# VITAMIN C CONTENT OF LABORATORY ANIMALS, AS SHOWN BY HISTOCHEMICAL METHODS

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Evidence has been published that certain drugs affect the ascorbic acid content of animal organs. It has been shown that aspirin, sulfanilamide, and potassium iodide cause a fall in the vitamin C content of human blood, and that administration of sulfanilamide to guinea pigs and albino rats leads to accumulation of ascorbic acid in the liver [4, 5]. Medicinal doses of quinine hydrochloride, sodium salicylate, and calcium chloride cause depletion of body ascorbic acid [2], while barbiturate derivatives stimulate biosynthesis of vitamin C [1].

H. Steege and R. Pirtkin [10], using a histochemical method, have shown that the ascorbic acid content of the adrenal glands rises after administration of thiosemicarbazones.

On the strength of these published data, we decided to apply the histochemical method to the detection of ascorbic acid in animal organs, and to use this as a test in the comparative study of the action of drugs.

## EXPERIMENTAL METHODS

We applied the method of Giroud and Leblond [7] to the detection of ascorbic acid in tissue sections. Small fragments of tissue are immersed in a 10% silver nitrate solution, made acid with 1% acetic acid, and reduction of silver nitrate takes place within 15-30 minutes. The tissue is then withdrawn from the solution, washed with distilled water, and fixed in 3% hyposulfite solution for 15-30 minutes, after which it is again washed, and embedded in paraffin in the usual way. Black granules of silver are seen in the tissue sections at the locations of ascorbic acid in the fresh tissue. G. I. Roskin [3] and Burne [6] regard the reaction as being specific for ascorbic acid, under the given conditions. All manipulations involving silver salts were performed in a dark room, using a red light.

We know of only very few Soviet and foreign papers on the histochemical study of the distribution of vitamin C in animal and human organs [3, 8, 9], and they do not give a complete picture of the morphological distribution of ascorbic acid in organs.

The present paper gives an account of an investigation of the distribution of ascorbic acid within the cells of various organs of laboratory animals.

### Results of Histochemical Studies

We examined the organs of 30 guinea pigs, 20 white rats, and 14 cats.

We found high ascorbic acid contents in the intestine, liver, adrenals, hypophysis, and gonads, and less in the nervous system, myocardium, and skin; it was absent from skeletal muscle, connective tissue, and thyroid gland.

In the alimentary tract, the highest ascorbic acid contents were found in the mucosa of the duodenum and of the higher levels of the jejunum; the mucosa of the stomach and the colon contained little ascorbic acid.

The distribution of ascorbic acid in the mucosa of the small intestine varied for different animals. In guinea pigs maintained on a fully adequate diet, vitamin C is indicated by small or large deposits of silver: fine, dust-like granules of silver are seen in the protoplasm and cuticles of the mucosal epithelium, in smooth muscle fibers, and in intramural ganglion cells; both fine and coarse granules are seen in mesenchymal cells of the reticular stroma of the villi and lymphatic follicles, and at the poles of the nuclei of endothelial cells of the blood and lymph vessels (Fig. 1, a).

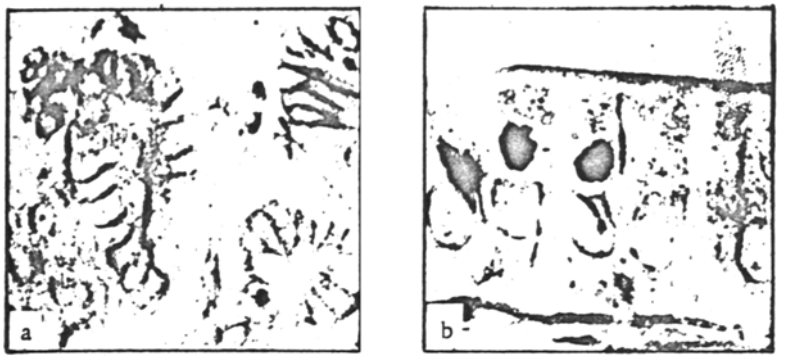


Fig. 1. Deposition of silver granules at the sites of concentration of ascorbic acid in the epithelium of an intestinal gland of a normal guinea pig (a), and in the Golgi apparatus of an albino rat (b).
Method of Giroud and Leblond. For (a) ocular × 10, obj. × 10; for (b) ocular × 10, obj. × 90.

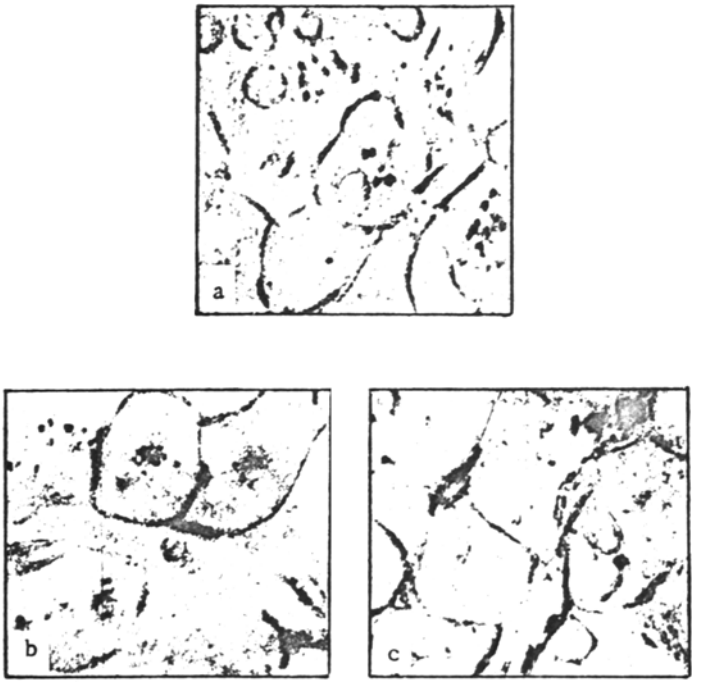


Fig. 2. Various structures of silver deposits at sites of concentration of vitamin C: a) in guinea pig liver cells; b) in cat liver cells; c) in Kupfer cells.
Method of Giroud and Leblond. Magnification: for a) and c), ocular 10 ×, obj. 40 ×; for b) oc. 10 ×, obj. 90 A.

A similar, but denser, distribution of ascorbic acid in cells of the intestinal wall of albino rats was found. In addition, we observed dense aggregations of granules in the Golgi apparatus of epithelial cells of the intestinal glands; these formations resemble those described by Tonutti (Fig. 1, b).

Various forms of deposits were seen in the liver. The granules are diffusely distributed in the parenchyma cells of guinea pigs, and are very small; complex structures made of granules and spherules appear in groups of cells (Fig. 2, a). Polygonal masses were seen in the cytoplasm of hepatic cells of albino rats and cats (Fig. 2, b).

The Kupfer cells of guinea pig liver contained isolated small granules, and of albino rats and cats large granules, singly, or in aggregates (Fig. 2, c).

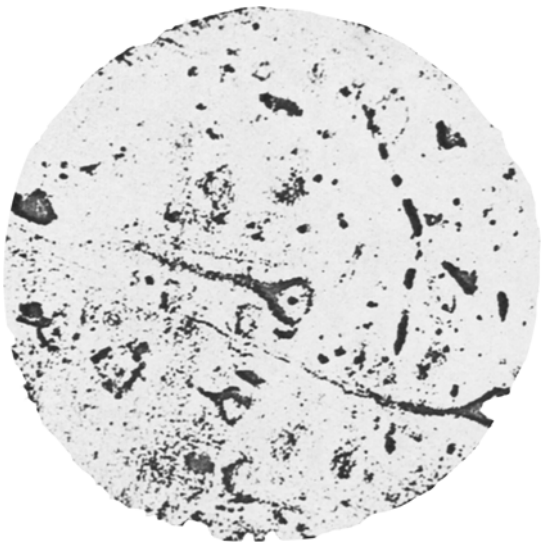

Fig. 3. Silver deposits at sites of concentration of vitamin C in cells of the cerebral cortex of a normal guinea pig.
Method of Giroud and Leblond. Magnification: oc. 10 ×, obj. 90 ×.

Vitamin C was encountered in reticular cells of the spleen follicles and pulp, sometimes in large amounts in albino rats. It is present in the cytoplasm of epithelial cells of the convoluted tubules of the kidney, and less frequently in that of the straight tubules. It is encountered in the myocardium, chiefly in the myofibrils and in the endothelium of the blood vessels (as granular structures).

The ascorbic acid content of the skin varied according to the sampling site; high concentrations were found in the skin of the nose and of the plantar surfaces of the extremities of albino rats, and lower ones in skin taken from the trunk or from other parts of the limbs. The granules were present in the cytoplasm of the glandular epithelium, the fibroblasts, and the fat cells, and fine granular structures were seen in the ground substance.

A survey of the endocrine glands of adult animals showed that ascorbic acid could not be detected in the cells of the pancreas and the thyroid, but was present in the adenohypophysis, in the interstitial cells of the testes, and in luteal and follicular cells of the ovary, as shown by silver granules. Its distribution in the adrenal glands has been described by Giroud [8, 9] and others, but in insufficient detail. We found that ascorbic acid was present in both the cortex and the medulla of the adrenal glands of normal animals; its distribution in the cortex was not uniform. The ascorbic acid content of the cells of the glomerular zone varied widely in different segments. In the fascicular zone it was not infrequently concentrated around the capillaries, the lumina of which contained small amounts of ascorbic acid. The highest concentrations were found in the cells of the reticular zone, some of which showed a few granules only, while in others the cytoplasm was filled with granules and aggregates, to such an extent that they obscured the nucleus. Vitamin C appeared in the form of very fine to medium granules in the large cells of the adrenal medulla, and in the medullary sympathetic nerve cells. In addition, ascorbic acid could be detected in the reticular cells of the walls of the cortical capillaries, which contained more or less sparsely scattered granules.

Ascorbic acid was found in small amounts in the central nervous system of adult animals, chiefly in the cytoplasm of cortical nerve cells, in the cells of the subcortical nodes, and in the motor cells of the anterior horn of the spinal cord. Isolated small granules were seen in the endothelium and lumen of capillaries and blood vessels of the brain (Fig. 3).

We were never able to find ascorbic acid in the nuclei of cells of normal tissues.

Tonutti [11] believes that ascorbic acid is at first deposited in the form of large structures in the Golgi apparatus, from whence it is supplied to other parts of the cytoplasm as required. In his opinion, the Golgi apparatus serves as a bridge for the subsequent passage of ascorbic acid into the protoplasmic substance, which it protects from the effects of sudden rise in ascorbic acid content, due to uptake from the blood stream.

The finely granular vitamin C structures are encountered in the protoplasm and peripheral parts of the

endothelial cells of the intestinal glands, in which absorption of monosaccharides and synthesis of disaccharides takes place, in the liver cells, in muscle fibrils, and in the cytoplasm of nerve cells of the vegetative system and of the anterior horns of the spinal cord, which have a high metabolic rate.

It is conceivable that this finely granular form of vitamin C is associated with carbohydrate metabolism of the tissues.

The ascorbic acid content of cells is subject of considerable variations, depending on the functional state of the organ. We found high concentrations of ascorbic acid in the tissues and cells of embryos, including those of the pancreas, which is practically free of ascorbic acid in the adult.

Variations in the ascorbic acid contents of organs of experimental animals to which various drugs had been administered will be reported in subsequent communications.

## SUMMARY

Depositions of silver were revealed at the sites of appearance of ascorbic acid in the organs and tissues of white rats, guinea pigs and cats by the histochemical method of Giroud and Leblond. It was established that the distribution of ascorbic acid and its concentration in the organs was subjected to variations depending on introduction of different drugs. The multitude of forms of deposited ascorbic acid was demonstrated in different cells. It was found in the form of dust, granules, lumps, spheres and complicated figures. An attempt was made to find some association between these forms and various types of metabolism. It was established that the distribution of ascorbic acid and its concentration in the organs was subjected to variations depending on introduction of different drugs.

## LITERATURE CITED

[1] A. R. Valdman, Uspekhi Sovremennoi Biol., 32, No. 1 (4), 63 (1951).

[2] F. K. Menshikov and E. Ya. Verenkova, Vrach. Delo, No. 11 (1947).

[3] G. I. Roskin, Uspekhi Sovremennoi Biol., 30, No. 2, 194 (1944).

[4] M. G. Smirnova, Klin. Med., No. 6, 86 (1952).

[5] B. I. Yanovskaya and G. L. Maller, Farmakol. i Toksikol., 12, No. 6, 13 (1949).

[6] G. H. Burne, Nature, 1933, v. 131, p. 874.

[7] A. Giroud and C. P. Leblond, L'acide ascorbique dans les tissues et sa defection, Paris, 1936.

[8] A. Giroud and C. P. Leblond, Compt. rend. Soc. biol., 1934, v. 115, p. 705.

[9] A. Giroud, C. P. Leblond and M. Rabinowicz, Compt. rend. Soc. biol., 1934, v. 115, p. 1088.

[10] H. Steege and R. Pirtkin, Ztschr. ges. exper. Med., 1953, Bd. 121, No. 5, S. 461.

[11] E. Tonutti, Ztschr. f. mikrobiol.-anat. Forschung, 1940, Bd. 48, H. 1.