

## ASCORBIC ACID CONTENT OF HUMAN ARTERIAL TISSUE\*

G. C. WILLIS, M.D. and  
S. FISHMAN, Ph.D., *Montreal*

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.<sup>1, 2, 3</sup> 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.<sup>4</sup> 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.<sup>4</sup>

The ground substance depends on ascorbic acid for its formation<sup>5</sup> and under conditions of ascorbic acid depletion the ground substance undergoes depolymerization.<sup>6</sup> 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<sup>7</sup> and this has been our experience also.

\*From the University Clinic and the Department of Metabolism of the Montreal General Hospital and the Department of Medicine of the Queen Mary Veterans' Hospital.

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

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

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% trichloroacetic 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<sup>8</sup> 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.

TABLE I.

TO SHOW THE ASCORBIC ACID CONTENT OF THE AORTA IN CASES OF SUDDEN DEATH.  
ASCORBIC ACID EXPRESSED IN MGM. PER 100 GM. OF FRESH TISSUE.

Case	Diagnosis	Hours after death	Sex	Age	Ascorbic acid content of aorta
1	Fatal crush injury of neck	4	M.	25	3.0
2	Electrocution	10	M.	25	2.0
3	Drowning	3	F.	25	0.7
4	Fractured skull and ribs	12	M.	45	1.6
5	Fractured skull	10	M.	45	3.5
6	Poisoning, nature unknown	18	M.	48	0.7
7	Cause unknown	12	M.	49	1.5
8	Cause unknown	4	M.	50	0.5
9	Carbon monoxide poisoning	4	F.	51	1.4
10	Sudden death due to myocardial infarction	2	M.	56	1.3
11	Sudden death due to myocardial infarction	6	M.	60	1.0
12	Sudden death due to cerebral hæmorrhage	5	M.	65	0.9

TABLE II.

TO SHOW THE ASCORBIC ACID CONTENT OF THE AORTA IN HOSPITAL CASES TREATED WITH ASCORBIC ACID ANTE MORTEM.  
ASCORBIC ACID EXPRESSED IN MGM. PER 100 GM. OF FRESH TISSUE.

Case	Diagnosis	Ascorbic acid therapy	Hours after death	Sex	Age	Ascorbic acid content of aorta
13	Infectious hepatitis	2.5 gm. I.V. over 5 days	20	M.	40	2.8
14	Post-op. gastrectomy for bleeding ulcer	2.0 gm. I.V. over 4 days	2	M.	50	3.5
15	Tabes dorsalis. Bleeding gastric ulcer	12 gm. I.V. over 12 days	14	M.	55	1.9
16	Hepatoma	1.5 gm. I.V. in 1 day	8	M.	62	1.7
17	Carcinoma floor of mouth	0.5 gm. I.V. 5 days prior to death	15	M.	69	0.8

TABLE III.

TO SHOW THE ASCORBIC ACID CONTENT OF THE AORTA, CAROTID SINUS AND INTERNAL CAROTID ARTERY IN ROUTINE HOSPITAL AUTOPSIES. ASCORBIC ACID EXPRESSED IN MGM. PER 100 GM. OF FRESH TISSUE.

Case	Diagnosis	Hours after death	Sex	Age	Ascorbic acid content		
					Aorta	Carotid sinus	Int. carotid
18	Hæmangiopericytoma of vulva	7	F.	18	0.7	0.7	0.8
19	Staph. septicæmia	15	M.	19	0.2	0.3	0.8
20	Thalamic tumour	9	F.	19	1.0	—	—
21	Uræmia, chronic glomerulonephritis	13½	M.	23	0.6	0.7	1.4
22	Diabetes, Staph. septicæmia	20	F.	51	0.7	0	0
23	Bronchogenic carcinoma	16	M.	55	0.9	—	—
24	Carcinoma head of pancreas	12	M.	57	1.0	1.4	5.2
25	Bronchogenic carcinoma	15	M.	59	0	—	—
26	Myocardial infarct., hypercholesterolæmia	12	F.	59	0	0	0
27	Carcinoma of tongue	15	M.	60	0	—	—
28	Hypertension	12	F.	62	1.3	1.2	4.3
29	Bronchogenic carcinoma	41	M.	63	1.1	1.1	1.7
30	Diabetes, hypertension, cerebral hæmorrhage	26	F.	65	0.6	0.3	0.8
31	Thrombosis of r. int. carotid artery	—	M.	66	0.2	0.1	0
32	Uræmia, chronic glomerulonephritis	8	M.	66	0.5	—	0.7
33	Staph. pyogenes bronchopneumonia	13	F.	75	0	0	0
34	Thrombosis of r. int. carotid artery	7	M.	80	0	0	0
35	Cerebral astrocytoma	2	M.	80	0.4	0.1	0.6
36	Prostatic carcinoma	4	M.	82	0	0	0
37	Bronchopneumonia	11	F.	87	0	—	—

## DISCUSSION

*Significance of ascorbic acid depletion.*—The atherosclerosis which develops in the guinea-pig as a result of ascorbic acid depletion has all the characteristics of human atherosclerosis. The lesions are morphologically identical with those of human atherosclerosis. The plasma cholesterol levels of these animals are normal and there is no lipid deposit in the reticulo-endothelial system. Ascorbic acid deficiency is not usually considered to exist in subjects with atherosclerosis where nutrition seems good. The results in Table III indicate, however, that a gross deficiency does in fact exist in the arteries of many well-nourished autopsy subjects, except in cases 15, 27, 36 where the patient was cachectic.

Recent biochemical studies show that radioactive acetate is incorporated into cholesterol considerably more rapidly in tissues depleted of ascorbic acid.<sup>9</sup> The significance of this fact is that the aorta can synthesize cholesterol<sup>10</sup> and Table III shows that severe degrees of arterial ascorbic acid deficiency are commonly found. These observations are all integrated in the finding that atherosclerosis rapidly develops in guinea-pigs rendered scorbutic.<sup>4</sup>

*Plasma glucoproteins.*—Another manifestation of the disturbance of ground substance in atherosclerosis is the appearance of glucoprotein in the blood.<sup>11</sup> This release of glucoprotein is believed to result from the depolymerization of ground substance and is a phenomenon seen in scurvy.<sup>12</sup>

## MECHANISM OF ASCORBIC ACID DEPLETION

The fact that it takes about 150 days of a scorbutic diet to induce scurvy in man<sup>13</sup> makes it seem that the ascorbic acid deficiency noted in human arteries is not due to malnutrition. It is known that systemic stress, such as infection,<sup>14</sup> toxæmia<sup>15</sup> or burns,<sup>16</sup> is accompanied by a great increase in the ascorbic acid requirement. We presume that the stress of the various fatal diseases listed in Table III accounts for much of the ascorbic acid deficiency found in the arteries concerned.

*Localized ascorbic acid deficiency.*—Superimposed upon the systemic stress, our studies indicate the presence of a local form of stress peculiar to the artery.<sup>4</sup> In a previous paper<sup>17</sup> we reviewed the mechanical factors in the pathogenesis of atherosclerosis which comprise this

local stress. Very appreciable differences in mechanical stress can be demonstrated along the major arteries, and the sites where stress is greatest coincide with the localization of atherosclerotic plaques. A good example of the localization of atherosclerosis by mechanical stress occurs in the carotid artery. Because of the dilatation associated with the carotid sinus and bifurcation of the common carotid artery, this site is highly susceptible to atherosclerosis. The immediately adjacent internal carotid artery is only rarely involved by atherosclerosis. Our results suggest that the local point of excess mechanical stress in the carotid sinus is associated with a relative depletion of ascorbic acid in most instances as compared with the adjacent internal carotid artery.

*Ascorbic acid therapy.*—Our preliminary work on the efficacy of ascorbic acid therapy in terminal cases suggests that it is possible to restore the ascorbic acid content to normal in the arterial wall.

## CONCLUSIONS

1. A gross and often complete deficiency of ascorbic acid frequently exists in the arteries of apparently well-nourished hospital autopsy subjects. Old age seems to accentuate the deficiency.
2. The ascorbic acid depletion is probably not nutritional, but rather related to the stress of the fatal illness.
3. A localized depletion often exists in segments of arteries susceptible to atherosclerosis for reasons of mechanical stress. Adjacent segments, where mechanical stress is less, tend to have a higher ascorbic acid content and atherosclerosis here is rare.
4. The significance of this ascorbic acid depletion lies in the fact that scurvy in guinea-pigs results in the rapid onset of atherosclerosis. Furthermore it has been reported that the aorta can synthesize cholesterol and the incorporation of radioactive acetate into cholesterol in tissues is said to be several times more rapid in tissues depleted of ascorbic acid.
5. Ascorbic acid deficiency in arteries with resulting ground substance depolymerization may account for the release of glucoprotein noted in the blood of subjects with severe atherosclerosis.
6. Preliminary studies suggest that it is possible to replenish the ascorbic acid in arteries by ascorbic acid therapy.

REFERENCES

1. ASCHOFF, L.: Lectures on Pathology, Paul B. Hoeber Inc., New York, 1924, p. 131.
2. DUFF, G. L.: *Arch. Path.*, 20: 81, 1935.
3. MOON, H. D. AND RINEHART, J. F.: *Circulation*, 6: 481, 1952.
4. WILLIS, G. C.: *Canad. M. A. J.*, 69: 17, 1953.
5. WOLBACH, S. B. AND HOWE, P. R.: *Arch. Path. & Lab. Med.*, 1: 1, 1926.
6. GERSH, I. AND CATCHPOLE, H. R.: *Am. J. Anat.*, 85: 457, 1949.
7. YAVORSKY, M., ALMADEN, P. AND KING, C. G.: *J. Biol. Chem.*, 106: 525, 1934.
8. MINDLIN, R. L. AND BUTLER, A. M.: *J. Biol. Chem.*, 122: 673, 1938.
9. BECKER, R. R. *et al.*: *J. Am. Chem. Soc.*, 75: 2020, 1953.
10. SIPERSTEIN, M. D., CHAIKOFF, I. L. AND CHERNICK, S. S.: *Science*, 113: 747, 1951.
11. BERKMAN, J., RIFKIN, H. AND ROSS, G.: *J. Clin. Invest.*, 32: 415, 1953.
12. PIRANI, C. L. AND CATCHPOLE, H. R.: *A. M. A. Arch. Path.*, 51: 597, 1951.
13. PETERS, R. A. *et al.*: *Lancet*, 1: 853, 1948.
14. ABBASY, M. A., HARRIS, L. J. AND HILL, N. G.: *Lancet*, 2: 177, 1937.
15. HARRIS, L. J., PASSMORE, R. AND PAGEL, W.: *Lancet*, 2: 183, 1937.
16. ABBOTT, W. E. *et al.*: *Surgery*, 20: 284, 1946.
17. WILLIS, G. C.: *Canad. M. A. J.*, 70: 1, 1954.

RÉSUMÉ

Il existe souvent, chez des sujets apparemment bien nourris, une déficience marquée, voire même totale, d'acide ascorbique dans les spécimens d'artères prélevés à l'autopsie. La vieillesse semble accentuer cette déficience. Ce manque d'acide ascorbique est probablement associé au stress de la dernière maladie plutôt qu'à une mauvaise nutrition. Certains segments d'artères sujets à tension mécanique et portés à l'athérome accusent une déficience localisée; des segments adjacents, mais où la tension mécanique est moindre, montrent un taux d'acide ascorbique plus élevé et rarement des signes d'athérome. L'importance de la carence en acide ascorbique réside dans le fait que le scorbut chez le cobaye donne rapidement naissance à l'athérosclérose. Il a de plus été rapporté que l'aorte peut synthétiser le cholestérol et que l'inclusion d'acétate radioactif dans le cholestérol des tissus est sensée être considérablement plus rapide dans les tissus pauvres en acide ascorbique que dans les tissus normaux. La déficience d'acide ascorbique dans les artères, résultant en la dépolymérisation du ciment inter-cellulaire, peut être la cause de la libération des glucoprotéines observées dans le sang des sujets affligés d'athérosclérose avancée. Des travaux d'approche semblent indiquer qu'il soit possible de relever la teneur des artères en acide ascorbique par l'administration de cette substance. M.R.D.

FAMILIAL ADRENAL  
PHAEOCHROMOCYTOMA  
WITH SUSTAINED  
HYPERTENSION

R. C. YOUNG, M.D.\* and  
W. A. MURRAY, M.D.,† Halifax, N.S.

A FUNCTIONING TUMOUR of chromaffin tissue (phaeochromocytoma), most commonly situated in the adrenal medulla, has been considered in the past to be a very rare condition. Graham<sup>1</sup> was able to collect reports of only 198 cases in the world literature up to the middle of 1949. To this number he added nine cases of his own, making a total of 207 which formed the basis of a comprehensive review. In discussing the incidence he points out that an increasing number of cases are being discovered at the time of lumbo-dorsal sympathectomy for essential hypertension, so that exploration of the adrenal areas, at least, is now almost imperative in this type of surgery. In addition, the tumour as a cause of hypertension is being recognized more frequently because of the wide use of relatively new

diagnostic methods. He estimates that approximately 800 persons die annually in the United States from phaeochromocytoma, most of these being avoidable deaths. If this is so, the tumour is not as rare as formerly thought and is masquerading as essential hypertension in many instances.

Familial incidence of phaeochromocytoma is extremely rare. The primary purpose of this article is to report two cases occurring in a brother and sister, aged 15 and 12 years respectively, both of whom had a tumour of the left adrenal. One of these was treated successfully by surgery. Graham<sup>1</sup> encountered only one report of familial incidence in his broad survey. These cases were reported by Calkins and Howard<sup>2</sup> and were related as aunt and niece. Each had bilateral adrenal tumours which were surgically removed. Since Graham's review, Roth and co-workers<sup>3</sup> have reported on three additional cases, two sisters and a brother of the same family, who had bilateral adrenal phaeochromocytomas. In all these cases surgical removal of the tumours was successful. No other reports of familial incidence have been encountered by us.

A phaeochromocytoma may occur at any anatomical site where chromaffin tissue is found, but in approximately 80% of instances it is located in the right or left adrenal gland. In about 10%

\*Resident Physician on staff of Nova Scotia Sanatorium, Kings County, N.S.; formerly Resident in Internal Medicine, Victoria General Hospital, Halifax.

†Attending Physician, Victoria General Hospital, Halifax; Lecturer in Medicine, Dalhousie University Medical School.