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On the Possible Liquid Crystalline Structures in Artificial Thrombi Formed at Arterial Shear Rates: Effects of Disease, Proteins Concentrations, and ABO Blood Groups†

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Abstract—All characteristics of blood coagula, such as clotting times, viscosities of coagula, and their morphologies depend on the shear rate at which the clotting process takes place. At arterial shear rates only the red and white thrombi are formed. The apparent viscosity of artificial thrombi, formed in the variable-frequency thromboviscometer (VFTV) at 60 and 180 cycles/min (mean shear rates of 26 and 80 sec^{-1}), was studied in patients and fibrinogen–thrombin systems. Viscosity is always (even multifold) higher in patients suffering from hypertension, renal failure, unsuccessful kidney transplantation, or arteriosclerosis than in normals. In the thrombi, obtained from the fibrinogen–thrombin systems and from patients suffering from haemophilia, there is a sensible dependence of viscosity on the concentration of fibrinogen. In the thrombi of patients, suffering from the myocardial infarction or thyroid diseases, that is not the case and a reverse correlation might be present depending on the ABO blood group. A significant effect must be attributed to the protein ratios, namely the albumin/globulin, albumin/fibrinogen and globulin/fibrinogen ratios. Effect of these ratios on the viscosity of artificial thrombi appears to be greater than that of the actual concentrations of proteins. Nevertheless, the pattern obtained may be reverse (opposite) in the red and in the white thrombi (obtained at 60 and 180 cycles/min, respectively), and may be reverse in the patients of A and O blood groups.

It is suggested that the viscosity dependence of the thrombi (of basic shear-thinning or thixotropic rheology) on the ratios of plasma proteins and on the ABO antigens and antibodies indicates a presence of the liquid crystalline structures.

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1. Introduction

The fundamental role of the blood flow velocity and, specifically, of the velocity gradient on the coagulation of blood and on thrombus formation has been established during the last decade. The three important features of blood coagulation, the clotting time, the morphology of clots and thrombi, and viscosity of coagula are all determined by the shear rate present (or applied) during the clotting process.[1,2,3]

With an increase of the " casting " shear rate, the clotting times and viscosities decrease hundreds-fold and, in parallel, the morphology changes showing a spectrum[4,5,6] from red clots and red thrombi, to white thrombi, and suspension coagula or suspensions of microthrombi (Table 1). These findings were made possible by an

TABLE 1 Effect of " Casting " Shear Rate on Clotting Time, Rates of Formation and Apparent Viscosity of Clots and Thrombi. A schematic representation.

Coagulation parameter	Shear rate sec^{-1} @0	10–30	60–100	above 400
latent clotting time, minutes and seconds	@10	2–6	1–4	0^{01}–0^{10}
total thrombus formation time, minutes and seconds	—	3–8	2–6	0^{10}–0^{40}
apparent visocotity, poises	@1000	@10–50	@1–8	@0.05
morphology	red clot	red thrombus	white thrombus	suspension coagulum

This table is compiled from data published in the book *Blood Microrheology: Viscosity Factors in Blood Flow, Ischaemia and Thrombosis* by L. Dintenfass. Publisher: Butterworths, London, 1971.

application of a technic based on the variable-frequency thromboviscometer[1,4,7] which is a large-amplitude oscillatory version of the cone-in-cone viscometer.

Recent investigations in the formation of artificial thrombi, analogous morphologically to the in vivo arterial thrombi, showed statistically significant and often multifold differences in their formation rates and viscosities between the normal controls and the

patients suffering from hypertension, renal failure, myocardial infarction, etc.[8,9,10]

In an attempt to explore the reasons for, and trace some patterns in such phenomena the data obtained in a series of patients studies, and in a series of fibrinogen-thrombin systems, have been correlated with the concentrations of plasma proteins, such as fibrinogen, albumin and globulin, and with the concentration of cholesterol. Furthermore, an evaluation was made of the effects of ABO blood groups.

It will be shown that the ratio of different proteins is of importance, and that the effect of the protein types, forming such a ratio, is different in blood samples of different blood groups. These effects may be influenced, and even reversed, by an application of a different shear rate during the formation of artificial thrombi.

It will be discussed that a greater importance of the protein ratios than of their actual concentrations suggests the presence of liquid crystalline structures. It will be further speculated that these liquid crystalline structures are affected by the presence of antigens or antibodies.

2. Experimental

A. Instrumentation

The variable-frequency thrombo-viscometer (VFTV) is an oscillatory version of the rotational cone-in-cone viscometer and has been described previously.[4,7,8,9] Only a summary is given here. The instrument consists of two coaxial converging cones of angles of 35 and 40 degrees towards the vertical axis. A schematic representation is shown by Fig. 1. The inner, solid cone, suspended on a torsion strip (Mallory 73 copper beryllium alloy, of cross-section 0.008 × 0.075 inches) is made of Teflon. The outer, rotating hollow cone is made of brass. The outer cone oscillates through an arc of 110 degrees, at frequencies of 60 and 180 cycles per minute. The corresponding mean shear rates are 26.8 and 80 sec^{-1}. These shear rates fall within the upper and lower limits of the shear rates observed in arteries.

Calibration of VFTV with the standard oils of known viscosity (obtained from the National Standards Laboratories, CSIRO) showed that at 60 cycles/min a deflection of 0.83 units (d.u.) observed

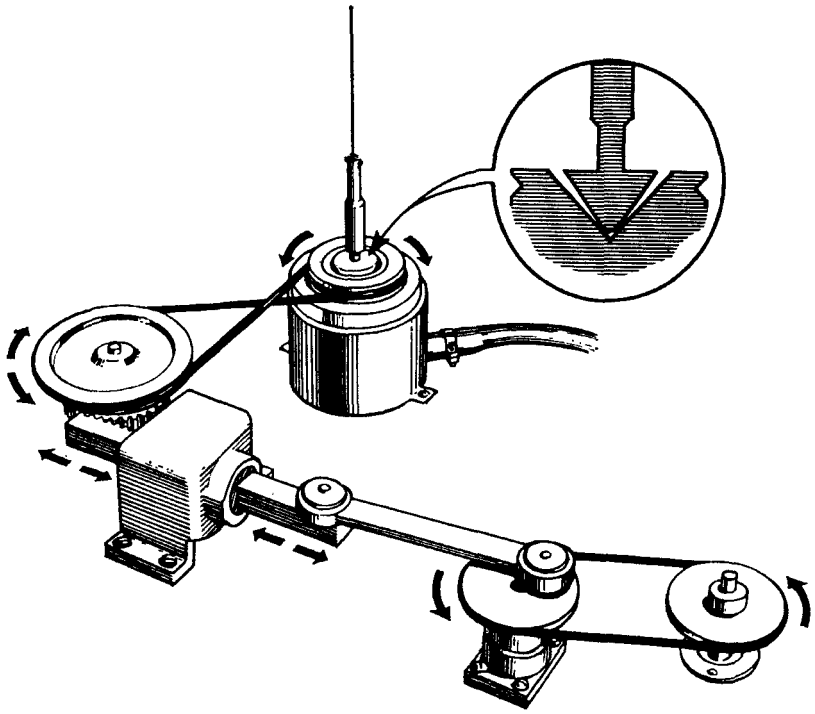

Figure 1. A schematic representation of the variable-frequency thrombo-viscometer (VFTV). The coaxial cones (see the insert) are thermostated. Oscillatory drive (arc of 110 degrees) is provided through a cam and a reciprocating drive. Blood sample is poured into the gap between the outer and inner cone.

on a semi-circular (radius 29.5 cm) plastic scale represents viscosity of one poise. At 180 cycles/min the viscosity of one poise corresponds to a deflection of 2.49 units of the scale. It must be stressed that due to the large amplitude of oscillation, it is quite easy to calibrate the instrument in the terms of viscosity (or apparent viscosity). A deflection of the torsion strip (and of the inner cone) is directly proportional to the viscosity of the fluid tested and to the frequency of oscillations (Fig. 2).

B. Methods and Materials

Blood samples are obtained by venepuncture, a two-syringe system being employed. Plastic disposable syringes are used.

Freshly shed blood is poured immediately into the gap between the two cones (about one ml of blood is needed), while the rest of the blood is utilized for other tests, such as blood groupings, micro-haematocrits, etc. Albumin and globulin concentrations are defined by means of Autoanalyzer, and cholesterol concentrations by titration

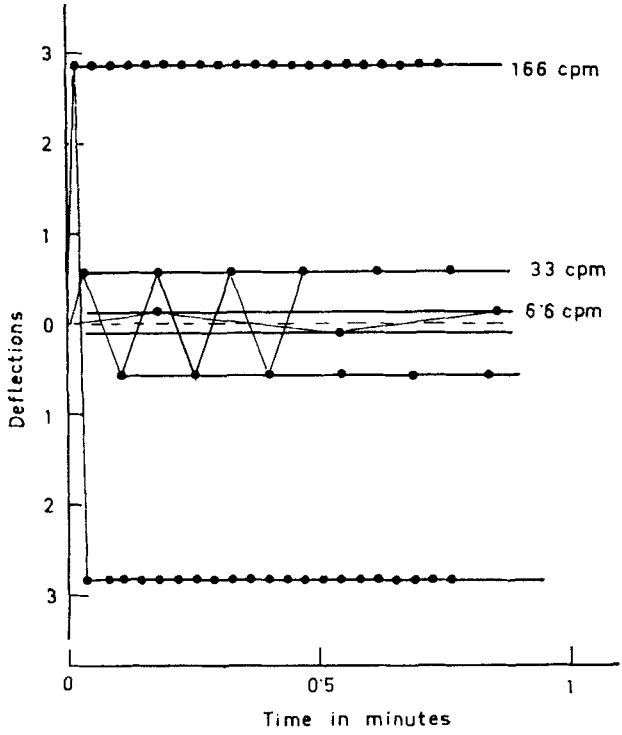

Figure 2. Calibration of the VFTV using medicinal paraffin oil. Deflection of the torsion strip is proportional to the frequency of oscillations (cpm).

methods, in the Biochemistry Departments. Fibrinogen is assayed by the method of Ratnoff and Menzie.[11] These assays were carried out by the Coagulation Laboratory (Head: Dr. G. P. McNicol) of the Glasgow Royal Infirmary, and by the Haematology Department (Head: Dr. Susan Gordon) of the Sydney Hospital.

Patients used for the study were hospitalized at the Glasgow Royal Infirmary and in the Sydney Hospital.

The series studies included patients suffering from hypertension, atherosclerosis, renal failure, myocardial infarction and haemophilia (in total 150 patients). The clinical descriptions of these groups have been either published[7,8,9,10] or are under preparation.

The fibrinogen-thrombin systems[12] were based on human fibrinogen obtained from the Commonwealth Serum Laboratories, Melbourne, and on the thrombin obtained from Parke-Davis Co. Thrombin was used in the concentration of 10 mg/100 ml.

The solution of fibrinogen, of different strength, were prepared in saline, or in albumin, or in albumin–globulin solutions. Albumin used was human albumin of 25% concentration, obtained from the Commonwealth Serum Laboratories (CSL), Melbourne. The other protein preparation, the stable plasma protein solution (S.P.P.S.), also obtained from CSL, contains 86% albumin and 14% globulin in the form of a 5% solution. All fibrinogen solutions were tested for concentration of fibrinogen by identical method as the blood samples.

Fibrinogen and thrombin solutions were used in volume ratios of 20 : 1. In different series different volumes of albumin or globulin were added.

All samples, both of the blood obtained from patients and of artificial solutions of fibrinogen were coagulated in the VFTV at 60 and 180 cycles/min at 37 °C.

During the latent period, when the blood (or solution) viscosity remains constant, the oscillations of the outer cone are transmitted only in a very small measure to the inner cone; the torsion strip and the galvanometer mirror attached to the strip remain nearly stationary. However, as the viscosity of coagulating system increases, larger and larger movements are transmitted to the inner cone. The deflections of the inner cone are observed by means of a lightspot on the semi-circular scale. A stop-watch is started at the moment when blood is poured (or fibrinogen–thrombin solution mixed and poured) into the VFTV, and all the subsequent events are timed.

All the parameters describing the formation and viscosity of artificial thrombi (or coagula) are described in the nomenclature following, in which the subscripts 60 or 180 refer to the frequency of oscillations.

The VFTV nomenclature.[7,8,9]

r_{60} and r_{180}:
the latent clotting time, measured from the moment when the sample is poured into the VFTV till the moment when viscosity starts to increase;

s_{60} and s_{180}:
the artificial thrombus formation time; it is timed from the moment when blood viscosity starts to increase till the moment when thrombus of maximum apparent viscosity is formed;

$r+s_{60}$ and $r+s_{180}$:
the total thrombus formation time; a sum of r and s;

A_{60} and A_{180}:
The maximum apparent viscosity of the thrombus;

A/s_{60} and A/s_{180}:
the rates of thrombus formation; these are given in the units of scale deflection per one minute of the thrombus formation time;

A/F_{60} and A/F_{180}:
the rates of thrombus degradation; these are given in units of scale deflection per minute of the degradation time measured from the moment when the thrombus achieves its maximum viscosity; the term A is here a shorthand for $A_{\max}-A_{\min}$.

3. Results

(a) *Artificial thrombi in different diseases*

Figures 3 and 4 show the VFTV dynamic coagulation curves drawn on the basis of means and standard deviation of VFTV parameters. All these values were calculated from log normal distribution. Figure 3 shows VFTV curves for artificial red thrombi (obtained at 60 cycles/min) and artificial white thrombi (obtained at 180 cycles/min) for normals and for patients suffering from hypertension, arteriosclerosis and renal failure. Figure 4 shows the VFTV curves for patients suffering from myocardial infarction and for patients on haemodialysis and after successful and unsuccessful kidney transplants.

The apparent viscosity of artificial thrombi is always higher at

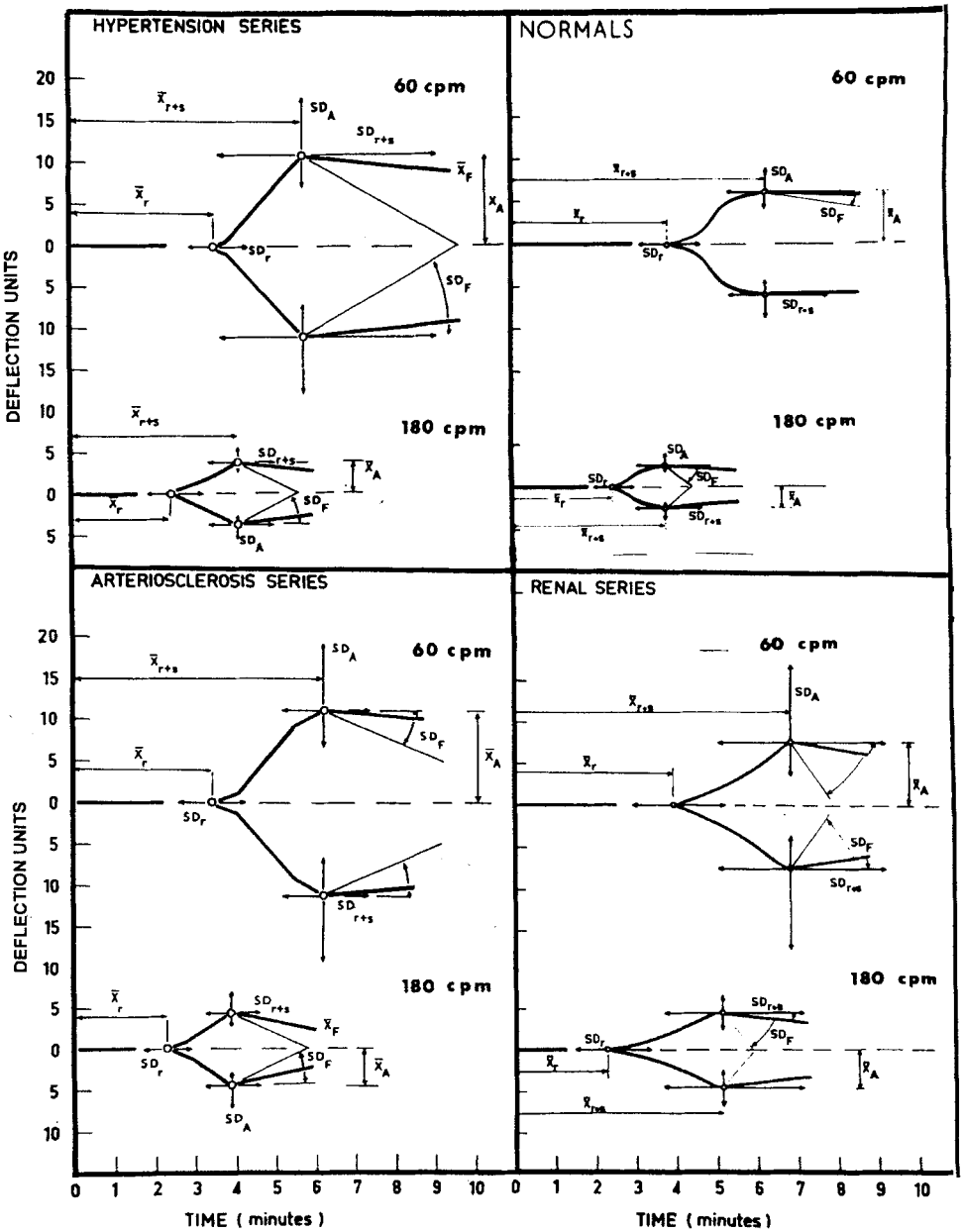

Figure 3. VFTV coagulation curves for four series of tests: hypertensive patients, normal controls, patients with arteriosclerosis and peripheral vascular disease, and patients with renal failure. These curves are based on arithmetic means and standard deviations of VFTV parameters obtained from log normal distributions. The upper curves (60 cpm) correspond to the red thrombi; the lower curves (180 cpm) correspond to the white thrombi. SD: standard deviation; $\bar{x}$: arithmetic mean. Subscripts refer to the parameters explained in the text of this paper.

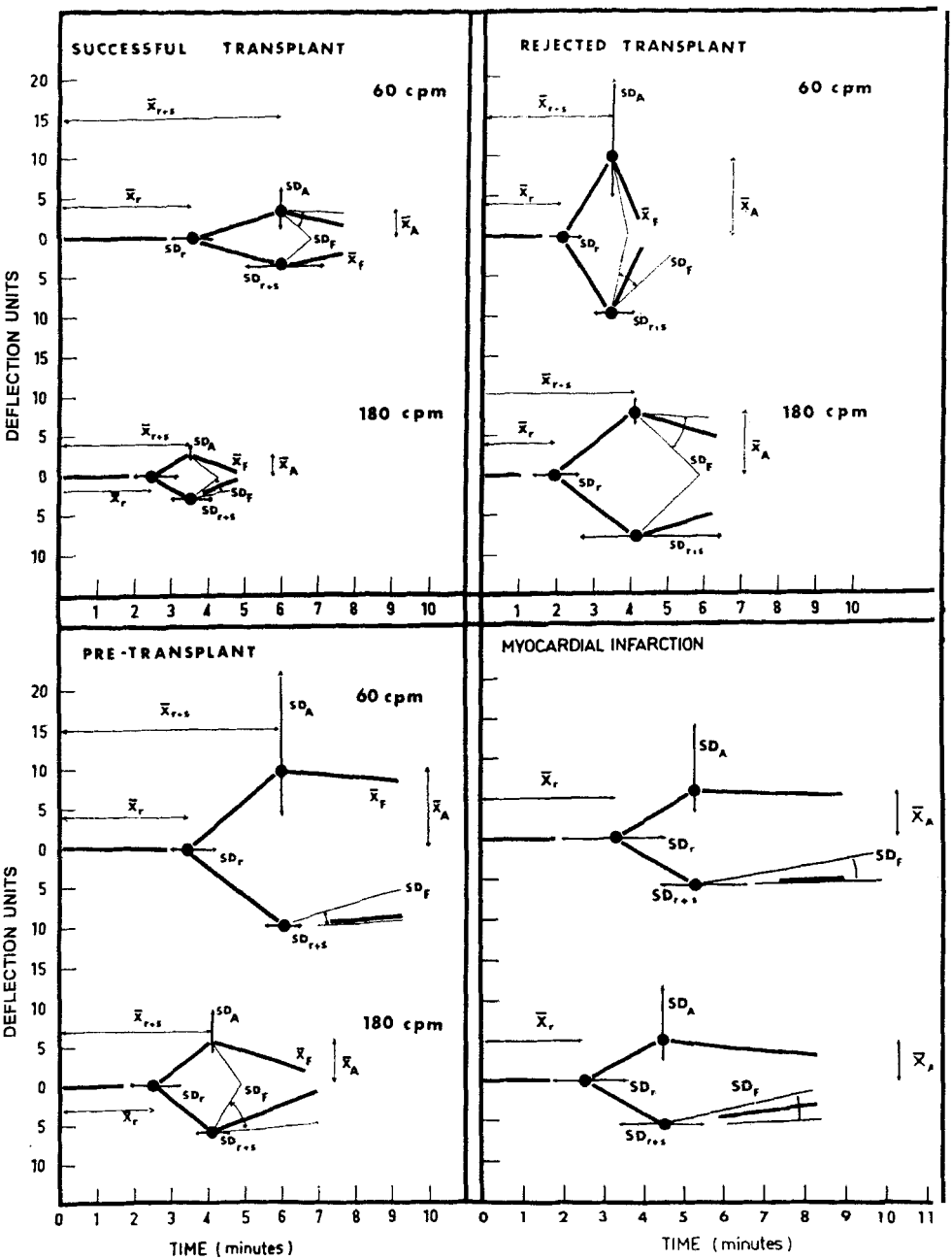

Figure 4. VFTV coagulation curves for four series of tests: patients after successful kidney transplantation ($n = 11$), patients after kidney rejection (or complication) ($n = 4$), patients on haemodialysis ($n = 6$), and patients suffering from myocardial infarction ($n = 23$). For further explanation see legend to Fig. 3.

60 cycles/min than at 180 cycles/min, in accordance with the fundamental patterns reported.[4,13,14] This is obvious even in the figures in which the ordinate is given in the deflection units of the scale. In order to compare these values in the viscosity units, the parameter $\bar{X}_A$ for 60 cycles/min should be multiplied by three (the ratio of frequencies used).

The apparent viscosity of artificial thrombi is much higher in patients than in normal controls or in patients who have undergone a successful kidney transplant. It appears that this elevation of viscosity of artificial thrombi in patients with cardiovascular disorders is a measure of the hypercoagulability or the hyper–thrombus–formation tendency in these diseases. A comparison with healthy normals indicated for each of the patients group a statistically significant elevation in viscosity ($P < 0.005$) versus the normal values.

TABLE 2 Effect of ABO Blood Groups on the VFTV Parameters in Different Diseases.

Disease & VFTV parameter	Significance limits A vs. B	A vs. O	B vs. O	Comment
Hypertension ($n = 30$)				
r_{180}	$P < 0.01$	n.s.	$P = 0.025$	B > A > O
A/s_{180}	$P < 0.05$	$P < 0.1$	$P < 0.05$	B > O > A
A/F_{180}	$P = 0.05$	$P = 0.1$	$P = 0.1$	B > O > A
Renal Failure ($n = 31$)				
r_{180}	n.s.	P@0.15	n.s.	O > B > A
$r + s_{60}$	$P < 0.1$	n.s.	$P < 0.05$	B > A > O
$r + s_{180}$	n.s.	$P < 0.05$	$P < 0.001$	B > A > O
A/s_{180}	n.s.	$P < 0.05$	$P < 0.1$	O > A > B
A/F_{180}	$P < 0.01$	n.s.	$P < 0.01$	B > O > A
Arteriosclerosis & Peripheral Vascular Disease ($n = 40$)				
r_{180}	n.s.	$P < 0.025$	$P = 0.025$	B > A > O
$r + s_{60}$	$P < 0.05$	$P < 0.05$	n.s.	A > O > B
A/s_{60}	n.s.	$P < 0.05$	n.s.	O > B > A
A_{60}	$P < 0.05$	n.s.	$P < 0.05$	O > A > B
A/F_{60}	$P < 0.05$	n.s.	$P = 0.05$	B > A > O
Myocardial Infarction ($n = 23$)				
A_{180}	—	$P < 0.05$	—	A > O

n.s. = not significant

Some significant differences were found when data for each of the patients group were broken down according to the ABO blood group of the patient. Some of these data[7,8,9,15] are compiled in Table 2. These data are not too exciting but they prepare a proper background for the results reported in the following sections of this paper.

(b) *Effect of fibrinogen and cholesterol on thrombus viscosity*

The results of these series of experiments are contained in Figs. 5, 6, 7, 8, 9, 10 and 11, and in Table 3. In order to obtain an information on the direct effect of fibrinogen in thrombus formation, artificial fibrinogen–thrombin systems were used in which the concentration of fibrinogen could be greatly varied. The ideal baseline would have been obtained in normals, were the differences in fibrinogen concentrations large enough. As this is not so, the series of haemophilia

TABLE 3 Effect of Concentration of Fibrinogen or Cholesterol on Thrombus Viscosity in Different Diseases in Patients of Blood Group O and A.

Concentration of protein or lipid vs. thrombus viscosity		linear regression and correlation		
		n	angle	r
Hypertension series				
cholesterol vs. A_{60}	bl.gr. A	7	0.0268	0.4637
	O	15	0.0329	0.2885
cholesterol vs. A_{180}	bl.gr. A	7	−0.0165	−0.5586
	O	15	−0.0022	−0.0691
Myocardial infarction				
fibrinogen vs. A_{60}	bl.gr. A	11	−0.0053	−0.1131
	O	12	0.0140	0.4270
fibrinogen vs. A_{180}	bl.gr. A	11	0.0063	0.1813
	O	12	−0.0069	−0.4161
Haemophilia series				
fibrinogen vs. A_{60}	bl.gr. A	8	0.0159	0.4223
	O	13	0.0452	0.8265[a]
fibrinogen vs. A_{180}	bl.gr. A	7	0.0100	0.3160
	O	13	0.0251	0.8849[b]
Fibrinogen–Thrombin in saline				
fibrinogen vs. A_{60}		18	0.0068	0.7660[a]
fibrinogen vs. A_{180}		18	0.0027	0.6419[c]

[a] significance limit $P<0.005$
[b] significance limit $P<0.001$
[c] significance limit $P<0.01$

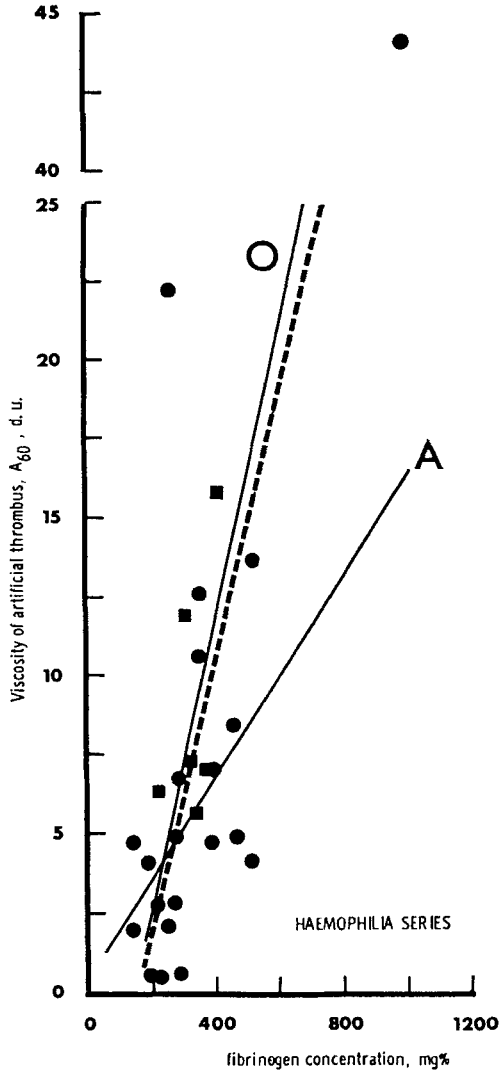

Figure 5. Viscosity of artificial thrombus, formed at 60 cycles per minute, as a function of the fibrinogen concentration in patients suffering from haemophilia. Dots refer to the patients with haemophilia A, squares refer to patients with Christmas disease. O and A mark linear regression lines for all patients with O and A blood groups, respectively. The broken line corresponds to the linear regression curve for all the data.

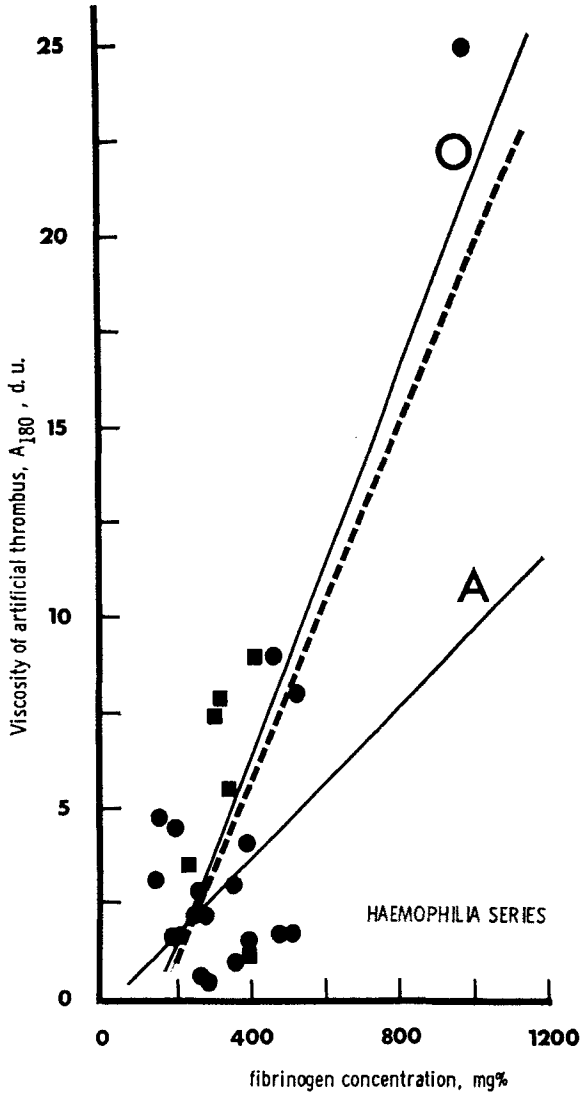

Figure 6. Viscosity of artificial thrombus, formed at 180 cycles per minute, as a function of the fibrinogen concentration in patients suffering from haemophilia. Legend as in Fig. 5.

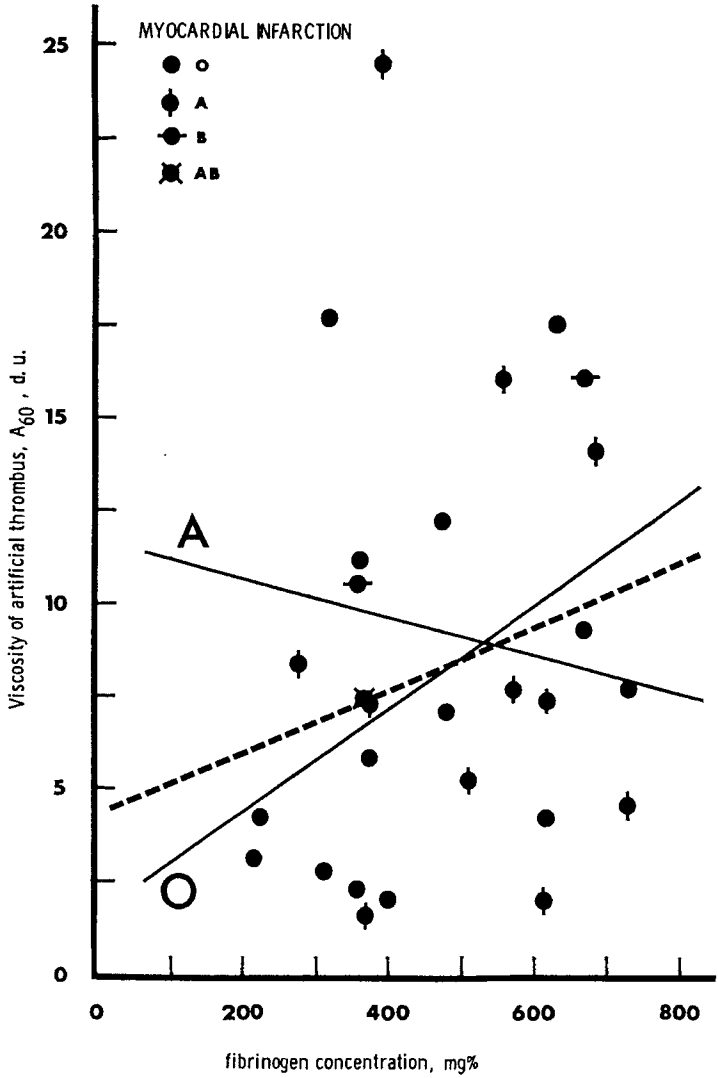

Figure 7. Viscosity of artificial thrombus, formed at 60 cycles per minute, as a function of fibrinogen concentration in patients with myocardial infarction. Experimental points are marked according to their blood group. A and O designations in the figure refer to the linear regression lines for all data of A and O blood groups, respectively. The broken line is the linear regression curve for all the data. Note the reverse sign of the A and O linear regression curves.

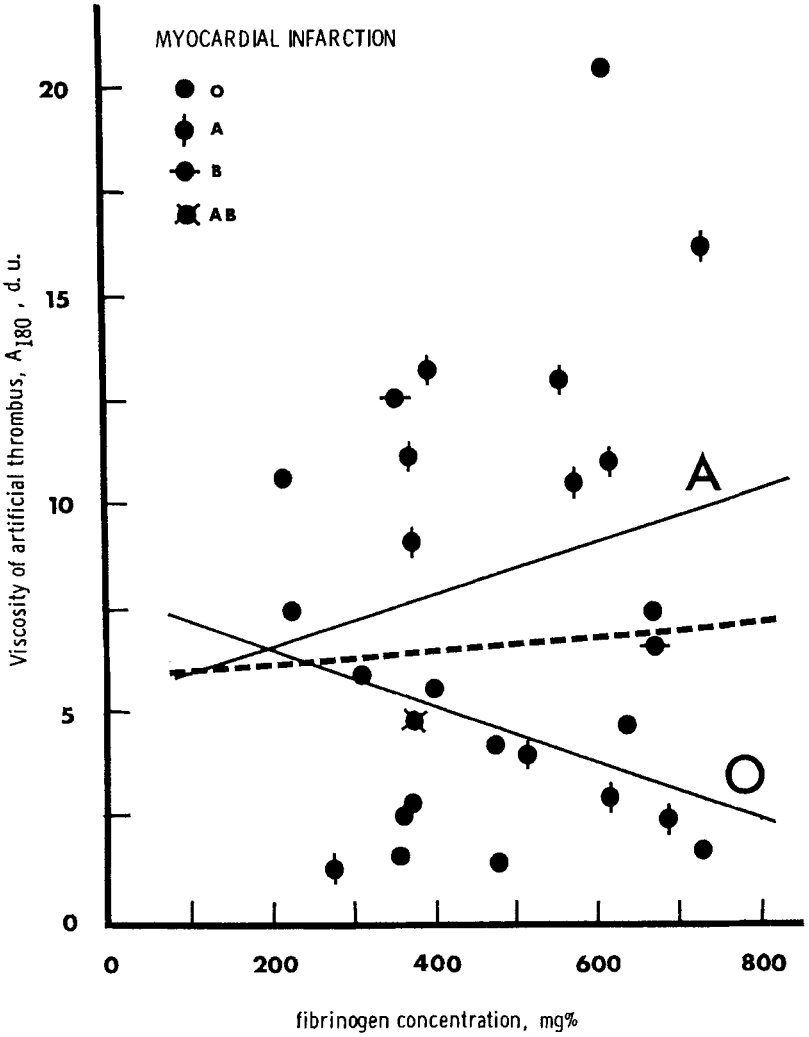

Figure 8. Viscosity of artificial thrombus, formed at 180 cycles per minute, as a function of fibrinogen concentration in patients with myocardial infarction. Legend as in Fig. 7. Note the reverse sign of the A and O linear regression curves.

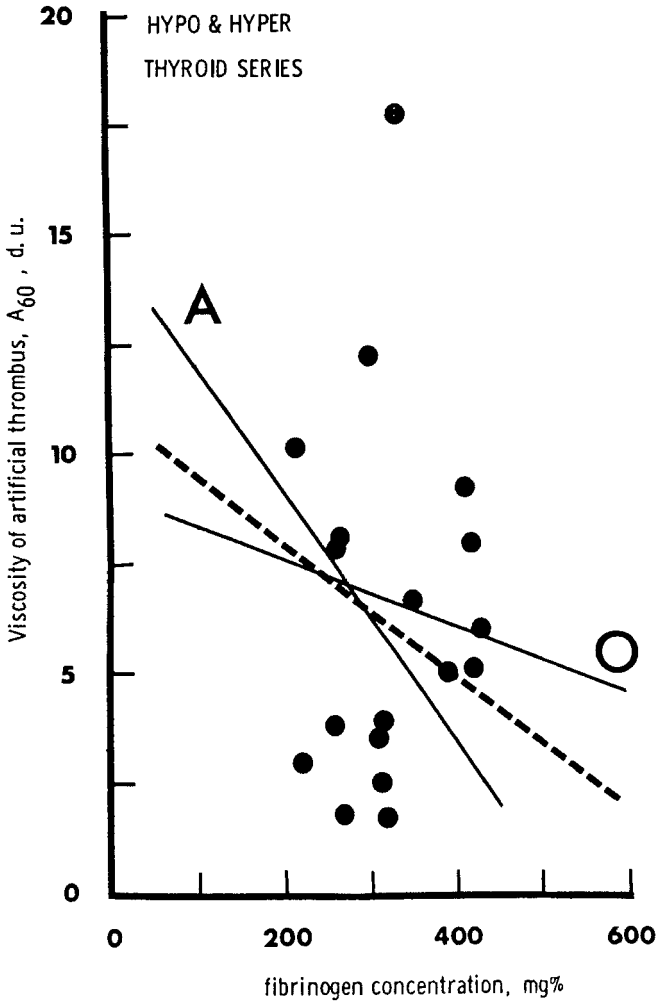

Figure 9. Viscosity of artificial thrombus, formed at 60 cycles per minute, as a function of fibrinogen concentration in patients with thyroid diseases. A and O mark the linear regression lines for all data of A and O blood groups, respectively. The broken line is a linear regression curve for all data. Note the negative angle of all linear regression curves.

patients was accepted as nearest to the proper baseline on the grounds that most of these patients were infused with many liters of normal plasma. As a result of these frequent infusions, their fibrinogen varied greatly. It could be noted (Table 3, Figs 5 and 6) that in haemophilics there are small differences only between patients with A and O blood groups.

A striking effect of ABO blood groups on the viscosity of artificial thrombi in the presence of different concentrations of fibrinogen are showed by experimental data for patients with myocardial infarction (Figs. 7 and 8, Table 3). In the both types (red and white) of thrombi the linear regression curves are quite different for patients of O and A blood groups. An increase in the fibrinogen concentration appears to lead to an increase of thrombus viscosity in the patients of blood group O, and a decrease in the patients of blood group A, when the thrombi are formed at 60 cycles/min; a reverse situation exists when these thrombi are cast at 180 cycles/min.

That an increase in the concentration of fibrinogen can actually lead to a decrease (and not increase) of thrombus viscosity is illustrated by few data obtained in patients with thyroid diseases (Fig. 9).

In a slightly analogous pattern, an increase in the total cholesterol concentration in patients with hypertension appears to lead to an increase of the A_{60} (viscosity of the thrombus cast at 60 cycles/min) but to a decrease of A_{180} (viscosity of the thrombus cast at 180 cycles/min.) The total cholesterol is quoted, which means that no distinction is made between the free cholesterol and the cholesterol esters (although it is realized that their physicochemical properties are vastly different). Results are given in Figs 10 and 11.

(c) *Effect of the protein ratios on viscosity of the artificial thrombi.*
It became quickly apparent that viscosity of artificial thrombi is affected not only by the concentration of fibrinogen but is also influenced by the presence of other plasma proteins, namely albumin and globulin. Linear regression and correlation analysis showed subsequently that this effect of other proteins is made more evident if instead of concentrations the ratios of proteins are used. Figures 12, 13 and 14 show the effect of such ratios in the haemophilia series, while Fig. 15 (which contains four figures in one) illustrates the same for the myocardial infarction series.

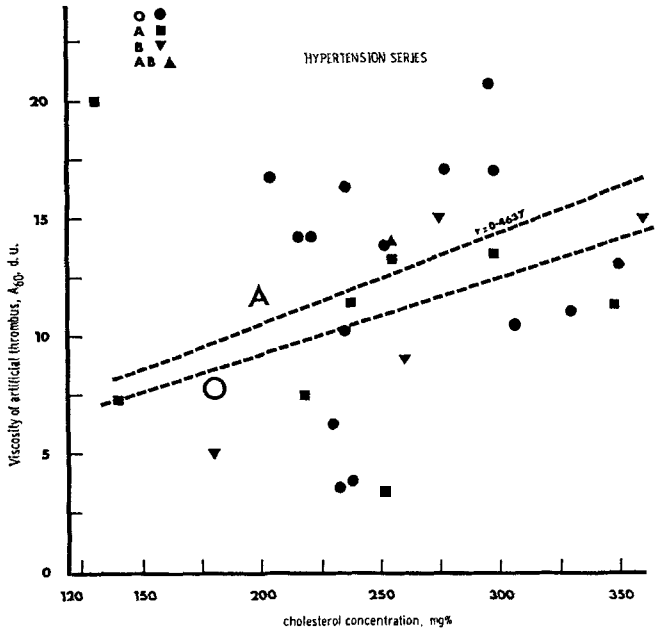

Figure 10. Viscosity of artificial thrombus, formed at 60 cycles per minute, as a function of cholesterol concentration in patients with hypertension. Differently shaped experimental points refer to different blood groups, as indicated in this Figure. A and O mark the linear regression curves for all patients with A and O blood groups, respectively. The correlation coefficient for blood group A is 0.4637; the same for blood group O is not significant (see Table 3).

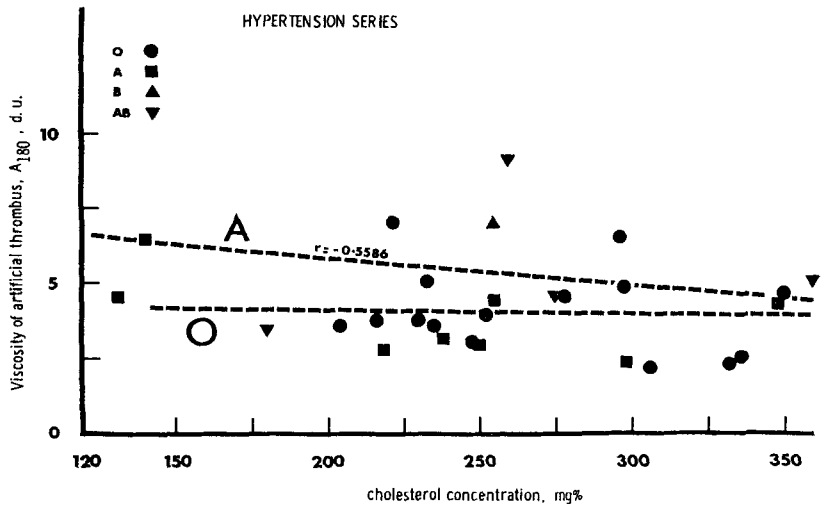

Figure 11. Viscosity of artificial thrombus, formed at 180 cycles per minute, as a function of cholesterol concentration. For legend see Fig. 11.

Linear regression and correlation analysis carried out on albumin/fibrinogen and S.P.P.S./fibrinogen ratios versus the viscosity of artificial thrombi in fibrinogen–thrombin systems (Table 4, Figs. 16 and 17) shows that an increase in the ratios leads to the decrease in the viscosity of artificial thrombi. Albumin/fibrinogen ratios have a

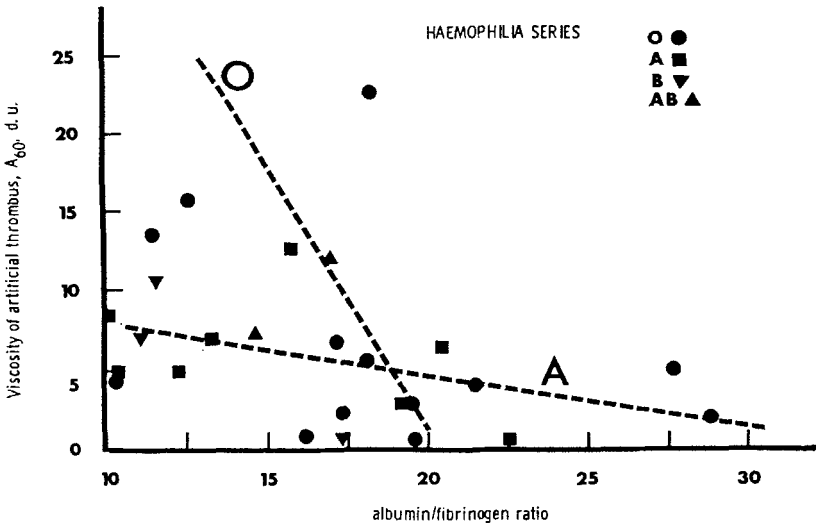

Figure 12. Viscosity of artificial thrombus, formed at 60 cycles per minute, as a function of albumin/fibrinogen ratio in patients with haemophilia. Different experimental points correspond to different blood groups as indicated in the Figure. A and O mark the linear regression lines for all patients with A and O blood groups, respectively.

similar effect on thrombus viscosity in haemophilia series, these results being as true for patients with blood group O as A. However, albumin/globulin and globulin/fibrinogen ratios have opposite results in haemophilics of the same blood group; and the signs are reversed for A and O blood groups (Figs. 13 and 14). In other words, in the globulin/fibrinogen vs. thrombus viscosity relationship the viscosity decreases with an increase of this ratio in blood group O, but increases in the series of patients of blood group A. Such a reverse response to the protein ratios is even more marked for the albumin/globulin results (Fig. 14).

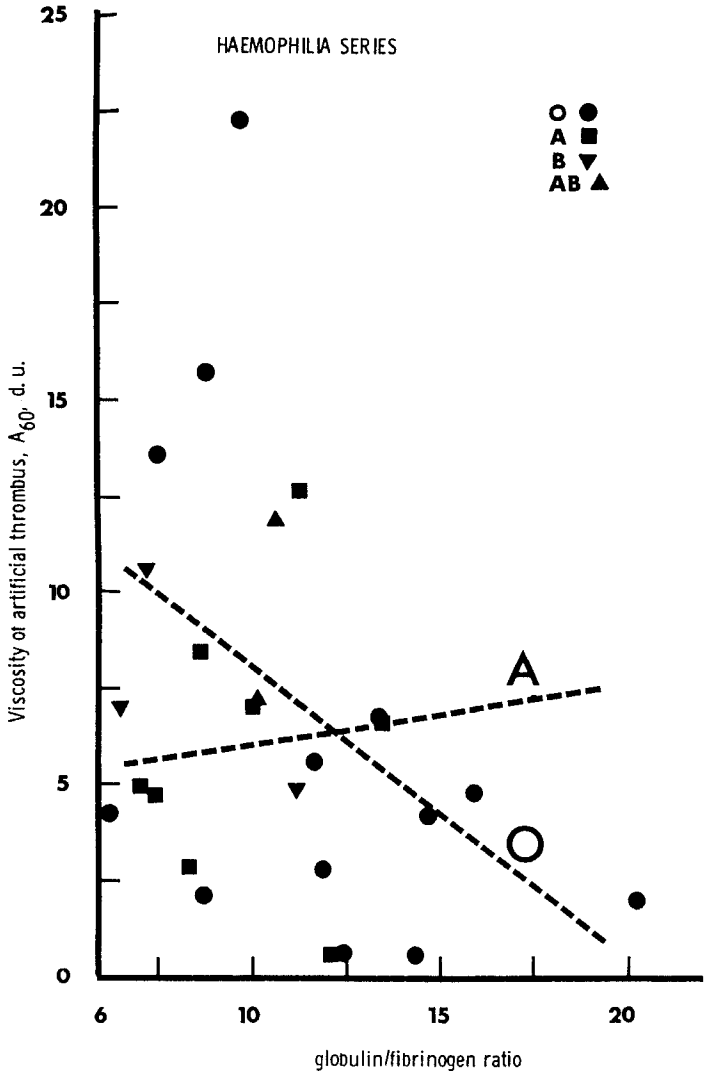

Figure 13. Viscosity of artificial thrombus, formed at 60 cycles per minute, as a function of globulin/fibrinogen ratio in patients with haemophilia. Legend as in Fig. 12.

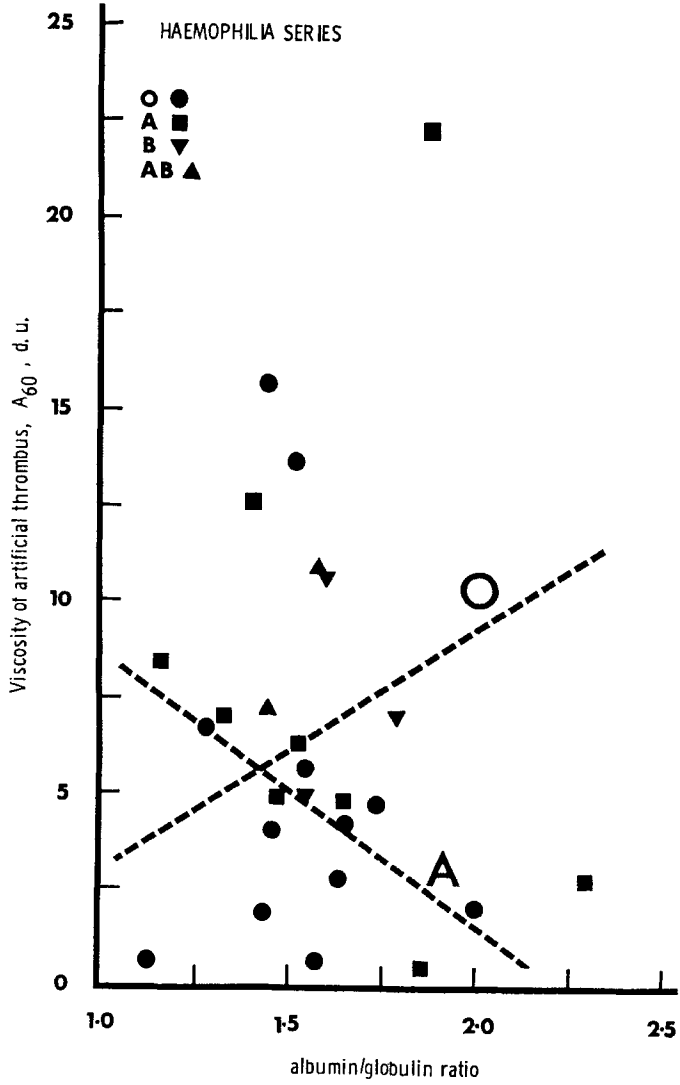

Figure 14. Viscosity of artificial thrombus, formed at 60 cycles per minute, as a function of albumin/globulin ratio in patients with haemophilia. Legend as in Fig. 12. Note the reverse signs of the linear regression curves for A and O blood groups.

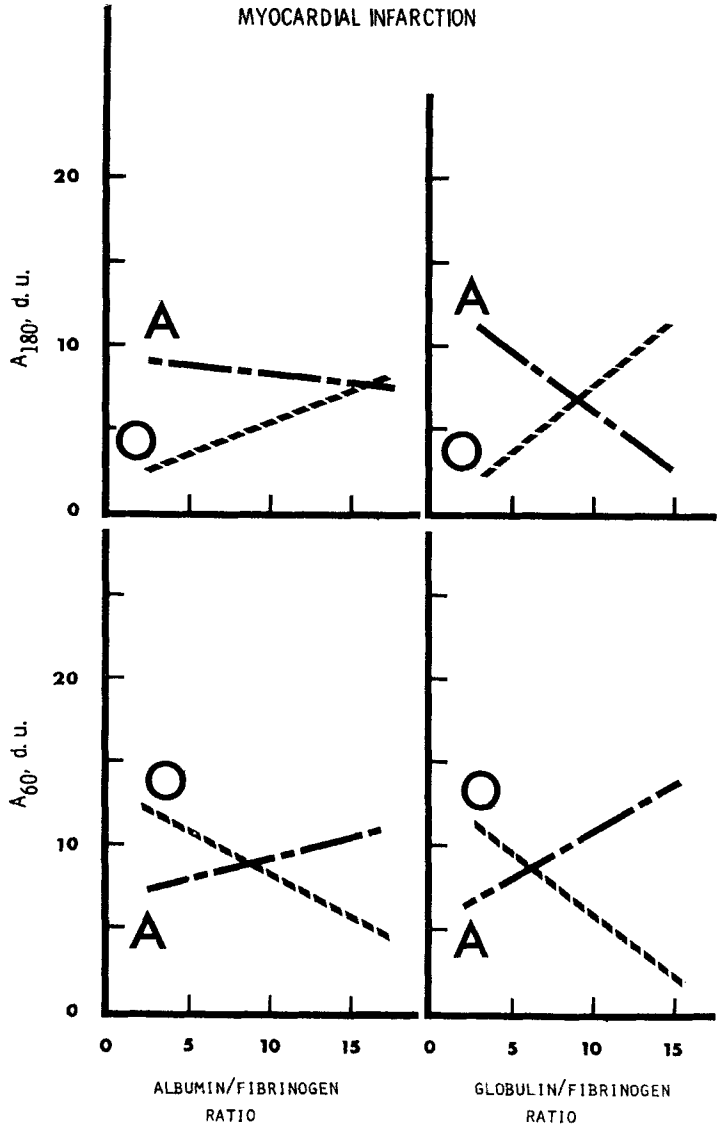

Figure 15. Viscosity of artificial thrombi, formed at 60 and 180 cycles per minute, as a function of the albumin/fibrinogen and the globulin/fibrinogen ratios in patients with myocardial infarction. A and O mark the linear regression lines for the patients with A and O blood groups, respectively. For additional details see Table 4. Note the reverse signs of O and A linear regression lines, and reversal of patterns for A_{60} and A_{180} thrombi.

TABLE 4 Effect of Protein Ratios on the Viscosity of Artificial Thrombi.

Protein ratio vs. thrombus viscosity			linear regression and correlation n	angle	r
Fibrinogen–Thrombin systems					
albumin/fibrinogen ratio					
vs. A_{60}			12	−0.1172	−0.4904
A_{180}			12	−0.0697	−0.4995
S.P.P.S./fibrinogen ratio					
vs. A_{60}			11	−1.6224	−0.5538
A_{180}			11	−0.3103	−0.5921
Haemophilia series					
albumin/fibrinogen					
vs. A_{60}	bl.gr.	A	8	−0.564	−0.4334
		O	13	−0.289	−0.3483
globulin/fibrinogen					
vs. A_{60}	bl.gr.	A	8	0.1579	0.1006
		O	13	−0.7679	−0.4456
albumin/globulin					
vs. A_{60}	bl.gr.	A	8	−7.085	−0.6895
		O	13	6.2419	0.2195
Myocardial infarction					
albumin/fibrinogen					
vs. A_{60}	bl.gr.	A	11	0.2458	0.1208
		O	12	−0.5050	−0.4891
vs. A_{180}	bl.gr.	A	11	−0.1439	−0.0946
		O	12	0.3509	0.4711
globulin/fibrinogen					
vs. A_{60}	bl.gr.	A	11	0.5677	0.2009
		O	12	−0.6988	−0.3274
vs. A_{180}	bl.gr.	A	11	−0.7445	−0.3529
		O	12	0.7377	0.4797

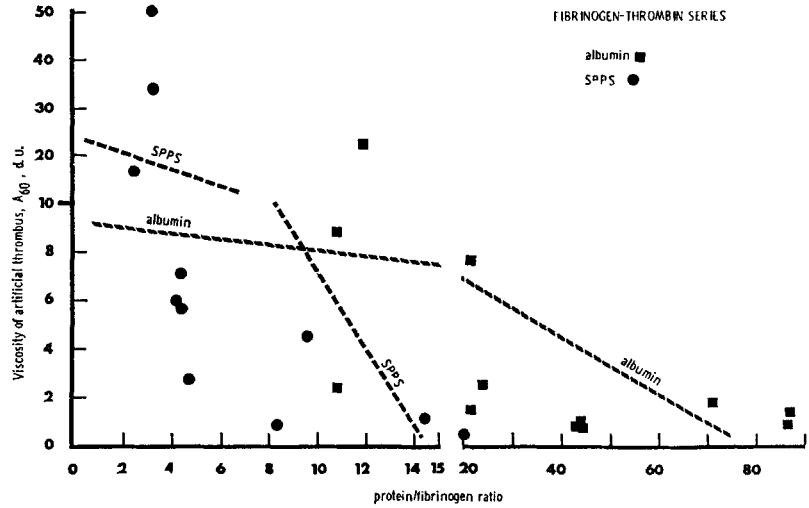

Figure 16. Viscosity of artificial thrombi, formed at 60 cycles per minute, as a function of protein/fibrinogen ratio, in fibrinogen–thrombin systems. The protein/fibrinogen ratios refer to the albumin/fibrinogen and S.P.P.S./fibrinogen ratios. S.P.P.S. is a mixture of albumin and globulin. For details see text. Linear regression lines are broken because scales of abscissa and of ordinate are changed in order to accommodate all the experimental data.

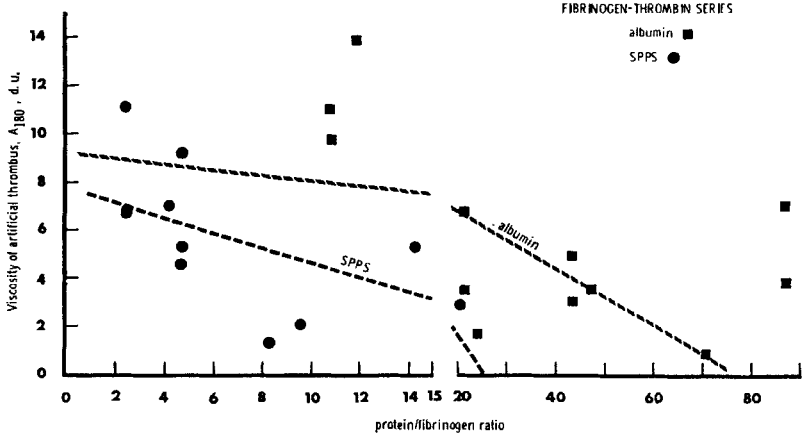

Figure 17. Viscosity of artificial thrombi, formed at 180 cycles per minute, as a function of protein/fibrinogen ratio, in the fibrinogen–thrombin systems. For legend see Fig. 16.

Linear regression curves plotted for the albumin/fibrinogen and globulin/fibrinogen ratios vs. thrombus viscosity for patients with myocardial infarction show a complex pattern (Fig. 15), Table 4. In the case of red thrombus (A_{60}), an increase in either the albumin/fibrinogen ratio or the globulin/fibrinogen ratio leads to an increase in the thrombus viscosity for patients of blood group A, but leads to a decrease in the viscosity for patients of blood group O. This pattern is reversed completely when the casting shear rate is increased threefold and the white thrombi are obtained (Fig. 15).

Although all these results show a consistent pattern of trends, the significance limits are only about or below 0.1.

4. Discussion

The viscosity, or the apparent viscosity, of artificial thrombi depends on a number of factors. The principal factor known[4,14] is the shear rate at which such thrombi are formed. Increasing shear rates lead to coagula in which platelet aggregation becomes increasingly important. The changes in the viscosities and morphologies of coagula cast at different shear rates were given[6,14,16,17] as due to (i) a progressive orientation of the fibrin network, a progressive fracture of this network and, finally, to the polymerization of fibrinogen into globular fibrin particles; (ii) a progressive release of the disaggregating red cells from the meshes of the fibrin network; (iii) a progressive aggregation of the platelets with increasing shear rates. A decrease in the clotting or thrombus formation times (at increasing casting shear rates) was suggested to be due to enhanced diffusion and collision frequencies of the coagulation factors and profactors, the diffusion and mixing being facilitated both by the increased flow velocity and by a simultaneous decrease in the viscosity of blood.

There is no question that patients with cardiovascular disorders do show an elevation in the viscosity of artificial thrombi. As the concentration of fibrinogen is elevated in these diseases, it would be fortunate if such an elevation of viscosity were due simply to an increased concentration of fibrinogen. This study shows, however, quite clearly that this is not the case. True, in the reconstructed systems and in the haemophilics (especially if treated with large

volumes of plasma infusion) there is an excellent correlation between the viscosity of thrombi and the fibrinogen concentration. That is not the case in patients suffering from myocardial infarction or from thyroid disease. The additional factors coming into play are the ABO blood groups, the concentrations of albumin and globulin and, even more important, the ratios of concentrations of these proteins. The effect of cholesterol is, at this stage, enigmatic as it was not possible in this study to separate the roles of the free cholesterol and the cholesterol esters.

That the fibrinogen *per se* is not the important factor in disease is shown by the fact that the viscosity of artificial thrombi can decrease with an increase in the concentration of fibrinogen.

The proteins ratios *per se*, although of great effect, do not show consistent pattern in the patients of different ABO blood groups.

It would appear that the important factor is a combination of the protein ratios and of the ABO blood groups; in other words, a combination of protein ratios and the ABO antigens and antibodies.

This behaviour could be explained by postulating liquid crystalline structures. It could be envisaged that different structures are formed depending on the protein ratios and antibodies or antigens present. Formation of these structures is affected by the shear rate at which they are formed. These structures (and artificial coagula and thrombi) are thixotropic as shown by Dintenfass.[1,14]

Artificial thrombi can show a netlike or microglobular structure. Perhaps there is a link here with the systems described by Sakamoto, Porter and Johnson.[18]

One could paraphrase G. T. Stewart[19] and ask whether the liquid crystalline state is an integral or merely an incidental feature of the blood coagulation and thrombus formation. If it is an integral feature of coagulation, it could play an additional role: namely, it might allow, lead to, or promote catalytic reactions during the coagulation process. One could wonder if some of the features of blood coagulation as studied by Seegers,[20,21,22] and specifically prothrombin, are not liquid crystals of catalytic power.[23]

Were it so, the pattern of phenomena would be—from the liquid crystalline point of view—not too remote from that taking place in the red cell membrane.[24]

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