Ability of Some Plasma Protein Fractions to Convert Venous Blood into Hemostatic Blood.*† (25655)

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We have used inability of venous blood to hemostatize the dog hind leg preparation(1) to show the hemostatic activity of many substances, since addition of such substances to venous blood has converted it into hemostatic blood. To investigate which factors in arterial blood were active in hemostasis, since under natural conditions only arterial blood can stop skin bleeding in the hind limb preparation (1), arterial plasma fractionation was performed and protein fractions added to venous blood to detect which of them were active in hemostasis. In this paper, besides the active arterial plasma fractions, other active and inactive fractions obtained from venous plasma and from lymph have been studied.

Methods. All blood samples employed for plasma fractionation or as nutrient for the hind leg preparation were taken from normal mongrel dogs obtained from the animal city pound. Hemostatic capacity of blood samples was tested in the dog hind leg preparation adapted for hemostasis observations(2). In each determination normal arterial blood was used before the sample being studied, to test the preparation for normal bleeding control, and afterwards to confirm the result found in the sample, since it was shown(2) that irrigation of the leg with a non-hemostatic blood sample deprived the preparation of its power to hemostatize, even if normal arterial blood was used afterwards.

Method 10 of Cohn *et al.*(3), *i.e.*, precipitation of protein by buffered alcoholic mixtures of known ionic strength at −5°C, was used, the precipitate spinned down in an adapted refrigerated centrifuge; all reagents were made according to Lever *et al.*(4). The resuspended protein precipitate was tested while as fresh as possible, although fractions could be kept active in the refrigerator for more than a week. Mixing of the fraction with venous blood, kept under petroleum jelly, was carried out just before the test to avoid possible deterioration with time.

Results. Table I shows a marked difference between proteins (from all sources: arterial, venous and lymph) that Cohn labelled I-II-III and the group of proteins called IV-V, as well as a striking difference of behavior of protein fractions from different sources, especially from arterial plasma, citrated lymph, and venous plasma. Except for conflicting results in Fraction IV-V of arterial plasma in which appropriate concentration in 10 determinations corrected the hemostasis of venous blood, and in 6 determinations failed to do so, all other fractions under study showed clear-cut results.

As active fractions were limited to the I-II-III group a further fractionation of this group into I-III and II was performed. The results are presented in Table II. Venous plasma fractions showed no activity in 44 determinations in all 4 groups studied (I-II-III, IV-V, I-III and II) in concentrations varying between 1 to 8 ml in 100 ml venous blood. Separation of active hemostatic proteins by further fractionation of I-II-III has failed, since activity of arterial plasma and citrated lymph fractions was about the same in Fraction I-III and in Fraction II. If we consider the variable results obtained in citrated lymph I-III fraction and the consistent results of Fraction II, it seems that this last fraction is more active, since active concentrations of Fraction II also produced results in agreement with this interpretation.

When an active fraction is added in a much higher concentration than in the range studied (0.25 to 4%) venous blood is no longer converted into hemostatic blood. For instance, Table II shows that with 10% of arterial plasma Fraction II an optimum amount is needed to attain full hemostatic effect.

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TABLE I. Hemostatic Behavior of Fractions I-II-III and IV-V of Lymph, Venous and Arterial Plasmas (Cohn Method 10), when Mixed with Venous Blood.

Fraction	Body fluid used	Fraction conc. in 100 ml venous blood (ml)	No. of determinations	Bleeding time (min.) Previous normal arterial blood	Bleeding time (min.) Venous blood plus fraction	Bleeding time (min.) Posterior normal arterial blood
I-II-III	Venous plasma	2	3	7 to 8	+30	+20
	Arterial plasma	2	5	5 to 9	5 to 9	6 to 8
	Citrated lymph	2	1	7	7	8
		1	2	7 to 8	6 to 7	10
IV-V	Venous plasma	4	3	6 to 9	+30	+20
		2	3	6 to 10	"	"
		1	4	6 to 10	+20	"
	Arterial plasma	4	3	7	+30	+20
		2	3	7	8 to 9	9 to 13
			3	7 to 8	+30	+20
		1	7	4 to 8	7 to 12	8 to 14
		.5	2	7 to 8	13 to 18	+20
	Citrated lymph	4	4	6 to 8	+30	+20
		2	3	7 to 8	"	"
					21	+30
		1	3	7 to 8	+30	"
		.5	1	7	+20	+20

Discussion. Interpretation of the results depends on behavior of dog plasma in the Cohn alcoholic fractionation. The only paper dealing with dog plasma fractionation by the Cohn method 10 is that of Russ and Raymunt (5). Detailed electrophoretic observations in the Tiselius apparatus were also carried out in our Institute on protein fractions of dog's plasma(6). It was found that the main constituents of dog plasma fractions were much the same as those described in human plasma, except that the greater part of the lipoprotein of dog plasma migrates with alpha globulins and not with beta globulin as in human plasma. Further qualitative differences were observed, such as an unusual distribution of dog beta lipoproteins and the impossibility with the Cohn method of obtaining from dog plasma a pure gamma globulin in Fraction II (a 50% contamination with beta globulin was consistently found).

As Fraction I-III is devoid of gamma globulin and is as active as Fraction II, the hemostatic substances seem to be connected with the only common factor in both of these sub-fractions, *viz.*, beta globulins and beta lipoproteins.

As beta globulin contaminating Fraction II contains cholesterol and phospholipids (10 to 20% of total lipids(7)), a lipoprotein could be the active protein involved in hemostasis.

The fact that substances able to convert venous into hemostatic blood, found in Fraction I-II-III, originated from fractionation of arterial blood and citrated lymph but never from venous plasma, regardless of concentration used (Tables I and II), shows that conversion of venous blood into hemostatic blood is not connected with an enrichment of substances previously present in small concentration in venous blood, but by addition or formation of substances found only in arterial blood and lymph, but absent in venous plasma. We were not able to separate the active principles by further fractionation of I-II-III fraction into Fraction I-III and Fraction II, since both of these last fractions showed a comparable hemostatic activity.

Involvement of the lipid moiety of lipoprotein in hemostasis agrees with our previous studies, which showed the activity of a glycero-phosphatide (cephalin)(8) and especially of one of the 4 of its components – phosphatidylethanolamine(9). Lipid participation in coagulation is shown by Poole(10) and still other facts pointing out participation of lipids in hemostasis will be published later.

That a relatively small amount of arterial plasma fraction when added to venous blood is able to convert this non-hemostatic blood

TABLE II. Hemostatic Behavior of Fractions I-III and II of Lymph, Venous and Arterial Plasmas (Cohn Method 10), when Mixed with Venous Blood.

Fraction	Body fluid used	Fraction conc. in 100 ml venous blood (ml)	No. of determinations	Bleeding time (min.) Previous normal arterial blood	Bleeding time (min.) Venous blood plus fraction	Bleeding time (min.) Posterior normal arterial blood
I-III	Venous plasma	8	5	7 to 12	+30	+30
		4	5	6 to 10	"	"
		2	4	7 to 9	"	"
		1	3	7 to 8	"	+20
	Arterial plasma	4	4	6 to 10	6 to 9	7 to 8
		2	4	6 to 7	7 to 12	8
		1	3	7	7 to 9	6
		.5	3	6	8	7
		.25	1		8	
		.10	1		+20	
	Citrated lymph	4	3	7 to 8	7 to 12	7
			5	7 to 8	+30	+30
		2	2	5	7 to 9	7
			6	7 to 8	+30	+30
		1	3	7 to 8	7	7
			1	7	+30	+20
		.5	3	7 to 9	"	"
II	Venous plasma	8	3	6 to 8	+30	+30
		4	4	6 to 7	"	"
		2	5	5 to 8	"	"
		1	2	6	"	"
	Arterial plasma	10	5	7 to 8	+30	+30
		4	12	5 to 10	6 to 9	8 to 10
		2	17	5 to 9	5 to 10	7 to 9
		1	10	7 to 9	6 to 10	7
		.5	2		11	+15
	Citrated lymph	10	2	6 to 7	7 to 11	7 to 8
		4	5	6 to 8	6 to 9	7 to 8
		2	4	6 to 8	6 to 8	8
		1	8	6 to 8	5 to 8	8
		.5	5	7 to 9	8 to 9	6 to 16
		.25	3		7 to 9	7
		.1	3	9	+30	7 to +30

sample into full hemostatic blood, suggests that a small imbalance between plasma proteins could have great significance in hemostasis.

Summary. Only protein fractions of arterial blood and lymph are able to convert venous into hemostatic blood. Protein fractions taken from venous plasma lack this property. Fractions IV-V (Cohn method 10) are inert, the hemostatic substances being distributed in Fraction I-III and in Fraction II. There is some indication that plasma constituent able to convert non-hemostatic venous blood into hemostatic blood is related to arterial plasma or lymph beta-lipoprotein.

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