Content of Collagen, Elastin, and Water in Walls of the Internal Saphenous Vein in Man

By Jiří Švejcar, C.Sc., Ivo Přerovský, C.Sc., Jiří Linhart, C.Sc., and Jiří Kruml

■ Quantitative analysis of the composition of the venous wall has not yet been attempted by chemical analysis. Aside from histological data, much of which was collected by Kügelgen,[1] the venous wall has so far escaped the attention recently devoted to the arterial wall.[2-5] In the course of studies of the pathogenesis of varices,[6-8] it was found that the normal composition of the venous wall had first to be determined, particularly in the internal saphenous vein. We have been particularly interested in whether differences in hydrostatic pressure on the venous wall are correlated with chemical differences, and to this end we analyzed material from ambulatory adults and from children below the age of ambulation.

Methods

MATERIAL (VENOUS SAMPLES)

For control experiments we used freshly prepared samples of the femoral vein and aorta, the latter without any obvious atheromata. Further analysis involved carefully sectioned samples of the internal saphenous vein (ISV) both from the superior portion of drainage into the femoral vein and from the inferior segment at the level of the ankle. These samples were removed 4 to 24 hours post mortem from cadavers with no signs of varices, in which the cause of death was accident. The ages of the adult group varied from 16 to 40 years, children from 20 hours to 8 months. In the latter group was one child three years old; however, this child had never walked due to the nature of its basic disease (congenital spastic diplegia).

CHEMICAL TREATMENT OF DRIED TISSUE

After careful removal of clotted blood and surrounding connective tissue, samples of from 80 to 120 mg. were accurately weighed, cleared of fat and water by extraction in acetone and ether, and dried in an oven at 105 C. Water content was determined by weight change on drying. The analyzed constituents were then calculated on the basis of fat-free dry weight.

DETERMINATION OF COLLAGEN AND ELASTIN

Chvapil's[9] modification of the method of Neumann and Logan[10] was used. Hydroxyproline content was estimated by the method of Stegemann.[11] Elastin was determined by two methods: hydroxyproline content and the gravimetric method of Lowry.[12] With the given analytical methods, we found that the hydroxyproline content of elastin of human vessels was 1.23 per cent. To calculate elastin from hydroxyproline content, therefore, a factor of 81.2 was used.[7]

MUSCLE AND OTHER TISSUES

By numerical subtraction of the per cent content of collagen and elastin from 100, a number results which we assumed to represent muscle and other cellular elements in the vessel wall. Histologically, most of this fraction would appear to be muscle in origin. The histological slices were prepared from paraffin embedding and stained with trichrome. The width of the media was calculated as the average of four measurements from various venous segments.

Results were analyzed using Fisher's *t*-test (for paired values).

Results

To test the accuracy of the methods used, we first compared the known composition of the aortic wall with that of the femoral vein. Values for both connective tissue components, collagen and elastin, and the ratios of these components in both vessels are shown in figure 1 (mean of six observations). The ratio of the sum of both connective tissue components and muscle in our analyses is given in comparison with values obtained from planimetric analysis of histological preparations presented by Kügelgen[1] in table 1.

Further tables show the scatter of the estimated values from various aspects. Table 2

From the Central Biochemical Laboratory of Thomayer Hospital and Institute for Cardiovascular Research, Praha-Krc; and Pathologic-Anatomical Department of Bulovka Municipal Hospital, Praha, Czechoslovakia.

Received for publication February 28, 1962.

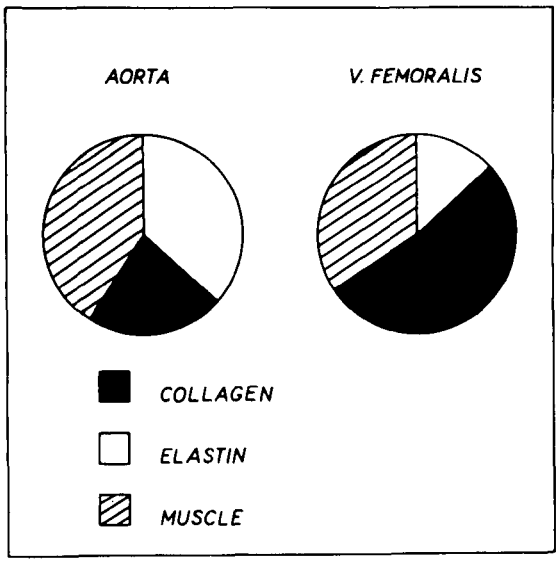

FIGURE 1

Comparison of the contents of collagen, elastin, and muscle in the aorta and femoral vein (mean of six observations).

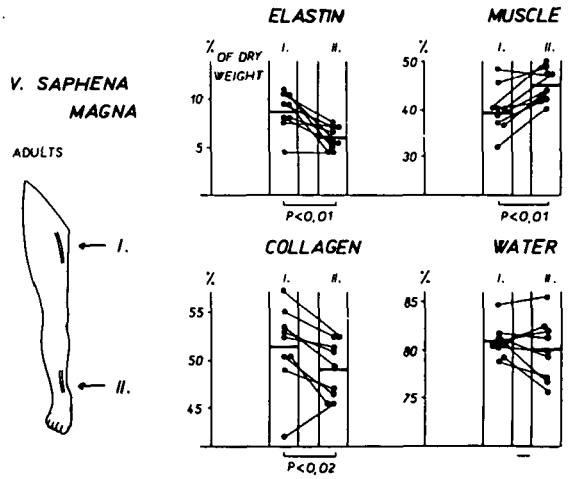

FIGURE 2

Differences in the composition of the superior and inferior segments of the internal saphenous vein in adult subjects.

shows average and extreme values from 32 estimations for collagen and elastin contents in the ISV without regard to the site of the sample. These results are given either in per cent collagen and elastin or in per cent measured hydroxyproline. An example of the detailed analysis in neighboring samples of ISV tissue is shown in table 3, where per cent content of collagen and elastin is given in three neighboring samples of the upper segment of the ISV and in three similar samples from the inferior segment. There was a small variation between neighboring samples, but in table 4 we can see much greater differences when the analyses of the various sites are compared with those of other sites. Left to right differences were small, but interindividual differences were significant in terms of water, collagen, and elastin.

Figure 2 shows upper segment composition compared with that of the lower segment. The superior segment contained more collagen and elastin than the lower, which, on the other hand, contained more muscle. These data were complemented by histological estimation of the thickness of the media, as shown in figure 3, where it can be seen that the inferior venous segments had a thicker muscular layer than the superior. This latter difference is mainly due to the circular muscle of the media, which bears the main brunt of hydrostatic pressure. Such hydrostatic differences would not be expected to operate in children, and this is confirmed by the data in figure 4.

TABLE 1

Ratio of Connective Tissue and Muscle in the Present Data (Mean of Six Observations) and in the Histological Data of Kügelgen[1]

		Collagen + elastin (per cent)	Muscle (per cent)	
			Circular	Longitudinal
Kügelgen:	ISV superior	53	38	9
	inferior	55	45	
	femoral vein	68	25	7
Švejjar et al.:	ISV superior	60	40	
	inferior	56	44	
	femoral vein	69	31	

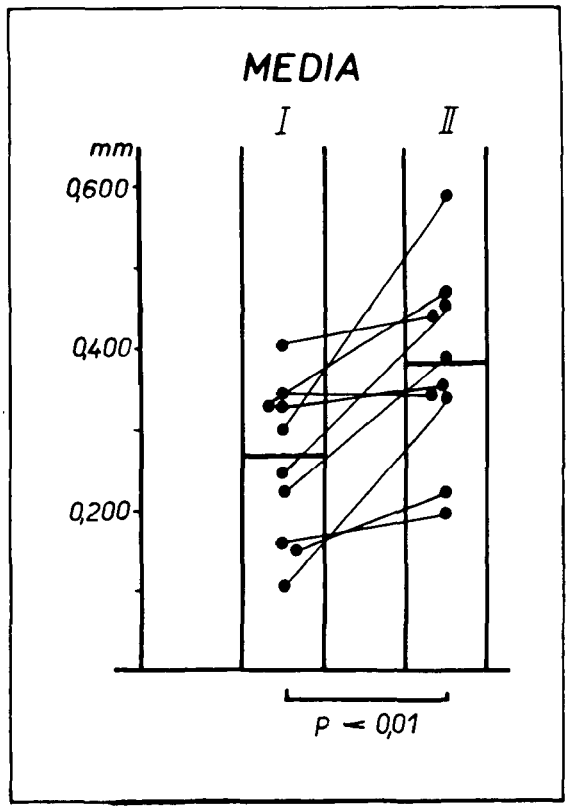

FIGURE 3

Differences in the width of the media in the superior and inferior segments of the internal saphenous vein in adult subjects.

Although there is less elastin in the lower venous segment in infants, there were no such differences for collagen and muscle.

Discussion

The values for collagen and elastin content of the aorta are in agreement with those of other authors,[3] which strongly suggests that the methods used here for both arterial and venous tissue were adequate. Moreover, the methods used here agreed with the planimetric measurements of Kügelgen.[1]

The reasons for the considerable scatter of the extreme values were analyzed in detail. Despite the fact that venous composition was far more variable than arterial,[1] the present results show that the composition of neighboring segments of the ISV show little variation. The greatest factor in scatter was interindividual differences. Age dependence within our given groups was not apparent. When superior and inferior segments of the ISV are compared in a given individual, however, significant differences appear, and these differences would appear to be of physiological significance in terms of the different hydrostatic loads applied, particularly in the standing or upright adult. The higher muscle content of the lower venous segments may well be a reflection of this pressure gradient, and these differences are in agreement with histological estimates of the thickness of the medial layers in these two segments. In particular, the circular muscle layer of the media comes into focus as the greatest variable as a function of pressure gradient. On the other hand, the inferior segment contained less collagen and elastin, and it is a general phenomenon that elastic tissue components decrease in the vessel wall in a direction to the periphery. It is not known whether this also applies to collagen fibers. Analysis of infant venous material shows that the elastin differences are present even before the organism begins bipedal locomotion. However, the lack of a difference in muscle content in superior and inferior venous segments in the infant material suggests that this latter difference in the adult is a function of the upright posture and bipedal locomotion.

There were no differences in tissue water content. Even if absolute values of tissue

TABLE 2

Extreme and Average Values for Collagen and Elastin in the Human Internal Saphenous Vein, Superior and Inferior Segments (Thirty-two Observations)

	mg. Hydroxyproline/100 mg. FFDS*	Per cent collagen or elastin
Collagen	4.60 –8.65 (6.65)	34.3–64.5 (49.6)
Elastin	0.056–0.189 (0.123)	4.5–15.3 (10.0)

*FFDS = fat-free dry substance.

TABLE 3

Scatter of Values of Collagen, Elastin, and Water in Various Samples of Two Segments of Internal Saphenous Vein in One Individual

Segment	Collagen	Elastin	Water
Superior	47.5	15.6	83.2
	48.0	15.5	83.6
	47.0	14.9	82.8
Average	47.5	15.3	83.2
Inferior	42.0	13.0	84.6
	40.5	13.8	82.1
	45.0	12.2	84.6
Average	42.5	13.0	83.8

water might be expected to change post mortem, since two venous segments were compared with one another in relative terms, these differences would tend to cancel out.

Summary

Chemical analysis of the collagen, elastin, and water content of various segments of the internal saphenous vein in the human has been carried out. Normal values were obtained to give a basis for comparison with pathological material from varicosities. The following observations were made: (1) The ratio of connective tissue elements in the internal saphenous vein (ISV) is the reverse of that in the aortic wall; the ISV contains more collagen than elastin. (2) The total scatter of elastin and collagen values in the venous wall is large, mainly due to interindividual differences. No particular dependence on age was observed in the ambulant group. (3) Analysis of neighboring samples of vein in a given individual showed little variation. (4) There was a consistent difference in venous composition in a given individual between the superior segment at the insertion of the ISV into the femoral vein and the inferior segment at the level of the ankle. There was more collagen and elastin superiorly, and more muscle inferiorly. This corresponded to histological analysis showing a thicker layer of circular muscle in the media of the inferior segment. (5) The same differences in elastin content were found in infants who had not yet begun to walk; however, the infant group showed no superior-inferior differences in collagen and muscle composition of the venous wall. It is suggested that this latter difference is a function of upright posture and bipedal locomotion, and the attendant gradient of hydrostatic pressure.

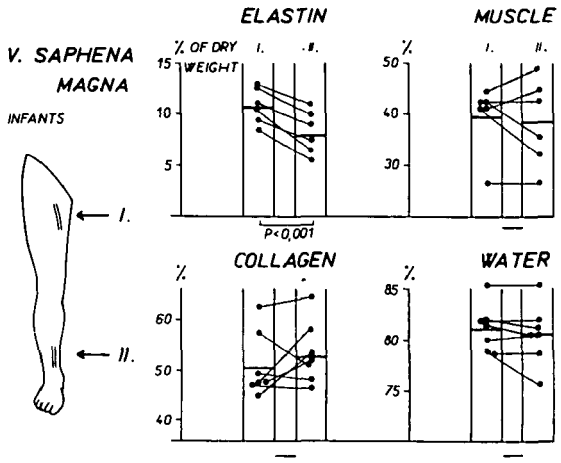

FIGURE 4

Differences in the composition of the superior and inferior segments of the internal saphenous vein in infants.

TABLE 4

Content of Collagen, Elastin, Muscle, and Water in the Superior Segment of the Internal Saphenous Vein of Several Individuals (Single Values)

Subject	Age	Side	Collagen	Elastin	Muscle	Water
B	16	right	57.3	10.5	32	78.8
		left	53	10.5	36.5	80.7
L	18	right	49.5	4.5	46	80.9
		left	50.5	10	39.5	80.6
T	39	right	53.5	8	38.5	84.8
		left	42	9.5	48.5	81.9
S	40	right	53.5	8	38.5	80.5
		left	52.5	7.5	40	81.5

References

1. KÜGELGEN, A. V.: Über das Verhältnis von Ringmuskulatur und Innendruck in menschlichen grossen Venen. Z. Zellforsch. 43: 168, 1955.
2. PAGE, I. H.: Connective Tissue, Thrombosis and Atherosclerosis. London, Academic Press, 1959.
3. LANSING, A. I.: The Arterial Wall. Baltimore, Williams & Wilkins, 1959.
4. CEMBRANO, J., LILLO, M., VAL, J., AND MARDONES, J.: Influence of sex difference and hormones on elastine and collagen in the aorta of chickens. Circulation Research 8: 527, 1960.
5. KAO, K. Y. T., AND MCGAVACK, T. H.: Connective tissue: I. Age and sex influence on protein composition of rat tissues. Proc. Soc. Exper. Biol. & Med. 101: 153, 1959.
6. PREROVSKÝ, I., LINHART, J., AND DEJDAR, R.: Störungen der tiefen Venen der unteren Gliedmassen. Jena, VEB Gustav Fischer Verlag, 1960.
7. PREROVSKÝ, I., LINHART, J., DEJDAR, R., ŠVEJCAR, J., KRUML, J., AND VAVREJN, B.: Research of varicose veins in the Institute of Cardiovascular Research. To be published in Czechoslov. Medicine, 1962.
8. ŠVEJCAR, J., PREROVSKÝ, I., AND LINHART, J.: Chemical composition of the venous wall of the lower limbs. Cor Vasa 3: 90, 1961.
9. CHVAPIL, M.: Možnosti kvantitativního stanovení kolagenních bílkovin a elastinu v biologickém materiálu. Čsl. Fysiol. 8: 81, 1959.
10. NEUMANN, R. E., AND LOGAN, M. A.: Determination of collagen and elastin in tissues. J. Biol. Chem. 186: 549, 1950.
11. STEGEMANN, H.: Mikrobestimmung von Hydroxyprolin mit Chloramin-T und p-Dimethylaminobenzaldehyd. Hoppe Seyler Z. Physiol. Chem. 311: 41, 1958.
12. LOWRY, O. H., GILLIGAN, D. R., AND KATERSKY, E. M.: Determination of collagen and elastin in tissues with results obtained in various normal tissues from different species. J. Biol. Chem. 139: 715, 1941.

Content of Collagen, Elastin, and Water in Walls of the Internal Saphenous Vein in Man

Jirí Svejcar, Ivo Prerovský, Jirí Linhart and Jirí Kruml

Circ Res. 1962;11:296-300
doi: 10.1161/01.RES.11.2.296
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571