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ASCORBIC ACID CONTENT OF HUMAN ARTERIAL TISSUE*

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FROM THE TIME OF VIRCHOW it has been considered by pathologists that the earliest demonstrable lesion in atherosclerosis is an alteration of the *ground* substance of the arterial intima.[1,2] This concept has been based upon the finding of metachromasia of the ground substance and upon the fact that lipid is deposited in the ground substance. Approaching the subject in a different way, we have shown that the ground substance disturbance resulting from *ascorbic acid deficiency in the guinea-pig* is accompanied by arterial lesions morphologically typical of atherosclerosis.[3] We have found that ascorbic acid given parenterally exerts a marked inhibitory effect upon the development of atherosclerosis induced by cholesterol feeding in the guinea-pig.[4]

The ground substance depends on ascorbic acid for its formation[5] and under conditions of ascorbic acid depletion the ground substance undergoes depolymerization.[6] Because of the influence of ascorbic acid upon atherosclerosis in the guinea-pig, it was decided to determine the ascorbic acid content of human arteries under various circumstances, and thus study the metabolism of arterial ground substance.

MATERIALS AND METHODS

Ascorbic acid was measured in arteries from the following three groups of cases: (1) Cases of sudden death.‡ (2) Routine hospital autopsy material.‖ (3) Cases treated in hospital with ascorbic acid for various lengths of time prior to death.‖

The arteries were removed as soon as possible after death. It has already been demonstrated that the decrease in the ascorbic acid content of human tissues is very slight during the first 24 hours[7] and this has been our experience also.

After carefully removing the adventitia, portions of the arteries (the proximal 4 or 5 cm. of the descending thoracic aorta in the case of the aorta) were placed in tared beakers containing a measured amount of a 20% solution of mixed acid (5% metaphosphoric acid and 15% trichloracetic acid) and weighed. The tissue was then ground to a pulp in a porcelain mortar and sufficient water was added to bring the concentration of acid to 5%. Usually about 5 gm. of tissue was ground with 5 ml. of the mixed acid and diluted to 20 ml. with water. After thorough mixing, the thin suspension was immediately filtered or centrifuged. It was found that the addition of a few ml. of peroxide-free ether to the pulp during the grinding ensured a clearer filtrate. If the extract was still cloudy after filtration, a further extraction with ether usually helped to clarify it. This treatment with ether does not interfere with the assay of ascorbic acid as described below.

The ascorbic acid was determined in the acid extract of the tissue by the method described by Mindlin and Butler[8] for the determination in plasma filtrates.

In this procedure a standardized sodium acetate-buffered solution of dichlorophenol indophenol is added to an aliquot of the filtrate, and after 30 seconds the excess unbleached dye is determined in the Evelyn colorimeter. This excess of dye is proportional to the amount of ascorbic acid in the filtrate. The readings were made against a blank identical with the test solution in all respects except for the absence of the dye.

Occasionally, the acid extract of calcified arteries yielded a precipitate of calcium phosphate on the addition of the sodium acetate-dye solution. This necessitated a preliminary removal of the calcium salts from the filtrate by neutralization with sodium acetate.

The ascorbic acid content of the arteries is expressed as milligrams of ascorbic acid per 100 gm. of fresh tissue.

RESULTS

The results in the three groups are given in Tables I, II and III.

The values for ascorbic acid in the arteries in Table I indicate the levels which may be found in sudden death from natural and violent causes. In comparison it will be noted that the ascorbic acid content of arteries from patients dying after various illnesses as shown in Table III is for the most part considerably lower. In seven of the 20 cases in this group, no ascorbic acid at all was found in the arteries. In the older age groups the depletion tended to be particularly marked. Two cases were studied in which the artery was thrombosed (Nos. 31 and 34). There was no ascorbic acid in the arterial wall in either instance.

Ascorbic acid depletion is often found in a segmental distribution in arteries. Thus, for example, the internal carotid artery usually has a higher ascorbic acid content than the adjacent carotid sinus.

The results in Table II suggest that it is possible to replace the ascorbic acid deficiency of arteries by ascorbic acid therapy prior to death.

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‡Most of these cases were obtained from the City of Montreal morgue through the kindness of Dr. R. Fontaine and Dr. J. M. Rousael.

‖Cases from the Departments of Pathology of the Montreal General Hospital and the Queen Mary Veterans' Hospital.