Thromboplastin Activities of Human Arterial and Venous Tissues.* (27369)

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Quantitative determinations of thromboplastin activity of human arterial and venous tissue were first reported by Astrup *et al.* (1,2) and by Witte and Bressel(3). The present study concerns the activity of this factor in the various layers of the walls of human aorta, pulmonary artery and inferior vena cava. For comparative purposes, thromboplastin activity was measured separately on normal and arteriosclerotic tissue portions of the same aortic samples.

Materials and methods. Samples were obtained at autopsy from 30 subjects ranging in age from 3 months to 87 years. An average time of 9 hours (0.5 to 18 hours) had elapsed between the death of the individuals and performance of autopsies; approximately similar thromboplastin values were recorded for samples collected 0.5 to 4 hours and 12 to 18 hours after death. Thromboplastin activity determinations were made by a modification of Astrup's(2) procedure. After removal of adhering blood by brief rinsing of the vascular samples, homogenates of the various layers of the blood vessels were prepared with isotonic sodium chloride solution using a Kontes Duall type tissue grinder. The homogenized samples were subsequently placed in a deep freeze for 24 hours to assure quantitative release of the thromboplastin from the vascular tissue. Shortly before the assay, the homogenates were thawed and rehomogenized.

The tests were conducted at a temperature of 30°C with use of reconstituted lyophilized human citrated plasma. Coagulation time determinations were made with the nichrome wire technic in 10 × 75 mm test tubes. 0.2 ml of tissue homogenate, 0.1 ml of Owren's buffer, pH 7.35, and 0.1 ml of 0.06 M $CaCl_2$ solution were placed in the tube. After 5 minutes of preheating, 0.2 ml of plasma was added to the sample. An electrical stop watch operated by a foot switch was employed for recording coagulation time. All thromboplastin determinations were performed with at least 3 different tissue concentrations (0.2, 0.5, and 2.0% homogenate); measurements were made in duplicate and these duplicate determinations generally showed very close agreement.

The quality of the reconstituted lyophilized plasma used in the tests was ascertained by frequent comparison of coagulation time values recorded for that plasma with those obtained under similar conditions with the Warner-Chilcott Co. Diagnostic Plasma. The Simplastin preparation of the same company was used as a thromboplastin reference standard. Tests with various dilutions of that preparation were run with each set of tissue thromboplastin determinations. Coagulation times observed with the Simplastin preparation were found to be highly reproducible. In order that thromboplastin activities of the vascular tissue samples could be expressed in percentages of that exhibited by human brain tissue, thromboplastin tests were similarly conducted with homogenates of the grey matter of brain tissue. The average thromboplastin activity found through assays of 10 different human brain samples was employed as the tissue reference standard for recording of the vascular activities.

For calculation of thromboplastin values of the vascular tissue samples, the conventional double logarithmic plot was used (Fig. 1). When the logarithms of tissue concentrations are plotted on the abscissa and the logarithms of coagulation times on the ordinate, a straight line is obtained. Since the plot of the vascular tissue values was parallel with the curves of the Simplastin preparation and of the brain tissue, at least for medium tissue concentrations, it was possible to calculate the activities of the vascular tissue samples in percentages of the standards by direct use of the double logarithmic plot.

It should be pointed out that Astrup's(2) thromboplastin values for human aortic tissue

* Supported by U.S.P.H.S. grant.

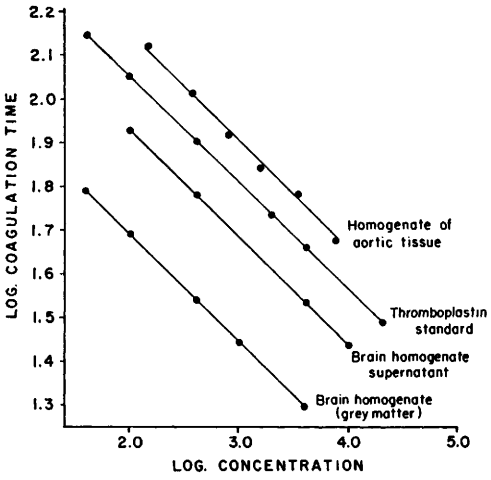

FIG. 1. Double logarithmic plot for calculation of thromboplastin activities of human vascular tissue samples. Tissue concentrations are expressed in μg of wet wt used and thromboplastin standard in μg of solution employed (properly prepared Simplastin suspension). Coagulation times expressed in seconds.

were expressed in percentages of the activity of the supernatant of a brain homogenate, although this is not specifically mentioned in his report. Since the activity of the brain supernatant is only about one-tenth of that exhibited by the total brain homogenate (Fig. 1), Astrup's values must be divided by 10 to make them comparable with those reported here.

TABLE I. Thromboplastin Activities of Human Blood Vessels.

	No. of samples	Mean thromboplastin activity*	S.E. of mean
Normal aorta:			
Intima	30	3.4	.45
Media	30	2.8	.43
Adventitia	27	1.8	.24
Arteriosclerotic aorta:			
Fibrous-lipid intima	22	2.0	.51
Fibrous-lipid media	22	2.3	.26
Ulcerated tissue	6	.2	—
Pulmonary artery:			
Intima	26	1.2	.25
Media	26	1.4	.36
Vena cava inferior:			
Intima	25	3.5	.45
Media	25	3.0	.49

* Values expressed in % of activity of human brain tissue (grey matter).

Results. The average thromboplastin values observed for the various layers of the blood vessels are listed in Table I. Approximately similar activities were found for normal aortic tissue portions and for the inferior vena cava, whereas lower values were observed for pulmonary artery tissue. The intimal layer of the aorta generally exhibited a higher thromboplastin activity than the media and the adventitia, but in no case was the difference statistically significant between adjacent areas.

TABLE II. Coefficients of Correlation between Age and Tissue Thromboplastin Activities.

	No. of samples	r	t	
Age / Normal aortic intima	30	−.14	.75	n.s.*
Age / Normal aortic media	30	+.03	.16	n.s.
Age / Adventitia	27	−.08	.39	n.s.
Age / Arteriosclerotic aortic intima	22	−.55	2.87	$p < .01$
Age / Arteriosclerotic aortic media	22	−.39	1.97	n.s.
Age / Pulmonary artery intima	26	+.38	2.05	$p < .05$
Age / Pulmonary artery media	26	+.19	.95	n.s.
Age / Vena cava intima	25	+.02	.09	n.s.
Age / Vena cava media	25	−.34	1.63	n.s.

* n.s. = not statistically significant.

The assays performed on fibrous-lipid arteriosclerotic tissue samples of the aortas usually showed lower thromboplastin values than those recorded for the normal tissue specimens. One interesting finding was the extremely low thromboplastin concentration of the ulcerated arteriosclerotic areas, which was only about one-tenth of that encountered in the fibrous-lipid tissue portions.

The correlation between age and vascular thromboplastin activities was calculated. The coefficients of correlation (Table II) show a significant decrease with age in thromboplastin activity of the arteriosclerotic (fibrous-lipid) aortic intimal tissue. This decrease may be related to an increased formation of hard fibrous tissue in connection with the progressive development of arteriosclerotic

changes with advancing age. In contrast to this decline in thromboplastin activity of the aortic arteriosclerotic tissue with age, thromboplastin values of the non-arteriosclerotic intimal layer of the pulmonary artery showed a tendency to increase with age.

Thromboplastin activities reported here for the intimal and media layers of normal aortic tissue are in good agreement with those reported by Astrup *et al.*(2) when the different brain tissue reference standards employed in the 2 studies are considered. Direct comparison of the aortic activity values with those reported by Witte and Bressel(3) is not possible because they used only a commercial reference standard.

Calculation of the data of Astrup *et al.*(2) reveals moderately lower activities of the arteriosclerotic aortic samples than of the normal samples, which is in accordance with our observations; the activity values listed by Astrup *et al.* show, however, somewhat greater variations than those encountered in the present investigation. A lower thromboplastin activity of fibrous arteriosclerotic tissue portions than of normal arterial tissue was similarly observed by Perlick(4).

Discussion. The thromboplastin activity of the arterial wall, particularly of the intimal layer, has received some attention in recent years in connection with Duguid's(5) theory about the pathogenesis of atherosclerosis. The actual significance of this tissue factor in the process of blood clotting remains, however, uncertain under conditions where the vascular wall is undamaged. In contrast to this, it is generally believed that when ulcerated areas exist in the intimal layer, the tissue thromboplastin may be in direct contact with the blood. Although the ulcerated portions of the arteriosclerotic tissue in the present study were found to contain only a low thromboplastin activity, such exposure of the vascular thromboplastin may initiate fibrin formation on the inner surface of the blood vessels. The finding by Essbach(6) that thrombus formation generally occurs at the borders of ulcerated tissue areas and not on the surface of non-ulcerated arteriosclerotic lesions is in accordance with this assumption.

The occasional appearance of intramural hemorrhages with fibrin formation in connection with arteriosclerotic vascular changes has been reported in detail by Paterson(7); it must be assumed that the thromboplastin of the vascular wall participates in the intramural blood clotting under such circumstances. A possible correlation between reduced thromboplastin activity of the fibrous arteriosclerotic tissue and localization of intramural hemorrhages has been suggested by Perlick(4).

Summary. Determinations were made of thromboplastin activities of the various layers of the human aorta, pulmonary artery, and inferior vena cava. The activity measurements were performed at 30°C by a modification of Astrup's procedure. Thromboplastin values of the vascular samples were expressed in percentages of average activity of the grey matter of human brain tissue, using the double logarithmic plot for the calculation. The mean activities found for the intima, media, and adventitia of normal aortic samples were, respectively, 3.4, 2.8, and 1.8% of human brain tissue (grey matter). Moderately lower values were observed for fibrous-lipid arteriosclerotic tissue portions, and extremely low thromboplastin activities were recorded for ulcerated tissue areas. The layers of the inferior vena cava usually showed activities of the same order of magnitude as those found for the aorta (intima, 3.5; media, 3.0), whereas significantly lower thromboplastin values were recorded for the intima (1.2) and media (1.4) of the pulmonary artery samples.

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Received January 8, 1962. P.S.E.B.M., 1962, v109.