THE VITAMIN C REQUIREMENT IN RHEUMATOID ARTHRITIS *

By MARSHALL G. HALL, M.D., ROBERT C. DARLING, M.D., and F. H. L. TAYLOR, PH.D., *Boston, Massachusetts*

IN normal healthy individuals the blood serum or plasma level and the urinary excretion of vitamin C are dependent largely upon the dietary intake of the vitamin.[1, 2, 3, 4] It has been shown by van Eckelen [5] and Heinemann [6] that normal adults have a daily requirement of 60 mg. of the vitamin.

The presence of infection is known to increase the requirements for vitamin C.[7] In patients suffering with various forms of infection the level of vitamin C in the blood plasma or serum may be reduced to the level found in patients with scurvy.[7] Under these circumstances the amount of vitamin C required to raise the blood plasma level of the vitamin to that of the kidney threshold and to maintain this degree of "saturation" is many times that accepted as the normal requirement of healthy individuals.[8]

Rinehart [9, 10] has shown that in patients with rheumatoid arthritis there is an apparent vitamin C deficiency as indicated by low concentration of the vitamin in the blood.

The present studies were originally undertaken to determine the incidence of lowered vitamin C content of the blood among patients with rheumatoid arthritis as compared with normal individuals on a similar dietary regime. At the same time information was sought which would indicate whether patients with rheumatoid arthritis had a greater requirement for vitamin C than normal people, and if so whether the satisfaction of such an increased demand would result in clinical improvement.

METHODS

Analytical. The method for the determination of cevitamic acid in the blood plasma is essentially that of Farmer and Abt [11] with slight modifications to prevent oxidation of the vitamin.

Five ml. of venous blood are withdrawn into a tube containing 0.05 ml. of potassium oxalate saturated solution and 1 drop of 5 per cent sodium cyanide solution. The blood is centrifuged and 2 ml. of the plasma are precipitated with 4 ml. of 5 per cent metaphosphoric acid and 4 ml. of distilled water. The protein is removed by either centrifugalization or filtration. Two ml. samples in duplicate of the filtrate are diluted with 3 ml. of distilled water and titrated with 2-6 dichlorophenol-indophenol solution

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From the Medical Service of the Robert B. Brigham Hospital; the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard) of the Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts.

which had been standardized on the same day. The first pink color persisting for 30 seconds is arbitrarily taken as the end point.

The determination of cevitamic acid in urine is made according to the method of Taylor et al.[12]

Standard Solutions. The 2-6 dichlorophenol-indophenol solution is made up fresh every week. Approximately 20 mg. of LaMott special indicator solution are weighed into a 100 ml. volumetric flask, dissolved in hot boiled water and diluted to the mark with cold boiled distilled water. (The solution is usually complete, but if any insoluble material is present it should be removed by filtration.) This stock dye solution is then kept in the icebox in a brown glass stoppered bottle.

The working standard is prepared by diluting 10 ml. of the stock dye solution to 100 ml. in a volumetric flask using freshly boiled distilled water.

Standardization of the Dye. Five hundred ml. of 2 per cent metaphosphoric acid are prepared by dilution with boiled distilled water. Approximately 15 mg. of cevitamic acid (Merck's Cebione) are weighed to four places on the analytical balance and transferred to a 100 ml. volumetric flask. The cevitamic acid is dissolved in 2 per cent metaphosphoric acid and diluted to the mark with the same reagent. Ten ml. of this stock standard are transferred to a second 100 ml. volumetric flask and made up to volume with 2 per cent metaphosphoric acid.

One ml. of this working standard is transferred to a titration flask and diluted with 4 ml. of 2 per cent metaphosphoric acid. The mixture is titrated with the dilute dye solution. A blank both for perception of end point and on reagents is made by titrating 5 ml. of 2 per cent metaphosphoric acid.

Clinical Material. The frequency of low levels of vitamin C in the blood plasma was determined from a study of 56 cases of rheumatoid arthritis. The cases were both early and late and had varying degrees of deformity and severity of symptoms. A control group of 12 normal adults living in the hospital on exactly the same diet was studied as a control. An intensive study of vitamin C metabolism was made in ten of the patients with rheumatoid arthritis of long duration. The vitamin C levels in the plasma of these individuals was less than 0.5 mg. per 100 ml. Four of these patients were placed on a vitamin C free diet for a period of from three to four weeks during which time urine analyses for vitamin C were made and periodic estimations of the amount of cevitamic acid in the circulating blood plasma. In addition the response of each of the four patients to the administration of a single oral dose of one gram of cevitamic acid was studied by following the changes in the level of the substance in the blood plasma and urine output of vitamin C. Daily red blood cell counts and hemoglobin determinations were made and the weights of the patients determined at frequent intervals. In addition close check was kept on any clinical changes in the patients including observations on the red blood cell sedimentation rate.

TABLE IA

Cevitamic Acid Level of Blood of 58 Unselected Cases of Rheumatoid Arthritis on Hospital Diet Containing Approximately 80 mg. per Day Cevitamic Acid

Patient No.	Age	Sex	Date	Cevitamic Acid mg./100 c.c. Blood Serum
1	44	female	June 14	2.320
2	27	male	Feb. 18	2.245
3	39	male	Feb. 17	2.023
4	57	female	June 2	1.650
5	40	female	Feb. 12	1.580
6	45	female	June 7	1.260
7	40	female	Feb. 12	1.260
8	49	female	May 6	1.160
9	37	female	Feb. 14	1.110
10	56	female	Feb. 18	1.035
11	29	female	Feb. 14	.950
12	39	female	Feb. 13	.890
13	51	female	May 21	.790
14	27	female	Apr. 27	.730
15	23	female	June 2	.720
16	47	female	Feb. 14	.700
17	50	female	June 18	.678
18	30	male	Apr. 22	.610
19	19	female	June 18	.604
20	62	female	June 18	.562
21	45	female	Apr. 23	.550
22	55	male	Feb. 16	.550
23	29	male	June 18	.548
24	19	male	Feb. 18	.521
25	30	male	May 12	.477
26	35	female	Mar. 10	.475
27	21	male	May 11	.465
28	57	female	Feb. 19	.450
29	13	female	Apr. 22	.429
30	42	female	Feb. 14	.420
31	22	female	Apr. 22	.401
32	23	male	Mar. 20	.393
33	27	female	Feb. 17	.362
34	41	female	May 12	.358
35	27	female	Mar. 20	.350
36	68	male	Apr. 21	.330
37	37	female	June 9	.329
38	51	female	Feb. 18	.329
39	57	female	Apr. 22	.321
40	18	female	Apr. 26	.317
41	61	female	Feb. 14	.302
42	70	female	June 2	.301
43	32	male	Feb. 18	.279
44	21	male	Feb. 20	.268
45	57	male	June 14	.257
46	27	female	May 16	.254
47	41	female	Feb. 18	.253
48	38	male	Feb. 17	.218
49	25	female	Apr. 26	.234
50	47	female	Apr. 27	.214
51	26	male	May 5	.193
52	41	male	May 5	.193
53	28	male	Apr. 27	.172
54	27	female	June 7	.158
55	27	male	Feb. 18	.152
56	17	male	Feb. 18	.152
57	23	female	Feb. 19	.150
58	50	female	May 12	.067

At the end of the control period each of the four patients was placed on a daily intake of 100 mg. of pure cevitamic acid for two weeks and the various observations made during the control period repeated. The patients were then given 200 mg. of cevitamic acid daily by mouth and the observations repeated.

In six of the ten patients the vitamin C free diet was replaced by an ordinary house diet on which the patients were maintained throughout the entire period of observation. In all other respects the studies on these cases were the same as on the other four.

At no time during the observations of any of the ten patients was there any abnormality in the basal metabolic rate and the patients' temperature showed the low fluctuations between 99 and 100° F. typical of the disease in its chronic stage.

Experimental Results

The Incidence of Lowered Blood Plasma Cevitamic Acid Level in Rheumatoid Arthritis. Observations on 24 cases were made shortly after admission to the hospital; the other 31 cases had been in the hospital for from one month to several years before the observations were commenced. The experimental data are summarized in table 1a. In summary, 14 or 25 per cent of these 56 patients showed a cevitamic acid level in the plasma of 0.8 mg. per hundred ml. or higher, which is within the accepted normal range.[4] Nine or 16 per cent had values between 0.5 and 0.8 whereas 33 or 59 per cent had levels of cevitamic acid in the blood plasma below 0.5 mg. per hundred ml. Five of the patients who had plasma cevitamic acid levels above 0.8 mg. had supplemented the hospital diet at the time of this survey with sufficient citrus fruits or orange juice to account for an additional intake of 80 mg. per day of cevitamic acid.

Table Ib

Cevitamic Acid Level of Blood of 12 Normal Individuals Maintained Exclusively on House Diet

Control No.	Age	Sex	Date	Cevitamic Acid mg./100 c.c. Blood Serum
1a	24	female	May 10	2.066
2a	27	female	May 6	1.990
3a	54	female	Mar. 11	1.620
4a	56	female	May 5	1.470
5a	23	female	May 5	1.150
6a	36	male	May 10	1.150
7a	35	male	May 3	.929
8a	49	female	May 6	.832
9a	43	female	May 6	.785
10a	36	female	May 5	.680
11a	21	female	Feb. 16	.560
12a	26	male	May 10	.206

The data obtained on these 12 normal subjects are given in table 1b. Ten or 83 per cent had levels of cevitamic acid in the blood plasma ranging between 0.9 and 2 mg. per hundred ml. Two subjects had values of 0.6 and 0.25 mg. per hundred ml. respectively. The individual with the lowest plasma ascorbic acid stated that he rarely ate fruits or uncooked vegetables included in the diet.

TABLE II

Cevitamic Acid Level of Blood of 29 Cases of Rheumatoid Arthritis after Maintenance on Cevitamic Acid Therapy

Patient No.	Age	Sex	Date	Cevitamic Acid mg./100 c.c. Blood Serum	Cevitamic Acid Excretion in Urine mg./24 Hours	Mg. Cevitamic Acid Orally per Day	No. Days of Therapy
16	47	female	June 2	1.82	137.0	100	12
						200	11
						300	
20	62	female	June 18	.56	17.2	200	16
24	19	male	June 18	1.18	109.6	200	32
25	30	male	June 21	2.13	20.5	200	28
						100	13
27	21	male	June 24	1.32	118.3	200	41
28	57	female	June 24	1.19	87.5	200	14
30	42	female	Apr. 27	1.10	96.0	300	53
31	22	female	June 23	1.27	32.9	200	11
32	23	male	June 2	1.25	100.0	100	11
						200	12
33	27	female	June 22	1.12	104.8	200	39
37	37	female	June 24	1.72	42.0	200	41
38	51	female	June 22	1.83	73.3	100	11
						200	31
39	57	female	June 14	2.16	123.2	200	31
40	18	female	June 23	1.30	100.4	200	30
41	61	female	May 25	1.03	126.0	100	11
						200	12
						300	
42	70	female	June 19	1.71	72.6	200	35
43	32	male	June 18	1.12	144.9	200	33
46	27	female	June 9	1.18	121.4	200	25
49	25	female	June 21	.91	83.3	200	31
50	47	female	June 18	1.19	87.5	200	14
51	26	male	June 18	1.20	124.0	100	11
						200	32
52	41	male	June 26	1.30	36.5	100	11
						200	35
53	28	male	June 21	1.19	35.3	200	28
						100	10
54	27	female	June 21	1.51	148.8	200	14
55	27	male	June 21	1.60	44.2	200	28
						100	13
56	17	male	May 31	.84	139.0	100	11
						200	51
57	23	female	June 3	1.60	90.0	100	11
						200	35
58	50	female	June 18	.96			
10	56	female	June 21	1.30	23.0	100	11
						200	30

The data shown in tables 1a and 1b may be compared with those of table 2 which gives data for cases of rheumatic arthritis after the daily ingestion of vitamin C in amounts varying between 150 and 200 mg. per day. In all of these cases the blood level of vitamin C was normal.

The Vitamin C Requirements of Patients with Rheumatoid Arthritis. The four patients maintained on a vitamin C poor diet showed a daily excretion of vitamin C in the urine below 20 mg. The six patients whose control period was the ordinary house diet showed an excretion which did not exceed 50 mg. per day. During the control period no essential change was observed in the amount of vitamin C in the blood plasma.

The administration of 1 gram of vitamin C to these ten patients was followed by a prompt rise in the level of the vitamin in the blood which returned to the pre-administration levels in 48 hours.

All 10 patients were then placed on a daily intake of 100 mg. of vitamin C per day given in two doses and the urines analyzed daily for vitamin C. Blood samples were taken fasting and before the administration of the vitamin at frequent intervals. With the dosage of 100 mg. there was no significant rise in the blood cevitamic acid level during the two weeks of therapy above that of the control period, nor did the urine cevitamic acid rise remarkably above that obtained for the control period. A typical series of observations on one of these patients is shown in figure 2.

After the patients had taken for two weeks a dosage of 100 mg. per day they were given 200 mg. per day in four doses. Under these circumstances the blood levels rose progressively in 10 to 12 days reaching levels between 1.0 to 1.8 mg. per hundred ml. of blood and remained essentially constant. At the same time the daily urinary excretion increased markedly, ranging from 30 mg. to 100 mg. per day.

In three of the patients the vitamin C administered was increased by an additional 100 mg. The blood level of ascorbic acid did not increase beyond that obtained when 200 mg. were given each day but the daily urinary excretion was increased by 40 to 70 mg.

At the end of the period of 200 mg. level of administration vitamin C was omitted for two days and the response of the patient's blood and urine to a single oral dose of 1 gram was again determined. Table 3 shows a comparison of the response of the patient's excretion of vitamin C following the oral administration of 1 gram of the vitamin during the control period and after the administration of vitamin C. It will be observed that following the generally accepted ideas of saturation with the vitamin that the patients after therapy at a level of 200 mg. per day of the vitamin have become saturated.

These observations would indicate that the requirements of an arthritic individual for vitamin C were between 100 and 200 mg. per day or in other words between two and four times that of a normal individual.

Clinical Effects of Vitamin C in Rheumatoid Arthritis. None of the patients showed symptoms of the type associated with scurvy itself, in spite

TABLE III

Comparison of Amount of Cevitamic Acid Excreted in Urine Following an Oral Dose of 1000 mg. Before and After Cevitamic Acid Therapy in Patients with Rheumatoid Arthritis

Patient No.	Age	Sex	Control Period		Therapy			After Therapy		Per cent of Cevitamic Acid fed to the amount excreted
			Cevitamic Acid mg./100 c.c. blood serum	mg. Cevitamic Acid excreted in 48 hours	Per cent Cevitamic Acid fed to amount excreted in urine	mg. Cevitamic Acid per day	No. of Days	Cevitamic Acid mg./100 c.c. blood serum	mg. Cevitamic Acid excreted in 48 hours	
10	56	female	.320	15.76	1.58	100 200	11 30	1.300	252.64	25.26
16	47	female	.240	72.00	7.20	100 200 300	11 12 8	1.938	466.00	46.60
30	42	female	.700	145.00	14.50	300	16	1.230	460.00	46.00
32	23	male	.190	6.49	.65	100 200	11 11	1.24	523.66	52.37
38	51	female	.380	6.08	.61	100 200	11 12	1.43	356.42	35.64
41	61	female	.380	55.00	5.50	100 200 300	11 12 8	1.15	471.00	47.10
51	26	male	.190	18.00	1.80	100 200	11 14	1.33	178.20	17.82
52	41	male	.110	7.00	.70	100 200	11 8	1.03	515.52	51.55
56	17	male	.140	9.00	.90	100 200	11 15	.85	409.00	40.9
57	23	female	.310	6.43	.64	100 200	11 8	1.23	447.28	44.73

of the fact that many had cevitamic acid levels below that usually present in this disease. In one case, there was a tendency to bleeding gums, but this did not clear up after the blood became saturated with vitamin C. Capillary fragility tests carried out by the method described by Wright and Lilienfeld [13] (i.e. by counting the number of petechiae present in a uniform area on the forearm after 15 minutes of tourniquet pressure held at halfway between systolic and diastolic pressures) showed no significant increase when the plasma level of vitamin C was low, or decrease when the blood was saturated.

After eight months, during which time the patients were given vitamin C daily and their blood known to be saturated with vitamin C, no clinical improvement which could be attributed to the ingestion of vitamin C was observed. Some cases improved during this period, but others continued unchanged or became worse as judged by the condition of their joints, failure to gain weight or hemoglobin, and slowing of the red cell sedimentation rate.

No increase in the red blood cell count was found in any of the patients although occasionally sporadic but slight increases in the reticulocytes were observed.

This study indicates that the ordinary hospital diet was inadequate in its vitamin C content to supply the increased demands of the rheumatoid arthritic and may lead to a general revision of diets in institutions devoted to the care of this disease. Further investigation may discover additional deficiencies in the dietary requirements for other vitamins and essential nutritional substances.

Summary

Seventy-five per cent of 56 cases of rheumatoid arthritis had a subnormal content of vitamin C in the blood. Fifty-nine per cent had levels below 0.5 mg. per 100 ml. These findings are confirmatory of those of Rinehart.[9, 10]

Although some of the patients had had diets containing vitamin C well below the amount usually required for normal people, none of them presented clinical evidence of scurvy.

Patients with rheumatoid arthritis actually have a much greater demand for vitamin C than the normal individual. From a study of 10 patients with rheumatoid arthritis it was shown that these individuals could tolerate an intake of over 100 mg. and usually 200 mg. without marked excretion into the urine.

Following this investigation all patients with rheumatoid arthritis in the Hospital were placed on an intake of 200 mg. of vitamin C per day for 8 months. No improvement has been noted that could be attributed to the effect of the vitamin.

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