

## Vitamin C Requirement of the Guinea Pig.\*

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The assumed daily requirement of ascorbic acid for the guinea pig has gradually been increased from 0.5 mg to 1.5 mg (Szent-Györgyi.<sup>1</sup>) In fact, Zilva<sup>2</sup> showed that even on a dosage of 1.5 mg per day saturation of the tissues did not occur, and that about 20 times this amount was necessary to maintain saturation of the tissues. However, since Zilva's animals apparently thrived on 1.5 mg per day, this author assumed that this amount sufficed to assure undisturbed growth and health.

Szent-Györgyi,<sup>1</sup> however, believes that the true physiologic daily dose of the vitamin is that amount which the animal consumes in its native environment. He calculated that in his natural habitat the guinea pig ingests about 30 mg of ascorbic acid daily, and that this amount compares favorably with the amount needed to maintain a maximal saturation of its tissues. During the course of the experiments on the relationship of vitamin C to complementary activity,<sup>3-5</sup> 870 blood-serum ascorbic-acid determinations were made. The guinea pigs were given varying amounts of ascorbic acid.

This report deals with the relationship of the blood-serum ascorbic-acid level to the daily intake of varying amounts of the vitamin.

*Methods.* All the animals were maintained on a stock diet of Purina mixed rabbit chow. This mixture is supposed to contain all the essential elements needed for full growth of the animals with the exception of vitamin C. In fact, guinea pigs develop scurvy when maintained on this diet for periods longer than 21 days.

Crystalline ascorbic acid was administered either as a commercial sodium salt known as "Cevalin" or as ascorbic acid neutralized

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<sup>1</sup> Szent-Györgyi, A., *Deutsch. med. Wchnschr.*, 1937, **63**, 1789.

<sup>2</sup> Zilva, S. S., *Biochem. J.*, 1936, **30**, 1419.

<sup>3</sup> Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., *J. Immunol.*, 1938, **34**, 19.

<sup>4</sup> Pillemer, L., Seifter, J., Kuehn, A. O., and Ecker, E. E., to be published.

<sup>5</sup> MacDonald, F., and Johnson, H., to be published.

before injection with 0.1 N NaOH. All injections were given intramuscularly.

A modified Tillman's procedure was used for the estimation of the ascorbic-acid content of the serum. The method follows: 4 to 5 cc of blood were drawn by cardiac puncture and allowed to stand for 15 minutes and centrifugalized. Two cc of the serum was then added to 4 cc of freshly prepared 20% trichloroacetic acid. The mixture was allowed to stand for 5 minutes and again centrifugalized. Three cc of the clear supernatant liquid was then titrated against 2,6-dichloro-phenolindophenol (Hoffbann-LaRoches tablets). Each cc of the dye solution is equivalent to 0.02 mg of ascorbic acid. All titrations were done in duplicate and triple distilled water was always employed.

TABLE I.  
Daily Dose of Ascorbic Acid and Blood-serum Ascorbic Acid in the Guinea Pig.

No. of determinations	Daily dose, mg	Blood-serum ascorbic acid in mg%		
		min.	max.	med.
40	0	0	.05	.02
220	0.5	0	.21	.09
218	1.0	.05	.36	.13
47	2.0	.12	.52	.33
117	5.0	.39	.74	.57
143	10.0	.83	1.44	1.03
85	20.0	.87	1.73	1.08

*Results.* The table gives a composite protocol of the results of 870 blood-serum ascorbic-acid values.

It is seen that the maximal concentration of vitamin C in the serum of the guinea pig only occurs when a daily dose of from 10 to 20 mg is given. Since it has been shown that serum-complement reaches its maximal activity at a level of about 1 mg of ascorbic acid per 100 cc, it is suggested that an amount of 10 mg per day be given to maintain the normal activity of the blood-serum proteins since a full complementary activity is an expression of the normal behavior of these proteins. Cohen<sup>6</sup> found that 5 mg of ascorbic acid per day was needed in a tooth-protective ration.

*Summary.* Analysis of a series of 870 blood ascorbic-acid values reveals that a daily intake of from 10-20 mg of ascorbic acid is needed to secure a 1 mg per 100 cc concentration in the blood serum of the guinea pig. Under these conditions a normal complement-value is obtained.

<sup>6</sup> Cohen, M. B., *J. Allergy*, 1938, **10**, 15.