

Original Communications

## Experimental Scurvy in Man

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DESPITE MUCH INVESTIGATION, there are still considerable differences of opinion regarding the amount of vitamin C needed in the diet of normal adults (1). In the United States, the National Research Council has recommended an intake of 70 mg (recently revised to 60 mg) daily (2), whereas in the United Kingdom, the daily intake recommended by the Committee on Nutrition of the British Medical Association (3) was 20 mg except during the second half of pregnancy when the recommended daily intake was 40 mg (recently revised to 30 and 60 mg, respectively). The Medical Research Council Sheffield study (4) produced evidence that a daily dose of 10 mg of ascorbic acid not only prevented scurvy but quickly relieved the signs and symptoms in scorbutic volunteers.

Because of the opinion of many authorities that "tissue saturation" provides "added protection against scurvy, promotes wound healing, preserves enzyme activity, favors cellular proliferation and increases the resistance to common stresses such as those induced by bacterial toxins, low temperature and fatigue," the Food and Nutrition Board of the National Research Council has recommended a relatively high intake of ascorbic acid for all Americans (2).

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It is surprisingly difficult to obtain objective evidence for or against the desirability of perpetuating this practice. The Committee on Nutrition of the British Medical Association (3) reported in 1950 that they were unable to find any trustworthy evidence that maintenance of saturation of the blood with ascorbic acid was desirable in the interests of health and they thought that the large allowances recommended by the National Research Council (U.S.) were unnecessary. Accordingly, we designed and conducted a study in healthy prison volunteers that essentially reproduced the clinical aspects of the Sheffield study but utilized the precise control of a metabolic ward and the sophisticated biochemical and radioisotopic techniques now available.

The objectives of this study were: 1) to measure, by labeling with L-ascorbic-1-<sup>14</sup>C acid, the body pool of vitamin C and to study its rate of depletion during complete ascorbic acid deprivation; 2) to induce deficiency of ascorbic acid in healthy men; 3) to estimate the minimal requirements for ascorbic acid; 4) to observe the relationship between body pool size of ascorbic acid and clinical signs of scurvy; 5) to observe the effects of a deficiency of ascorbic acid on certain physiologic responses including wound healing, resistance to infection, blood coagulation, basal metabolism, and electrical activity of the heart and brain; and 6) to ascertain the amount of ascorbic acid necessary to replete the body

pool and alleviate clinical signs and symptoms of scurvy.

#### METHODS

The subjects were six prisoners selected from volunteers (5) at the Iowa State Penitentiary in Fort Madison, Iowa. They ranged in age from 33 to 44 years and were in good health as judged by a medical history and physical examination. There was no history suggestive of coronary artery disease. The only defects found on initial examination were minor ST segment and T wave abnormalities in the electrocardiograms of two of the men.

The subjects were housed on the metabolic ward where they remained under constant nursing supervision throughout the entire study. All food consumed was prepared by the metabolic ward dietitians in accordance with the following research protocol.

#### *The Plan of the Study*

The study began on November 1, 1966, and after a brief period of instruction in metabolic ward methods, the men were taught to swallow a gastric tube through which they later administered, three times daily, a liquid formula diet totally deficient in ascorbic acid but adequate in all other nutrients (Table 1). This diet was fed from November 8, 1966, *day 1*, until *day 113*.

A labeling dose (0.86 mg) of L-ascorbic-1-<sup>14</sup>C acid (containing a total activity of 23.9  $\mu$ Ci) was given orally to each of the men on December 1, 1966 (5). (Originally it was planned to administer the labeling dose at the beginning of the deficiency period but, because of unavoidable delays in obtaining permission to administer this material, it was not given until the 23rd day of deficiency.)

The deficient diet was fed until convincing evidence of mild clinical scurvy appeared and until, on the 99th day, the body pool of ascorbic acid had been largely depleted. (Details of ascorbic acid metabolism are reported separately (6).) On the 100th day, repletion with ascorbic acid was begun using controlled levels of intake of low specific activity carbon 14-labeled L-ascorbic acid ranging from 4 to 64 mg daily. This enabled us to determine the amount of ascorbic acid retained and excreted in response to repletion at different levels of intake. During the

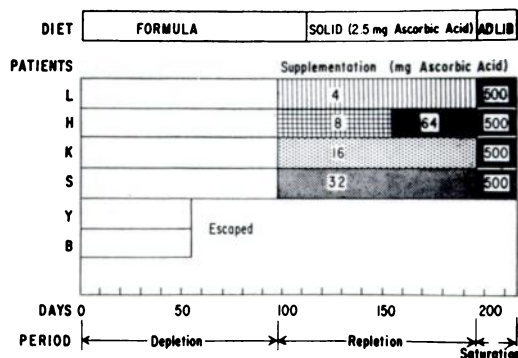


FIG. 1. The experimental design. Study originally involved six men but two of them, Y and B, escaped on the 54th day. Period of deficiency continued through the 99th day and the formula diet was fed through the 114th day. Thereafter, a solid diet containing 2.5 mg of ascorbic acid was given throughout the period of repletion. A final recovery period permitted a diet of free choice supplemented by 0.5 g of ascorbic acid daily.

first 2 weeks of the repletion phase, the men continued to receive the ascorbic acid-free formula diet. At the beginning of the 3rd week of repletion (*day 113*) a modified solid diet was fed that supplied 2.5 mg of ascorbic acid daily in addition to the radioactively labeled supplement. The design of the study is shown in Fig. 1.

#### *Diet*

The experimental diets mentioned above were of two basic types: one completely devoid of ascorbic acid and the other containing 2.5 mg in a day's ration. The ascorbic acid-free formula diet (Table 1) was composed of vitamin-free casein, purified carbohydrates, and fats. This diet was supplemented by the amounts of all essential minerals and vitamins recommended by the National Research Council (2) with the exception of ascorbic acid. The modified solid diet provided only 2.5 mg of ascorbic acid daily and was composed of soy protein foods that resemble meat products,<sup>2</sup> various cereal and pastry foods, cooked rice, egg white, and soft drinks (Table 1). Throughout the study, half of the subjects received a major portion of their carbohydrates as sugar whereas the other half received most as starches. This

<sup>2</sup>"Bontrae" was generously supplied by General Mills, Inc., Minneapolis, Minn.

permitted us to study any possible interrelationships between types of carbohydrate and metabolic changes in scurvy.

We have learned from previous metabolic studies of vitamin deficiencies that any formula diet becomes monotonous and intolerable if the subjects are required to drink it three times daily for a prolonged period of time. Accordingly, we have developed a technique (7) for introducing formula directly into the stomach (Fig. 2). The men soon learn to swallow a gastric tube without nausea or discomfort and to administer their formula through this tube by means of a rubber bulb which introduces air pressure into a 3-liter plastic bottle. This method of feeding has been well tolerated for periods up to 9 months.

Six prisoners commenced the study but two (*Y* and *B*) escaped on the 54th day of deficiency. The remaining 4 subjects consumed the deficient formula diet for 114 days. Repletion with ascorbic acid began on *day 100* by administering controlled daily doses of *l*-ascorbic 1-<sup>14</sup>C acid ranging from 4 to 32 mg daily. Thus, *subject L* received 4 mg; *subject N*, 8 mg; *subject K*, 16 mg; and *subject S*, 32 mg daily. On *day 156* the repletion dose for *subject N* was increased from 8 mg to 64 mg daily. On the 113th day of the study (14th day of repletion), the subjects were changed from the formula diet to the solid diet, which provided 2.5 mg ascorbic acid daily.

*Physical Activity*

Because specific deficiency diseases are less likely to become manifest in the presence of restricted calorie intake and calorie expenditure, we felt it was essential to stimulate calorie intake in our subjects by maintaining a high level of physical activity. Accordingly, they were required to walk approximately 10 miles daily (and wear a pedometer at all times).

*Routine Examinations*

The nursing staff recorded temperature, pulse and respiratory rates, body weight, and blood pressure daily. A physical examination checklist was kept for each of the subjects who were examined three times daily, twice by nurses and once by a physician. We specifically looked for changes in the skin and mucous membranes for swollen or bleeding gums, for other hemor-

TABLE I  
The formula diet<sup>a</sup>

Ingredients	Amount to be consumed per subject per day
Vitamin-free casein (92% protein)	122 g (112.9 g protein)
Sucrose or Milo oxidized starch	338 g
Peanut oil	64 g
Cocoa butter	56 g
Safflower oil	13 g
Iodized salt	5 g
Basic mineral and cystine mixture	1 bottle
Basic vitamin mixture (vitamin C-free)	1 capsule
Additional minerals and vitamins	1 bottle
H <sub>2</sub> O	1,000-1,500 cc
Basic mineral and cystine mixture	
Ferrous fumarate	30 g
Calcium acid phosphate	4.1 g
Potassium chloride	3.8 g
Dihydrogen sodium phosphate	850 mg
Anhydrous magnesium hydroxide	480 mg
DL-cystine	0.6 g
Basic vitamin mixture (vitamin C-free)	
Thiamine hydrochloride	1.1 mg
Pyridoxine hydrochloride	1.7 mg
Calcium pantothenate	10.9 mg
Riboflavin	1.6 mg
Niacinamide	17.0 mg
Folic acid	0.15 mg
Biotin	150 μg
Vitamin B <sub>12</sub>	3 μg
Vitamin K	1 mg
Vitamin E	10 mg
Vitamin D	400 IU
Vitamin A	5,000 IU
Additional minerals and vitamins	
Folic acid	0.35 mg
Vitamin E	70 mg
Pyridoxine hydrochloride	0.8 mg
Manganese sulfate	4.40 mg
Aluminum potassium sulfate	2.15 mg
Copper sulfate	11.30 mg
Zinc chloride	3.15 mg

<sup>a</sup> This contained absolutely no ascorbic acid but was adequate in all essential nutrients. Vitamin C from synthetic formula, 3,000 kcal (15% protein, 40% fat, 45% carbohydrate).

TABLE II  
The solid diet supplying 2.5 mg ascorbic acid daily

Day I	Day II
<b>Breakfast</b>	<b>Breakfast</b>
Pancakes	Pancakes
Baked egg whites with isolated soy protein bacon bits	Baked egg whites with isolated soy protein ham
Brown sugar syrup	Brown sugar syrup
<b>Lunch</b>	<b>Lunch</b>
Isolated soy protein chicken casserole	Isolated soy protein beef casserole
Homemade noodles	Homemade noodles
Chicken bouillon, marjoram, thyme	Beef bouillon
Butterscotch pudding with marshmallows and meringue	Butterscotch pudding with marshmallows and meringue
Strawberry Kool-Aid with sugar or Sucaryl	Strawberry Kool-Aid with sugar or Sucaryl
Chocolate candy	Chocolate candy
Graham crackers	Graham crackers
Muffin	Muffin
<b>Dinner</b>	<b>Dinner</b>
Isolated soy protein seafood casserole	Isolated soy protein chicken casserole
Rice and red pepper	Rice
Gingerbread	Gingerbread
Meringue cookie	Meringue cookie
Chocolate candy	Chocolate candy
Graham crackers	Graham crackers
Biscuit	Biscuit
Sorghum	Sorghum
<b>H.S.</b>	<b>H.S.</b>
Graham cracker	Graham cracker
Kool-Aid with sugar	Kool-Aid with sugar
Chocolate candy	Chocolate candy

Calories, 3,000; protein, 113 g (15%); fat, 133 g (40%); carbohydrates, 336 g (45%). Half of the subjects received 30% of their carbohydrates as simple carbohydrate and 70% as complex carbohydrate. The other half received the reverse.

rhagic phenomena such as petechial hemorrhages and for edema of the feet and ankles. Attempts to have the men record their thoughts and emotional reactions in a daily diary were unsuccessful.

#### *Collection of Specimens*

Venous blood was collected once weekly in a fasting state for biochemical determinations. Complete collections were made of all urine and feces. Urine was collected for 24-hr periods in containers kept under constant refrigeration. As a preservative, concentrated hydrochloric acid, 1 ml/liter of urine, was added. Food aliquots were collected weekly for determination of vitamin content (vitamin A, thiamine, pyridoxine, and ascorbic acid). Feces were collected

over 7-day periods and stored at  $-15^{\circ}\text{F}$  in weighed 1-gal metal paint cans. After completion, each weekly specimen was allowed to thaw, the can and its contents weighed, and an amount of water equivalent to the weight of feces was added. Six large metal washers were added and the lid tightly sealed. The can was then shaken for 30 min in a commercial paint shaker in order to emulsify the feces. An aliquot of this emulsion was taken for analysis.

#### *Biochemical Determinations*

In addition to routine urinalysis (specific gravity, pH, glucose, protein, and microscopic examination) and hematologic measurements (white blood cell count, red blood cell count, hemoglobin, hematocrit, and erythrocyte sedi-

mentation rate), we periodically measured, by standard methods, total serum cholesterol, serum triglycerides, total serum proteins and electrophoretic fractions, serum creatinine, plasma amino acids, plasma ascorbic acid, plasma vitamin A and carotene, and erythrocyte hemolysis (as an index of vitamin E nutrition). In addition, sucrose tolerance tests were performed by administration of 100 g of sucrose orally and measurement of blood glucose initially and at 30-min intervals in order to ascertain whether scurvy affected intestinal disaccharidase activity or induced any alteration in the rate of absorption of carbohydrates across the intestinal wall. Urine was assayed for its content of ascorbic acid, thiamine, pyridoxine, total nitrogen, and creatinine.

For each subject, the daily total of  $^{14}\text{C}$  activity excreted in the urine was measured. In addition, metabolic end products of ascorbic acid excreted in the urine were studied (6).

#### Physiological Tests

Periodically electrocardiograms and electroencephalograms were performed to determine whether scurvy caused any abnormalities. The basal metabolic rate was also measured at monthly intervals. After deficiency was well established and the repletion phase started (*day 100*) a surgical wound, 5 cm in length, through the skin down to the fascia lata, was made on the lateral aspect of the left thigh of each subject. The edges of the incision were closed by four or five sutures. One week and two weeks later, punch biopsies were taken from the healing wound edges. Tissue samples were examined by standard histologic and by electron microscopic techniques to evaluate the healing process as influenced by scurvy and by repletion with differing doses of vitamin C.<sup>3</sup> Coagulation studies were performed, including platelet counts, bleeding and clotting times, clot retraction and clotlysis, prothrombin (one and two stage), plasma clotting time, thromboplastin generation test, prothrombin consumption test, platelet adhesiveness and aggregation response to adenosine diphosphate.<sup>4</sup>

Several of the men developed spontaneous

<sup>3</sup> We are indebted to Dr. C. E. Radcliffe and Dr. J. C. Hoak for performing the biopsies and the microscopic studies.

<sup>4</sup> Dr. Hoak also performed the coagulation studies.

Figure II

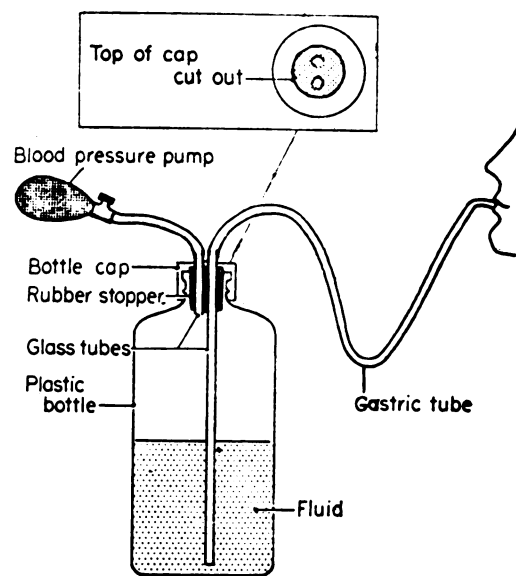


FIG. 2. Formula diet was administered through a gastric tube by means of a pressure bottle. Note that a plastic bottle was used to avoid explosive hazards. Formula was fed by this means for a period of 114 days.

infections, including a common cold in one, a sore throat in a second, and a severe staphylococcal external otitis accompanied by fever and regional lymphadenopathy in a third man. This provided an opportunity to evaluate the effect of the stress of acute infection on the rate of catabolism of ascorbic acid. One other form of physiologic stress, emotional trauma, occurred spontaneously. As inevitably happens in a group of prison volunteers, there were periods of emotional unrest and depression. In the present study, a much more severe degree of emotional stress occurred in one subject as a result of the escape of two of his roommates. After questioning by prison officials he disclosed the details of the escape. He later reflected on his disclosure and realized that he would be subjected to severe censure and might even suffer physical harm when he returned to the prison because of the "code of ethics" that prevails among prisoners. He then became exceedingly anxious and apprehensive requiring constant observation and the administration of a tranquilizer (Meprobamate). This

period of anxiety persisted for 3 weeks but subsided after prison officials were able to arrange for his protection until he could become eligible for parole. Later, when his parole again seemed unlikely, he again became apprehensive and emotionally disturbed until he was assured of parole.

#### OBSERVATIONS—CLINICAL

##### *General Symptoms*

In any prolonged metabolic study it is difficult to differentiate between subjective complaints and actual symptoms of deficiency. This is especially true of prisoners who have greater social and emotional problems than most people. They are inclined to complain of trivial conditions, and to exaggerate any discomfort. Nonetheless, the following signs and symptoms were judged by two clinicians (REH and JH) to be worthy of note and to occur probably as a result of deficiency (Table III).

Fatigability, especially of the lower limbs, and mild general malaise began insidiously about the time of development of objective manifestations of scurvy. Two subjects, *L* and *S*, complained of dull aching muscular pain in both legs. *Subject S* also complained of muscular aching in the shoulder region. The fatigability of the legs was present in all four subjects and necessitated a reduction in the length of their daily walks.

Mental and emotional changes occurred in two of the four men. *Subject N* developed symptoms of depression after the 30th day and expressed suicidal thoughts on the 52nd day. Because of the nature of his crime this man had been ostracized by his companions. On the advice of a psychiatrist, he was treated with an antidepressant, amitriptyline. *Subject L*, as mentioned before, developed a severe anxiety reaction following his testimony concerning the escape of two companions. There was no definite evidence to suggest that these emo-

tional responses were in any way related to scurvy.

On the 112th day, *subject L* complained of feeling alternately hot and cold. This was followed by vertigo, faintness, and profuse sweating. His electrocardiogram was normal and his subsequent stools were negative for occult blood. On *day 130*, he complained of left side pleuritic pain, lasting about 12 hr. An X-ray of the chest, an electrocardiogram, and a Master's exercise test were normal.

##### *Hemorrhagic Manifestations on the Skin*

The first petechial hemorrhages were noted in three subjects on the 26th day of depletion: a single one on the dorsum of the hand of *subject N* and several above the ankles in *subjects Y* and *S*. These were not characteristically perifollicular in site and faded quickly. On the 29th day another petechial hemorrhage appeared on the back of *N's* hand and on the 32nd day a few petechiae, unrelated to follicles, appeared below the left knee of *Y*. On the 45th day the first truly perifollicular hemorrhage was seen above the ankle of *B*, and on the 52nd day a similar hemorrhage was seen behind the knee of *S*. All of these disappeared within 2–4 days. From time to time, small ecchymoses appeared as a result of minor trauma. On the 131st day, hemorrhagic staining appeared around a developing furuncle in *L* but subsided promptly. *Subject L*, who habitually sat with crossed legs, was noted to have a bluish-red telangiectatic area behind the right knee on *day 152* and a similar lesion behind the left knee a week later. Both faded after 14–21 days.

##### *Hemorrhagic Spots in the Eyes*

Small superficial bilateral red spots resembling bulbar conjunctival hemorrhages were noted in three of the four men between the 84th and 91st days. These lesions were located mainly near the limbus. In *subject N* on the 92nd day two larger sub-

conjunctival hemorrhages appeared near the inner canthi. These lesions, which cleared rapidly in *S* and more slowly in *N* after ascorbic acid repletion, are reported elsewhere (8).

#### *Hemorrhagic Lesions Involving the Mouth and Gums*

We selected subjects who had their own teeth. They also had varying degrees of periodontal disease from the start. This was most marked in *K*, who had considerable gingivitis and pyorrhea. He developed some slight increase in gum swelling after 42 days, but this did not progress and his gums did not become hemorrhagic. The first gingival hemorrhage appeared in *subject Y* on the 43rd day, in association with periodontal inflammation. *Subject N*, who initially had swollen gums that bled after he brushed his teeth, had an increase in gum swelling by the 38th day, and by the 76th day his gums were intensely congested and swollen. By the 91st day he also had pronounced swelling of the interdental papillae. Within 1 month of repletion with 10.5 mg of ascorbic acid daily his gums had returned to their original condition. In *subject L*, redness of the gum margins first appeared on the 105th day and swelling around the molars occurred a few days later. He was given 4 mg of ascorbic acid daily from *day 100–day 114* and 6.5 mg daily thereafter. No obvious changes were noted during the 2 weeks of 4 mg daily, but his gums returned to normal by *day 180* with a daily intake of 6.5 mg ascorbic acid. *Subject S* had slight bleeding of his gums on the 83rd day but developed no other gum changes throughout the study. The gingival lesions characteristically began along the gum margins and later involved the interdental papillae. Small sublingual petechial hemorrhages were seen in three subjects; in *B* on *day 36*, in *L* on *day 90*, and in *K* on *day 189*. These cleared within 1–4 days.

*Subject S* had two teeth extracted on

*day 36* (a molar and a premolar) for acute pulpitis. No scorbutic gum changes were presented at this time. Bleeding was not excessive after extraction and healing was complete by the 79th day. X-Rays of the teeth of all subjects at the peak of deficiency showed that the periodontal membrane remained intact.

#### *Follicular Hyperkeratosis and Congestion*

Two of the men, *S* and *L*, initially had some hyperkeratosis, but this increased by the 60th and 77th days, respectively. The other two men, *K* and *N*, developed hyperkeratosis on the 84th and 88th days. The lesions appeared first on the posterior aspect of the calves and later involved the anterior and lateral aspects of the thighs, buttocks and the skin over the patellae and along the inner aspect of the legs above the ankles. Hyperkeratosis did not occur on the forearms of any of the subjects and the arms were involved on the posterior aspect in only one subject (*S*) in whom hyperkeratosis also involved the shoulder region on the 94th day.

In *subject S*, pustule formation developed around the hyperkeratotic follicles on the calves on the 80th day. Two days later these follicles became congested and bluish-red in appearance. In *subject L*, congestion around the follicles on the legs appeared on the 129th day during the period of clearing of the hyperkeratosis. In *subject N*, slight congestion of the follicles on the calves was noted on *day 99*. No congested follicles were noted in *subject K*. Coiled hairs, especially on the back of the thighs and on the buttocks, were marked in one man (*L*). All four subjects had mild acne on the chest and back, but no exacerbation occurred during deficiency.

Hyperkeratosis and congestion of the follicles cleared at varying rates after ascorbic acid repletion was begun. In two of the subjects, increased perifollicular congestion was noted during clearing of

TABLE III  
Clinical signs and symptoms of scurvy depicted on a 0 to 4+ scale

Clinical signs	Sub- jects	Depletion phase										Repletion phase										
		10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210
Hyperkeratosis	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Y	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	L	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	K	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Congestion of fol- licles	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B																					
	Y																					
	L																					
	N																					
Petechial and other skin hem- orrhages	K																					
	S																					
	B																					
	Y																					
	L																					
Conjunctival lesions	N																					
	K																					
	S																					
	B																					
	Y																					



the hyperkeratosis, diminishing after loss of the hyperkeratotic plugs. In *subject S*, hyperkeratosis began to subside within 1 week of supplementation with 32 mg ascorbic acid daily, cleared from the calves in 21 days, but persisted longer on the thighs and buttocks. As the hyperkeratosis diminished, congestion around the follicles increased for about 30 days before gradually disappearing. The hyperkeratosis in *subject L*, who received daily supplements of 4 mg ascorbic acid, gradually cleared and returned to the initial state by day 170, although some redness persisted around the follicles for a further 21 days. This man, who habitually sat with his legs crossed, was noted to have a bluish-red telangiectatic area behind the right knee on the 152nd day and a similar area behind the left knee 1 week later. Both faded after 14–21 days. The very mild perifollicular congestion noted in *subject N* disappeared in 10 days with daily supplements of 8 mg ascorbic acid.

#### *Physiological Studies*

Initial electroencephalograms were normal and repeat tracings at the time of maximal depletion and after repletion were again essentially normal. Electrocardiograms were performed at intervals of 4 weeks throughout the study. Two men had slightly abnormal initial electrocardiograms, yet during the period of deficiency their tracings showed a lesser degree of abnormality. No changes in the PR intervals were noted. Basal metabolic rates were not appreciably altered by deficiency. Periodic measurements of the red blood count, white blood count, hematocrit, and erythrocyte sedimentation rate remained normal. Coagulation tests performed during the period of clinical scurvy were normal. Rumpel-Leede tests were positive in two of the four subjects (*L* and *S*), but the number of petechiae induced by venous occlusion bore no relation to the state of depletion or repletion.

Surgical incisions made on the thighs of the subjects on the 100th day were observed to heal at approximately equal rates despite differing rates of supplementation of their diet with ascorbic acid (4, 8, 16, or 32 mg daily). Punch biopsies taken from the wound margins after 1 and 2 weeks did not show convincing histologic differences in the rate of healing. The wounds made by the punch biopsies also healed equally well in all four men.

#### *Biochemical Changes*

Serum proteins and electrophoresis and creatinine levels did not show any significant change throughout the study. Measurements of the urinary excretion of nitrogen and creatinine suggested that during depletion there was negative nitrogen balance with reversal to positive balance during repletion (6). Serum carotene, as anticipated, decreased rapidly to very low levels, since the diet contained no carotene. Despite a daily intake of 5,000 IU of preformed vitamin A, the serum concentration of vitamin A in two of the men decreased during the period of ascorbic acid deficiency from a mean initial value of 33  $\mu\text{g}/100\text{ ml}$  to a mean of 16  $\mu\text{g}/100\text{ ml}$  and rose after repletion with ascorbic acid was commenced. No significant changes occurred in serum triglycerides. Serum cholesterol values in the subjects fed sucrose during the depletion period tended to rise, but the increase was not of statistical significance. No change occurred in the serum cholesterol level in subjects fed starch. Sucrose tolerance tests gave evidence of normal disaccharidase function (sucrase) and of normal intestinal absorptive function. Erythrocyte hemolysis tests for vitamin E adequacy were normal in all subjects at the peak of depletion.

Urinary excretion of vitamin C, as measured by thin-layer chromatographic methods, reached "zero" levels before the 23rd day of depletion but the urine still contained a substance that reacted to

dinitrophenylhydrazine (DNPH). This organic substance has not as yet been identified but chromatographic studies showed that it was not L-ascorbic acid. The concentration of ascorbic acid in plasma declined progressively until the 56th day of depletion by which time the test became invalid because of erratic results. Chromatographic studies of serum at this time indicated that there were organic reacting compounds present which were not L-ascorbic acid. Urinary excretion of vitamin C remained undetectable until the pool size had been repleted to a level of approximately 1.5 g at which time free *l*-ascorbic acid could once again be found in the urine by thin-layer chromatographic methods (6).

Measurements of urinary excretion of hydroxyproline were reported to show a significant rise during the period of depletion and to become normal after repletion (9).

#### *Radioassays*

The results of radiometric assays of urine permitted calculation of rates of depletion and repletion (6). These data provide a rational basis for estimates of the physiologic needs for ascorbic acid.

#### DISCUSSION

It was not our intention to induce a severe scorbutic state in these subjects. Symptomatically the men had few complaints other than fatigue and muscle cramps. Clinical signs of scurvy consisted of swelling, redness and bleeding of the gums, follicular hyperkeratosis of the skin, conjunctival hemorrhages, and perifollicular hyperemia.

It is evident that neither the urinary excretion of ascorbic acid nor the serum concentrations of this vitamin could be relied upon to give definitive evidence of scurvy. Although the Sheffield study and other studies reported measurement of ascorbic acid in white blood cells and platelets to

be a definitive means of diagnosing scurvy, we chose to omit this determination because the use of isotopically labeled ascorbate provided ample evidence of rates of depletion and repletion.

It is interesting to compare our results with those of the Sheffield study (4). In both studies, deficient subjects excreted an average of 24 mg daily of an unidentified compound that gave the same chemical reaction as ascorbic acid and hence rendered the test invalid. By thin-layer chromatography it was possible to determine that this substance was not *l*-ascorbic acid.

By giving radioactively tagged L-ascorbic acid we were able to observe the rate of destruction of ascorbic acid as evidenced by the urinary excretion of <sup>14</sup>C-labeled compounds (6). When one examines the data presented by the Sheffield group (4) and takes into consideration the probable intake of ascorbic acid and the earliest onset of symptoms of scurvy, it appears that their experience was remarkably similar to our own. Because their volunteers initially were receiving 50 mg of ascorbic acid daily, it is probable that their pool size was near maximal. The diets that they fed their subjects provided "not more than one mg" of ascorbic acid daily. Accordingly it might be anticipated that the onset of scurvy in their subjects would be delayed somewhat as compared with the present study in which the diet contained no ascorbic acid.

The idea of studying experimentally induced dietary deficiencies in man is not new. In 1789, Stark (10) induced scurvy in himself by subsisting on a diet of bread and water. After about 60 days, his gums became swollen and bled easily and one nostril was purple and painful. The earliest sign of scurvy noted by Crandon, Lund and Dill (11) in a study of experimental scurvy in a single subject was the appearance of perifollicular hyperkeratotic papules on the buttocks and calves after 132 days. After 161 days of deprivation, perifollicular hemorrhages were present on the

legs but no gross gum changes were noted. In the Sheffield study (4), some hyperkeratosis was present in a single subject at 110 days and in 6 of the 10 deprived volunteers after 147 days. After 182 days, hyperkeratosis was present in all subjects and hemorrhagic follicles were present in six. The follicles were hemorrhagic in 9 of the 10 subjects at 245 days. Pijoan and Lozner (12) reported petechial and perifollicular hemorrhages in five of six subjects and tender swollen bleeding gums in one after experimental deprivation of ascorbic acid for 150 days. In a short-term study lasting 84 days in a single subject, Van Eekelen (13) noted no clinical signs of scurvy.

Holst and Frölich (14) mentioned the occurrence of numerous skin hemorrhages and severe gum changes in Russian prisoners fed a deficient diet for 180 days and cited a report by Koren of a strict vegetarian who developed skin and soft tissue hemorrhages in the lower limbs after 225 days of a self-imposed diet of bread and water. Roberts (15) mentioned the appearance of early scorbutic signs after a period of only 2 months in the Antarctic when the diet consisted mainly of tinned foods supplemented by fish, penguin, and penguin eggs.

Although the men in the Sheffield study had an increased amount of acne, none of our subjects had an exacerbation of the mild acne on their chests and backs. The Sheffield study reported an increase in the number and distribution of the acne papules between the 112th and 210th days, some of the papules becoming bright red and hemorrhagic at the same time as the hair follicles (4). Hemorrhagic staining was noted around a developing furuncle in our *subject L* on *day 131*.

In our subjects, occasional perifollicular hemorrhages were seen early in depletion. Hyperkeratosis either increased or made its first appearance between the 60th and 88th days of deprivation and congestion of

the follicles on the legs first appeared on the 82nd day.

Slight gum changes were noted in our study from as early as the 38th day, becoming marked in two subjects, by *day 76* in *subject N* and *day 110* in *subject L*. In the Sheffield study, gum changes appeared between the 161st and 238th day of deficiency. Crandon, Lund and Dill (11) noted no gross gum changes after 161 days of deprivation although perifollicular hemorrhages were then present on the legs. Gingivitis was noted by Pijoan and Lozner (12) in only one of six subjects deprived of ascorbic acid for 5 months despite the presence of petechiae and follicular hemorrhages in five.

It is obvious that our men developed signs of scurvy earlier than in previous studies, probably as a result of feeding a diet totally deficient in ascorbic acid.

Although interruption of the lamina dura (periodontal membrane) has been reported after 168 days of ascorbic acid deprivation (11), no changes were noted in dental films taken in our subjects at the time of maximal depletion.

The conjunctival hemorrhages that we observed are not commonly associated with scurvy, although there are passing references to conjunctival hemorrhages occurring in this disease. The details of this observation will be published elsewhere (8).

Hemorrhagic manifestations of scurvy have generally been attributed to an intrinsic weakness of the vascular wall of capillaries, to a loss of "intercellular cement," or a decrease in fibrillary structure of surrounding connective tissue. The measurements of blood clotting activity and histologic and electron microscopic studies provided no explanation for the hemorrhagic phenomena noted in our study.

The commonly held view that ascorbic acid, when given in abundance, provides a protective mechanism against various tox-

ins, physical stress, extremes of temperature, the harmful effects of fatigue, or that it exerts beneficial effects on wound healing and resistance to infection, is remarkably poorly documented (16). Although our subjects occasionally had upper respiratory infections and one man, on two separate occasions, suffered from severe otitis externa accompanied by pain, fever, swelling, and regional lymphadenopathy, there was no change in the apparent rate of catabolism of ascorbic acid.

On the other hand, the one man who experienced severe emotional stress excreted more radioactivity than he ingested, thus indicating a net loss of ascorbic acid (6). Apparently this form of stress does increase catabolism of ascorbic acid.

It is interesting to note that the wound healing in our subjects proceeded to completion even though the amounts of ascorbic acid given as supplements were only 4, 8, 16, or 32 mg daily. Furthermore, there was no evidence that the rate of healing differed among these four men. Crandon, Lund and Dill (11) noted good healing of a surgical incision in one subject, after 3 months deprivation of ascorbic acid, with ample intercellular substance and capillary formation. A second incision in the same subject after 181 days deprivation, although appearing to heal well on the skin surface, when biopsied showed that no true healing had occurred and the gap in the incision was filled with unorganized blood clot. Biopsies taken from the healing wounds in our four subjects who received differing daily repletion doses of ascorbic acid when examined by both light and electron microscopy failed to show any significant abnormalities. Apparently a more severe degree of scurvy must exist before collagen synthesis is impaired.

Our studies of hydroxyproline excretion in the urine also gave results that differ from those of others (17). Theoretically, a deficiency of ascorbic acid should interfere

with hydroxylation of proline and with collagen synthesis. It is difficult to explain the increased rate of excretion of hydroxyproline by our scorbutic subjects, but perhaps differences in age and species are responsible.

Unfortunately, these studies do not answer the most fundamental questions relating to human scurvy; namely, the enzymatic roles of ascorbic acid in human metabolism and the optimal amount of vitamin C for maintenance of health in man. Our observations do suggest, however, that the British investigators who conducted the Sheffield studies were correct when they estimated that the adult human requirement for vitamin C was in the region of, or perhaps somewhat below, 10 mg daily. They reasoned that triple this amount, or 30 mg daily, should provide an ample allowance.

#### SUMMARY

Six apparently healthy men from the Iowa State Penitentiary volunteered for metabolic studies of human scurvy. They were hospitalized on the metabolic ward and given a diet totally devoid of vitamin C but adequate in all other essential nutrients. Although two of the prisoners escaped, the remaining four developed clinical signs of scurvy; follicular hyperkeratosis of the thighs, buttocks, calves, and the posterior aspects of the arms; swollen bleeding gums; perifollicular hemorrhages and congested follicles; and conjunctival hemorrhages. Biochemically there was virtually complete disappearance of ascorbic acid from the blood and urine, although an interfering substance gave erratic results once deficiency occurred.

Carbon-14 labeled L-ascorbic acid was used to estimate the pool size in these subjects and to measure the rate of catabolism of this vitamin. These results are reported elsewhere (6).

Blood counts, numerous biochemical

measurements, the rate of wound healing, and physiologic tests including electrocardiograms, electroencephalograms, basal-metabolism rates, and blood coagulation studies, showed no abnormalities as a result of induced scurvy.

It is estimated that clinical symptoms began to appear when the total body pool had decreased to approximately 300 mg and when the rate of catabolism of vitamin C had fallen to less than 2.5 mg daily.

The present studies do not provide sufficient evidence to estimate the optimal daily allowance of ascorbic acid, nor do they answer the question of what enzymatic roles are supplied by ascorbic acid, but they are in close accord with the British Sheffield study (4) in which a supplement of 10 mg ascorbic acid daily was found to cure and to prevent clinical scurvy.

#### REFERENCES

1. KING, C. G. Present knowledge of ascorbic acid. *Nutr. Rev.* 26: 33, 1968.
2. Recommended Dietary allowances. *Natl. Acad. Sci-Natl. Res. Council Publ. 1146*. (6th rev. ed.) 1964, p. 23-24.
3. Report of the Committee on Nutrition, British Medical Association, London, 1950.
4. BARTLEY, W. H., A. KREBS AND J. R. P. O'BRIEN. *Med. Res. Council Special Rept. Ser.* no. 280. London: H. M. Stationery Office, 1953.
5. HODGES, R. E., AND W. B. BEAN. The use of prisoners for medical research. *J. Am. Med. Assoc.* 202: 513, 1967.
6. BAKER, E. M., R. E. HODGES, J. HOOD, H. E. SAUBERLICH AND S. C. MARCH. Metabolism of ascorbic-1-<sup>14</sup>C acid in experimental human scurvy. *Am. J. Clin. Nutr.* 22: 549, 1969.
7. HODGES, R. E., W. B. BEAN, M. A. OHLSON AND R. E. BLEILER. Factors affecting human antibody response. III. Immunologic responses of men deficient in pantothenic acid. *Am. J. Clin. Nutr.* 11: 85, 1962.
8. HOOD, J., AND R. E. HODGES. Ocular lesions in scurvy. *Am. J. Clin. Nutr.* 22: 559, 1969.
9. BURKLEY, K. Urinary hydroxyproline excretion in scurvy (Master's Thesis). Iowa City: University of Iowa, 1968.
10. DRUMMOND, J. C., AND A. WILBRAHAM. William Stark, M.D., an eighteenth century experiment in nutrition. *Lancet* 2: 459, 1935.
11. CRANDON, J. H., C. C. LUND AND D. B. DILL. Experimental human scurvy. *New Engl. J. Med.* 223: 353, 1940.
12. PIJOAN, M., AND E. L. LOZNER. Vitamin C economy in the human subject. *Bull. Johns Hopkins Hosp.* 75: 303, 1944.
13. VAN EEKELEN, M. On the amount of ascorbic acid in blood and urine. The daily human requirements for ascorbic acid. *Biochem. J.* 30: 2291, 1936.
14. HOLST, A., AND T. FRÖLICH. Über experimentellen Skorbut. Ein Beitrag zur Lehre von dem Einfluss einer einseitigen Nahrung. *Z. Hyg. Infektionskrankh.* 72: 1, 1912.
15. ROBERTS, J. M. The Falkland Islands Dependencies Survey. *Brit. Med. J.* 2: 863, 1949.
16. GOLDSMITH, G. A. Human requirements for vitamin C and its use in clinical medicine. *Ann. N.Y. Acad. Sci.* 92: 230, 1961.
17. MARTIN, G. R., AND S. E. MERGENHAGEN. Influence of scurvy and lathyrism (odoratism) on hydroxyproline excretion. *Nature* 191: 1008, 1961.