

CHANGES IN THE ASCORBIC ACID CONTENT OF THE WHITE BLOOD AS AN INDEX OF THE VITAMIN C CONDITION OF THE BODY

A. A. Anisova

From the State Research Institute of Vitaminology of the Ministry of Health of the USSR, Moscow

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In a number of papers by foreign authors there are indications of the existence of a connection between the ascorbic acid content of the white cell-platelet layer of the blood and the intake of vitamin C. From the point of view of method, the investigations described differ in the method of separation of the white cell-platelet layer (from exudate [4, 7], from whole blood [2, 8] etc.) and in the method of estimation of the ascorbic acid (by means of 2-4-dinitrophenylhydrazine [1, 7] and others and 2-6-dichlorophenolindophenol [2, 4] and others). The results obtained by these authors cited showed that the ascorbic acid content in the white cell-platelet layer fell when the dose of vitamin C was reduced.

Butler and Cushman [2] observed that the ascorbic acid content of the white cell-platelet layer of the blood was the best index of saturation of the animal body with the vitamin. Lubschez [6] found greater constancy in the ascorbic acid content of the white cell-platelet layer of the blood than in the plasma. In the paper by Crandon [3] it is pointed out that the development of signs of scurvy is accompanied by a fall in the ascorbic acid content of the white cell-platelet layer of the blood. Lowry [5] came to the conclusion that the ascorbic acid content of the white cell-platelet layer of the blood could be used as a criterion of the vitamin C requirement. The aim of the present research was to make as detailed an investigation as possible of the changes in the ascorbic acid content of the white cell-platelet layer of the blood in relation to the dose of vitamin C in experiments of long duration on animals, so that if definite results were obtained, observations on human subjects could subsequently be made.

EXPERIMENTAL METHOD

Observations were made on guinea pigs. Blood was taken from the heart of the animals. The ascorbic acid content was estimated by means of 2-4-dinitrophenylhydrazine by the method suggested by Bessey [1], except that the blood was taken in larger volume: 0.6 ml instead of 0.1 ml. Furthermore, Bessey obtained the white cell-platelet layer by slow centrifugation, whereas the method we used was as follows: by rapid centrifugation of whole blood in a specially shaped tube for 10 minutes, the red cell and white cell-platelet layers of the blood were separated. The tube consisted of three parts: an upper wide part (internal diameter 7 mm, height 40 mm), a middle narrow part (diameter 3 mm, height 35 mm) and a lower spherical part (diameter 5 mm).

The red cell layer settled in the spherical part and occupied an insignificant volume of the narrow part of the tube. The white cell-platelet layer settled in the narrow part of the tube (above the red); it was collected in a Pasteur pipette with a rubber balloon (under these circumstances a small portion of the red cell layer entered the pipette), after which it was transferred to a hematocrit and centrifuged once again. Next the white cell-platelet layer was extracted by means of a capillary pipette, transferred to a tube and estimated by Bessey's method.

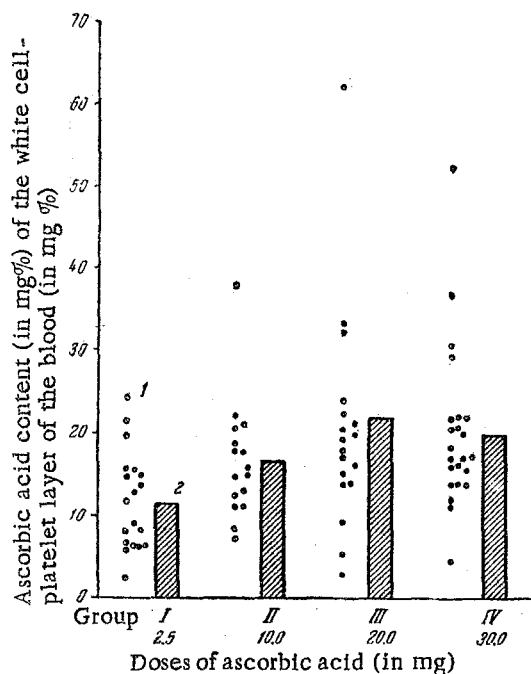


Fig. 1. Ascorbic acid content (in mg%) of the white cell-platelet layer of the blood of guinea pigs at the end of an experiment in which large doses of ascorbic acid were given daily to the animals for a period of 6 months. First experiment. 1) Individual results; 2) mean results for the groups.

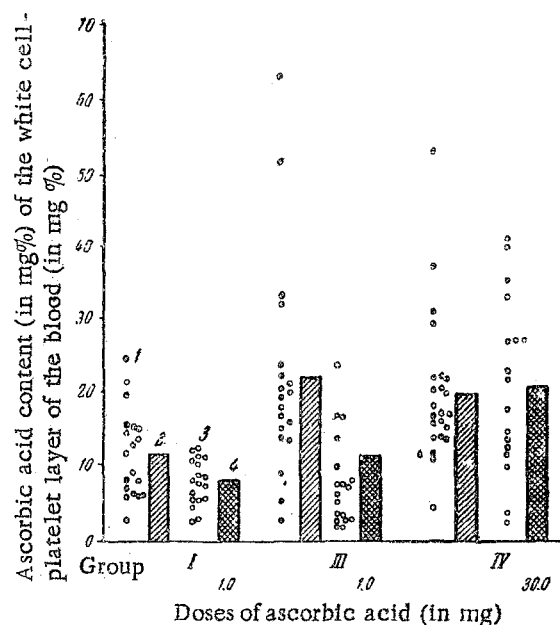


Fig. 2. Ascorbic acid content (in mg%) of the white cell-platelet layer of the blood of guinea pigs at the beginning and end of the experiment. Duration of experiment 2 months. 1) Individual results at the beginning of the experiments; 2) mean results for the groups at the beginning of the experiment; 3) individual results at the end of the experiment; 4) mean results for the groups at the end of the experiment.

Experiments were carried out on young male guinea pigs weighing 300-320 g. The guinea pigs arrived at the laboratory weighing 200 g. In the period preceding the experiment the guinea pigs received a scorbutic diet, consisting of oats, bran, hay and water. Separately from the diet the animals were given ascorbic acid daily in a dose of 25 mg in aqueous solution, 833 i. u. of vitamin A and 15 i. u. of vitamin D in sunflower oil. Animals whose weight had reached 300-320 g were selected for the experiment and were divided into groups. The experiment lasted 6 months. During the course of the experiment the guinea pigs received the same diet and the same doses of vitamins A and D, but the different groups were given different doses of ascorbic acid: 2.5, 10, 20 and 30 mg. The ascorbic acid content of the white cell-platelet layer of the blood was determined at the end of the experiment.

EXPERIMENTAL RESULTS

With comparatively large doses of ascorbic acid, the white cell-platelet layer of the blood of the guinea pigs was rich in vitamin C: at a dose of 20 mg of ascorbic acid, the content was almost twice (75% higher) that at a dose of 2.5 mg. There was no difference between the ascorbic acid content of the white cell-platelet layer of the groups receiving 20 and 30 mg of vitamin C (Fig. 1). Mathematical treatment of the results showed that the difference between the groups receiving 2.5 and 20 mg of ascorbic was close to significant ($t = 2.9$).

The second experiment was a continuation of the first; it lasted 2 months.

The fourth group, which in the first experiment received 30 mg, remained as the control, and the animals in it continued to receive the same dose of vitamin C as during the second experiment, and it was found that the mean ascorbic acid content of this group was unchanged; 20 and 21 mg% respectively (Fig. 2).

The first group of animals was changed from a dose of 2.5 mg to one of 1 mg of ascorbic acid. In this group the mean vitamin C content of the white cell-platelet layer of the blood fell slightly (from 12 to 8 mg%).

The third group was changed from a dose of 20 mg to one of 1 mg of ascorbic acid. Under these circumstances there was a sharp fall in the vitamin C content of the white cell-platelet layer (from 22 to 11 mg% on the average for the group).

From an analysis of the experimental results it may be concluded that between dosage limits of 1 and 20 mg of ascorbic acid, there is a connection between its contents in the white cell-platelet layer of the blood and the dose of vitamin C administered. The dose of ascorbic acid at which its means content in the white cell-platelet layer in guinea pigs reaches its maximum value is 20 mg (first experiment).

The findings described suggest that the determination of the ascorbic acid content in the white cell-platelet layer of the blood may be employed as one of the criteria of the vitamin C state of the body.

SUMMARY

In experiments on guinea pigs a relation has been established between the ascorbic acid content in the white cells and the dose of ascorbic acid administered. This permits us to conclude that the ascorbic acid content in the white cells may serve as a criterion of the vitamin C condition of the body.

LITERATURE CITED

- [1] O. A. Bessey, *The Biochemistry and Physiology of the Vitamins*, 4, pp. 113-150. Moscow, 1951 [Russian translation].
- [2] A. M. Butler, M. Cushman, *J. Clin. Invest.*, 1940, v. 19, p. 459-467.
- [3] I. H. Crandon, C. C. Lund, D. B. Dill, *New England J. Med.*, 1940, v. 223, p. 353-359.
- [4] J. Lebrun-Pages, *Compt. rend. Acad. sc.*, 1953, v. 236, p. 1381-1383.
- [5] O. H. Lowry, O. A. Bessey, M. J. Brock et al., *J. Biol. Chem.*, 1946, v. 166, p. 111-119.
- [6] R. Lubschez, *J. Clin. Invest.*, 1945, v. 24, p. 573-578.
- [7] W. J. Nungester, A. M. Ames, *J. Infect. Dis.*, 1948, v. 83, p. 50-54.
- [8] B. F. Steele, R. L. Liner, Z. H. Pierge, et al., *J. Nutrition*, 1955, v. 57, p. 361-368.