

II<sup>nd</sup> COMMUNICATION. THE ASCORBIC ACID CONTENT

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Ascorbic acid, as is well known, plays an important part in the oxydative-reductive processes. In view of this fact it seemed worth while to study the ascorbic acid content in relation to Arthus' phenomenon. Biochemical investigations have shown that ascorbic acid plays a part in the processes of sensitization and desensitization and, in particular, that it exerts a desensitizing influence upon the development of anaphylactic shock [3, 5, 6, 7]. V. I. Kachurets [2] also came to the conclusion that preliminary injection of 50 mg ascorbic acid into the skin of a rabbit or its external application onto the skin prevents the development of Arthus' phenomenon; in the areas exposed to vitamin C, the skin changes were of normergic character. V. P. Bezuglov and E. N. Domontovich [1], conversely, established that ascorbic acid has no desensitizing effect upon the Arthus' phenomenon.

It was the aim of the present paper to investigate histochemically the content and the distribution of ascorbic acid in the skin of rabbits during the development of the Arthus' phenomenon.

## METHODS

Five ml of normal horse serum was injected under the skin of the back of rabbits at intervals of 6 days. 6 days after the last injection skin biopsy was performed and the animal was killed. In addition to the skin, the myocardium, the liver, the lungs and blood films were investigated. The Arthus' phenomenon usually developed after the 5th or 6th injection in the shape of a marked infiltration, accompanied by necrosis. Two rabbits which proved to be less sensitive had to be given 9-10 injections. A total of 20 experiments was carried out. Two rabbits served as control animals. All animals were kept on a mixed diet. The investigation was carried out in the summer months. The tissue sections were stained with hematoxylin-eosin, and by van Gieson's method; the histochemical investigation of ascorbic acid was carried out by the method of Giroud and Leblond [4].

## RESULTS

Histochemical investigation of the skin in the control rabbits revealed that the protoplasm of the cells in the stratified squamous epithelium and in the epithelium of the hair follicles contained ascorbic acid (in diffuse or perinuclear location). In the corium proper ascorbic acid could be demonstrated along the connective tissue fibers, the fibroblasts, and in the muscle fibers of the skin along the dark discs.

During the sensitization and after the injection of the shock dose of horse serum, changes in the content and the distribution of ascorbic acid could be observed. After 3-4 injections the stratified squamous epithelium and the epithelium of the hair follicles still contained considerable quantities of ascorbic acid. In the corium proper the ascorbic acid content decreased in those areas where the collagenous fibers showed a slight swelling and a weak basophilic and picrinophilic staining; the granular structure turned into a fine dust and, parallel to the gradual derangement of the connective tissue and to the appearance of a mucoid substance, the granules became invisible. Within the muscle fibers ascorbic acid was located along the dark discs; only in a few places ascorbic acid was found in an irregular arrangement in the sarcoplasm (Fig. 1). Besides, isolated neutrophilic and eosinophilic leucocytes, containing ascorbic acid, could be seen.

After the 5th - 6th injection further changes in the content and the distribution of ascorbic acid developed against a background of fibrinoid necrosis in the connective tissue, and of an inflammatory, exudative and proliferative reaction. In the stratified squamous epithelium and in the preserved hair follicles, ascorbic acid could be found

in considerable quantities. In the corium proper only small quantities of ascorbic acid could be found in those areas where the disorganization of the connective tissue was far advanced; in areas of fibrinoid necrosis no ascorbic acid could be demonstrated. In the foci of exudative reaction ascorbic acid could be demonstrated in the protoplasm of the eosinophilic and neutrophilic leucocytes. In areas of proliferative reaction ascorbic acid could be found in the epitheloid cells, in the fibroblasts, and particularly at the sites where connective tissue fibers developed and in the vascular epithelium. We were unable to establish histochemically the presence of ascorbic acid in blood films. After 9-10 injections severe destructive processes could be observed in the skin of the rabbits and large quantities of ascorbic acid were found in the protoplasm of the eosinophilic and neutrophilic leucocytes and in the areas of proliferative reaction. In the parenchymatous organs (liver, myocardium, lungs) we found no marked changes in the content of ascorbic acid during the period of sensitization and during the development of the Arthus' phenomenon.

It thus appears that the content of ascorbic acid decreases during the sensitization and during the development of the Arthus' phenomenon in rabbits in areas where the connective tissue becomes disorganized; this process culminates in the complete disappearance of ascorbic acid from the areas of fibrinoid necrosis. In the inflammatory infiltrate (in the protoplasm of neutrophilic and eosinophilic leucocytes) accumulation of ascorbic acid could be observed. In the formed elements of the blood (in films) no ascorbic acid could be demonstrated. The increase in the ascorbic acid level of the blood observed by V. P. Bezuglov and E. N. Domontovich [1] was apparently due to its accumulation in the plasma rather than in the formed elements. In the areas of proliferative reaction ascorbic acid was found in various cell elements of the proliferating connective tissue, a fact which can apparently be explained with the increased ascorbic acid requirements of these cells. In the muscle fibers of the skin ascorbic acid was found in the dark discs and in the shape of diffuse accumulation in the sarcoplasm (Fig. 2) which developed simultaneously

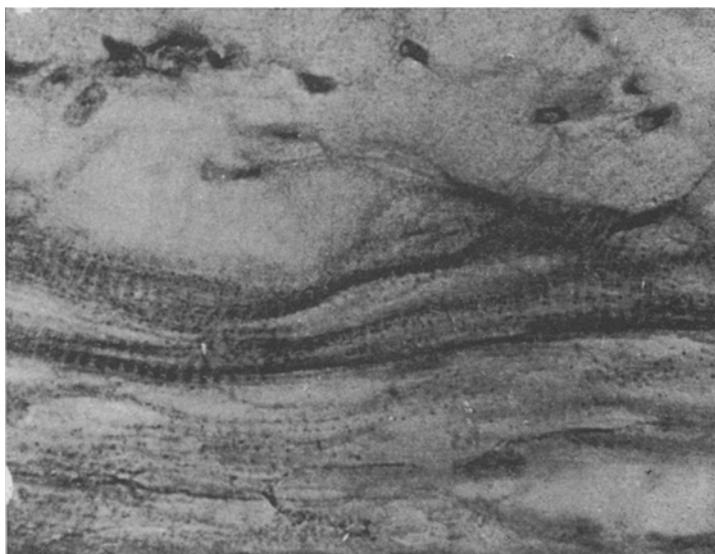


Fig. 1. Distribution of ascorbic acid along dark disks and in separate portions of rabbit's sarcoplasm. Investigation made after 4th injection. Stained according to Giroux and Leblanc. Magnification: objective 40 $\times$ , ocular 15 $\times$ .

with the sensitization and the Arthus' phenomenon. The fact that vitamin C exerts a desensitizing influence upon the development of anaphylactic shock [3, 6] and that preliminary injection of vitamin C prevents the development of Arthus' phenomenon [2], as well as our histochemical findings: — decrease or disappearance of ascorbic acid from those areas where the connective tissue becomes disorganized and from the areas of fibrinoid necrosis, the changes in the distribution of ascorbic acid in the muscle fibers and its accumulation in neutrophilic and eosinophilic leucocytes — warrant the conclusion that ascorbic acid plays a part in the pathogenesis of Arthus' phenomenon.

#### SUMMARY

Ascorbic acid content was examined according to Giroux-Leblon method. As established, during the development of Arthus' phenomenon caused by repeated horse serum administration there occur shifts in the ascorbic acid

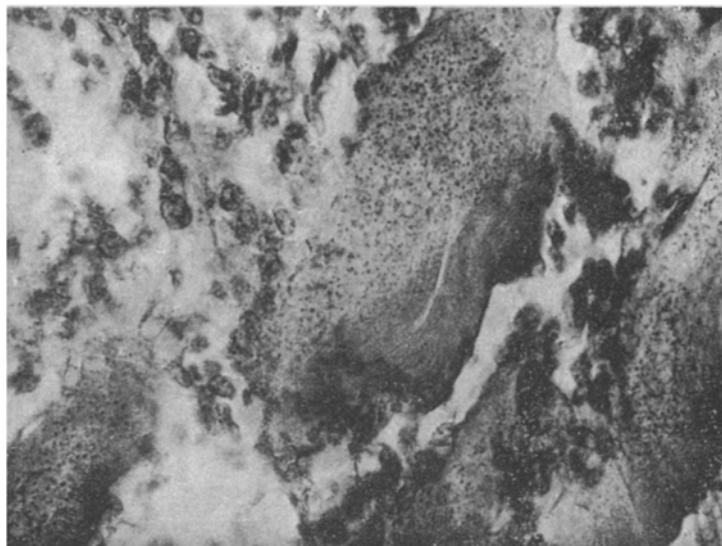


Fig. 2. Irregular distribution of ascorbic acid in sarcoplasm of a rabbit in the region of inflammatory infiltration. Investigation made after 5th injection. Stained according to Giroux and Leblanc. Magnification: objective 90  $\times$ , ocular 10  $\times$ .

content and distribution. In the skin proper the ascorbic acid content decreases at the sites of connective tissue disorganization following the 3rd and 4th injections. After the 5th - 6th injection it disappears at the sites of fibrinoid necrosis, being localized in the muscle fibers not along the dark discs, but diffusely - in the sarcoplasm. Ascorbic acid appeared and accumulated at the sites of exudative and proliferative reaction. Evidently, vitamin C participates in the pathogenesis of Arthus' phenomenon.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

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