Clinical manifestations of ascorbic acid deficiency in man[1, 2]

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The metabolism of ascorbic acid has been studied in normal men receiving adequate intakes of this vitamin by Baker et al. (1, 2). Prior to 1966, no study utilizing isotopic techniques had been performed in experimental human scurvy to define the metabolism of this vitamin in the face of severe dietary deficiency. We recently designed and completed two separate studies of experimentally induced scurvy in man. The results of the first have been published (3–5) and the observations made during the second study constitute the substance of this report.

Earlier studies of experimental scurvy by Pijoan and Lozner (6) suggested that after initial saturation with vitamin C, the body stores were sufficient to protect against scurvy for a period of deprivation lasting as long as 5 to 6 months. The British Medical Research Council (7) reviewed previous experimental studies and concluded that healthy men have stores of ascorbic acid sufficient to enable them to exist on deficient diets for periods ranging from 160 to 200 days without developing overt scurvy. In our two studies during which the subjects developed scurvy, it was apparent that the onset of this disorder began in less than 90 days, even though the participants were initially fed a diet containing 77.5 mg vitamin C. During the second study, we compared the body pool size with the blood and plasma levels of ascorbic acid and with the onset of clinical scurvy.

The British Medical Research Council demonstrated that a daily dose of 10 mg L-ascorbic acid was sufficient to prevent scurvy for as long as 424 days (7) and that the same dose was enough to cure scurvy once it had appeared. In a study performed by the Royal Canadian Air Force, Johnstone et al. (8) did not mention the development of clinical signs of scurvy in a group of 33 recruits who received a daily intake of 7.9 mg ascorbic acid for a period of 6 to 8 months. We previously observed (3, 4) that an intake of 6.5 mg ascorbic acid daily was sufficient to cause slow but steady disappearance of scurvy in a single subject. This low intake of ascorbic acid (except during periods of emotional stress) was enough to cause a gradual repletion in the body pool of ascorbic acid in one man.

The major objectives of this second study of experimental scurvy in man were to obtain additional information regarding *1*) the size of the body pool of ascorbic acid, *2*) the rate of catabolism of this vitamin, *3*) the correlation between body pool size and blood plasma levels, and *4*) the relationship of all three to clinical signs and symptoms of scurvy. In addition, by giving both tritium-labeled ascorbic acid and ^{14}C-labeled ascorbic acid, we were able to study the metabolism of this vitamin more precisely. Finally, we wished to observe the rate of repletion of body pool size and the cure of scurvy in men receiving a greater range of daily doses of this vitamin.

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TABLE 1
List of subjects, period of depletion and level of intake on repletion

Volunteer	Age	Height, cm	Initial weight, kg	Period of complete depriva-tion of ascorbic acid, days	Daily intake of ascorbic acid from diet and supplements	
					Day	*mg*
H	26	184.2	84.1	91	1 to 13	77.5
					14 to 104	0
					105 to 110	4
					111 to 227	6.5
					228 to 258	600
P	33	175.3	87.3	91	1 to 13	77.5
					14 to 104	0
					105 to 110	4
					111 to 227	6.5
					228 to 258	600
S	37	182.9	82.7	97	1 to 13	77.5
					14 to 97	0
					98 to 110	2.5
					111 to 227	66.5
					228 to 258	600
R	52	167.6	65.2	97	1 to 13	77.5
					14 to 110	0
					111 to 227	66.5
					228 to 258	350
Mc	34	170.2	68.9	84	1 to 13	77.5
					14 to 97	0
					98 to 110	128
					111 to 227	130.5
					228 to 258	350

Methods

Volunteers from the Iowa State Penitentiary at Fort Madison, Iowa, were recruited and their informed consent was obtained. They then were housed on the Metabolic Ward of University Hospitals in Iowa City, Iowa, for the duration of the experiment. This second study began with six volunteers, but one subject requested to withdraw from the experiment prior to the development of clinical scurvy. The remaining five men were deprived of ascorbic acid for periods ranging from 84 to 97 days following an initial control period (*days 1* to *13*) during which they were fed a solid diet of soy protein products containing 2.5 mg ascorbic acid daily and supplemented by an additional 75 mg ascorbic acid. On *day 14* their diet was changed to a liquid formula containing essentially no ascorbic acid. This formula, based on vitamin-free casein, has been previously described by Hodges et al. (3).[8] Because of the unpalatability of this formula, the men took it thrice daily via polyethylene gastric tube (3). Fifteen percent of their calories were supplied from protein, 40% from fat, and 45% from carbohydrates. Absence of L-ascorbic acid and L-dehydroascorbic acid from the diet was confirmed by chemical analysis (4). Mineral supplements and the amounts of essential vitamins recommended by the National Research Council (9) (with the exception of ascorbic acid) were given daily. The mineral supplement was inadvertently omitted from the diets during the first 34 days of the depletion period (*days 14* to *47*).

Isotopic labeling of the body pools with L-ascorbic-1-^{14}C acid was accomplished in all men 1 week before commencing depletion. Two men (*S* and *P*) received, in addition, tritium-labeled L-ascorbic acid as described elsewhere (10).

After the period of complete deprivation of ascorbic acid, and at a time when the men had obvious evidence of clinical scurvy, we began to replete them with differing intakes of vitamin C. A solid diet based on soy protein foods and containing 2.5 mg L-ascorbic acid was fed from *day 98* to *day 227* to *subject S* and from *day 111* to *227* to all the other men. The percent of calories derived from protein, fats, and carbohydrates remained the same in the soy protein diet as in the liquid formula diet. In the final phase of the study, beginning on *day 228* and lasting until *day 258*, the men were fed a regular diet providing approximately 100 mg ascorbic acid plus a supplement of either 250 or 500 mg daily and an ad libitum intake of calories. The duration of deprivation and the total daily intakes of ascorbic acid from the diet and from the oral supplements are summarized in Table 1.

[8] The formula contained 30 mg ferrous fumarate, not 30 g as shown in Table 1 of reference 3.

TABLE 2
Signs and symptoms of scurvy in the five subjects

H	Hyperkeratosis
	Congested follicles
	Petechiae
	Ecchymoses
	Subconjunctival hemorrhage
	Joint effusions
	Dyspnea on exertion
	Edema
	Femoral neuropathy
Mc	Hyperkeratosis
	Congested follicles
	Petechiae
	Ecchymoses
	Swollen gums
	Arthralgia
P	Hyperkeratosis
	Congested follicles
	Coiled hairs
	Petechiae
	Bleeding swollen gums
	Joint effusions
R	Hyperkeratosis
	Congested follicles
	Coiled hairs
	Ecchymoses
	Joint effusions
	Dyspnea on exertion
S	Hyperkeratosis
	Redness of gum margins
	Petechiae

Temperature, pulse, and respiratory rates were recorded four times daily and blood pressure twice daily. The men were examined each day by an internist and at intervals by an ophthalmologist (11). Chest X-rays and electrocardiograms were taken at intervals. Twenty-four hour collections of urine and feces were made daily for determinations of urinary and fecal nitrogen and for radiometric assay (10). Samples of expired air were collected for radiometric assay (10) subsequent to administration of the isotopically labeled L-ascorbic acid. Weekly hematologic studies included hemoglobin, white and red cell counts, hematocrit, and erythrocyte sedimentation rate.

Aliquots of the diet were assayed weekly for ascorbic acid content (4). Plasma and whole blood ascorbic acid levels were determined weekly (4). The daily rate of excretion of the labeled ascorbic acid or metabolites in the urine was measured (4) and serum vitamin A and carotene levels were determined weekly (12). Additional measurements included total serum proteins and serum protein electrophoresis, cholesterol, creatinine, erythrocyte hemolysis and tryptophan load tests (13). Insulin tolerance tests (0.1 unit/kg body wt subcutaneously) and adrenalin tolerance tests (0.0025 mg/kg body wt subcutaneously) were performed at monthly intervals. During these tests the concentration of glucose was measured in the blood at 0, 30, 60, 90, 120, 180, and 240 minutes.

The men were exposed in a cold climate-control room (50 F, 45% humidity) over a 4-hr period each day for 1 to 4 days beginning on *day 112*. Psychological tests were administered and measurements of psychomotor performance were made at intervals (14). Vascular reactivity was studied during deficiency and again after repletion (15). The immunologic response to injections of typhoid antigens was assessed during deficiency and after repletion; agglutination titers were determined by the method of Widal (16).

Results (clinical)

Despite a somewhat shorter period of deprivation in the second scurvy study, (84 to 97 days) than in the first (99 days), the subjects in the second study developed a more severe degree of scurvy. Joint effusions, petechial hemorrhages, small ecchymotic lesions, a large subconjunctival hemorrhage and edema and dyspnea were observed in addition to the hyperkeratosis, perifollicular congestion, gum changes, and occasional petechial hemorrhages observed in the first study. The signs and symptoms of scurvy noted in each subject are listed in Table 2.

The increased severity of scurvy that occurred in the second study can be accounted for by individual differences in the rate of depletion of the body pool(s) of ascorbic acid (Table 3).

In the first study, the body pool(s) of ascorbic acid were labeled isotopically by oral administration of L-ascorbic-1-^{14}C acid

TABLE 3
Rate of catabolism of ascorbic acid

	Subject	Percent available body pool of ascorbic acid catabolized/day
Second study	*H*	4.1
	P	3.5
	Mc	3.2
	R	2.8
	S	2.6
First (4) study	*N*	2.7
	K	2.5
	S	2.4
	L	2.2

on the 23rd day of depletion (4). In the second study, isotopic labeling was accomplished 7 days prior to the beginning of the depletion period and again on the 14th, 15th, and 16th days of deprivation (10).

The most rapid rate of depletion of ascorbic acid, 4.1% of the available body pool/day, occurred in *subject H* who also developed the most severe degree of clinical scurvy. Initially the pool size of each man approximated 1,500 mg.

The first sign of scurvy to appear in both studies was petechial hemorrhage. These petechiae occurred in four of the five men in the second study and appeared as early as the 29th day of depletion. They first appeared when the plasma ascorbic acid ranged from 0.13 to 0.24 mg/100 ml and the pool size from 96 to 490 mg. Spontaneous ecchymoses occurred in four of the men, but they were always small in size and appeared only on the legs.

Coiled hairs were observed in two of the men and first appeared on the 42nd and 74th days, respectively. Gum changes appeared only in the four men who had their own teeth; there were none in the one subject who was edentulous. As in the first study, these changes were most marked in those men who initially had gingivitis. The subject who developed the most severe scurvy initially had healthy gums and he showed only minor gum changes at the peak of deficiency. The first definite abnormalities of the gums appeared between the 43rd and 84th days of depletion and progressed after the plasma ascorbic acid levels fell below 0.16 mg/100 ml and the pool size below 360 mg.

Hyperkeratosis, which was present in all five subjects, was marked in three of them and slight in two. This first appeared or became more apparent between the 45th and 100th days. Follicular congestion also developed in all five of the men between the 49th and 90th days.

Although none of the subjects in the first scurvy study developed arthralgia, this was a complaint in four of the five men who participated in the second scurvy study. The onset of joint pains began between the 67th and 96th days. Joint effusions appeared between the 68th and 103rd days by which time the pool size was below 110 mg and the plasma ascorbic acid level was less than 0.16 mg/100 ml. Joint pain occurred in the knees of three of the five men, in the ankles of two, and in the elbows, wrists, and shoulders of one man each. Effusions in both knees occurred in three men, one of whom also had swelling of both of his ankles.

The onset of signs and symptoms, the levels of plasma and whole blood ascorbic acid, and the size of the body pool(s) of this vitamin are summarized in Table 4. It is apparent that arthralgia and joint effusions were relatively late manifestations of clinical scurvy and that the onset of joint involve-

TABLE 4
Onset of signs of scurvy

Signs of scurvy	Day of study	Day of depletion	Plasma ascorbic acid, mg/100 ml	Whole blood ascorbic acid, mg/100 ml	Pool size, mg
Petechiae 4/5	42 to 79	29 to 66	0.13 to 0.24	0.32 to 0.40	96 to 490
Ecchymoses 4/5	49 to 116	36 to 103	0.06 to 0.30	0.27 to 0.73	19 to 438
Coiled hairs 2/5	55 to 87	42 to 74	0.14 to 0.17	0.33 to 0.50	94 to 166
Gum changes 4/5	56 to 97	43 to 84	0.09 to 0.16	0.30 to 0.50	63 to 360
Hyperkeratosis 5/5	58 to 113	45 to 100	0.00 to 0.16	0.29 to 0.47	64 to 342
Congested follicles 5/5	62 to 103	49 to 90	0.00 to 0.16	0.20 to 0.40	32 to 324
Sicca syndrome 5/5	71 to 136	58 to 123	0.06 to 0.18	0.30 to 0.42	24 to 138
Dyspnea 2/5	76 to 103	63 to 90	0.16 to 0.16	0.34 to 0.37	34 to 78
Arthralgia 4/5	80 to 109	67 to 96	0.04 to 0.16	0.30 to 0.50	45 to 217
Joint effusions 3/5	81 to 116	68 to 103	0.07 to 0.16	0.20 to 0.34	39 to 110
Neuropathy 1/5	84	71	0.15	0.37	80
Marked edema 1/5	114	101	0.15	0.47	67

Numbers after signs of scurvy refer to the number of men out of the total (5) in whom a particular symptom appeared.

ment was always preceded by other signs of scurvy.

Edema was noted in three of the men, but in two of them there was only minimal pretibial pitting on pressure or some slight puffiness of the feet. Beginning on the 88th day of deprivation there was a rapid increase in weight followed by swelling of the legs in the third man (*H*), who had the most severe degree of scurvy. Repletion with 4 mg ascorbic acid day was commenced 4 days later on *day 105* (see Table 5). Oliguria followed and his urinary output dropped to 340 ml/day on *day 109*. His intake of ascorbic acid was then increased to 6.5 mg/day on *day 111* and after a brief initial increase in edema, he had a profound diuresis with complete disappearance of edema by *day 133*.

An unusual complication of scurvy that occurred in one subject (*H*) was bilateral femoral neuropathy (17). The onset of this neuropathy occurred early in the course of scurvy on the 71st day of deprivation. At this time his plasma ascorbic acid was 0.15 mg/100 ml and his body pool size was only 80 mg. Except for two perifollicular hemorrhages on one ankle between the 67th and the 73rd days, he had no other evidence of scurvy until the 89th day of depletion when effusions appeared in both knees. His neuropathy was attributed to hemorrhage into the sheaths of both femoral nerves. Recovery was complete 4 months after the beginning of repletion with ascorbic acid.

Another interesting clinical observation made during the second scurvy study was the development of Sjögren's (sicca) syndrome (11), a condition usually associated with one of the diffuse connective tissue disorders. All five men in the second study developed one or more of the component features of this syndrome and two men (*H* and *P*) developed the complete syndrome. *Subject H* had xerostomia and keratoconjunctivitis sicca and *subject P* had enlargement of his submandibular salivary glands in addition to keratoconjunctivitis sicca. Dryness of the eyes was present in a third subject (*S*) and another

TABLE 5
Water retention in *subject H*

Day of study	Day of deprivation	Daily intake ascorbic acid, mg	Urine output, ml	Weight, kg	Ascorbic acid		
					Pool size, mg	Plasma, mg/100 ml	Whole blood, mg/100 ml
0				84.1			
101	88		720	84.7	37		
102	89	0	760	85.8	34		
103	90		710	86.5	32		
104	91		760	86.8	30		
105			895	86.9	33	0.14	0.2
106			560	86.0	37		
107			500	86.5	42		
108		4	490	85.8	46		
109			340	86.1	50		
110			410	86.6	54		
111			600	86.0	57		
112			790	86.9	60	0.15	0.47
113			990	87.8	64		
114			1,200	88.8	67		
115		6.5	2,200	88.8	70		
116			1,910	87.8	73		
117			2,900	86.7	76		
118			3,180	84.9	79		
119			2,260	82.9	83	0.10	0.27
120			2,160	81.9	86		
121			2,270	81.0	89		
133				77.2	128	0.18	0.30

man (*Mc*) had transient parotid gland enlargement. Other features of the sicca syndrome included dental caries, recurrent loss of new dental fillings and tenderness of the buccal mucosa. Cutaneous changes included pruritus, scaling ichthyotic or eczematous appearance, and pigmentation of the ichthyotic areas. The men also noted excessive loss of hair.

Hematologic studies showed a slight degree of anemia as manifested by a decline of hemoglobin and hematocrit values. In *subject H,* this decline was greater when he had water retention and returned to normal after diuresis.

Urinary excretion of ascorbic acid declined rapidly during depletion and was essentially zero after 1 month. Average values for plasma ascorbate fell rapidly during the first month of depletion. Subsequently the majority of the values obtained remained below 0.2 mg/100 ml until repletion was begun. Results for *subjects R* and *S* who were repleted with 66.5 mg of ascorbic acid daily are shown in Fig. 1. In Fig. 2, one can see that the rate of depletion of ascorbic acid in *subjects P* and *H* was similar to that in Fig. 1. However, the plasma levels for *subject H* did not remain below 0.2 mg/100 ml until after the second month of depletion. During repletion on a level of 6.5 mg ascorbic acid/day, the plasma levels of *subjects P* and *H* generally remained low.

Serum cholesterol values initially averaged 280 mg/100 ml on blood drawn at the prison. During the control period the average serum cholesterol value fell to about 200 mg/100 ml and continued to fall during the depletion period to an average level of about 180 mg/100 ml. Beginning with the repletion period, the cholesterol value began to rise before there was any other change in diet and continued to rise until the end of the study when it reached 300 mg/100 ml. Of course, during the final 30 days of the study the men ate foods that would be expected to increase serum cholesterol values (Fig. 3).

The men all had been previously immunized with typhoid antigen during military service. They were given "booster injec-

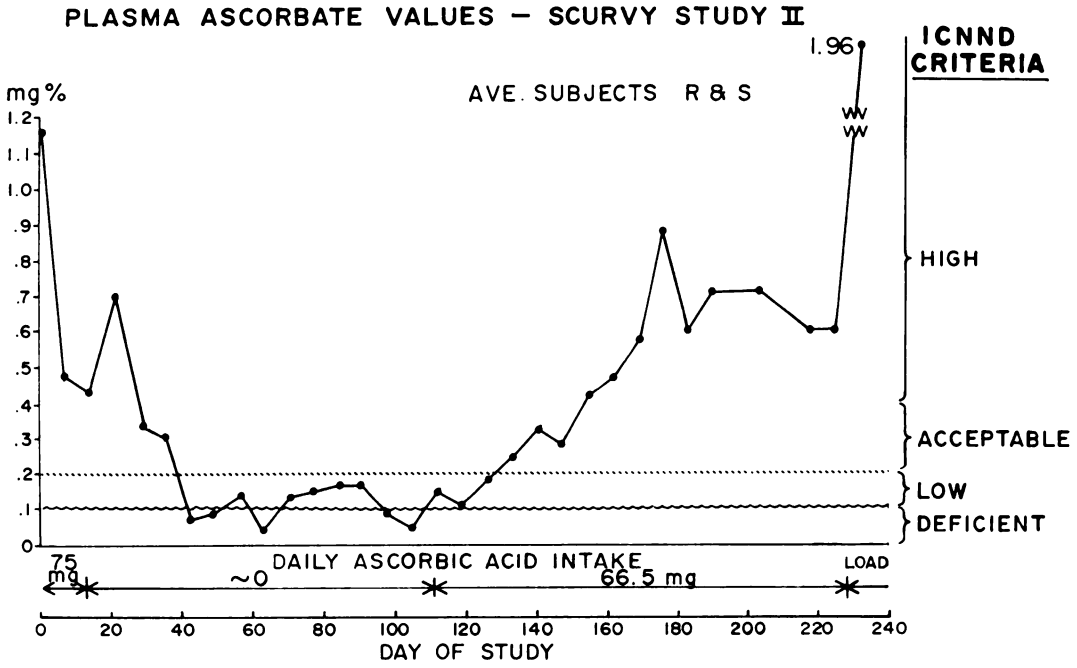

FIG. 1. Average plasma ascorbate values of two men during depletion and repletion. Note variability during period of depletion. Repletion with 66.5 mg daily resulted in a rapid rise in plasma levels. Note also that during deficiency many of the plasma values fell within the low range. The ICNND criteria (24) are based upon serum ascorbate values. In our laboratories parallel studies indicated no significant difference between serum and plasma ascorbate values under the conditions of the study.

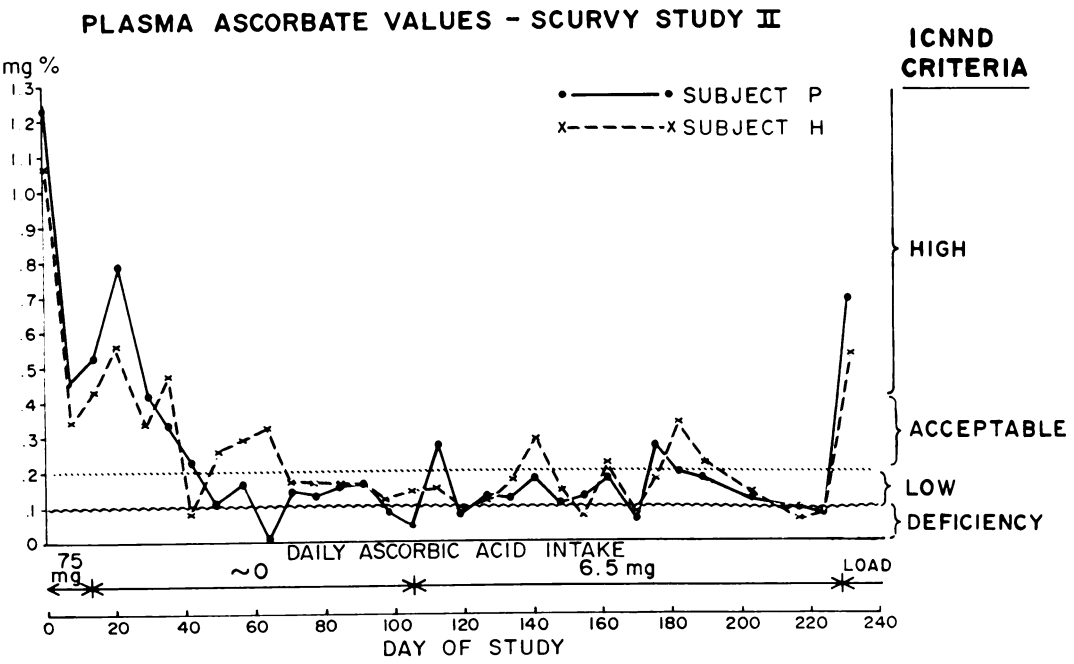

FIG. 2. Average plasma ascorbate values of two men repleted with only 6.5 mg ascorbic acid daily. Repletion with this amount had a negligible effect on plasma levels. Note the frequency with which plasma values fell above the deficiency range.

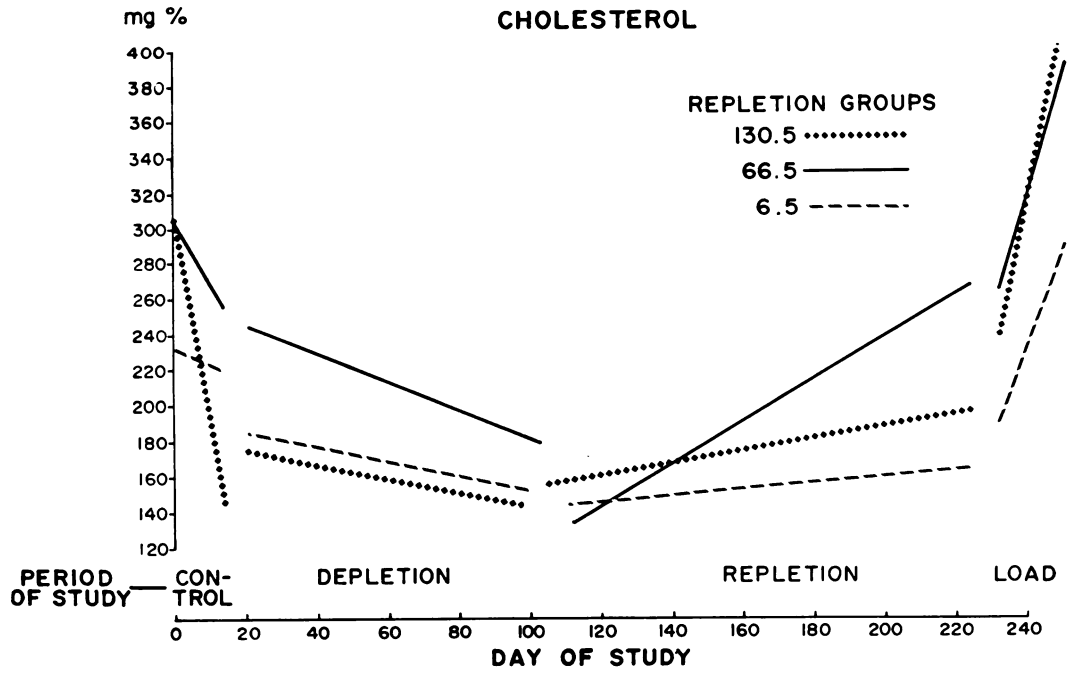

FIG. 3. The regression curves of the cholesterol values obtained during the control, depletion, repletion, and load phases of the study are depicted. The coefficient of correlation for the four respective experimental phases for groups receiving 6.5, 66.5 and 130.5 mg ascorbic acid/day were as follows: 6.5 mg—0.276, 0.642, 0.307, 0.768; 66.5 mg—0.363, 0.813, 0.732, 0.883; and 130.5 mg—0.954, 0.535, 0.468, 0.905.

tions," 0.5 ml of antigen (typhoid vaccine, USP, Eli Lilly and Company, Indianapolis, Indiana) subcutaneously, at the time of maximal depletion and again during the period when saturation doses of vitamin C were given. Their response to immunization during clinical scurvy was normal with a quadruple rise in antibody titer (Fig. 4).

Insulin tolerance curves during scurvy showed a significant decrease in blood glucose values at 2, 3, and 4 hr. These changes were reversed by repletion with ascorbic acid (Fig. 5).

Adrenalin tolerance tests also showed a significant decrease in blood glucose values throughout a period of 240 min during which the men were scorbutic, yet the glycogenolytic response remained intact. Repletion with ascorbic acid resulted in a rise in glucose values throughout the test period (Fig. 6).

Discussion

Comparison between the two Iowa City studies and the British Medical Research Council study gives evidence that very small amounts of ascorbic acid may protect against the onset of scurvy for rather long periods

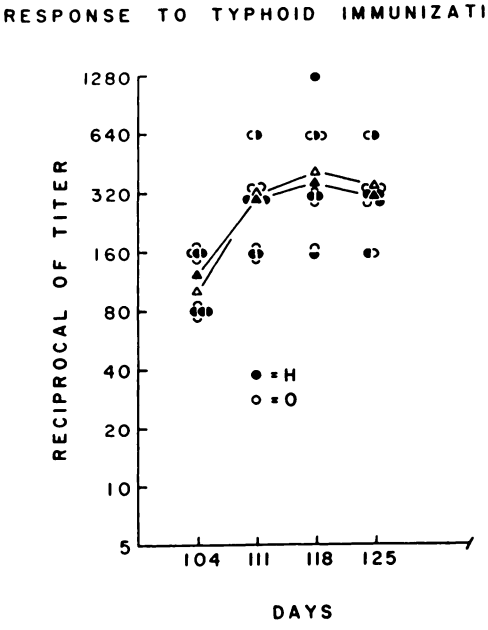

FIG. 4. Antibody response to typhoid antigen during end of depletion and the early phase of repletion was normal.

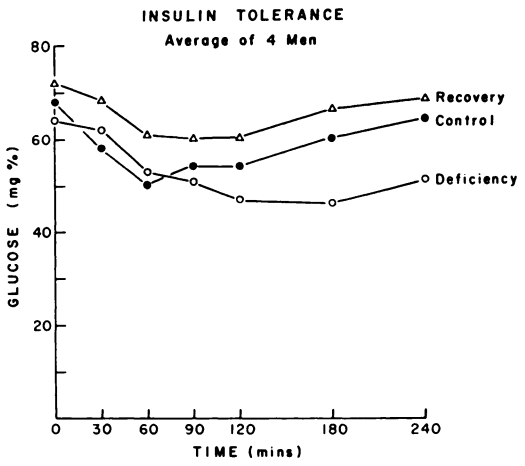

FIG. 5. Insulin tolerance curves during scurvy showed a statistically significant decrease in blood glucose at 2, 3, and 4 hr. *Subject R* became clinically hypoglycemic. These changes were reversed by repletion with ascorbic acid.

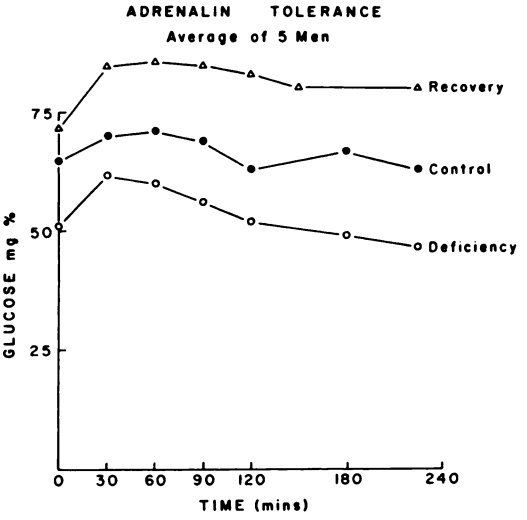

FIG. 6. Blood glucose values declined significantly during scurvy and rose again after administration of ascorbic acid, yet the glycogenolytic effect of adrenalin persisted.

of time. The British had estimated that their diets contained approximately 1 mg ascorbic acid in a day's ration, yet it required a much longer period of time for their subjects to develop the same signs and symptoms than those that appeared in our subjects (Table 6). These observations suggest the possibility that the British diet might have contained substantially more than the estimated 1 mg

TABLE 6
Comparison between Sheffield and Iowa City studies

	Sheffield study	Iowa City studies I and II
No change	Up to 119 days	Up to 29 days
Petechial hemorrhages (not perifollicular)		26 to 66 days
Follicular hyperkeratosis	82 to 149 days	45 to 100 days
Aching of limbs	149 to 168 days	64 to 96 days
Swollen or bleeding gums	163 to 254 days	38 to 105 days
Perifollicular hemorrhage	182 to 238 days	49 to 90 days
Acne	114 to 210 days	No change
Joint effusions	210 to 216 days	68 to 103 days
Dyspnea	210 to 228 days	63 to 90 days
Edema, slight to marked		33 to 101 days
Neuropathy		71 days
Sicca syndrome		58 to 123 days
Ocular hemorrhages		84 to 95 days

ascorbic acid in a day's ration. We know that a dose of 6.5 mg daily was barely enough to ameliorate scurvy in three subjects. We also observed that the catabolism of the vitamin was more rapid at the beginning of the period of deprivation when the subjects were deprived of the mineral supplement than it was later in the experiment when the subjects received an adequate mineral intake (10).

The Iowa City studies confirm the previous reports that the principal signs and symptoms of scurvy consist of follicular hyperkeratosis, hemorrhagic manifestations, fatigue, muscular aches and pains, swollen joints, swollen bleeding gums, and peripheral edema. Other little-recognized syndromes made their appearance and are reported elsewhere. In the first scurvy study (5), one of us (JH) noted the appearance of minute hemorrhages and small aneurysms in the bulbar conjunctivae. Ocular lesions are reported uncommonly in scurvy, perhaps as a result of the rather slow loss of ascorbic acid from the eye. Another little-known feature of scurvy was observed in the subjects participating in Scurvy II. This has been referred to variously as the sicca syndrome or Sjögren's syndrome (11). The occurrence of peripheral neuropathy in one man was an unexpected finding (17). His recovery was gradual and complete after restoration of ascorbic acid.

In this second study of scurvy in man, we have repeated the experiment conducted in Scurvy I (3), but extended it and achieved a more severe state of clinical scurvy, as evidenced by *subject H* who became almost unable to walk as a result of the rapid onset of arthropathy superimposed on bilateral femoral neuropathy. The onset of scurvy signaled a period of potentially rapid deterioration. The relatively short time that elapsed between the onset of illness in one subject (*H*) and the development of serious evidences of scurvy warns against procrastination in treatment of this condition.

Historical accounts of scurvy have repeatedly made mention of mental depression and other emotional changes occurring in this disorder (18). Psychological studies conducted by experimental psychologists from the U. S. Army Medical Research and Nutrition Laboratory in Denver did indeed confirm abnormalities in emotional responses. These results will be reported separately (14).

Administration of radioactively tagged L-ascorbic-1-^{14}C acid permitted us once again to observe the rate of destruction of this vitamin and to estimate its pool size in previously healthy men. Comparison of the pool size and the blood levels of ascorbic acid with the development of signs and symptoms was thus made possible. Repletion was accomplished at several dose levels ranging from 6.5 to 130.5 mg daily and once again demonstrated that a certain pool size must be attained before urinary excretion of the free reduced L-ascorbic acid occurs (10). Furthermore, the rate of recovery from signs and symptoms of scurvy seemingly was proportional to the repletion dose.

Although other investigators have reported abnormal vascular reactivity and vasomotor and structural abnormalities of autonomic ganglion cells in scorbutic animals, no previous studies of vascular reactivity have been reported in scorbutic man. In Scurvy II the appearance of cyanosis and edema of the lower extremities, incident to exposure in a cold room, triggered investigations that demonstrated abnormal vascular reactivity without evidence of significant depletion of endogenous norepinephrine or defective synthesis of norepinephrine (15). It was obvious that forearm vascular responsiveness to intraarterial norepinephrine and tyramine and to lower body negative pressure became abnormal. The mechanisms, however, are not yet clear. These abnormalities conceivably might help to explain the sudden death so often reported in historical accounts of scurvy.

Another interesting point relates to the differences between experimental and spontaneous scurvy. Scurvy, as it occurred historically and as it still appears in scattered areas throughout the world, generally is more severe and involves more systems than experimental scurvy. One might speculate that this results from multiple deficiencies or from climatic extremes plus a high level of physical activity or from associated infectious diseases. Nonetheless, the similarities between experimental and spontaneous scurvy are remarkably close.

In Scurvy I, we reported the characteristics of follicular hyperkeratosis, perifollicular erythema, petechial hemorrhages, and swollen bleeding gums. In Scurvy II, the same signs and symptoms were again present, but were more marked than in the first study. Joint swelling and pain made themselves evident in Scurvy II, but had not been observed in the subjects participating in Scurvy I.

The observations made in *subject H* demonstrate another curious function of ascorbic acid. During the time when he was developing dependent edema of his lower extremities, he had accompanying oliguria and a progressive rise in body weight. Subsequent to administration of enough ascorbic acid to reverse some of his scorbutic symptoms, he had prompt diuresis accompanied by weight loss and subsidence of edema. No doubt this phenomenon had been observed by others and presumably may have led to the oft-quoted statement that ascorbic acid has a diuretic effect (19). In this instance, it definitely did.

Nutritionists have endeavored to set arbitrary levels which would enable differentiation between well-nourished, adequately nourished, poorly nourished, and deficient individuals. In the case of ascorbic acid, great reliance has been placed on the Interdepartmental Committee on Nutrition for National Defense (24) Standards for Nutrition Surveys which are; high $= > 0.4$, acceptable $= 0.2$ to 0.4, low from 0.1 to 0.2, and deficient < 0.1 mg/100 ml. It is important to note in Figs. 1 and 2 that the subjects in Scurvy II had plasma values well above the deficiency level throughout the time they had frank scurvy. Furthermore, a comparison between plasma levels of ascorbate and pool sizes showed a very poor correlation. Whole blood values were somewhat more dependable in providing an estimate of pool size (10). In general, it is fair to say that scurvy appeared when the body pool size fell below 300 mg (10) and the whole blood level below 0.3 mg/100 ml.

The mild anemia may have resulted from the volume of blood drawn for laboratory purposes. There was little evidence of correlation between the severity of scurvy and the degree of anemia.

The changes in cholesterol are the opposite of those that one might expect from reports by Russian investigators of a reduction in serum cholesterol concentrations as a result of therapy with ascorbic acid (20). The men in the present study had a fall in serum cholesterol values probably as a result of the formula diet that contained no cholesterol and large amounts of polyunsaturated fats. At a time when they were being repleted, while still on a cholesterol-free diet, they had a significant rise in cholesterol levels. This rise became more marked at the close of the study when they were fed a diet of choice, high in cholesterol and saturated fats. Bronte-Stewart (21) also noted low serum cholesterol levels in scorbutic Bantus, and found that cholesterol

levels rose with the administration of ascorbic acid alone.

Changes in serum proteins have been reported in scorbutic animals to show a decrease in albumin and a rise in alpha-1 globulin (22). The similar fall in albumin in our subjects was accompanied by a rise in alpha-2 and gamma globulins (10). The observation in Scurvy I that nitrogen losses in the urine were greater than normal suggests that impaired protein metabolism is a feature of scurvy. The immune responses of scorbutic animals have been reported to be normal (23). This observation was confirmed in the present human study.

Our observations in a total of nine subjects with experimentally induced scurvy suggest that rather small doses of ascorbic acid can cure scurvy, given sufficient time. Certainly the administration of modest doses (66.5 mg) resulted in rapid disappearance of symptoms and signs of scurvy and in prompt repletion of the body pool. Since this vitamin is readily absorbed from the gastrointestinal tract there seems little reason to treat scurvy with parenteral ascorbic acid or with massive oral doses.

Summary

Six healthy volunteers from the Iowa State Penitentiary at Fort Madison, Iowa, participated in studies of human scurvy. They were hospitalized on the Metabolic Ward of University Hospitals in Iowa City, Iowa, and fed a diet totally devoid of vitamin C.

One of the men withdrew from the study because of personal reasons. The remaining five subjects developed clinical scurvy in 84 to 97 days, manifested by signs and symptoms of fatigue, hemorrhagic phenomena, swollen joints, swollen bleeding gums, follicular hyperkeratosis, muscular aches and pains, and emotional changes.

Urinary ascorbic acid rapidly declined to undetectable levels early in the course of depletion and blood levels progressively became too low to measure accurately. Serum protein abnormalities appeared that consisted primarily of a decrease in albumin and an increase in alpha-2 and gamma globulins. Other changes occurred in serum lipids.

Radioisotopic studies indicated progressive depletion of the body pools during the depletion phase of the study and repletion in proportion to the amount of ascorbic acid administered daily. This study confirms and extends the observations made in our earlier study that the full clinical syndrome does not appear until the normal body pool has been depleted to less than 300 mg.

The minimal amount of ascorbic acid necessary to prevent or cure scurvy appears to be slightly less than 10 mg daily. Once again our observations are in accord with those of the British Medical Research Council. Estimates of the optimal intake of ascorbic acid must be made on the basis of these data plus a knowledge of the biological and physiological variables of mankind.

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