A Method for the Isolation of Intima–Media Samples from Arteries[1] (35052)

HARVEY WOLINSKY AND MARIE M. DALY[2]
(Introduced by T. H. Spaet)

Departments of Medicine and Biochemistry and the Unit for Research in Aging, The Albert Einstein College of Medicine, Bronx, New York 10461

The adventitial layer of large systemic arteries does not appear to contribute directly to the mechanical properties of the normal vascular wall (1, 2). The primary role of this layer rather seems to be the support of auxiliary mural structures, such as vasa vasorum and nerves. Furthermore, vascular diseases nearly always spare the adventitial layer of large vessels (3). Morphological differences among adventitia, media, and intima with respect to cell types and the distribution of extracellular proteins are also well recognized (1).

For the purpose of relating metabolic or chemical properties of the vessel wall to its function under physiological and pathological conditions, it is desirable to study the intimal and medial layers independent of the adventitial layer; this would prevent the adventitial contribution from masking or distorting the metabolic and chemical characteristics of the functional portions of the wall. Others have attempted to deal with this problem by removing as much adventitia as possible from the outside of the vessel (4–8) but, many such "adventitia-free" preparations have not been documented histologically or if demonstrated, have shown persistence of variable amounts of adherent adventitia. Complete removal of the adventitia of the abdominal aorta has been noted to be particularly difficult (5, 8).

In our initial efforts to isolate the intima and media of the thoracic and abdominal aorta using the usual external approach for the studies described below, difficulty was encountered in removing the adventitia completely, especially from the abdominal segment. No matter how carefully the adventitia was picked away, histological sections showed remaining adventitial strands, often of considerable thickness, adherent to the intima–media portion. This preparation was unsuitable for the quantitative analyses of isolated media and intima which we sought to accomplish. Faced with this problem, a method was devised which we were unable to find described heretofore. This report describes the method in detail and presents results of two types of analyses which are significantly influenced by its application.

Materials and Methods. The vessel segment is opened longitudinally and laid flat with the intimal surface facing upward (Fig. 1a). Two pairs of forceps are used, one pair to hold down the vessel wall and the other to tease a horizontal tear in the relatively yielding intima and media. This tear is continued through the thickness of the wall until the medial–adventitial junction is reached (Fig. 1b). This plane is easily recognized by the considerable resistance offered by the adventitia compared to the rubbery consistency of the relatively friable intimal and medial layers. The tear is continued in a radial direction until a completely circumferential strip several millimeters in width is formed. Now using one pair of forceps to hold down the adjacent wall while the other is used to lift off successive intimal-medial strips, the intima and media are separated from the adventitia (Fig. 1b). The microscopic appearance

[1] This work was supported by U.S. Public Health Service Grants HE 12766, HE 05143, and HD 00674; and New York City Health Research Contracts U-2041 and I-253.

[2] Career Scientist, Health Research Council of the City of New York.

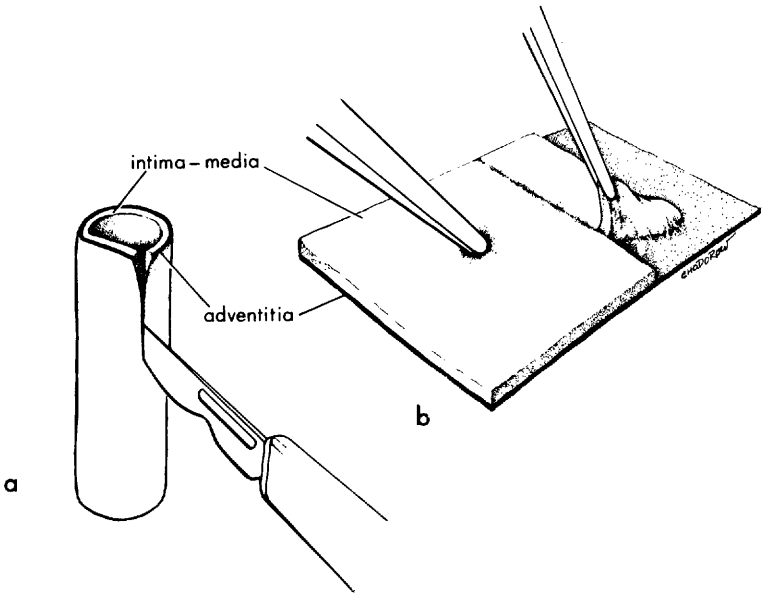

FIG. 1. Diagrammatic representation of the method used to isolate intima–media preparation from arteries. The vessel segment is first opened longitudinally (a); and, with the intimal surface facing upward, intima–media strips are removed from the adventitia (b).

of the cross-sections of rabbit thoracic and abdominal aortas which had the adventitia removed using the usual external approach are shown in Fig. 2A and C. Microscopic sections of the same segments of rabbit aorta prepared using our method, described above, which can be called the internal approach, are shown in Figure 2B and D. It is obvious that adventitia (A) persisted in vessels prepared by the external approach and was absent from those prepared by the internal approach. The adventitia was invariably removed completely by the latter method; the number of elastin lamellae in the isolated intima–media portion corresponded closely to those present in the intact vessel wall (Fig. 2) indicating that little, if any media, remained adherent to the adventitia.

The external and internal methods were compared in two types of studies:

Lipid studies. Male Carworth Farms-Nelson rats, weighing 350–450 g, were used and the segment of aorta between the heart and the diaphragm was removed. Four segments were prepared by the external approach and four segments were prepared by the internal approach. The aortic tissue was homogenized and extracted by the method of Folch *et al.* (9). The extracts were analyzed for cholesterol content by the method of Zlatkis *et al.* (10) and for triglycerides by the method of Van Handel (11).

Fibrous protein studies. Thoracic and abdominal aortic segments from adult female New Zealand white rabbits (3.5–4.5 kg of body wt) were used; thoracic segments were delimited exactly by the left subclavian and celiac arteries and abdominal segments by the left renal artery and iliac bifurcation. The external approach and the internal approach were each used in three animals. Protein fractions were prepared from the samples for amino acid analysis by the method of Lansing (12); amounts of collagen and elastin were calculated by a modification of the method of Spackman *et al.* (13). The details have been presented elsewhere (14). The Student's *t* test was used to test for significance of results.

Results and Discussion. Lipids. Cholesterol and triglyceride concentrations in the rat thoracic aortas are shown in Table I. Aortas

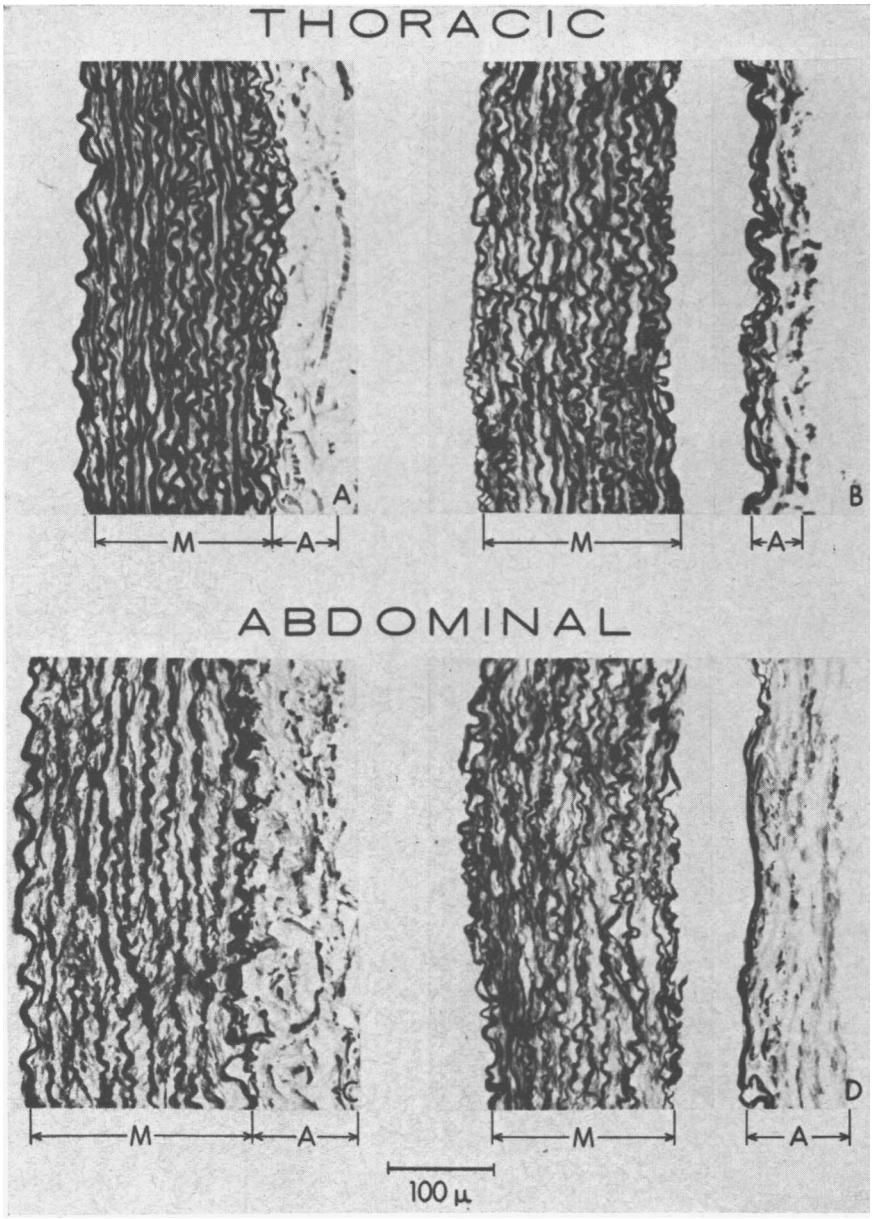

FIG. 2. Comparison of microscopic appearance of cross-sections of rabbit thoracic and abdominal aortic walls obtained after the usual external approach (A and C) and using the internal approach described here (B and D). The intima and media (M) are separated completely from the adventitia (A) by the latter method. Note the difference in relative amounts of adventitia in thoracic and abdominal segments. Weigert-van Gieson strain; ×270.

prepared by external dissection contained a similar concentration of cholesterol, but a fivefold greater concentration of triglycerides than aortas prepared by our "internal" approach. The excess triglycerides in the "external" preparations are components of the adventitia, and their contribution clearly could distort the picture of their true concentration in the media and intima.

Fibrous proteins. Similarly, fibrous protein

TABLE I. Lipid Content of the Rat Thoracic Aorta.

	Lipid (μg/mg of tissue nitrogen)[a]		
	External	Internal	p
Cholesterol	36.6 ± 3.0 (4)	34.3 ± 2.2 (4)	>0.5
Triglyceride	61.9 ± 7.8 (4)	15.9 ± 1.7 (4)	<0.01

[a] Mean ± standard error; number of determinations in parentheses.

contents of aortas freed of adventitia by the two methods were quite different (Table II). Aortic elastin content was significantly higher and collagen content significantly lower in the thoracic segment obtained by the internal method compared to that prepared by external approach, though total fibrous protein contents were not significantly different. In the abdominal segment, only the difference in collagen contents was significant. Using the internal method, however, it is clear that although a slight fall in the concentration of elastin and a slight rise in collagen content takes place with progression distally along the aorta, a reversal in relative contents of these two proteins between the two segments which would be suggested by the results from the external approach (Table II) does not occur. Others, studying the aortas of a variety of mammalian species (4–6), including the rabbit (5), prepared by the external approach, have reported the tendency for a reversal to occur between the two segments, with the transition taking place abruptly near the level of the diaphragm.

TABLE II. Fibrous Protein Content of the Rabbit Aorta.

	External	Internal	p
	Thoracic		
Elastin[a]	34.56 ± 0.70	41.97 ± 1.32	<0.01
Collagen[a]	22.54 ± 0.73	16.85 ± 0.70	<0.01
Total[a]	57.10 ± 1.11	58.82 ± 2.00	>0.4
	Abdominal		
Elastin[a]	25.47 ± 2.53	32.43 ± 1.63	>0.05
Collagen[a]	37.73 ± 2.60	23.47 ± 1.50	<0.01
Total[a]	63.20 ± 1.07	55.90 ± 3.13	>0.05

[a] Percentage; mean ± SE.

The variation in agreement of results obtained by the two methods for thoracic and abdominal segments might reflect a segmental difference in the amount and composition of adventitia which remains after the "external" approach and which is removed by the "internal" method (Fig. 2). Supporting this conclusion is the finding by Cleary (5) of a strikingly higher collagen and lipid content in *loose* adventitial tissue than in the remaining vessel wall. Data on fibrous protein content obtained here using the two methods can be translated into absolute amounts of each protein in the two types of aortic segment by using initial defatted, dry weights of the identical segments as reference points. The differences between the two approaches in the absolute amount of each fibrous protein, presumably reflect the contribution of the closely adherent adventitia seen in Fig. 2. The comparison is shown in Table III. It can be concluded that the difference (= adventitia) between the methods consists of approximately 50% elastin and 50% collagen in the thoracic aorta and approximately one-third elastin and two-thirds collagen in the abdomi-

TABLE III. Amounts of Elastin and Collagen in the Rabbit Aorta.

	External	— Internal	= Difference	p
		Thoracic		
Elastin[a]	29.88 ± 1.53	19.55 ± 1.18	10.33	<0.01
Collagen[a]	17.32 ± 0.93	7.84 ± 0.40	9.48	<0.001
		Abdominal		
Elastin[a]	7.05 ± 1.14	3.81 ± 0.36	3.24	>0.05
Collagen[a]	10.30 ± 1.05	2.76 ± 0.29	7.54	<0.01

[a] Weight in milligrams; mean ± SE.

nal segment. In both segments, these compositions are strikingly different from those found for the intima–media preparations using the internal method. Clearly, the true composition of the intima and media, and probably changes in composition associated with a variety of physiological and pathological conditions, are masked or seriously distorted by the presence of considerable amounts of "contaminant" adventitial tissue of very different composition.

We have found this method for the isolation of intimal and medial layers to be applicable in studies of elastic and large muscular arteries of several mammals, including man. It has already proved to be useful for our evaluation of the response of vascular tissue to several pathological states, including hypertension (14). Its applicability to other types of vessels should be confirmed histologically because of occasional deviations of certain segments from usual mammalian vascular structure (5, 15).

Summary. A method is described for the preparation of aortic intima–media samples which are free of adventitia. Advantages of its use over the usual method of isolation are described in two types of application.

1. Burton, A. C., Physiol. Rev. **34**, 619 (1954).

2. Wolinsky, H., and Glagov, S., Cir. Res. **14**, 400 (1964).

3. Roberts, J. C., Jr., and Stauss, R., eds., "Comparative Atherosclerosis." Harper and Row, New York (1965).

4. Harkness, M. L. R., Harkness, R. D., and McDonald, D. A., Proc. Roy. Soc., London **146**, 541 (1957).

5. Cleary, E. G., M.D. thesis, University of Sydney, 1963.

6. Grant, R. A., J. Atheroscler. Res. **7**, 463 (1967).

7. Gan, J. C., Murthy, P. V. N., Nichols, C. W., Jr., and Chaikoff, I. L., J. Atheroscler. Res. **7**, 629 (1967).

8. Wirtschafter, J. T., and Sandberg, L. B., A. M. A. Arch. Pathol. **85**, 631 (1968).

9. Folch, J., Lees, M., and Sloane Stanley, E. H., J. Biol. Chem. **226**, 497 (1957).

10. Zlatkis, A., Zak, B., and Boyle, A. J., J. Lab. Clin. Med. **41**, 486 (1953).

11. Van Handel, E., Anal. Biochem. **11**, 266 (1965).

12. Lansing, A. I., *in* "Transactions Second Conference on Connective Tissue" (Ragan, C., ed.). Josiah Macy, New York (1951).

13. Spackman, D. H., Stein, W. H., and Moore, S., Anal. Chem. **30**, 1190 (1958).

14. Wolinsky, H., Circ. Res. **26**, 507 (1970).

15. Wolinsky, H., and Glagov, S., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **26**, 357 (1967).

Received May 18, 1970. P.S.E.B.M., 1970, Vol. 135.