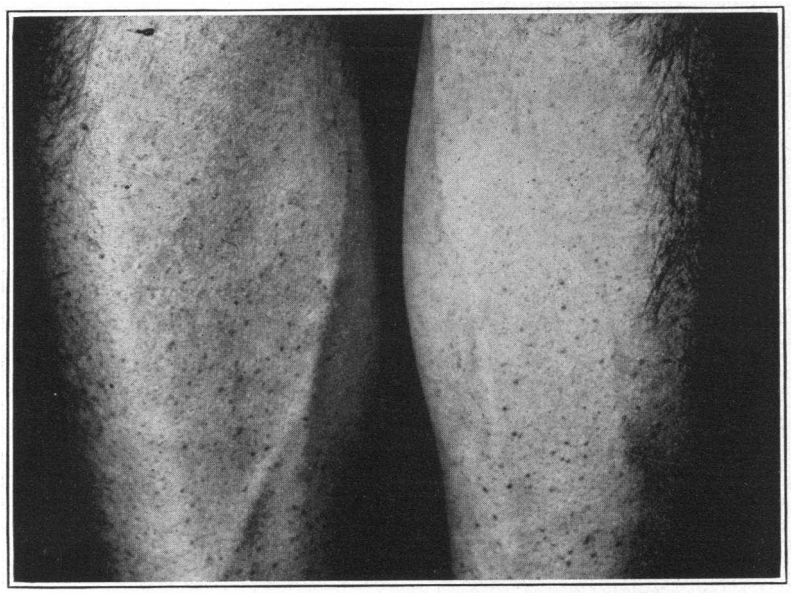

FIGURE 4. *Anterior View of the Lower Legs after Six Months of Vitamin C-Free Diet.*
Note the petechiae.

Teeth and Gums

During the first five months of the diet no changes were grossly apparent in the teeth or gums. A competent dentist pronounced the gums to be normal in appearance at the end of this time.

At the end of six months, when clinical scurvy, as manifested by the perifollicular hemorrhages over the legs, had been present for three weeks, examination of the gums and the teeth was made by Drs. A. P. Young and P. E. Boyle, of the Harvard Dental School. They found that the gums were slightly more boggy on pressure than usual, but no other gross changes could be seen. A biopsy specimen of the gingiva at this time was absolutely normal. Of interest is the fact that although the gross findings were negative, x-ray films of the teeth taken at this time showed occasional interruptions of the lamina dura.

Anemia

During the period of vitamin C deficiency there were four episodes of acute blood loss by venesection (Fig. 1). There was, moreover, mild, chronic loss of blood as a result of various blood determinations. In spite of this loss, which over the entire period of vitamin C deficiency amounted to slightly over 6000 cc., no anemia developed.† The hemoglobin showed a slight fall during the third month of the diet, but rose to normal after the intake of 0.5 gm. of ferrous sulfate daily (Fig. 1). During the week of intravenous vitamin C therapy the hemoglobin dropped sharply to 79 per cent as a result of a blood loss of about 120 cc. daily for plasma and white-cell-platelet determinations over a five-day period. This level was maintained for another week, in spite of cessation of blood loss and although there was a good reticulocyte response. With a normal diet, which included large amounts of orange juice daily, the hemoglobin thereafter rose rapidly, reaching 101 per cent after another ten days.

Leukopenia

At the onset of the experiment the white-cell count averaged around 5000, and no appreciable variation from this figure occurred until after one hundred and thirty-two days of the diet (Fig. 1). At this time the count fell to 3500, the differential count being normal. Except for slight rises following an episode of acute blood loss and after the last experimental wound, the white-cell count fluctuated between 3200 and 5000 during the rest of the experiment, remaining for the most part at the lower level. Following the administration of vitamin C there was a rise in the white-cell count to 5000 and later to 9000.

Infection

There was almost complete freedom from respiratory infection throughout the experimental period, covering the months of October to May. Only two very transient and mild attacks of coryza were noted, each lasting but a few days. In previous winters it has been not uncommon for the subject to suffer from frequent, severe upper-respiratory infections. Throughout the period of deficiency

†Hemoglobin determinations and other blood studies were done through the courtesy of Dr. E. L. Lozner, of the Thorndike Memorial Laboratory, Boston City Hospital.

there developed an occasional furuncle over the back of the neck, but each cleared up in normal time.

Blood-complement determinations* were done at the end of the third, fourth, fifth and sixth months of the diet, the last after clinical scurvy had been apparent for three weeks (Fig. 1). Each determination showed a perfectly normal titer as compared with a control.

Weight Loss

There was a gradual and continual weight loss for the first five and a half months of the experimental period, reaching a maximum of 27 pounds at the end of this time. This drop in weight can be criticized as adding an additional unknown factor to the experiment. We believe it difficult, however, for anyone to remain on a diet absolutely free of vitamin C over a long period about the mechanisms involved, the subject performed two grades of work on a motor-driven treadmill (walking and running). After ten days, during which the subject received vitamin C intravenously but remained on the deficient diet, the experiment was repeated. A control test was made seven weeks after the normal diet had been resumed. The data of Robinson[23] covering the same age group were used for comparison.

During a period of moderate work, — a walk for four and a half minutes on a grade of 8.6 per cent at a rate of 3.5 miles per hour, — both before and ten days after the beginning of vitamin C therapy, the subject's heart rate was nearly maximal, being about 40 beats per minute higher than on any of the men studied by Robinson. In the control test seven weeks later the heart rate after the same work was within normal limits. Throughout this moderate work the oxygen consumption was within normal limits in each experiment.

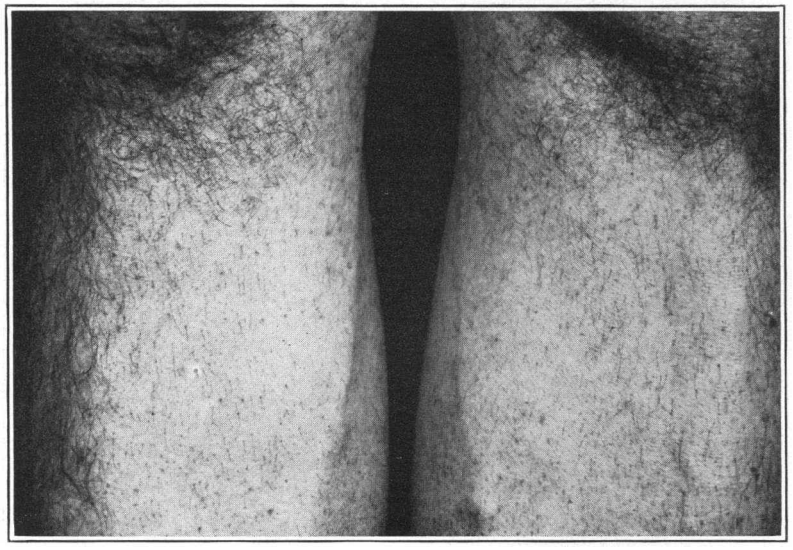

FIGURE 5. *Anterior View of the Lower Legs after a Week of Intravenous Vitamin C Therapy.*

Note the fading of the petechiae as compared with those in Figure 4.

without losing weight, since in order to avoid traces of the vitamin the diet must be restricted essentially to eggs, cheese, bread, butter and sucrose.

Fatigue

From the beginning of the third month of the diet there developed a feeling of fatigue which became progressively more marked. Measurement of this weakness was at first attempted by an ergograph. The number of contractions at the rate of one per second, made by the right hand against a standard resistance, showed only a slight drop during the period of vitamin C deficiency, falling from 59 at the outset to 42 at the end of the experiment. A week after institution of the ascorbic acid therapy, while the subject was still on the diet, the number of contractions increased slightly to 49, a rise which can hardly be considered significant.

At the end of the six months a fatigue test was carried out of the Fatigue Laboratory of Harvard University (Table 1). In order to determine the extent to which the capacity for work was reduced and to learn something

During a harder grade of work, — a run at a rate of 7 miles an hour to exhaustion, — the subject's performances while in the scorbutic state and directly after the vitamin C therapy showed considerable differences. In the state of complete vitamin C deficiency he was able to run for only 16 seconds, whereas following ascorbic acid therapy he ran for 66 seconds, in spite of the fact that at this second experiment the hemoglobin was 13 per cent lower and the oxygen capacity was 7.6 millimol. per liter as compared with 8.0 millimol. in the first test. In the control experiment after the normal diet had been resumed the subject ran for 84 seconds. This performance was still inferior to the 270-second average of other men of this age. Both before and after vitamin C therapy the maximal heart rates were about the same, reaching 190 beats per minute; and in both experiments during this maximal work the minute-oxygen consumption per kilogram of body weight was but three fourths the average found by Robinson. These were normal in the control experiment performed seven weeks later. This indicates an incapacity for aerobic work unrelated to vitamin C deficiency,

*Blood-complement determinations were done through the courtesy of Dr. C. P. Emerson, of the Thorndike Memorial Laboratory, Boston City Hospital.

but does not diminish the significance of the better performance following vitamin C therapy. Despite his inability to reach a normal level of oxygen consumption while on the diet, the subject's capacity for anaerobic work, as measured by a hand ergograph, was undiminished, even while in the scorbutic state (Table 1).

The subject accumulated a lactate concentration of 82 mg. per 100 cc. before and 104 mg. after the administration of vitamin C. The oxygen debts were 3.29 and 4.97 liters, respectively. These higher figures after the vitamin C therapy were associated with the increased performance in the run. This may have been the result in part of improved skill, but it should be pointed out that the capacity for supplying oxygen was somewhat lowered in the second test.

TABLE 1. *Fatigue Tests.*

DATA	APRIL 26 (SCORBUTIC STATE)	MAY 6 (AFTER VITAMIN C THERAPY)	JUNE 26 (CONTROL)	NORMAL RANGE
Duration of work (min.)				
Moderate work	4.5	4.5	4.5	15
Maximal work	0.27	1.1	1.4	3–5
Ergograph (lb.)	52.0	49.0		40–70
Oxygen consumption (cc./kg./min.)				
Moderate work	29.9	27.7	27.3	25–30
Maximal work	37.8	36.7	40.3	40–50
Oxygen debt (liters)	3.29	4.97	5.55	
Heart rate (beats/min.)				
Moderate work	182	178	140	120–140
Maximal work	180	191	180	180–195
Recovery (1 min.)	161	180	140	140–160
Recovery (10 min.)	105	108	112	80–120
Recovery				
Oxygen consumption (liters)				
0–3 min.	2.58	2.87	3.48	
3–15 min.	5.02	5.54	5.52	
Blood lactate (mg./100 cc.)				
5 min.	82.0	104.4	104.0	
10 min.	65.1	105.5	93.3	
15 min.	51.6	87.6	75.6	
30 min.	28.1	51.6	35.6	
45 min.	22.4	22.4	32.2	
60 min.	24.7	15.7		
Blood sugar (mg./100 cc.)				
5 min.	127	122	125	
10 min.	94	115	114	
15 min.	100	113	120	
30 min.	100	104	110	
45 min.	109	99	118	
60 min.	106	106		
Insulin-tolerance curve (sugar in mg./100 cc.)				
2 min.	88	100		
10 min.	83	90		
15 min.	76	80		
20 min.	74	72		
25 min.	66	65		
35 min.	67	70		
45 min.	69	83		
60 min.	74	100		

Of considerable interest are the blood lactate levels following the fatigue test performed when the subject was in a scorbutic state. There was a normal rate of disappearance of the blood lactate for the first fifteen minutes of the recovery period. During the following half hour the rate of disappearance was much decreased, being about half the normal rate. After the vitamin C therapy the rate of disappearance of the blood lactate was normal throughout the recovery periods of the tests made while the subject was still on the diet and also following the control run seven weeks later.

Studies made on samples of arterial blood, drawn when the subject was in the basal state, before and after treatment, showed no abnormalities except for a slight anemia (Table 2).

TABLE 2. *Arterial Blood Studies Before and After Vitamin C Therapy with the Subject in a Basal State.*

DATA	APRIL 26 (SCORBUTIC STATE)	MAY 6 (AFTER VITAMIN C THERAPY)	NORMAL RANGE
Carbon dioxide content (millimol./liter)	21.8	21.5	20.0 – 23.0
Hemoglobin oxygen concentration (millimol./liter)		7.10	8.0 – 9.5
Hemoglobin oxygen capacity (millimol./liter)	8.04	7.60	8.52– 9.44
Hemoglobin oxygen saturation (per cent)		93.5	93.0 – 97.0
Serum carbonic acid (milliequiv./liter)	2.92	3.10	2.4 – 3.2
Serum bicarbonate (milliequiv./liter)	25.3	23.8	24.0 – 27.0
Arterial carbon dioxide pressure (mm.Hg)	42.0	44.4	35.0 – 45.0
Plasma pH	7.40	7.34	7.35– 7.45
Lactate (milliequiv./liter)	1.0		1.0 – 1.5
Sugar (mg./100 cc.)	111.0		90.0 –110.0
Plasma chloride (milliequiv./liter)	107.1	106.4	103.0 –107.0
Plasma protein (gm./liter)	62.0	65.2	63.0 – 68.0
Plasma inorganic phosphorus (milliequiv./liter)	2.4	1.7	1.6 – 3.2

The performance of the subject while in the scorbutic state placed him in the same category as the Group X of Robinson, consisting of men in the eighth decade of life, for whom the five-minute walk was maximal work. The heart rate during recovery was higher than that observed by Robinson. Following treatment with ascorbic acid the rate fell more rapidly during recovery, despite a slightly higher initial value, a higher blood lactate concentration and a greater oxygen debt.

Basal Metabolic Rate

Basal metabolic rates were frequently determined by the Benedict-Roth method, beginning prior to the dietary period and continuing during the administration of parenteral vitamin C, while the subject remained on the diet (Fig. 1). During the dietary period the metabolic rate fell as low as —22 per cent, but after the administration of vitamin C was started, while the subject was still on the diet, values as low as —18 per cent were obtained. At the Harvard Fatigue Laboratory, where the metabolic rate was determined by the open-circuit gasometer method, there was an increase from —6 to —3 per cent after the institution of vitamin C therapy. It is doubtful, therefore, whether the fall in basal metabolic rate could in any way be attributed to vitamin C deficiency. It was probably due to weight loss or inanition or both. Basal metabolic rates were done daily after the parenteral ascorbic acid was started, both before its administration and one and a half to two hours thereafter. It is interesting that during the first four days the basal metabolic rate uniformly fell after the administration of 1000 mg. of ascorbic acid intravenously (Table 3). During this time the subject was becoming saturated with the vitamin (see below). Following this period of saturation, the basal metabolic rate two hours after the administration of ascorbic acid was consistently higher than it was before its injection.

Capillary Fragility

During the entire experiment the capillary fragility test, as described by Göthlin,[24, 25] remained absolutely negative (Fig. 1). At the end of five months, when the petechiae were beginning to appear over the legs, a pressure of 100 mm. of mercury was applied to the arm for ten minutes by means of a blood-pressure cuff. This brought out twenty-two petechiae in a measured area 6 cm. in diameter. Among 8 controls, 7 showed more petechiae at the end of ten minutes than did the subject. Dr. C. J. Farmer, at Northwestern University, performed a negative-pressure (Dalldorf[26]) test on the arm of the subject after five months and two weeks of dieting. This also was perfectly normal, 450 mm. (mercury) of negative pressure being required to produce any petechiae.

FIGURE 6. *Biopsy Specimen Removed after Three Months of Vitamin C-Free Diet: Eosin and methylene blue stain (× 650).*

Note the numerous capillaries and the abundance of intercellular substance in the granulation tissue.

TABLE 3. *Effect of Intravenous Ascorbic Acid (1000 mg.) on the Basal Metabolic Rate of the Scorbutic Subject.*

Date	Basal Metabolic Rate: Before the injection of vitamin C %	Basal Metabolic Rate: 1½ hr. after the injection of vitamin C %
April 28	0.0	− 3.0
April 29	−15.4	−16.4
April 30	− 7.5	−11.2
May 1	0.0	−18.7
May 2	−16.7	− 9.0
May 3	− 3.3	− 3.3
May 4	−12.3	− 8.3

Blood Pressure

The blood pressure remained at a constant level of 120 systolic, 70 diastolic, until immediately following the fourth episode of blood loss on the one hundred and fifty-fifth day of the diet, when it fell to 90/60. During the next week the systolic pressure rose to 98, but it never exceeded this level until the ascorbic acid was started, when it promptly rose.

Muscle Phosphorus and Phosphagen

At the time of biopsy of the second experimental wound a portion of sacrospinalis muscle was removed and subsequently analyzed for total soluble phosphorus and for phosphagen phosphorus content.* The total phosphorus content was low, being only 111.5 mg. per 100 gm. of muscle. The phosphagen phosphorus content was high, being 50.4 mg. per 100 gm. of muscle, or 45 per cent of the total phosphorus content. In normal muscle the phosphagen is only about 35 per cent of the total soluble phosphorus.

After a ten-day period, during which the subject remained on the same vitamin C-free diet but daily received 1000 mg. of ascorbic acid intravenously, another piece of muscle was analyzed, this time from the thigh. The total soluble phosphorus was considerably increased, being 145.9 mg. per 100 gm., while the phosphagen phosphorus was only 45.6 mg. per 100 gm., or 31 per cent of the total muscle phosphorus.

Miscellaneous

Two insulin-tolerance tests were done (Table 1).† Each was obtained following the intravenous injection of 3.1 units of insulin. The tests were made under identical conditions, and while the subject was in the basal state. It is apparent that vitamin C had no influence on insulin tolerance. Glucose-tolerance tests were also normal.

A gastric analysis done after five months of the diet showed only 1 unit of free acid and only 3 units of total acid, and after histamine 22 units of free acid and 27 units of total acid. After eleven days of intravenous administration of ascorbic acid, while the subject was still on the diet, analysis of the gastric contents again showed only 1 unit of free acid and 3 units of total acid, but this time, after the administration of histamine, there were 33 units of free and 42 units of total acid.

*Muscle-phosphorus and muscle-phosphagen-phosphorus determinations were done through the courtesy of Dr. Steven Horvath, of the Harvard Fatigue Laboratory.

†Insulin-tolerance tests were done through the courtesy of Dr. John W. Thompson, of the Harvard Fatigue Laboratory.

Other determinations, which included serum proteins, lipids, sedimentation rates, hematocrits, bleeding and clotting times, examination of stools and urines for blood (with complete analysis of the latter), chest x-ray films and electrocardiograms, were within normal limits (Fig. 1). There was a slight fall of the blood-sodium level from 142 to 136 milliequiv. per liter just before the ascorbic acid was begun. Following six days of ascorbic acid therapy the blood sodium regained the normal level.

An electroencephalogram made between the fourth and fifth months of the diet was negative.*

Saturation of Vitamin C

Following the first biopsy of the second experimental wound, saturation with vitamin C was begun, the same vitamin C-free diet being continued. For this purpose 1000-mg. doses of ascorbic acid were given daily after the manner of Wright, Lilienfeld and MacLenathen.[27] It was believed that the intravenous route was the best method of saturation, since oral doses may introduce unknown and perhaps variable absorption factors[28–30] as well as a time factor in the ensuing saturation curves. After preliminary plasma and white-cell-platelet determinations, the vitamin was injected, following which hourly plasma and urine determinations were done. The plasma and urine determinations are represented in Figure 11.

Early in the experiment it had been found that even with a plasma of zero and no intake of the vitamin, the titration method of Harris[11] gave considerable values in the urine, undoubtedly owing to the presence of reducing substances other than vitamin C.[31] For this reason the recently described method of Evelyn, Malloy and Rosen[12] was used.

It can be seen on examination of Figures 11 and 12 that on the first day the plasma C content fell very rapidly during the first three hours after injection of the vitamin, reaching zero at the end of five hours. On each succeeding day the initial plasma and also the white-cell-platelet levels were higher, the latter being 3, 16 and 28 mg. per 100 cc. respectively on the first three days of the saturation regime. This almost normal white-cell-platelet value on the third day, together with the very gradual drop in the plasma level of the vitamin on that day, may be taken as indication that saturation of the *blood* occurred somewhere between the third and fourth days.

Examination of the urinary output of the vitamin immediately reveals that the amount, as determined by the Evelyn–Malloy[12] method, is much smaller than has been obtained by the Harris titration method, if the subject may be considered saturated after the fourth day. In determining the values in the urine, great care was taken in our laboratory to avoid any ceiling in the method by multiple dilutions of the urine samples.

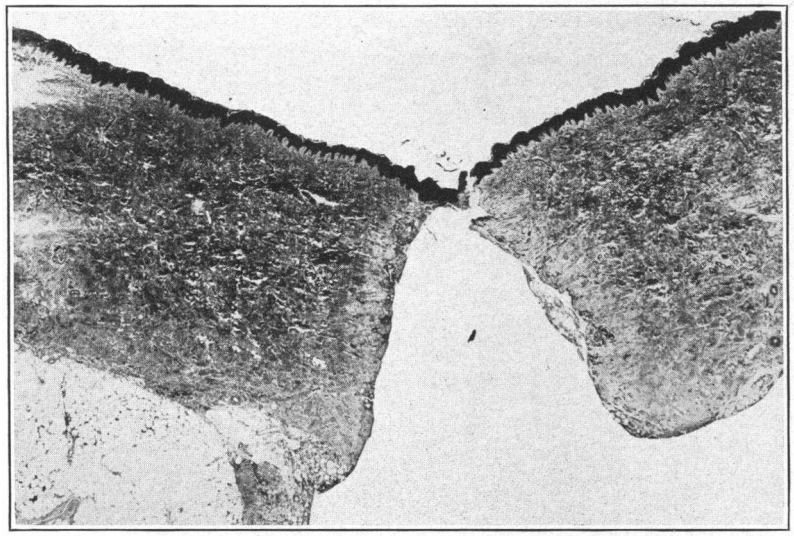

FIGURE 7. *Biopsy Specimen Removed after Six Months of Vitamin C-Free Diet: Eosin and methylene blue stain (× 15).*

This section shows complete lack of healing. The large space was occupied by an unorganized blood clot.

It becomes evident, on examination of Figure 11, that there is an apparent renal threshold, as described by Faulkner and Taylor,[32] using the Harris titration. According to our findings, however, this threshold does not occur at a plasma value of 1.4 mg. per 100 cc., as described by them and others, but rather with a plasma value of about 0.85 mg. in the normal adult, as shown by the April 30 figures. Moreover, the plasma saturation and urinary excretion curves suggest that in the totally vitamin C-deficient state this renal threshold may be lower, and that as saturation with vitamin C takes place it rises to normal (April 27 to 30 figures). On the other hand, the appearance of ascorbic acid in the urine coincidental with low plasma levels (in the first three curves) may be due to a lag phenomenon.

Examining the curves again, it may be noted that during the process of saturation with vitamin C the output of the vitamin in the urine was on two occasions greater during the second hour than during the first hour (April 30 and May 2). On these two days the volume of urine excreted was considerably greater during the second hour than during the first hour. On the other days during the saturation period the volume was greater in the first than in the second hour, and it will be noted that the excretion of the vitamin was also always greatest during

*Electroencephalography was performed through the courtesy of Dr. F. A. Gibbs, of the Boston City Hospital.

the first hour. This would suggest that while the organism is in a vitamin C-deficient state the output of ascorbic acid may depend not only on its plasma level and on the renal threshold but also on the amount of urine excreted. Of interest in this respect is the finding of Sendroy and Miller[33] that renal function is a factor in the excretion of ascorbic acid, and that of Wright and MacLenathen [34] that vitamin C may be excreted slowly in cases of kidney damage. Following saturation of the subject, however, the excretion of vitamin C was always greatest during the first hour after the injection, no matter how the urinary output was made to vary.

Effect of Vitamin C Therapy

Within twenty-four hours after the administration of the vitamin a subjective improvement was noted, there being a marked alleviation of weakness and languor. This improvement seemed to progress during the next four or five days. It is possible, of course, that considerable or even all the effect was due to suggestion.

The skin changes were remarkable. At the end of six days the petechiae had faded considerably, and the skin had returned from a rough to a smooth state, although hyperkeratotic papules were still present. Between twelve and fourteen days after the institution of the vitamin C therapy the petechiae had entirely disappeared, but traces of the papules remained over the buttocks for three weeks.

Although the hemoglobin fell following the commencement of vitamin C therapy, it is of interest that the blood pressure, which had been somewhat below the subject's normal since the last episode of acute blood loss, almost immediately began to rise after the vitamin C had been given.

On the third day after the vitamin was begun a slight blurring of vision was noticed when the eyes were focused on distant objects. This cleared up in twenty-four hours, and may have been due to diffusion of ascorbic acid into the lens.

Except for the increased ability to do aerobic work, as measured by the fatigue test, and, of course, the wound healing, there were no noteworthy changes during the vitamin C therapy while the subject remained on the diet. There was no gain in weight during this time.

Comment

Wound Healing

Since the emphasis by Wolbach and Howe[2] that the fundamental lesion of scurvy is a deficiency of intercellular substance and capillary formation, there has been increased interest in the surgical significance of the vitamin. The experimental work of Lanman and Ingalls[4] and of Taffel and Harvey,[35] showing that the wounds of scorbutic guinea pigs healed slowly and with poor tensile strength, received support from the clinical work of certain investigators,[36–39] notably Wolfer, and Archer and Graham. The clinical evidence for the necessity of the vitamin for wound healing lay somewhat open to question, however, since the cases reported were, of necessity, poorly controlled with respect to other variable factors important in wound healing, notably other avitaminoses, infection, surgical technic and so forth. Although it is to be remembered that only one case is considered here, all such variables were excluded, and the lack of wound healing must be attributed to ascorbic acid deficiency alone. The mere fact that the biopsy sections showed striking resemblance to those from the wounds of scorbutic guinea pigs would appear as conclusive evidence that the lack of healing was due not to infection, in-

FIGURE 8. *Biopsy Specimen Removed after Six Months of Vitamin C-Free Diet: Eosin and methylene blue stain (× 350).*

Note the newly formed fibroblasts having no intercellular substance between them. Old collagen may be seen at the bottom and extending down and to the right from the upper left-hand corner.

creased tension or other such factors but to vitamin C deficiency.

Significance of Plasma-Vitamin C Levels

It should be kept in mind that normal wound healing occurred after three months of vitamin C deficiency, when the plasma ascorbic acid had been zero for as long as forty-four days.[15] This, together with the other negative findings at that time, suggests that the plasma ascorbic acid is a poor index of the vitamin C status of the patient.

Lack of controlled cases may possibly be responsible for the general belief that the plasma levels of the vitamin are a fair index of deficiency and that levels below 0.5 mg. per 100 cc. are dangerously low.[39, 40] As pointed out by Butler and Cushman,[19] it would seem that the white-cell-platelet level of the vitamin in the centrifuged blood is a much more accurate measure of deficiency. In this case the level reached zero just ten days before the hyperkeratotic papules appeared, and thirty days before the appearance of the petechiae, at which time the plasma had been zero for one hundred and twenty days.

Why did so much time elapse before clinical scurvy appeared in this subject? The answer to this question must lie in the fact that there were no complicating factors such as growth, infection or multiple avitaminosis. That Stark[14] showed signs of scurvy within ten weeks on a diet almost totally deficient in all the vitamins suggests that multiple avitaminosis may bring on clinical vitamin C deficiency more rapidly. And it is to be remembered, of course, that clinical vitamin C deficiency is practically always associated with some deficiency of vitamin B, and perhaps also of the other vitamins. There is a known increased disappearance of vitamin C from both plasma and white cells in cases with infection,[41–48] and when growth or hyperthyroidism is a factor.[49, 50] It is possible, moreover, that extreme muscular exertion may deplete the body of the vitamin more rapidly, and that scurvy would have appeared in the experimental subject much sooner had he been constantly subjected to extreme muscular fatigue.

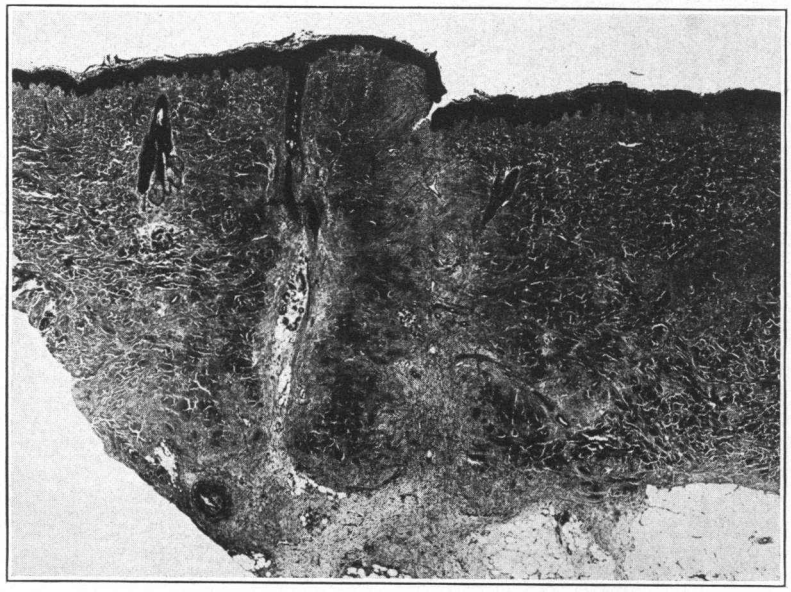

FIGURE 9. *Biopsy Specimen Removed after Ten Days of Intravenous Vitamin C Therapy: Eosin and methylene blue stain (× 15).*

This section shows complete healing of both the original wound and that of the biopsy ten days later.

Human Requirement

Much has been written on the vitamin C requirement of human adults, and many methods[17, 18, 31, 51–56] have been evolved and employed for its estimation. The majority are necessarily based on the assumption that saturation is the optimal state for the individual, a postulate having little evidence for its support. Estimations of the daily requirement have varied between 20 and 100 mg. per 100 cc., the majority of workers setting 30 mg. as the daily minimum.[54]

It is unfortunate that in this experiment the point at which the subject became saturated cannot be accurately determined. Although the blood level of the vitamin had returned to normal by

the fourth day of the saturation period, the urinary output was not maximal by the sixth day. Taking into consideration the total amount lost in the urine (about 1 gm.) 4 to 6 gm. may be considered as roughly the maximal amount of ascorbic acid necessary to resaturate this individual. Since the first signs of scurvy appeared after one hundred and thirty-two days of total vitamin C deficiency, the maximal daily utilization of the vitamin must have been between 30 and 45 mg. It is possible that the true requirement lies somewhat below this figure. On the other hand, this estimate would of course be elevated if infection, multiple avitaminoses, hyperthyroidism and possibly increased exertion had been factors in the experiment.

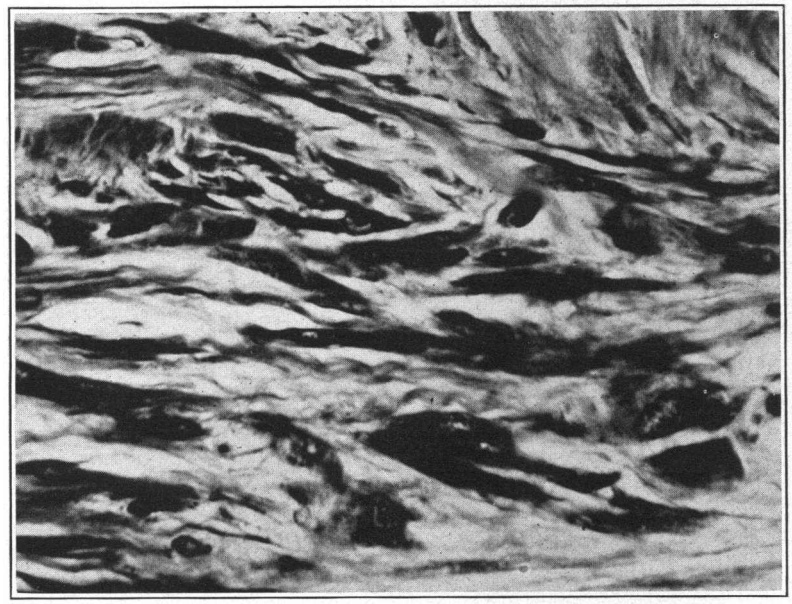

FIGURE 10. *Biopsy Specimen Removed after Ten Days of Intravenous Vitamin C Therapy: Eosin and methylene blue stain (× 600).*

Note the abundant, newly formed fibroblasts, with collagen between them.

Teeth and Gums

Grossly the teeth and gums showed no marked changes during the entire dietary period, although very slight bogginess was found one week before the institution of vitamin C therapy. On brushing the teeth there was no trace of bleeding at any time. Immediately after the fatigue test, while in the scorbutic state, however, the subject did show for the first and only time a small hemorrhage at one gingival margin, a finding which suggests that had he been exposed to such fatigue throughout the experiment, signs of scurvy might have appeared much earlier. The lack of changes in the gums and teeth during the entire period raises the question, however, as to whether those commonly described[57] as typical of scurvy may not be at least in part due to pre-existing or superimposed gingivitis or dental caries or both. Supporting this hypothesis is the fact that in edentulous scorbutic patients no gingival changes are commonly seen. Of interest are the x-ray films of the teeth taken during the scorbutic state, particularly since they are consistent with the alveolar lesions found by Boyle in scobutic guinea pigs.[58–60] In these films slight but definite interruptions of the lamina dura can be made out, which presumably result from atrophy of alveolar bone and its replacement with collagen-free fibrous tissue. Slight but definitely noticeable changes toward the normal could be discerned in the roentgenograms taken five weeks after the subject had resumed a normal diet. It is possible that x-ray films showing such interruptions of the lamina dura may be one of the best criteria for the diagnosis of incipient scurvy.

Anemia

The fact that no appreciable anemia occurred in the subject until the administration of ascorbic acid was begun, in spite of a blood loss of over 6000 cc. during the six-month period, is not in accord with the conclusions of Mettier, Minot and Townsend[61] and of Parsons and Hawksley[62] — which have already been challenged by several workers, notably Cooley[63] and Gingold[64] — to the

effect that vitamin C deficiency per se may cause diminished hematopoiesis.

Resistance and Infection

There is at present a widespread belief that vitamin C deficiency is an important cause of lowered resistance to infection. This is based in part on the well-known fact that there is much more rapid disappearance of the vitamin from

Leukopenia

During the dietary period there was a slight fall in the white-cell count, but since the normal count for the subject was apparently only 5000, this cannot be considered as significant. The rise to 9000 occurring after the beginning of vitamin C therapy is consistent with the findings of other workers,[73] who have noted moderate leukocytosis

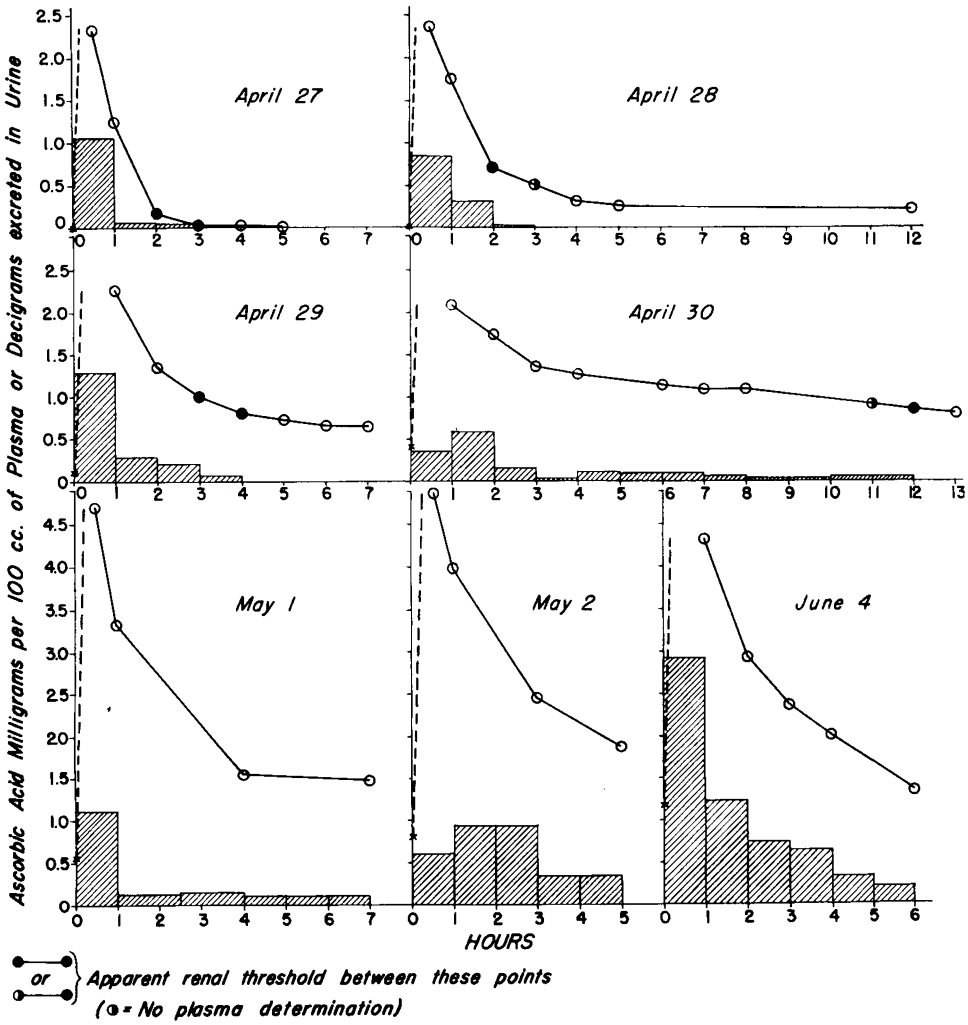

FIGURE 11. *Plasma and Urine Vitamin C Levels Following Saturation with Ascorbic Acid.*

both plasma and tissues in cases of infection, and in part on results in experimental animals.[65–69] Moreover, Ecker[70, 71] and others[72] have recently reported lowered titers of blood complement in scurvy. Although the literature supporting the value of vitamin C in sepsis is extensive, the complete lack of infection in this experimental subject over the period of deficiency raises doubt as to whether vitamin C per se is a marked factor in lowering the resistance of an adult. Moreover, blood-complement titers showed no fall whatsoever even after the appearance of frank scurvy. following the intravenous administrations of ascorbic acid in cases of leukopenia.

Fatigue

The belief that scurvy is characterized by languor and incapacity for work is well borne out by this experiment. Subjectively, weakness was first noticed at about the end of the third month, becoming progressive as time went on. Although no marked change could be found in the muscular activity of the forearm as measured by an ergograph, both for a single contraction and for

contractions each second to exhaustion, the results when the subject ran on a treadmill were striking. Although a considerable part of his inability to run over 16 seconds must be attributed to factors other than vitamin C deficiency, he was able to run 66 seconds following the parenteral administration of ascorbic acid while on the same diet, even though the hemoglobin was 13 per cent lower. Of interest, also, is the fact that the heart rate fell more rapidly during the recovery period following the administration of the vitamin. Moreover, it will be noted that the blood lactate curve was definitely abnormal in the scorbutic

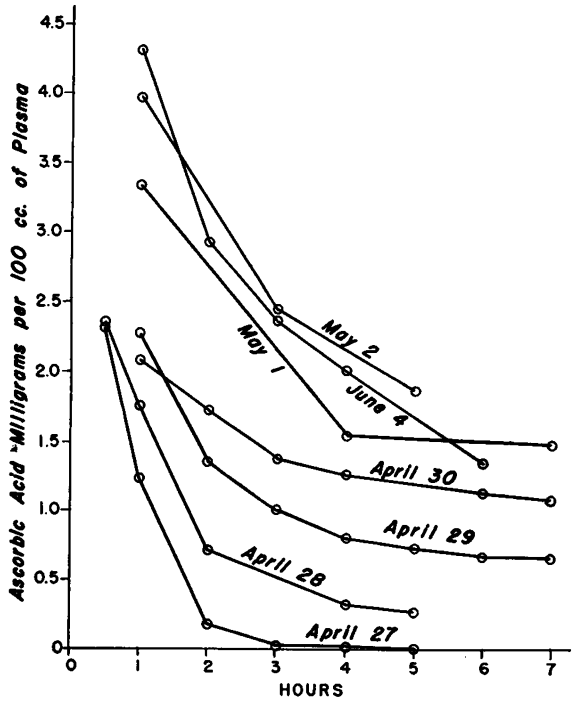

FIGURE 12. *Plasma Vitamin C Saturation Curves.*

state. No hint as to the underlying physiologic mechanisms responsible for these differences before and after therapy could be gleaned from the laboratory determinations made coincidentally with these fatigue tests.

Capillary Fragility

It should not be concluded that capillary fragility is not part of the underlying pathology of scurvy merely because the Göthlin and Dalldorf tests were negative throughout the experiment. The appearance of the petechiae in the lower extremities after a long period of standing suggests that hydrostatic pressure and capillary fragility were factors in their etiology. The experimental results suggest, however, that neither the Göthlin nor the Dalldorf test is a good measure of capillary fragility in early scurvy. Certainly this would seem to throw serious doubt on the contention that such tests are a good index of subclinical vitamin C deficiency,[74] in spite of the fact that frequently in cases of scurvy such tests are positive, and that in many such cases vitamin C therapy rapidly clears up all signs of such abnormal fragility. Lack of correlation between capillary fragility and vitamin C deficiency is also being found by other workers,[75] and notably Farmer.[76]

Blood Pressure

Of possible significance was the fall in blood pressure which occurred and persisted after the last episode of acute blood loss (one hundred and fifty-fifth day), since it returned to normal after the administration of vitamin C, in spite of continuation of the vitamin C-free diet and blood loss. Pertinent in this respect are the facts that in animals relatively large amounts of vitamin C are stored in the adrenal glands, and that scorbutic guinea pigs have diminished adrenocortical hormone[77] and at autopsy show atrophy of the adrenal cortex.[78] Toward the end of the experiment, almost coincidentally with the fall in blood pressure, the blood sodium fell to 136 milliequiv. per liter, rising to 142 milliequiv. directly after vitamin C therapy. Whatever the cause of this lowered systolic pressure, it was not due to diminished blood volume, for this was normal at the end of the six-month period, as measured by the Gibson method.

Glucose and Insulin Tolerance

There are at present many conflicting views as to the effect, if any, of vitamin C on carbohydrate metabolism. It has been reported that there is a lowered glucose tolerance in scorbutic guinea pigs[79] and that the administration of vitamin C results in increased sensitivity to insulin both in normal subjects and in diabetic patients.[80–84] In contrast to these findings are the entirely negative results of other workers[85–87] who have been unable to show any relation between vitamin C and carbohydrate metabolism. In agreement with the latter conclusions are the insulin-tolerance and glucose-tolerance curves of the experimental subject, which were within normal limits while he was in the scorbutic state.

Basal Metabolic Rate

Because vitamin C has relatively strong reducing properties, there is a widespread belief that it serves primarily as a "hydrogen transport agent."[88] Here again the experimental findings are in conflict, some investigators[89, 90] reporting a decrease in metabolic rate in scorbutic guinea pigs, others[91] an increase.

Since the basal metabolic rate of the experimental subject dropped to almost as low a level following the beginning of vitamin C therapy as during the period of deficiency, the variations during this period cannot be considered significant. These findings are in accord with the results of other workers.[92] Of interest, however, is the fact that during the saturation of the subject with vitamin C, the basal metabolic rate fell slightly directly after each injection of the vitamin, whereas after saturation had been completed no such fall occurred. Although this fall was constant during the saturation period it was generally very slight, and its significance may therefore be open to question. A significant drop in metabolic rate following the administration of parenteral ascorbic acid to a scorbutic individual would, of course, suggest that by the introduction of the vitamin the individual's efficiency for utilization of oxygen was increased.

Metabolism of Vitamin C

What part does vitamin C play in the physiology of the human organism other than as a prerequisite for the formation of intercellular substance? This question must at present remain unanswered. The abnormal blood lactate curves during the recovery period of the scorbutic subject after the fatigue test, the variations of the metabolic rate and the abnormal phosphorus and phosphogen contents of the striated muscle, all may serve as leads for future investigations into the metabolism of vitamin C.

Summary and Conclusions

A normal active adult placed himself on a vitamin C-free diet supplemented by the other known vitamins for a period of six months. The findings in this state of pure vitamin C deficiency, that is, in the absence of factors such as multiple avitaminoses, infection, growth or other stress, were as follows:

One hundred and thirty-two days of a diet totally deficient in vitamin C were required for the first abnormal clinical signs — hyperkeratotic papules — to appear; 161 days were necessary for the appearance of the perifollicular hemorrhages of scurvy.

The plasma-ascorbic acid level was zero for thirteen weeks before the first evidence of clinical scurvy was manifest. It is not necessarily, therefore, a good index of the vitamin C status of the individual.

The vitamin level in the white-cell-platelet layer of the centrifuged blood was a good index of the vitamin C status of the subject. This level fell to zero shortly before the appearance of clinical scurvy.

Adequate wound healing occurred after the plasma-ascorbic acid had been zero for forty-four days and when the white-cell-platelet ascorbic acid level was 4 mg. per 100 cc.

With total vitamin C deficiency, failure of wound healing occurred. The tissues under these circumstances showed microscopically a lack of intercellular substance. Parenteral vitamin C alone brought about good healing, and considerable intercellular substance appeared within ten days.

Hyperkeratotic papules containing ingrown hairs appeared over the buttocks and posterior aspects of the legs as a result of vitamin C deficiency; indeed, they may be the first sign of such a deficiency.

There were no gross changes in the gums or teeth (with good pre-existing oral hygiene). Although the mouth was grossly negative, x-ray films of the teeth showed interruptions of the lamina dura in early acute scurvy. Such an x-ray picture may be one of the better diagnostic criteria in early scurvy.

Vitamin C deficiency did not produce anemia.

After prolonged vitamin C deficiency there was inability to perform aerobic work, although the capacity for anaerobic work was undiminished. After a period of aerobic work in the scorbutic state the rate of disappearance of the blood lactate was abnormally slow.

During a six-month period of total deficiency and after a month of clinical scurvy the blood-complement titer was still normal. Over this period there was no evidence of lowered resistance to infection.

The Göthlin, Dalldorf and Rümpel–Leeds tests were negative, even in the presence of frank scurvy. These tests must therefore be poor indices of subclinical scurvy, even though they may in some cases produce petechiae which are cleared up by ascorbic acid therapy.

With severe vitamin C deficiency there was a fall in the blood pressure.

There was a lowering of the total phosphorus content of striated muscle, with an increase in the phosphagen phosphorus.

All the signs and symptoms of scurvy rapidly disappeared following the intravenous injection of ascorbic acid.

When the state of deficiency was complete the plasma-ascorbic acid level fell to zero in five hours after injection of 1 gm. of the vitamin.

Although the blood became completely saturated (as measured by plasma saturation curves and white-cell-platelet levels) after 3 or 4 gm.

of ascorbic acid had been given intravenously, the tissues were not completely saturated at this time, since the urinary output of ascorbic acid was still well below maximal over a six-hour period.

References

1. Holst, A., and Frölich, T.: Ueber experimentellen Skorbut: ein Beitrag zur Lehre von dem Einfluss einer einseitigen Nahrung. *Ztschr. f. Hyg. u. Infektionskr.* 72:1-120, 1912.
2. Wolbach, S. B., and Howe, P. R.: Intercellular substances in experimental scorbutus. *Arch. Path. & Lab. Med.* 1:1-24, 1926.
3. Wolbach, S. B.: Controlled formation of collagen and reticulum: a study of the source of intercellular substance in recovery from experimental scorbutus. *Am. J. Path.* 9:689-700, 1933.
4. Lanman, T. H., and Ingalls, T. H.: Vitamin C deficiency and wound healing: experimental and clinical study. *Ann. Surg.* 105:616-625, 1937.
5. *The Genuine Works of Hippocrates.* Edited by Francis Adams. Vol. I. 466 pp. London: Sydenham Society, 1849. Pp. 196, 267.
6. Lind, J.: *Treatise on the Scurvy.* 476 pp. London: A. Millar, 1757.
7. Aschoff, K. A. L., and Koch, W.: *Der Skorbut.* 122 pp. Jena: Gustave Fischer, 1919.
8. Farmer, C. J., and Abt, A. F.: Determination of reduced ascorbic acid in small amounts of blood. *Proc. Soc. Exper. Biol. & Med.* 34:146-150, 1936.
9. Mindlin, R. L., and Butler, A. M.: Determination of ascorbic acid in plasma: macromethod and micromethod. *J. Biol. Chem.* 122:673-686, 1938.
10. Butler, A. M., and Cushman, M.: Distribution of ascorbic acid in the blood and its significance. *J. Clin. Investigation* 19:459-467, 1940.
11. Harris, L. J., and Ray, S. N.: Diagnosis of vitamin-C subnutrition by urine analysis, with a note on the antiscorbutic value of human milk. *Lancet* 1:71-77, 1935.
12. Evelyn, K. A.; Malloy, H. T., and Rosen, C.: The determination of ascorbic acid in urine with the photoelectric colorimeter. *J. Biol. Chem.* 126:645-654, 1938.
13. Hirsch, A.: *Handbook of Geographical and Historical Pathology.* Vol. 2. 681 pp. London: Sydenham Society, 1885. Pp. 531-533.
14. Drummond, J. C., and Wilbraham, A.: William Stark, M.D.: eighteenth century experiment in nutrition. *Lancet* 2:459-462, 1935.
15. Crandon, J. H., and Lund, C. C.: Vitamin C deficiency in an otherwise normal adult. *New Eng. J. Med.* 222:748-752, 1940.
16. Lund, C. C., and Crandon, J. H.: Human experimental scurvy and the relation of vitamin C deficiency to postoperative pneumonia and to wound healing. *J. A. M. A.* (in press).
17. Ralli, E. P.; Friedman, G. J., and Sherry, S.: The vitamin C requirement of man: estimated after prolonged studies of the plasma concentration and daily excretion of vitamin C in three adults on controlled diets. *J. Clin. Investigation* 18:705-714, 1939.
18. Van Eekelen, M.: On the amount of ascorbic acid in blood and urine: the daily human requirements for ascorbic acid. *Biochem. J.* 30:2291-2298, 1936.
19. Frazier, C. N., and Hu, C. K.: Cutaneous lesions associated with a deficiency in vitamin A in man. *Arch. Int. Med.* 48:507-514, 1931.
20. Dalldorf, G.: The pathology of vitamin C deficiency. *The Vitamins: A symposium arranged under the auspices of the Council on Pharmacy and Chemistry and the Council on Foods of the American Medical Association.* 637 pp. Chicago: American Medical Association, 1939. Pp. 339-348.
21. Keil, H.: The follicular lesions of vitamine A and C deficiencies: critical survey. *Am. J. Digest. Dis.* 5:40-48, 1938.
22. Jeghers, H.: The degree and prevalence of vitamin A deficiency in adults with a note on its experimental production in human beings. *J. A. M. A.* 109:756-762, 1937.
23. Robinson, S.: Experimental studies of physical fitness in relation to age. *Arbeitsphysiol.* 10:251-323, 1938.
24. Göthlin, G. F.: Outline of a method for the determination of the strength of the skin capillaries and the indirect estimation of the individual vitamin C standard. *J. Lab. & Clin. Med.* 18:484-490, 1933.
25. *Idem:* When is capillary fragility a sign of vitamin-C subnutrition in man. *Lancet* 2:703-705, 1937.
26. Dalldorf, G.: A sensitive test for subclinical scurvy in man. *Am. J. Dis. Child.* 46:794-802, 1933.
27. Wright, I. S.; Lilienfeld, A., and MacLenathen, E.: Determination of vitamin C saturation: a five-hour test after an intravenous test dose. *Arch. Int. Med.* 60:264-271, 1937.
28. Stepp, W.: Vitaminmangel als Ursache und Folge von Magen-Darmerkrankungen. *München. med. Wchnschr.* 83:1119-1123, 1936.
29. Kendall, A. I., and Chinn, H.: The decomposition of ascorbic acid by certain bacteria. CIX. Studies in bacterial metabolism. *J. Infect. Dis.* 62:330-336, 1938.
30. Borsook, H.; Davenport, H. W.; Jeffreys, C. E. P., and Warner, R. C.: The oxidation of ascorbic acid and its reduction in vitro and in vivo. *J. Biol. Chem.* 117:237-279, 1937.
31. Heinemann, M.: On the relation between diet and urinary output of thiosulphate and ascorbic acid. II. Human requirements for vitamin C. *Biochem. J.* 30:2299-2306, 1936.
32. Faulkner, J. M., and Taylor, F. H. L.: Observations on the renal threshold for ascorbic acid in man. *J. Clin. Investigation* 17:69-75, 1938.
33. Sendroy, J., Jr., and Miller, B. F.: Renal function as a factor in the urinary excretion of ascorbic acid. *J. Clin. Investigation* 18:135-140, 1939.
34. Wright, I. S., and MacLenathen, E.: Vitamin C saturation: kidney retention after an intravenous test dose of ascorbic acid. *Proc. Soc. Exper. Biol. & Med.* 38:55-59, 1938.
35. Taffel, M., and Harvey, S. C.: Effect of absolute and partial vitamin C deficiency on healing of wounds. *Proc. Soc. Exper. Biol. & Med.* 38:518-525, 1938.
36. Archer, H. E., and Graham, G.: The subscurvy state in relation to gastric and duodenal ulcer. *Lancet* 2:364-366, 1936.
37. Wolfer, J. A., and Hoebel, F. C.: The significance of cevitamic acid deficiency in surgical patients. *Surg., Gynec. & Obst.* 69:745-755, 1939.
38. Holman, E.: Vitamin and protein factors in preoperative and postoperative care of the surgical patient. *Surg., Gynec. & Obst.* 70:261-268, 1940.
39. Bartlett, M. K.; Jones, C. M., and Ryan, A. E.: Vitamin C studies on surgical patients. *Ann. Surg.* 111:1-26, 1940.
40. Abt, A. F., and Farmer, C. J.: Vitamin C: pharmacology and therapeutics. *J. A. M. A.* 111:1555-1565, 1938.
41. Harde, E.; Rothstein, I. A., and Ratish, H. D.: Urinary excretion of vitamin C in pneumonia. *Proc. Soc. Exper. Biol. & Med.* 32:1088-1090, 1935.
42. Rinehart, J. F.; Greenberg, L. D., and Baker, F.: Reduced ascorbic acid content of blood plasma in rheumatoid arthritis. *Proc. Soc. Exper. Biol. & Med.* 35:347-350, 1936.
43. Rinehart, J. F.; Greenberg, L. D., and Christie, A. V.: Reduced ascorbic acid content of blood plasma in rheumatic fever. *Proc. Soc. Exper. Biol. & Med.* 35:350-353, 1936.
44. Faulkner, J. M., and Taylor, F. H. L.: Vitamin C and infection. *Ann. Int. Med.* 10:1867-1873, 1937.
45. Abbasy, M. A.; Harris, L. J., and Hill, W. G.: Vitamin C and infection: excretion of vitamin C in osteomyelitis. *Lancet* 2:177-180, 1937.
46. Abbasy, M. A.; Harris, L. J., and Ellman, P.: Vitamin C and infection: excretion of vitamin C in pulmonary tuberculosis and in rheumatoid arthritis. *Lancet* 2:181-183, 1937.
47. Martin, G. J., and Heise, F. H.: Vitamin C nutrition in pulmonary tuberculosis. *Am. J. Digest. Dis. & Nutrition* 4:368-374, 1937.
48. Lauber, H. J.; Bersin, T., and Nafziger, H.: Der Ascorbinsäurebedarf bei chirurgischen Infektionen. *Klin. Wchnschr.* 16:1274-1276, 1937.
49. Sure, B., and Theis, R. M.: Hyperthyroidism and vitamin C. *Proc. Soc. Exper. Biol. & Med.* 37:646-648, 1938.
50. Lewis, R. A.: The effect of hyperthyroidism upon the metabolism of vitamin C. *Bull. Johns Hopkins Hosp.* 63:31-40, 1938.
51. Göthlin, G. F.: A method of establishing the vitamin C standard and requirements of physically healthy individuals by testing the strength of their capillaries. *Skandinav. Arch. f. Physiol.* 61:225-270, 1931.
52. *Idem:* Human daily requirements of dietary ascorbic acid. *Nature* 134:569, 1934.
53. Göthlin, G. F.; Frisell, E., and Rundqvist, N.: Experimental determinations of the indispensable requirements of vitamin C (ascorbic acid) of physically healthy adult. *Acta. med. Skandinav.* 92:1-38, 1937.
54. Report by Technical Commission on Nutrition on work of the third session. *Bull. Health Organ., League of Nations* 7:475, 1938.
55. Smith, S. L.: Human requirements of vitamin C. *J. A. M. A.* 111:1753-1764, 1938.
56. Kellie, A. E., and Zilva, S. S.: The vitamin C requirements of man. *Biochem. J.* 33:153-164, 1939.
57. Weisberger, D.; Young, A. P., and Morse, F. W., Jr.: Study of ascorbic acid blood levels in dental patients. *J. Dent. Research* 17:101-105, 1938.
58. Boyle, P. E.; Bessey, O. A., and Wolbach, S. B.: Experimental production of the diffuse alveolar bone atrophy type of periodontal disease by diets deficient in ascorbic acid (vitamin C). *J. Am. Dent. A.* 24:1768-1777, 1937.
59. Boyle, P. E.: Dietary deficiencies as a factor in the etiology of diffuse alveolar atrophy. *J. Am. Dent. A.* 25:1436-1446, 1938.
60. *Idem:* The effect of ascorbic acid deficiency on enamel formation in the teeth of guinea pigs. *Am. J. Path.* 24:843-848, 1938.
61. Mettier, S. R.; Minot, G. R., and Townsend, W. C.: Scurvy in adults: especially the effect of food rich in vitamin C on blood formation. *J. A. M. A.* 95:1089-1093, 1930.
62. Parsons, L. G., and Hawksley, J. C.: Studies in anaemias of infancy and early childhood: anhaematopoietic anaemias (deficiency diseases of erythron): nutritional anaemia, and anaemias of prematurity, scurvy and coeliac disease. *Arch. Dis. Child.* 8:117-144, 1933.
63. Cooley, T. B.: Brennemann's *Practice of Pediatrics.* 203 pp. Hagerstown, Maryland: W. F. Prior Co., 1937. P. 9.
64. Gingold, N.: L'action de l'acide ascorbique (vitamine C) sur l'appareil hématopoïétique normal et pathologique (en dehors des états hémorragiques). *Sang* 11:392-399, 1937.
65. Greenwald, C. K., and Harde, E.: Vitamin C and diphtheria toxin. *Proc. Soc. Exper. Biol. & Med.* 32:1157-1160, 1935.
66. King, C. G., and Menten, M. L.: The influence of vitamin C level upon resistance to diphtheria toxin. *J. Nutrition* 10:129-140, 1935.
67. Thaddea, S., and Hoffmeister, W.: C-Vitamin und Infektabwehr. *Klin. Wchnschr.* 16:856-858, 1937.
68. Perla, D., and Marmorston, J.: Role of vitamin C in resistance. *Arch. Path.* 23:683-712, 1937.
69. Greene, M. R.; Steiner, M., and Kramer, B.: The role of chronic vitamin-C deficiency in the pathogenesis of tuberculosis in the guinea pig. *Am. Rev. Tuberc.* 33:585-624, 1936.
70. Ecker, E. E.; Pillemer, L.; Wertheimer, D., and Gradis, H.: Ascorbic acid and complement function. *J. Immunol.* 34:19-37, 1938.
71. Ecker, E. E., and Pillemer, L.: Complement and ascorbic acid in human scurvy: experimental study. *J. A. M. A.* 112:1449-1452, 1939.
72. Chu, F. T., and Chow, B. F.: Correlation between vitamin C content and complement titer of human blood plasma. *Proc. Soc. Exper. Biol. & Med.* 38:679-682, 1938.
73. Schnetz, H.: Vitamin C und Leukocytenzahl. *Klin. Wchnschr.* 17:267-269, 1938.
74. Sloan, R. A.: Comparison of methods for detecting and grading subclinical scurvy. *J. Lab. & Clin. Med.* 23:1015-1026, 1938.

75. Liebmann, J.; Wortis, H., and Wortis, E.: Note on lack of correlation of capillary fragility with vitamin C content of blood, spinal fluid and urine. *Am. J. M. Sc.* **196**:388-392, 1938.
76. Farmer, C. J.: personal communication.
77. Giroud, A., and Santa, N.: Absence d'hormone corticale chez les animaux carencés en acide ascorbique. *Compt. rend. Soc. de biol.* **131**:1176-1178, 1939.
78. Bessey, O. A.; Menten, M. L., and King, C. G.: Pathologic changes in the organs of scorbutic guinea pigs. *Proc. Soc. Exper. Biol. & Med.* **31**:455-460, 1934.
79. Sigal, A., and King, C. G.: The relationship of vitamin C to glucose tolerance in the guinea pig. *J. Biol. Chem.* **116**:489-492, 1936.
80. Roller, M.: Ueber den Einfluss der Vitamine A und C auf die Glykosurie bei Diabetes mellitus. *Med. Klin.* **32**:898, 1936. Ueber den Einfluss der Vitamine A und C auf den alimentären Blutzuckeransteig. *Ibid.* **32**:1007-1009, 1936.
81. Stoïcesco, S., and Gingold, N.: Action de l'acide ascorbique (vitamin C) sur le métabolisme des hydrates de carbone chez l'homme normal. *Bull. Acad. de méd. de Roumanie* **1**:130-132, 1936.
82. Stepp, W.; Schroeder, H., and Altenburger, E.: Vitamin C und Blutzucker. *Klin. Wchnschr.* **14**:933, 1935.
83. Schroeder, H.: Beziehungen der wichtigsten Vitamine zum Kohlehydratstoffwechsel. *Ztschr. f. d. ges. exper. Med.* **101**:373-403, 1937.
84. Mosonyi, J., and Aszodi, Z.: Beeinflussung der Langerhanschen Inseln durch den Vagus mittels der Vitamine B_1 und C. *Klin. Wchnschr.* **17**:337-344, 1938.
85. Kreitmair, H.: Beiträge zur Pharmakologie der Ascorbinsäure (Vitamin C). *Arch. f. exper. Path. u. Pharmakol.* **176**:326-339, 1934.
86. Armentano, L.; Bentsáth, A.; Hámori, A., and Korányi, A.: Die Wirkung der Ascorbinsäure auf den Stoffwechsel und auf das Blut. *Ztschr. f. d. ges. exper. Med.* **96**:321-327, 1935.
87. Sebesta, V.; Smith, R. M.; Fernald, A. T., and Marble, A.: The vitamin C status of diabetic patients. *New Eng. J. Med.* **220**:56-60, 1939.
88. King, C. G.: The chemistry of vitamin C. The physiology of vitamin C. *The Vitamins.*[20] Pp. 323-338.
89. Söderström, N., and Törnblom, N.: Vergleichende Untersuchungen über die Sauerstoffaufnahme bei skorbutischen und normalen Meerschweinchen. *Skandinav. Arch. f. Physiol.* **66**:67-77, 1933.
90. Baucke, J.: Das Verhalten des Gasstoffwechsels Vitamin C-frei ernährter Meerschweinchen. *Arch. f. d. ges. Physiol.* **241**:392-401, 1939.
91. Fidlar, E.; Sheppard, M., and McHenry, E. W.: The physiological properties of ascorbic acid: the increase in metabolism of guinea pigs on a diet deficient in ascorbic acid. *Biochem. J.* **33**:344-346, 1939.
92. Altenburger, E., and Diehl, F.: Vitamin C und Grundumsatz. *Arch. f. exper. Path. u. Pharmakol.* **193**:10-13, 1939.

ALPHA AND BETA NEUTRAL KETOSTEROIDS (ANDROGENS)*

Preliminary Observations on Their Normal Urinary Excretion and the Clinical Usefulness of their Assay in Differential Diagnosis

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THIS paper reports preliminary data on the twenty-four-hour urinary excretion of alpha, beta and total neutral ketosteroids‖ by normal individuals of both sexes and of varying ages and by patients showing male sexual precocity or virilism.

The available evidence[1–7] indicates that the neutral ketosteroids (17-ketosteroids) arise from substances produced by the adrenal glands and male gonads. The total output is therefore considered to be a rough index of the combined activity of these two glands. The isolation of individual ketosteroids by Callow et al.,[2–5, 7, 8] Butler and Marrian,[10] Hirschmann[11] and Dorfman, Cook and Hamilton[12] affords evidence that whereas the alpha neutral ketosteroids probably arise from adrenal and gonadal secretions, the beta neutral ketosteroids are an excretion product only of the cells of the adrenal cortex.

Accepting this evidence as a working hypothesis, the rate of beta neutral ketosteroid excretion by the subjects mentioned above has been determined by a relatively simple but directly quantitative colorimetric method reported elsewhere.[13]¶ The data obtained not only augment the evidence favoring the adrenal cortex as the source of beta ketosteroids, but also provide specific diagnostic criteria for the differentiation of adrenocortical hyperplasia, Cushing's syndrome without adrenal tumor and adrenocortical carcinoma.

RESULTS

The data obtained on normal subjects (Table 1) show the following:

The average daily excretion of total neutral ketosteroids of children under seven years of age was very low (1.3 mg.). It increased with age, reaching an average of 4.0 mg. between seven and twelve years and 8.2 mg. between twelve and fifteen years. Adult men tended to excrete more ketosteroids (15.0 mg.) than did adult women (10.2 mg.). The level of total excretion of pregnant women (15.1 mg.) approximated that of adult men.

The average daily alpha neutral ketosteroid excretion paralleled the total output. The aver-

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‖The terms "alpha" and "beta" refer to the spatial position of the 3-hydroxyl group. The chemical separation of alpha and beta neutral ketosteroids as discussed here depends on the fact that beta ketosteroids (which include dehydroisoandrosterone and isoandrosterone) may be precipitated with digitonin, leaving the other, or alpha, ketosteroids (such as androsterone and etiocholane-3-[α]-ol-17-one) in solution. The difference in the ketosteroid content of solutions before and after precipitation, therefore, gives the beta ketosteroid content.

¶In any comparison of the data obtained by this method, due consideration should be given to the procedure employed for hydrolyzing and extracting the urines. The data reported here were obtained on individual twenty-four-hour specimens which were hydrolyzed by the commonly employed procedure of boiling for ten minutes with 150 cc. of hydrochloric acid per liter of urine, followed by immediate extraction of the hormones in a rapid, benzene[14] or carbon tetrachloride extractor.[15] Recent experiments in our laboratory indicate that this method of hydrolysis is not optimal. It results in some destruction of unconjugated hormone and some replacement of the beta hydroxyl group, on which the estimation of the beta fraction depends. Consequently, the results reported may include an underestimate of the total excretion and of the beta fraction and an overestimate of the alpha fraction. These errors will be considered in detail in a forthcoming paper.[33] They do not alter the significance of the data reported here.