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Cevitamic Acid Excretion in Pneumonias and Some Other Pathological Conditions.

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Previous studies by Harde,[1] and Harde and Philippe,[2] Harde and Benjamin,[3] Harde and Greenwald,[4] have shown a lowering of vitamin C content of the tissues of laboratory animals in many infections and intoxications. King[5] and his associates noted a similar

* P represents a preliminary, C a complete manuscript.

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1 Harde, E., *C. R. de l'acad. des Sc.*, 1934, **199**, 618.

2 Harde, E., and Philippe, *C. R. de l'acad. des Sc.*, 1934, **199**, 738.

3 Harde, E., and Benjamin, H. R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 651.

4 Greenwald, C., Harde, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1157.

5 Yavorsky, M., Almaden, P., and King, C. G., *J. Biol. Chem.*, 1934, **106**, 525.

fact in studies of human autopsies, and later in work on guinea pigs.[6]

These observations suggested to us that vitamin C in infectious diseases unrelated to scurvy, such as pneumonia, may be lowered. The examinations of urines for vitamin C from pneumonia and other pathological conditions permitted verification of this hypothesis.

A reducing substance in the urine of normal individuals, probably in large part cevitamic acid, has been studied by Hess and Benjamin[7] and by Birch, Harris and Ray.[8] The latter authors found a relation between vitamin C content of the diet and the urinary excretion of the reducing substance. On a balanced diet, normal adults excrete

TABLE I.
Pneumonia Cases.

Case No.	Diagnosis	Date	Mg. cevitamic acid excretion per cc. per diem	Mg. cevitamic acid excretion per diem	Dosage Mg. Cevitamic acid (Merck)	Remarks
1	Type II Serum	2/18	.015	5.05		Peak not reached until 8 days after dosing. Recovered.
		2/19	.006	12.75	200 mg.	
		2/20	.010	13.42	100 mg.	
		2/21	.009	6.90	200 mg.	
		2/22	.007	16.14	400 mg.	
		2/23	.012	10.58	200 mg.	
		2/24	—	—	400 mg.	
		2/25	.048	59.35	200 mg. 8 oz. orange ju.	
2	Type VIII Serum	2/25	.012	1.63	400 mg.	No saturation after 10 days dosing. Recovered.
		2/26	.010	2.31	6 oz. or. ju.	
		2/27	.012	4.49	400 mg.	
		2/28	.013	4.53		
		—	—	—	—	
		—	—	—	—	
		—	—	—	—	
		3/6	.007	4.59	400 mg.	
3	Type I *B. Friedlander* A Serum	2/28	.00	0.00	2×1-gm.	Massive dosing did not cause immediate saturation. Died.
		3/1	.00	0.00	3×1-gm.	
		3/2	.017	13.66	None	
		3/4	—	—	,,	
		3/5	.110	90.00	,,	
		3/6	.110	68.95	,,	
4	Type VII Serum			*		Recovered
		3/12	.016	4.80	1-gm.	
		3/13	.017	6.12	,,	
		3/14	.033	15.17		

[6] Bessey, O. A., and King, C. G., *J. Nutrition*, in press.

[7] Hess, A. F., and Benjamin, H. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 855.

[8] Birch, T. W., Harris, and Ray, *Biochem. J.*, 1933, **27**, 590.

TABLE I. (Continued).

Case No.	Diagnosis	Date	Mg. cevitamic acid excretion per cc. per diem	Mg. cevitamic acid excretion per diem	Dosage Mg. Cevitamic acid (Merck)	Remarks
5	Type V Serum	3/7	.018	8.55	No dosing	Recovered.
		3/8	.041	13.65		
		3/14	.012	7.60		
		3/15	.010	3.55		
6	Type VIII Serum	3/11	.018	12.85	2 500-mg.	Recovered.
		3/12	.028	36.70	2 500 "	
		3/13	.020	24.00	2 500 "	
		3/14	.039	33.03		
7	Type III Bacteremia Serum	2/20	.003	1.98	16 oz. orange juice daily	No saturation.
		2/21	.003	2.10		Recovered.
		2/22	.010	1.75		
		2/23	—	—		
		—	—	—		
		—	—	—		
		3/1	.008	2.40		
8	Type III Serum	2/24	—	—	400 mg.	Died
		2/25	.018	21.55	400 "	
		2/26	.021	17.85		
9	Type V Serum	5/1	.019	4.28	1 gm. 4 doses	Recoverd.
		5/2	.000	0.00		
		5/3	.000	0.00		
		—	—	—		
		—	—	—		
		5/7	.009	2.25		
		5/8	.005	3.00		
10	Type I Serum	4/24	.007	1.05	1 gm. 3 doses	No excretion.
		4/25	.000	0.00	1 " 3 "	Recovered.
		4/26	.000	0.00	1 " 3 "	
		4/27	.000	0.00		
11	Type I Non-Serum	2/18	.022	7.46	3 100-mg. doses	Recovered.
		2/19	.009	3.36		
		2/20	.008	1.12		
		2/21	.000	0.00		
12	Type VIII Non-Serum Influenza complications	3/29	.008	2.64	250 mg.	Following peak patient continued to overflow with low dosing, then dropped to normal. Recovered.
		—†	—	—		
		—	—	—		
		4/2	.017	4.69	500 "	
		4/4	.063	44.97	500 "	
		—	—	—		
		4/30	.045	22.88		

*Single urine specimen.
†Time elapsed.

0.02 to 0.03 mg. per cc. of this substance or a total of 15 to 30 mg. per 24 hours.

According to Harris and Ray, a peak of saturation may be reached normally within 3 to 5 hours following a very large dose of cevitamic acid. We found that this did not occur in most patients suffering from pneumonia. Very large doses for 3 to 4 days were occasionally required before a peak was reached. In some cases (Table I, Case 2) saturation was not observed even after continued dosing for 8 to 10 days. In all cases when administration of the vitamin was stopped an immediate drop was noted. A second peak could be obtained by an additional relatively small dose (Chart 1).

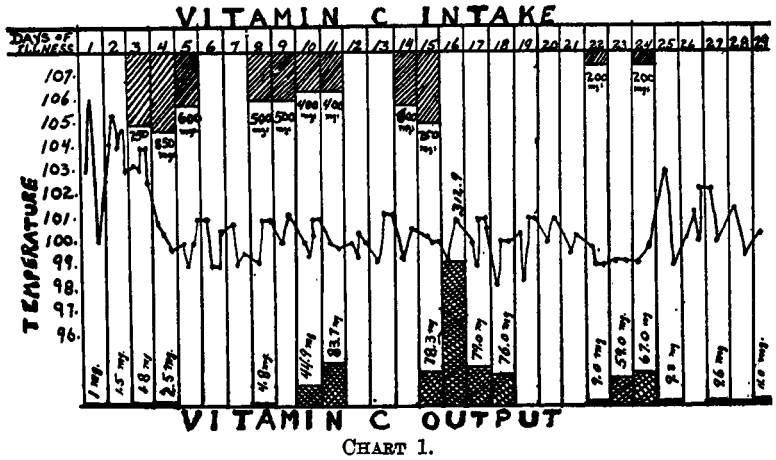

CHART 1.

This fact suggests that at least a portion of the reducing substance in the urine is present as a form of cevitamic acid, and that the overflow results from the saturation of the tissues by the vitamin.

Our experience with 29 pneumonia patients is in accordance with that of Schroeder,[9] who found that vitamin C excretion is decreased in pneumonia. He also found it diminished in cystitis, typhus, and tuberculosis. In our patient with tuberculosis the vitamin C excretion was reduced but on prolonged dosage a high peak was reached which rapidly fell when dosing was discontinued. (Table II, Case 4.) According to Harris,[10] the decrease in vitamin C excretion in pneumonia patients may be due to the increased metabolic rate. Most of our patients were undernourished by reason of poverty.

A preliminary report[11] was given by us on the study of urinary excretion of vitamin C in 10 cases of pneumonia. For our present

[9] Schroeder, *Klin. Woch.*, 1935, 484.

[10] By private communication from L. J. Harris.

[11] Harde, E., Rothstein, I. A., and Ratish, H. D., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1088.

TABLE II.
Cases Other Than Pneumonia.

Case No.	Diagnosis	Date	Mg. cevitamic acid excretion per cc. per diem	Mg. cevitamic acid excretion per diem	Dosage Cevitamic acid*	Remarks
1	Influenza	3/29	.050	23.75		Normal output.
		3/30	.050	11.25		
		4/1	.065	31.20		Recovered.
		4/2	.050	17.16	200 mg.	
		4/3	.075	30.00	100 "	
2	Serum Sickness	5/8	.008	6.50	600 mg. 3 doses	Recovered.
		5/9	.020	13.80	"	
		5/10	.017	12.25	"	
		5/11	.018	9.25	"	
		5/14	.000	0.00	"	
3	Amputation of leg caused by gangrene	5/10	.035	15.75	8 oz. orange juice daily	5/11 before amputation.
		5/11	.07	15.75		5/16 after amputation.
		—	—	—		
		—	—	—		
		5/16	.000	0.00		Died.
		5/18	.026	3.90		
		5/20	.000	0.00		
		5/21	.025	3.75		
4	Tuberculosis. Pneumonia Type VI	2/27	.024	21.45	6 oranges	Died.
		2/28	.024	40.35	"	
		†	†	†	†	
		3/19	.068	49.80	3/18-600 mg.	
		3/20	.40	200.00	3/19-600 mg.	
		†	†	†	†	
		4/9	.022	14.61‡	Dosing discontinued	
		4/10	1 P.M.—.03	3.00		
			4 P.M.—.00	0.00		
		†	†	†	†	
		4/12	.35	167.50	4/11 to 4/12 750 mg. in 3 doses	
		4/16	1 P.M.—.011	1.10		
			8 P.M.—.00	0.00		
5	Arthritis	2/22	.033	10.14		Recovered.
		2/23	.009	2.65		
		—	—	—		
		—	—	—		
		3/4	.15	22.50		
		3/5	.11	86.10	600 mg. 3 doses	
6	Rheumatic Fever	3/14	.060	24.52	No dosing	Patient reports 1 pint orange juice daily for past 2 years.
		—	—	91.75		
		3/19	.10			
7	Coryza	4/3	.075	34.38	600 mg. 4 doses	Recovered.
		4/4	.10	133.65		
		4/5	.10	55.60		

*Both Merck & Co. and the Hoffman-LaRoche Co. generously supplied the pure cevitamic acid used in these studies.

†Time elapsed.

‡Note reduction in excretion.

work the vitamin C excretion of 38 instances of various pathological conditions were studied. The technique of Harris and Ray[12] was followed. This method is based on the titration of the vitamin C against the dye, 2-6 dichlorophenolindophenol. (Modification of Tillman's Method.) The urine was titrated against .02-0.1 cc. of the dye.

Fresh solutions of the dye were prepared every 24 hours. Occasionally the dye was kept for 48 hours. In this case, it was standardized every 24 hours. The dye solution was kept in an amber bottle, which was placed in the refrigerator (50°F.) whenever it was not in use. The dye was standardized against a solution of 10 mg. of pure cevitamic acid in 50 cc. of 10% acetic acid. The urines, as voided, were collected in bottles containing 15 cc. of glacial acetic acid and placed, as soon as possible, in the refrigerator. Only titrations made within 12 hours are considered in this report, because Harris and Ray reported that urines preserved by 10% acetic acid will retain vitamin C for 10-12 hours.‡

We have occasionally had difficulty in determining the end point in highly colored urine, even when such urines were diluted as recommended by Harris and Ray.[12]

Doses of cevitamic acid were given in quantities varying from 100 mg. to 1 gm. depending upon age and vitamin excretion of the patient.

The urinary titration of cevitamic acid has confirmed previous work with tissues of experimental animals, namely, the diminution of vitamin C in the course of many intoxications and pathological conditions. These states do not suggest scurvy and may be designated hypovitaminosis accompanying, or etiologically related to the pathological conditions with which they occur.

In the pneumonias studied we have been unable to determine any striking correlation between the clinical condition of the patients and their vitamin C excretion. In the greater number of our cases it was noted that when the temperature was high the excretion was low. When saturation occurred it was very often during a drop in the temperature. This seems to support the contention of Harris that increased metabolism is associated with increased destruction of the vitamin in the tissues. The pulse rate showed no significant change. In many of the convalescent cases excretion of vitamin C

[12] Harris, L. J., and Ray, S. N., *Lancet*, 1935, 228.

‡ We have found it impossible to have an exact 10% solution of acetic acid as recommended by Harris and Ray. Experiments with various quantities of urine have shown that the addition of 15 cc. of the glacial acetic acid to the specimen yield approximately a 10% solution.

was low. In one case (Table II, Case 1) with an ordinary balanced hospital diet there was a normal excretion in spite of administering large amounts of vitamin C. This may have been due to the restoration of vitamin C to the tissues depleted during illness. At no time did we note an 80% excretion of the vitamin C intake as described by Harris and Ray for normal individuals.

We have found that administration of crystalline cevitamic acid is preferable to the large amounts of orange juice required to furnish an equivalent quantity of vitamin C, as orange juice in very large quantities may cause diuresis and diarrhea.

Summary. Hypovitaminosis, as determined by delayed saturation, occurs while the temperature is elevated and especially during high fever in pneumonia. Urinary examination after oral administration of large quantities of cevitamic acid in divided doses permits rapid determination of the degree of saturation of the tissues.

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Color Reactions of Keturonic Acids and a Color Test Differentiating α- and β-Glucosides.*

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Color reactions of the keturonic acids have recently become of importance to biology and medicine. In previous papers we have shown that a series of these acids can be prepared from carbohydrates by oxidation of their aqueous solutions with bromine.[1, 2] The solutions of the keturonic acids used in the experiments reported here were prepared as follows:

1% solutions of pure carbohydrates were placed in glass-stoppered flasks together with enough bromine to provide a small excess of liquid bromine throughout the experiments. These mixtures were kept in the dark for 42 days at 25°C. They were then aerated with washed air (and at times with carbon dioxide) to remove the excess bromine and then neutralized with potassium hydroxide to pH 7,

* Aided by a grant from the Research Appropriation of the University of Oklahoma Medical School.

1 Everett, M. R., Edwards, B. G., and Sheppard, F., *J. Biol. Chem.*, 1934, **104**, 11.

2 Sheppard, F., and Everett, M. R., *J. Biol. Chem.*, 1934, **105**, lxxx.