Nagoya J. med. Sci. 31: 25-50, 1967.

STUDIES ON BLOOD VISCOSITY DURING EXTRACORPOREAL CIRCULATION

HISASHI NAGASHIMA

1st Department of Surgery, Nagoya University School of Medicine
(Director: Prof. Yoshio Hashimoto)

ABSTRACT

Blood viscosity was studied during extracorporeal circulation by means of a cone in cone viscometer. This rotational viscometer provides sixteen kinds of shear rate, ranging from 0.05 to 250.2 sec^{-1}.

Merits and demerits of the equipment were described comparing with the capillary viscometer.

In experimental study it was well demonstrated that the whole blood viscosity showed the shear rate dependency at all hematocrit levels. The influence of the temperature change on the whole blood viscosity was clearly seen when hematocrit was high and shear rate low.

The plasma viscosity was 1.8 cp. at 37°C and Newtonianlike behavior was observed at the same temperature. In the hypothermic condition plasma showed shear rate dependency.

Viscosity of 10% LMWD was over 2.2 fold as high as that of plasma.

The increase of CO_2 content resulted in a constant increase in whole blood viscosity and the increased whole blood viscosity after CO_2 insufflation, rapidly decreased returning to a little higher level than that in untreated group within 3 minutes.

Clinical data were obtained from 27 patients who underwent hypothermic hemodilution perfusion.

In the cyanotic group, whole blood was much more viscous than in the non-cyanotic.

During bypass, hemodilution had greater influence upon whole blood viscosity than hypothermia, but it went inversely upon the plasma viscosity.

The whole blood viscosity was more dependent on dilution rate than amount of diluent in ml/kg.

The venous mixture was a little higher as to its viscosity than that of the oxygenated whole blood and the above phenomenon became more pronounced within low shear rate range.

Peripheral circulation was discussed in relation to the blood viscosity.

PREFACE

In recent years a considerable number of reports has been published on open heart surgery. However, few viscometric studies during extracorporeal circulation using a rotational viscometer have been made.

長 嶋 久
Received for publication September 27, 1967.

The need for a large volume of blood for initial priming of the pumpoxygenator has presented a difficult problem for the patient with cardiac disease; thus the ideas such as reduced flow, moderate hypothermia, and hemodilution, have been under investigation in regard to perfusion technics[1], while these three factors have great influences on the blood viscosity.

On the other hand, blood has been understood as a non-Newtonian fluid from the rheological point of view. The rotational viscometer is the most adequate apparatus to measure the viscosity of fluid of that nature.

The present report deals with the effect of hypothermic perfusion with hemodilution and the related factors on blood viscosity measured by means of a cone in cone viscometer.

HISTORICAL REVIEW

Within recent years, many reports on the methods of extracorporeal circulation have accumulated indicating a growing interest in the field of cardiovascular surgery.

In 1937, a heart-lung machine was introduced as an experimental device by Gibbon[2]. The first clinical application of the artificial circulation by Dennis, in 1951[3], resulted in failure. Since Gibbon reported the first successful clinical use of the apparatus[4], many successful cases have been reported by Crafoad[5], Lillehei[6] and Kirklin *et al.*[7].

Hypothermia, by surface cooling, has extensively been studied by Bigelow[8] and Swan[9]. Lewis[10] made successful closure of the atrial septal defect with the aid of hypothermia in 1953.

Cooling of the blood by extracorporeal circulation is an efficient way of lowering body temperature. Clinical use of the blood cooling method was studied by Ross[11], and Brock and Ross[12]. After an efficient heat exchanger was devleoped by Brown *et al.*[13] in 1958, the combination of hypothermia with a heart-lung apparatus greatly promoted cardiac surgery.

Well known difficulties in the procurement of a large volume of blood for initial priming and increased risks by blood transfusions have animated many attempts to overcome these obstacles. After experimental studies Neptune and others[14] developed a method of priming the pump-oxygenator without the need for the donor blood and this method was successfully applied on clinical cases, and 500 to 1,000 ml of the physiologic saline solution was used for initial priming.

Zuhdi[15], Long[16] and their coworkers stressed usefulness of hemodilution with 5% dextrose in water or low molecular weight dextran (LMWD).

From the rheological stand point, Poiseuille's equation[17] was applicable to the dynamics of flow in rigid tubes. Because of the anomalous flow properties of blood as a non-Newtonian fluid[18], the Poiseuillian law can not be directly

applied to capillary blood flow. By Whittaker[19], apparent viscosity of blood changes as velocity of blood changes.

Fahraeus and Lindqvist[20] investigated the viscosity of the blood in narrow capillary tubes and found that when the size of the tube is less than 1 mm in diameter, the calculated viscosity does not agree with the measured value. Dix and Scott-Blair[21] explained similar phenomena in colloidal suspension as sigma effect. Wells and Merrill[22] estimated the shear rate at the wall of the aorta in a resting normal man roughly as 100 inverse second (sec^{-1}). The shear rate at the wall of the blood vessel vary unrestrictedly according to the differences of the radius of the vascular tree. Therefore viscometry done at a fixed shear rate is not adequate for determining human blood viscosity. In 1961 Wells[23] introduced a cone plate rotational viscometer for measuring viscosity of biologic fluid. This type of instrument provides a wide ragne of shear rates and suits for the study of non-Newtonian fluid. In discussing the colloidal structures of blood utilizing a cone in cone viscometer, Dintenfass[24] stressed the importance of the shear rates lower than 10 sec^{-1}.

MATERIALS AND METHODS

The report is divided into two parts: part I, experimental studies and part II, clinical studies.

Part I

Fresh blood was obtained from donor dogs heparinized in a dose of 4 mg/kg of body weight. To carry out series of viscometry on blood samples of different hematocrit values, the blood was withdrawn into a sterile 500 ml vials and was centrifuged at 3,000 rpm for 15 minutes. Then plasma was pipetted to reconstitute the desired hematocrit level. After viscometry, actual hematocrit of each sample was confirmed using the Wintrobe tube, and samples not within $\pm 0.5\%$ of the planned hematocrit value was rejected from this study.

Samples were prepared in water-bath at a desired temperature prior to studies, and the water-jacketted sample cup of the viscometer were also kept at the same temperature ranging within $\pm 0.1°C$. Viscometry was carried out on the blood at various temperatures and hematocrit levels, and was also performed on several kinds of diluent.

To see the relationship between the blood gas and the blood viscosity, a further experimental study was carried out. At the bottom of a deep bottle blood sample was placed, then pure CO_2 was insufflated without bubbling until partial pressure of CO_2 reached 40 to 90 mm Hg at 37°C. With this sample the viscosity and blood gas content were determined. In other 10 experiments, after exposure to pure CO_2 at a partial pressure of 90 mm Hg viscosity was measured continuously every minute for 15 minutes with a single shear rate

of 31.1 sec^{-1}.

Part II

Clinical data were obtained from 27 patients who underwent hypothermic hemodilution perfusion for the correction of cardiovascular defects at the Nagoya University Hospital (Table 1). The cases were as follows: tetralogy of Fallot, 13 cases; ventricular septal defect, 5; mitral valve lesion, 4; atrial septal defect, 2; pulmonic stenosis, 2; and aortic valve lesion, 1.

TABLE 1

	Case No.	Sex	Age	Body weight (kg)	Lesion	Op. Procedure	Perfusion time (min)	Lowest Temp (°C) Mid-esoph	Lowest Temp (°C) Rectal	Results
Group 1	1	M	11	33.5	TF	Total correction	114	25.5	25.2	Alive and well
	2	F	7	20.2	TF	Total correction	110	27.3	29.3	Alive and well
	3	F	27	47.5	TF	Total correction	149	24.2	25.1	Alive and well
	4	M	25	45.9	TF	Total correction	177	26.0	24.0	Alive and well
	5	F	27	52.9	TF	Total correction	148	23.4	27.7	Alive and well
	6	M	5	17.7	TF	Total correction	94	24.0	25.5	Alive and well
	7	M	8	23.4	TF	Total correction	149	24.2	25.0	Alive and well
	8	F	10	32.4	TF	Total correction	126	25.0	24.9	Alive and well
	9	M	6	14.7	TF	Total correction	115	21.3	23.5	Alive and well
	10	F	14	35.2	TF	Total correction	108	27.0	23.5	Alive and well
	11	M	13	35.8	TF	Total correction	189	24.5	24.2	Alive and well
	12	M	15	40.8	TF	Total correction	132	25.4	25.5	Aline and well
	13	F	18	37.0	TF	Total correction	148	24.2	24.5	Died, P.O. 2nd D.
Group 2	14	F	16	37.7	AI+VSD	Annuloplasty Teflon patch	198	24.0	27.2	Died, P.O. 1st. D.
	15	M	5	17.2	PS	Commissurotomy	29	27.4	30.0	Alive and well
	16	M	5	16.8	VSD	Direct suture	74	26.8	28.5	Alive and well
	17	F	24	46.6	ASD	Direct suture	60	27.0	27.6	Alive and well
	18	F	5	13.5	VSD+PH	Teflon patch	133	23.5	26.6	Alive AV block
	19	F	22	43.5	MSI	Mitral valve repl	100	24.5	24.6	Alive and well
	20	F	5	12.5	VSD+PH	Teflon patch	91	24.9	27.9	Alive and well
	21	M	24	58.8	ASI	Aortic valve repl	166	25.4	24.6	Alive and well
	22	M	15	34.5	PS	Out flow patch	73	28.5	27.1	Alive and well
	23	F	6	18.5	ASD	Direct suture	46	28.8	28.3	Alive and well
	24	F	20	39.1	ASI+MS	Aortic valve repl Mitral val. commissurotomy	175	23.2	25.0	Died, P.O. 5th. D.
	25	M	4	13.9	VSD	Direct suture	80	26.0	26.3	Alive and well
	26	M	16	41.0	MSI	Mitral valve repl	199	26.2	26.1	Died. P.O. 1st. D.
	27	F	43	59.2	MSI	Mitral valve repl	197	22.4	25.0	Alive and well

Legend: TF Tetralogy of fallot; VSD Ventricular septal defect; ASD Atrial septal defect; PS Pulmonary stenosis; PH Pulmonary hypertension; AI Aortic insufficiency; ASI Aortic steno-insufficiency; MS Mitral stenosis; MSI Mitral steno-insufficiency

All cases were anesthetized with N_2O, O_2 and ether by the semiclosed overflow method.

Pump-oxygenator system included two Debakey-type rotary occlusive pumps, a Kay-Cross disc oxygenator and a double helical heat exchanger (Fig. 1)[25)26)],

The extracorporeal circuit was primed according to the following formula: $B+\frac{(A-B)}{4}$ = amount of priming solution. $3/4(A-B)$ = amount of heparinized blood, where B equals 1/3 of the daily fluid requirement of the patient of about 30 ml/kg and A is the priming volume. Prior to cannulation, 2 mg/kg of bodyweight of heparin was given intravenously, and the same amount of heparin was also installed into the apparatus.

Using a blanket and blood cooling, intraesophageal and rectal temperatures were lowered to 25 to 30°C and the total bypass was established by tightening the caval tapes. When intracardiac correction was accomplished, rewarming was started with a blanket and assisted circulation. The caval tapes were re-

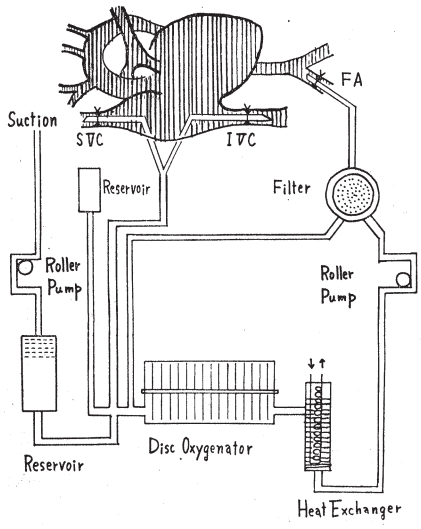


FIG. 1. Pump Oxygenator System

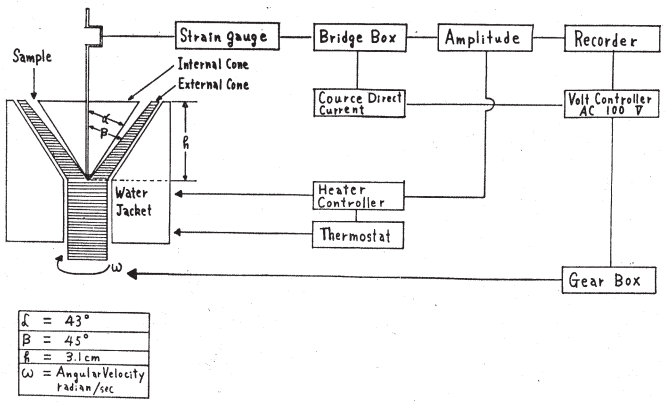


FIG. 2. Cone in Cone Viscometer

leased and partial perfusion was continued until the body temperature reached to 34°C.

Oxygen and carbon dioxide were insufflated into the disc oxygenator, with the flow rate of 5 l/min and 100 cm^3/min, respectively.

Blood samples were drawn by venipuncture before and one hour after the perfusion, employing a 20 ml glass-syringe containing 0.15 ml of heparin sodium, (7.5 U. per 1 ml of blood). The samples were collected as mixed venous blood and oxygenated arterial blood during total bypass and at the end of bypass. Additional samples were obtained at every 30 minutes intervals during total bypass when the perfusion was continued over one hour.

All glass-wares used in the study were siliconized.

The viscometer used in these studies was a coaxial cone in cone type, a kind of rotational viscometer, requireing 5 ml of sample placed between both cones (Figs. 2, 3 and 4)[27)28)].

The external cone was rotated by a constant speed motor which provides sixteen grades of rotating speeds, representing the following shear rates (Table 2). The angles of the internal cone (α) and the external cone (β) to the vertical were 43 and 45 degrees, respectively. The mean shear rates were calculated by the following equation:

$$D=(2w/\beta-\alpha)(\cot\alpha-\cot\beta)\left(\cot\alpha\ \mathrm{cosec}\ \alpha-\cot\beta\ \mathrm{cosec}\ \beta-\mathrm{Int}\cdot\frac{\tan\beta/2}{\tan\alpha/2}\right)^{-1},$$

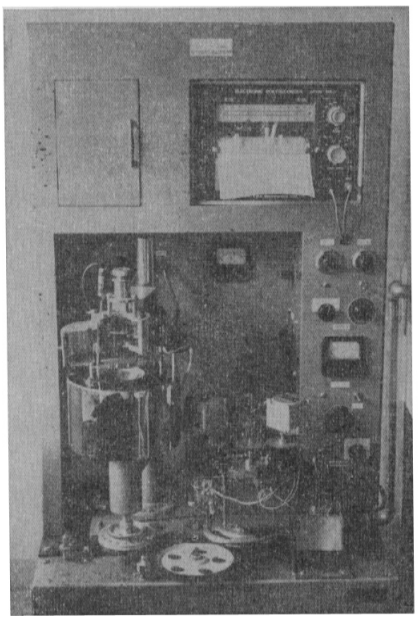


FIG. 3. General view of the viscometer.

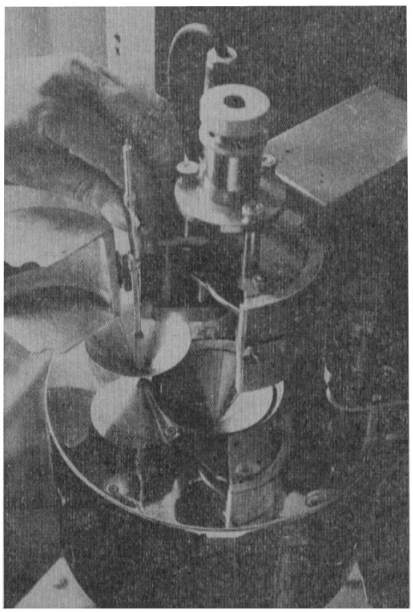


FIG. 4. External cone and internal cone surrounded by water jacket.

TABLE 2. Revolutions per minutes and shear rate

	R.P.M.	Shear Rate (SR) sec^{-1}		R.P.M.	Shear Rate (SR) sec^{-1}
1	0.025	0.05	9	2.0	4.17
2	0.05	0.10	10	3.0	6.26
3	0.1	0.21	11	6.0	12.51
4	0.2	0.42	12	12.0	25.0
5	0.25	0.52	13	15.0	31.3
6	0.5	1.04	14	30.0	62.6
7	1.0	2.09	15	60.0	125.1
8	1.5	3.13	16	120.0	250.2

where w is the angular velocity of the external rotating cone in radians per second. Temperature control was done by a water jacket that surrounded external cone.

Apparent viscosity was expressed in C.G.S. units of centipoise (cp.=1/100 poise). Shear rate was defined as the velocity gradient of two fluid planes divided by the distance between them $\left(\frac{cm/sec}{cm} = sec^{-1}\right)$, and shear stress as the tangential force per unit area of fluid plane (dyn/cm^2). Viscosity, by definition, is shear stress divided by shear rate ($dyn \cdot sec/cm^2$ or poise).

Calibration of the instrument was made using standard viscosity fluid of JS 10, the viscometer calibrating liquid prepared by Showa Oil Co. Ltd., providing constant viscosity of 8.537, 6.083, 4.828 and 3.508 cp. at 20.0, 30.0, 37.8 and 50.0°C, respectively.

Viscosity determination on each sample was carried out beginning from the lower shear rate toward the higher shear rate, keeping the sample at the same temperature as it was withdrawn.

The total protein concentration was determined by a refractometer manufactured by Hitachi Manufac. Co. Ltd. at room temperature. Plasma albumin and globulin were measured by the auto-analyser manufactured by Technicon Co. Ltd. U.S.A. and A/G ratio was calculated. Plasma fibrinogen was determined by the tyrosin method[29].

Blood gas analysis was carried out by Van Slyke Neil method[30].

RESULTS

Part I

Relationships among whole blood viscosity, hematocrit and temperature are presented graphically on log-log paper in Figs. 5, 6, 7, 8, 9, and 10. At hematocrit level between 20 and 40%, there was a relatively linear rise in viscosity with increasing hematocrit. Over 40%, viscosity increased more sharply and exponentially. For example, at the shear rate of 250 sec^{-1} at 37°C, the viscosity of the whole blood showed 3.50 cp. when hematocrit is 20%, whereas it became 6.95 cp. when hematocrit increased to 70%. However, at the low shear rate of 1.04 sec^{-1}, viscosity varied from 9.10 to 63.0 cp.

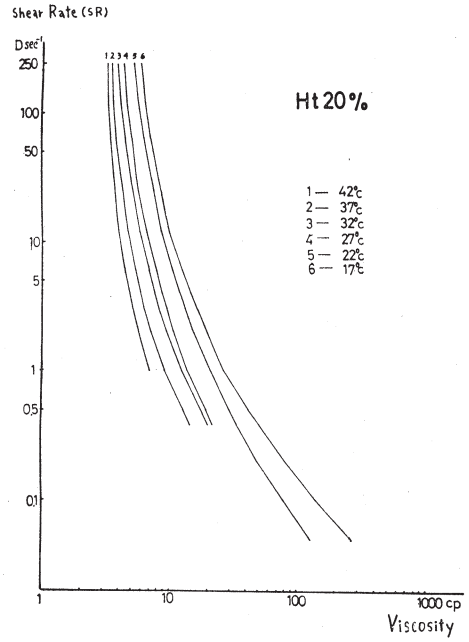


FIG. 5

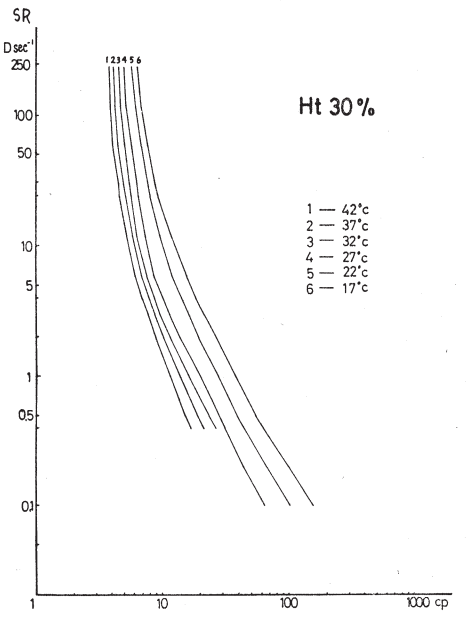


FIG. 6

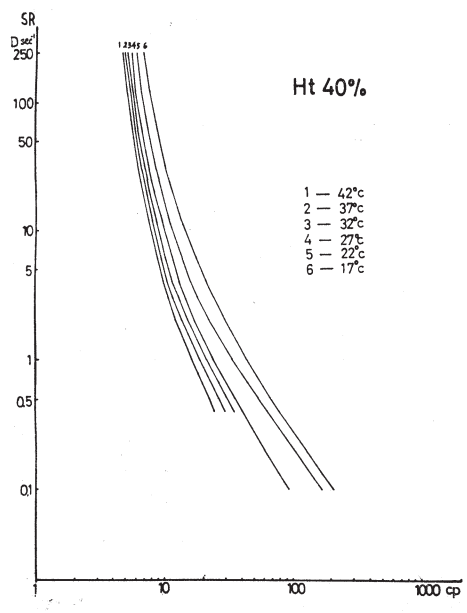


FIG. 7

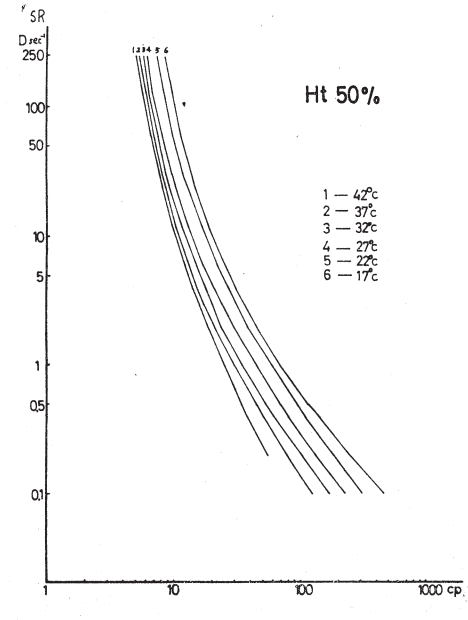


FIG. 8

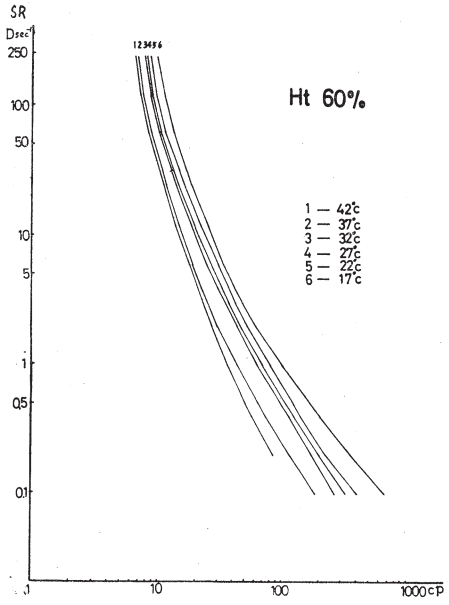


FIG. 9

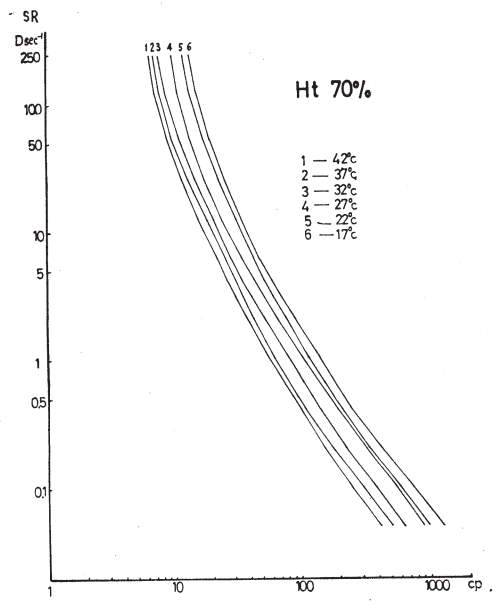


FIG. 10

FIGs. 5, 6, 7, 8, 9 and 10. Relationship among whole blood viscosity, hematocrit and temperature are plotted on log-log paper. Shear rate dependency of the whole blood viscosity as a non-Newtonian fluid is apparent and this tendency is more clearly manifested in the lower temperature and higher hematocrit range.

on the same sample with the similar change in condition. The influence of the temperature was remarkable when hematocrit was high and at the same time, the shear rate was low. The sample with hematocrit of 70%, shear rate of 250 sec^{-1}, and temperature of 42°C had viscosity of 6.50 cp. and the same sample showed viscosity of 13.5 cp. at 17°C. And at the shear rate of 1.04 sec^{-1} viscosity increased from 58.2 to 143 cp. as temperature was lowered from 42 to 17°C. These results indicate that the temperature affects the whole blood viscosity but not as markedly as hematocrit or the shear rate does. These was also clearly demonstrated the shear rate dependency, that is, non-Newtonian behavior of whole blood viscosity.

The influence of blood gas on whole blood viscosity was well detected in canine studies (Figs. 11 and 12). Oxygen and CO_2 content were determined just before viscometry. These figures showed a linear correlation that the rise in CO_2 content increased whole blood viscosity, while the O_2 content lowered whole blood viscosity. Whole blood viscosity immediately after CO_2 insufflation was 9.2 cp. with hematocrit of 40%, the shear rate of 31.3 and temperature of 37°C, whereas viscosity of the untreated sample was 6.7 cp. (Fig. 13). This ncreased whole blood viscosity after CO_2 insufflation rapidly decreased within

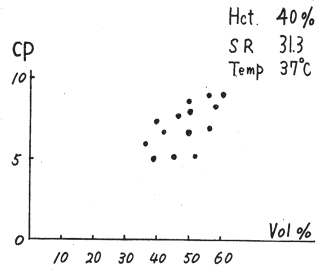


FIG. 11

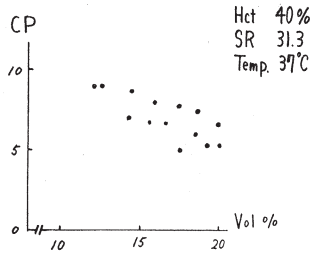
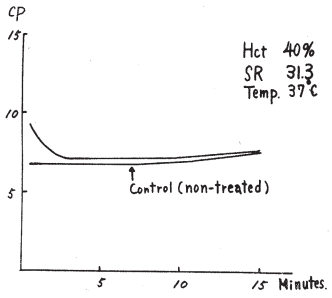


FIG. 12

Effect of CO_2 Content on Whole Blood Viscosity in Dog

FIGS. 11 and 12. Increase of CO_2 content results in a linear increase of the whole blood viscosity. O_2 content had an inverse relationship with whole blood viscosity.



Elapsed Time and Change in Whole Blood Viscosity after CO_2 Insufflation (Mean of 10 samples)

FIG. 13. Increased whole blood viscosity after CO_2 insufflation decreased within 3 minutes to a little higher level than that of untreated samples.

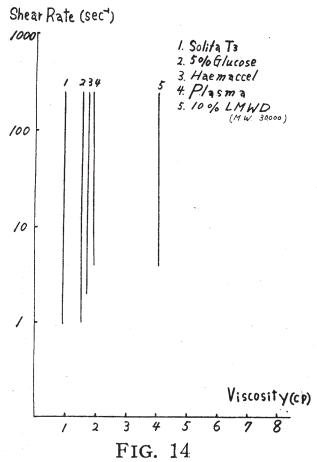
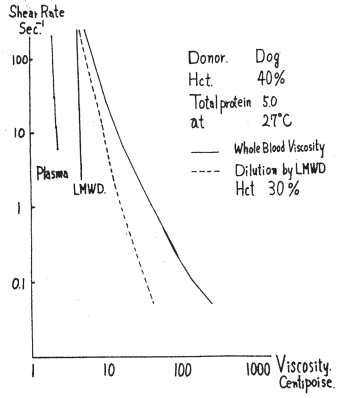


FIG. 14



Effect of LMWD (10% MW 30,000) on Whole Blood Viscosity

FIG. 15

FIG. 14. Viscosity of the Plasma and the Diluent at 37°C.

FIG. 15. Ten percent LMWD had a viscosity over 2.2 fold as high as that of the plasma.

3 minutes to a little higher level than that of untreated sample and then gradually increased again to 7.6 cp. This late increase might be explained by drying up of the blood in the sample cup.

Viscosity of the plasma and the diluents at 37°C was presented in Fig. 14. All the diluents behavior were like that of Newtonian fluid at 37°C. The mean value of the mixed priming solution actually used in the perfusion procedure was 1.53 cp. Except 10% LMWD (mean molecular weight 30.000), the diluents and mixed priming solution showed viscosity lower than that of the plasma. At 37°C, the mean value of the commercially available products like Solita T-3, 5% glucose in water, Haemaccel, and 10% LMWD, and that of the human plasma were 0.95, 1.50, 1.71, 4.00 and 1.80 in centipoise, respectively. Ten percent LMWD had viscosity over twofold as high as that of the plasma, but it made whole blood less viscous by lowering hematocrit and by the plasma expanding effect through the tissue perfusion (Fig. 15).

Part II

The cases were divided into two groups; 13 cyanotic cases and 14 non-cyanotic. Four of 27 cases died postoperatively. Case 13 died of right ventricular dysfunction on the 2nd post-operative day (POD); Case 14 of ventricular fibrillation on the 1st POD; Case 24 of renal shut down on the 5th POD; and Case 26 of myocardial dysfunction on the 1st POD.

Perfusion data were presented in Table 3. The longest perfusion time was 199 minutes and the shortest was 29. Perfusion rate varied from 65.4 to 30.4 ml/kg/min (average: 51.51). The initial priming solution was prepared mainly with 5% glucose in water, 5 ml/kg of 10% LMWD, 7% sodium bicarbonate, 2.9 mEq of K, 2 mg/kg of heparin sodium, and 500 mg of vitamin C. In cases 12, 13, 23, 24, 25, 26 and 27, Solita T-3 was used instead of 5% glucose and in Cases 10 and 18 Haemaccel. In 4 cases, heparinized blood was obviated. Average value of the volume of the diluent and the dilution rate were 37.7 ml/kg and 0.636, respectively, where dilution rate was defined as the lowest hematocrit during total bypass divided by hematocrit before dilution. Viscosity of the mixed diluent was 1.53 cp. and no significant difference was noted between the 5% glucose group and other diluent groups.

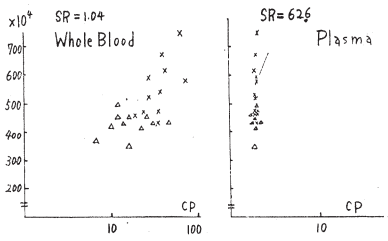
Polycythemia had a dominant influence on whole blood viscosity and little on the plasma viscosity (Fig. 16). In group 1, the whole blood was much viscous than in group 2, but as to the plasma itself there was no difference between two groups. The whole blood with polycythemia (RBC 746×10^4) had viscosity of 64 cp. at the shear rate of 1.04, sec^{-1} and the plasma viscosity was 2.1 cp. at the shear rate of 62.6 sec^{-1}.

The plasma viscosity in man was also affected by temperature (Fig. 17). The human plasma showed viscosity of 1.8 cp. at 37°C and was approximately that of Newtonian fluid characteristics but indicated the shear rate dependency

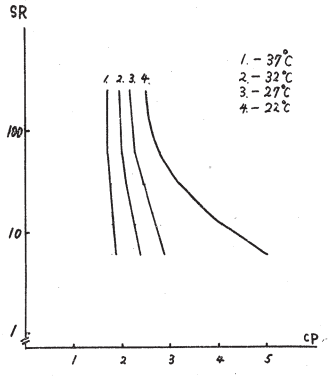
TABLE 3. Perfusion Data

Case No.	B.W. (kg)	Perf. Time (min)	Perf. Rate (cc/kg/min)	Initial Priming (cc)		Diluent (cc/kg)	Dilution Rate	Arterial Pressure (mmHg)	PVR (mmHg/cc/sec)
				Solution	Blood				
1	33.5	114	44.7	1160	600	34.7	0.645	38	1.52
2	20.2	110	59.4	860	800	42.6	0.685	40	2.00
3	47.5	149	52.6	1560	400	32.9	0.566	60	1.44
4	45.9	177	65.4	1770	200	38.6	0.512	50	1.00
5	52.9	148	47.3	1540		29.2	0.686	60	1.44
6	17.7	94	56.5	690	800	39.0	0.586	40	2.40
7	23.4	149	50.5	870	600	37.2	0.666	30	1.50
8	32.4	126	61.7	1150	400	35.5		50	1.50
9	14.7	115	61.2	850	600	57.8	0.617	20	1.33
10	35.2	108	54.2	1170	400	33.3	0.650	55	1.74
11	35.8	189	50.3	1100	500	30.7	0.700	60	2.00
12	40.8	132	39.3	1300	200	31.9	0.712	38	1.43
13	37.0	148	43.3	1200	400	32.5	0.587	65	2.44
14	37.7	198	53.1	1360	200	36.1	0.586	40	1.20
15	17.2	29	58.4	770	750	45.2	0.524		
16	16.8	74	59.6	700	800	41.6			
17	46.6	60	42.8	1520		32.6	0.640	30	0.90
18	13.5	133	59 3	770	600	57.0			
19	43.5	100	46.0	1400	600	32.2	0.787	65	1.95
20	12.5	91	48.1	600	600	48.2	0.708	44	4.40
21	58.8	166	45.9	2120		36.1	0.620	60	1.33
22	34.5	73	43.6	1175	400	34.1	0.666	40	1.60
23	18.5	46	43.3	780	600	42.2	0.602	30	2.25
24	39.1	75	38.4	1280	200	32.8	0.571		
25	13.9	80	43.1	680	600	48.7	0.648	38	3.80
26	41.0	199	43.9	1200	400	29.3	0.631	30	1.00
27	59.2	197	30.4	1480		25.0	0.666	40	1.33

Legend: B.W. body weight. Perf. perfusion. PVR peripheral vascular resistance.



Blood Viscosity and Red Blood Cell
Pre-Perfusion at 37°C
Group 1 × Group 2 △
FIG. 16



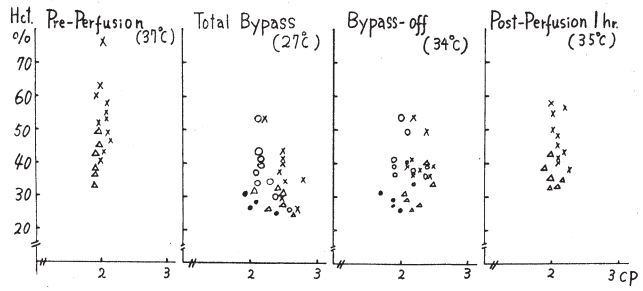
Plasma Viscosity in Man
FIG. 17

FIG. 16. Polycythemia had a dominant influence on whole blood viscosity and little on plasma viscosity.

FIG. 17. Plasma viscosity was also affected by temperature, and indicated shear rate dependency in the hypothermic condition.

in the hypothermic condition. Hypothermic condition, not hemodilution, made a little change in plasma viscosity. At the lowest temperature during total bypass plasma viscosity gave the highest value, and then gradually decreased to the normal level. The difference between group 1 and group 2, and arterio-venous (A-V) difference were not measured, but further investigation will make it clear.

The whole blood viscosity was more dependent on the dilution rate than on the amount of the diluent used [ml/kg], especially at the lower shear rate (Figs. 19 and 20), and there were no significant difference between group 1 and 2.

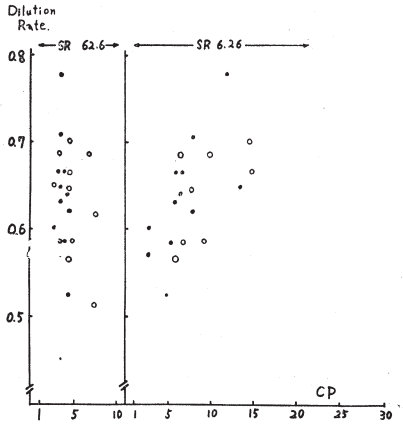


Plasma Viscosity SR=62.6 Arterial Bl. Venous Bl.

Group 1 ○ ×

Group 2 ● △

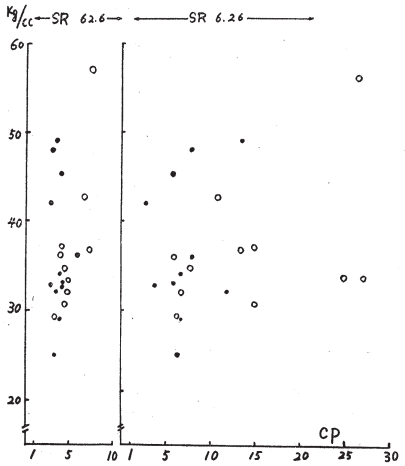
FIG. 18. During total bypass plasma viscosity shows its highest value at the lowest temperature range.



Whole Blood Viscosity and Dilution Rate

Group 1 ○ Group 2 ●

FIG. 19



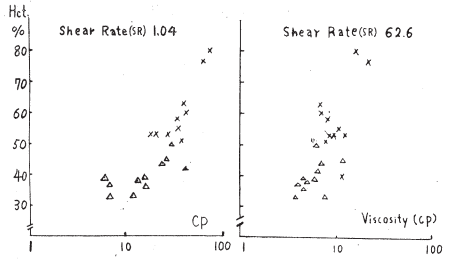
Whole Blood Viscosity and Hemodilution

Group 1 ○ Group 2 ●

FIG 20

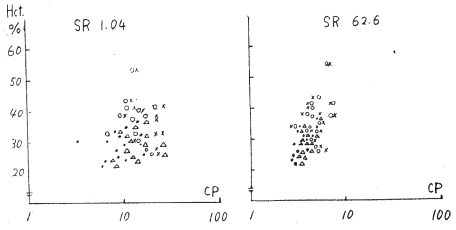
FIGs. 19 and 20. Whole blood viscosity was more dependent on dilution rate than on the amount of diluent, especially at the low shear rate area.

Clinically, hemodilution greatly influenced the whole blood viscosity (Fig. 21, 22, 23, and 24). When the dilution rate was the highest, the whole blood viscosity remained the lowest at the lowest temperature during total bypass. Both in arterial and venous blood, group 1 showed the higher level than group 2. The viscosity of the mixed venous blood was rather greater than that of the oxygenated blood. These findings were remarkable in the low shear rate range.



Whole Blood Viscosity Pre-Perfusion at 37°C
Group 1 ×
Group 2 △

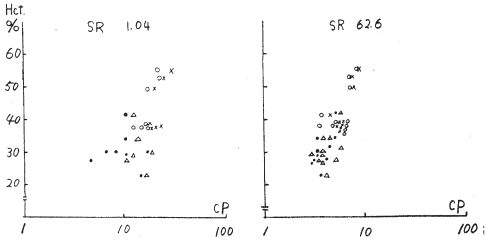
FIG. 21



Whole Blood Viscosity during Total Bypass (27°C)

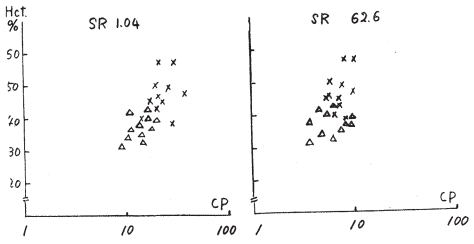
	Arterial Blood	Venos Blood
Group 1	○	×
Group 2	●	△

FIG. 22



Whole Blood Viscosity at the End of Bypass (34°C)
Group 1 ○ ×
Group 2 ● △

FIG. 23



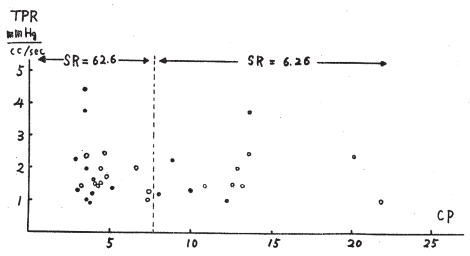
Whole Blood Viscosity one hour after Bypass (35°C)
Group 1 ×
Group 2 △

FIG. 24

FIGs. 21, 22, 23 and 24. During total bypass, the whole blood viscosity was the lowest when the temperature was the lowest. The whole blood viscosity of the Group 1 was higher than that of the Group 2.

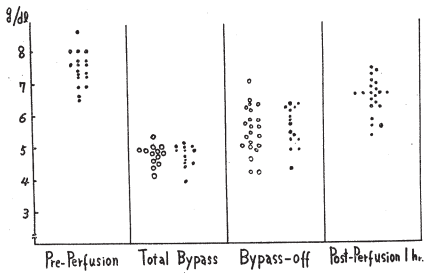
The total peripheral resistance during the total bypass was calculated as mmHg/ml/sec and had no significant effect on the whole blood viscosity (Fig. 25). There was no difference between group 1 and 2.

The plasma protein concentration showed almost no difference between the oxygenated and the mixed venous blood (caval mixture) throughout bypass (Figs. 26, 27, 28, 29 and 30). Generally, albumin, globulin, fibrinogen and total



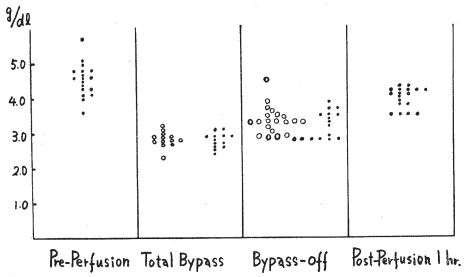
Whole Blood Viscosity and Total Peripheral Resistance during Total Bypass. Group 1 ○ Group 2 ●

FIG. 25. During total bypass, whole blood viscosity had no significant effect on the total peripheral resistance.



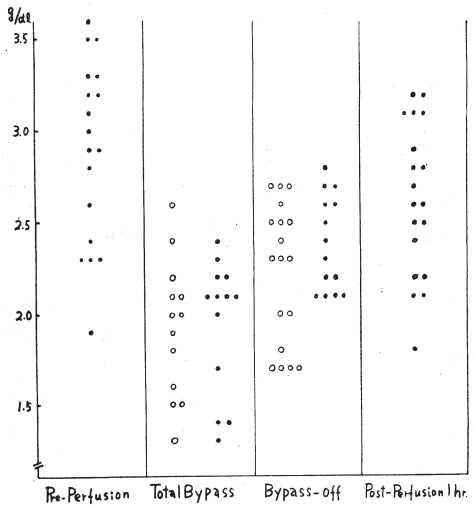
Total Protein oxygenated Blood ○
mixed venous Blood ●

FIG. 26



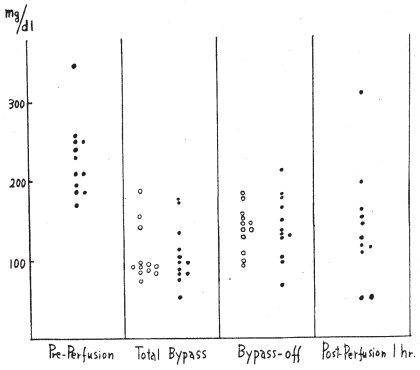
Albumin oxygenated Blood ○
mixed venous Blood ●

FIG. 27



Globulin oxygenated Blood ○
mixed venous Blood ●

FIG. 28



Fibrinogen oxygenated Blood ○
mixed venous Blood ●

FIG. 29

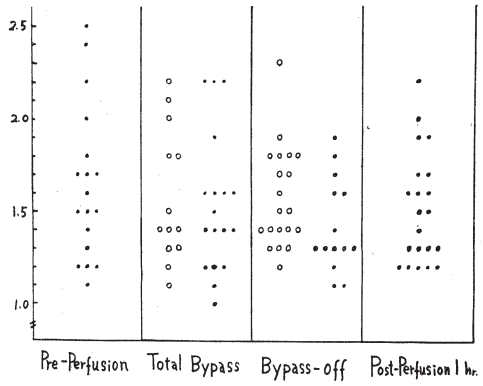


FIG. 30

FIGs. 26, 27, 28, 29 and 30. As to plasma protein concentration, there was no marked difference between oxygenated and mixed venous blood throughout bypass, and remained the lowest during total bypass.

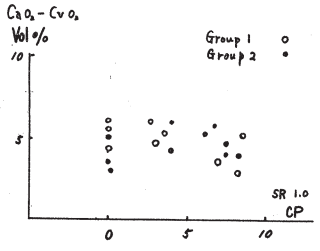


FIG. 31

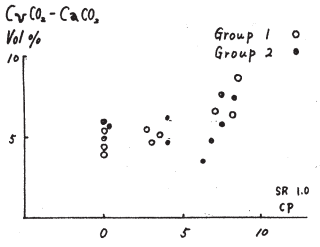


FIG. 32

A-V Difference of O_2 Content and of Whole Blood Viscosity during Bypass (27°C)

FIG. 31 and 32. Arterio-venous differenceof CO_2 content, not O_2 content, had a little effect on A-V difference of whole blood viscosity.

protein remained at the lowest levels during total bypass, and then returned to a little lower level than the pre-perfusion value 1 hour after perfusion. Mean values during total bypass were 2.8 g/dl, 1.9 g/dl, 106 mg/dl and 4.7 g/dl and those after bypass were 3.3 g/dl, 2.3 g/dl, 138 mg/dl and 5.6 g/dl, respectively. Albumin-globulin ratio (A/G) showed no significant change during perfusion.

The relationship between the blood gas and the whole blood viscosity was examined in clinical studies (Figs. 31 and 32). During bypass, the A-V difference of the whole blood viscosity was slightly affected by A-V CO_2 difference, and was not at all by A-V O_2 difference. The arterio venous difference of the whole blood viscosity became more pronounced when perfusion time was prolonged. There was no difference between two groups.

DISCUSSION

Since Wells and his colleagues[23] re-emphasized the flow property of the

blood as a non-Newtonian fluid, the viscometry by means of a capillary viscometer had its own practical limit because of the difficulty in deriving values in the lower shear rate range of below 100 sec^{-1} [31)32)]. First of all, the Poiseuillian equation has as its first condition that the fluid under study in the rigid tube be a Newtonian fluid which maintains a constant ratio of shear stress to shear rate[22)]. The tube radius has a pronounced effect on viscosity due to the Fahraeus Lindqvist effect. When whole blood passes through the narrow tube, accumulation of the blood cell to the central axis occurs, and so called "plasma skimming" is seen along the tube wall. This phenomenon makes it difficult to obtain an accurate estimation of viscosity. The second problem is to calculate the shear rate in a tube. Haynes and Burton[18)] have derived shear rate values for capillary tubes from analysis of the pressure-flow curves of red cell suspension. Virgilio[33)] used an Ostwald-Cannon-Fenske viscosimeter having capillary bores of 0.63 ± 0.02 mm which had the shear rates ranging from 750 to 50 sec^{-1} depending upon the nature of the heparinized sample and the temperature. He stated that the viscosity was relatively independent of shear rates in this range. His explanation was based on the report by Dintenfass. Using a cone in cone viscometer without anticoagulant, Dintenfass considered that the range below the 10 sec^{-1} was of the greatest importance in the determination of the colloidal structures of blood. He demonstrated a critical shear rate of normal blood to be 5 sec^{-1} above which viscosity was independent of further change in shear rate and abnormally thixotropic blood was characterized by the critical rates of shear of 30 to 60 sec^{-1}. And he also stressed that the importance and the extent of the thixotropy was not appreciated due mainly to the use of a capillary viscometer which was unable to permit determinations at the very low shear rate and also due to the use of anticoagulants which decrease and distort the thixotropy. The measurement or derivation of the shear rate below 200 to 100 sec^{-1} for non-Newtonian fluid in capillary tubes requires precise and microscopic measurements of the flow rate and the tube dimension[22)]. The arteriole has a shear rate of about 10 sec^{-1}, therefore, the shear rate of the capillary viscometer is too high to presume peripheral circulation and too complicated to calculate. The third is the pulsatile flow of the blood. It changes the radius of the blood vessel and flow velocity as well. Even with a static condition of the vascular tree, there may exist infinite variability, according to the vessel size. When the pulsatile change in every moment is considered, it becomes much more complicated to clarify viscosity of flowing blood.

The viscometer used in the present study was a cone in cone rotational type which provides sixteen different kinds of shear rate, ranging from 0.05 to 250.2 sec^{-1}. This range of shear rate was well reproducible with whole blood viscosity. However, when the plasma or whole blood of very low hematocrit is on determination, rotation of the internal cone caused by rotation of the

external cone at the revolution velocity of 1.5 rpm. is so slight that the observational error may increase. The above mentioned phenomenon may be due to the plastic characteristic of whole blood and this critical point observed could be intepreted as the yield value. But Gelin[34] stressed that blood behaved like pseudoplastic fluid. Anyway, the measurement of these less viscous samples was carried out at the range from 6.26 to 250.2 sec^{-1}. Supposing that plasma has a dominant function on the hemodynamics in the peripheral circulation, there is room for further improvement of the equipment.

Thixotropy as an isothermal sol-gel transformation, in which system viscositiy is dependent on time and shear rate, was clearly demonstrated by Dintenfass without using anticoagulant in the blood sample[24]. Because all samples were heparinized in this study, it was not adequate to determine the critical shear rate. From the Figs. 5, 6, 7, 8, 9 and 10, however, the flow curves were approximately parallel to the shear rate axis with the shear rate of over 10 sec^{-1} in the samples with lower hematocrit, and when hematocrit was over 40%, the flow curves shifted to the right as the shear rate decreased. Dintenfass summarized that the significance of thixotropy of blood was due to its intimate relationship with the aggregation of red cells and suggested that it was also related to the proneness of the thrombus formation[24)35].

Concerning sampling the donor blood, there are some discussions on the use of anticoagulant. Dintenfass[24] studied the thixotropy of blood and also its proneness to the thrombus formation in the absence of anticoagulants. He stressed that thixotropy was decreased and distorted due to the use of anticoagulants. Wells and Merrill[36] documented that the blood and plasma viscosity was altered by adding heparin of 20 U/ml, but Rand *et al.*[37] have not noted such an effect, employing a smaller amount of 7.5 U/ml. In this study the same dose as Rand *et al*'s was used, because it is effective with minute quantities by which sample is negligibly diluted.

The relationship between the corpuscular concentration and blood viscosity observed in this studies was demonstrated in Fig. 16. As to whole blood viscosity, it was more viscous in the polycythemic group than in the normocythemic group, but there was little difference between two groups in plasma viscosity.

Hemodilution is a reasonable procedure for polycythemic group because it reduces hematocrit, decreases whole blood viscosity, and diminishes the peripheral vascular resistance. Replogle[38], in his canine study, in which polycythemia was experimentally induced, observed an increase in the peripheral vascular resistance and a fall in cardiac output, and he noted that these results were associated with increased hematocrit and increased viscosity; and hemodilution brought about a reduction in the pressure load of the heart. In the present study the dilution rate had a positive correlation to whole blood viscosity at lower shear rate, but hemodilution in ml/kg appeared to have less correlation (Figs. 19 and 20). There were no differences among various

kinds of diluents used for priming. Total peripheral resistance during total bypass and whole blood viscosity had no significant relationship each other, as shown in Fig. 25.

Hypothermic perfusion with hemodilution has two dominant influences on blood viscosity, namely, hematocrit change and low temperature. These factors change the viscosity in more complex fashion, and more over, it is further complicated because of the non-Newtonian behavior of blood.

Shear rate dependency of the whole blood viscosity as a non-Newtonian fluid appeared clearly in Figs. 5, 6, 7, 8, 9 and 10, and this tendency became more conspicuous in the lower temperature and higher hematocrit region. Also the plasma itself showed its shear rate dependency in the lower temperature range. When the shear rate and the temperature were lower, the viscosity rapidly increased when the hematocrit value gradually became higher.

Rand[37] pointed out that a gradual and relatively linear rise in viscosity occurred as shear rates diminished from 212 to 106 sec^{-1} at all hematocrit levels and below this point of 106 sec^{-1} blood thickend dramatically. In the present study the thickening was pronounced in below 50 sec^{-1} shear rate and over 40% hematocrit region. When shear rate is less than 50 sec^{-1} and hematocrit over 40%, the shift of the flow curve to the right became clear. When the relationship between viscosity and the shear rate was checked on the log-log scale, temperature change caused viscosity change in a fixed manner at all hematocrit levels. The flow curve went precipitously downward and ended making a gentle slope. Virgilio[33] pointed out that there is a critical temperature range somewhere between 10 and 20°C, where there appeared to be a disproportionate increase in viscosity with both hemoconcentration and hypothermia, and he speculated that it might be the explanation for the poor capillary flow observed in the absence of intra-vascular aggregation in the same temperature range.

In the human vascular tree, there are innumerable variety of the flow velocity of blood, the diameter of the blood vessel and the shear rate. Under the assumption that the whole blood is one of Newtonian fluids, Wells[22] calculated the shear rate at the wall of the tube using the formula $4V/r$: where V represented the mean velocity in centimeters per second and r represented the radius of the vessel[39]. From his estimation, the aortic blood flow velocity of 35 cm per second and the radius of 1.3 cm would result in the shear rate of 108 sec^{-1}. And if one assumes that an end arteriole varies from 50 to 500 μ in diameter and the flow velocity of 0.11 mm per second[40], then in a vessel which bore is 100 μ from the formula shown above, blood in these arterioles would be subjected to the shear rate of about 10 sec^{-1}. These calculations are very instructive, but not exactly true as to the arterial stream because blood flow is pulsatile in nature, having influence over the flow velocity and the radius of the vessel.

Since blood viscosity is dependent on the shear rate, its variability is infinite according to the variation of shear rates which is usually seen through the aorta to microcirculation and then back to the venae cavae. It is clear that this non-Newtonian behavior of blood is significant in the smaller vessels leading to the capillary bed. At this level, the velocity of flow is much less than in the arteries, and blood viscosity is thought to be increased. However, splendid homeostasis is provided in flow mechanics. Fahraeus-Lindqvist found that with water the size of the tube did not alter the calculated viscosity, however, with blood, this value fell markedly if the diameter of the tube was less than 1 mm[21]. Plasma skimming or axial accumulation of the flowing blood cells plays a part. Along the vessel wall there may exist a zone free from the blood cell and it may result in the reduced resistance to flow. Actually hematocrit value of blood samples drawn from the capillaries is about 25% less than that of whole body blood[41]. From these facts, blood viscosity *in vivo* is much less than the measured viscosity *in vitro*.

During hypothermic-hemodilution perfusion, the effects of hypothermia and hemodilution on the whole blood viscosity is counteracting. A greater influence by the latter rather than the former upon whole blood viscosity is noted, but upon the plasma viscosity it went inversely. While whole blood viscosity was the lowest during total bypass, plasma viscosity remained the highest regardless of the diminished serum protein concentration by dilution. These viscosity changes returned to the subnormal level at the end of bypass.

The plasma viscosity is affected by the plasma protein concentration, especially by fibrinogen[27]. In this study during perfusion, fibrinogen had little effect on plasma viscosity. Wright *et al.*[42] stated that continuous circulation of plasma through the screen oxygenator produced a small, though definite, increase in viscosity, and the increase was less in each of the experiment with the membrane oxygenator than in any other with the screen oxygenator. And the rised viscosity level was accompanied with the increase in turbidity and ultraviolet light absorption. Lee[43] also pointed out plasma protein denaturation by contact with surface-polarizing forces at the blood-gas interface in currently used disc, bubble and screen oxygenators. In his study, oxygenated plasma also increased viscosity. Contrary to above reports, the oxygenated blood was less viscous than the mixed venous blood in the present study. Hematocrit and serum protein were examined both on the mixed venous blood and on the blood oxygenated through the disc oxygenator. There was no significant difference between them. It may be because of the shorter duration of bypass with whole blood or of the influence of the CO_2 content in whole blood. The arterio-venous difference of whole blood viscosity through the disc oxygenator was due in some cases to insufflation with a gas mixture of 98% O_2 and 2% CO_2. If the concentration of CO_2 in this gas-mixture became greater, A-V difference of whole blood viscosity whould be increased. There was no clear

relationship between the A-V difference both in gas content and in whole blood viscosity (Figs. 31 and 32), this may well indicate that the ratio of gas mixture is adequate.

Plasma viscosity was 1.8 cp. at 37°C and showed Newtonian-like behavior at the same temperature. In the hypothermic condition plasma demonstrated shear rate dependency. These findings are very important in speculating peripheral circulation where the shear rate is low and the axial accumulation occurrs. The high plasma viscoisty, in case of tissue perfusion, would result in hypoxia.

Although LMWD was 2.2 times as viscous as plasma, it plays as a flow improver by expanding plasma volume, lowering hematocrit and increasing the negative charge of the red cell surface, thereby decreasing the aggregation tendency.

Koeppe[44] and Welsh[45] demonstrated that exposure of blood to CO_2 results in a marked increase in viscosity. Burch[46] confirmed this with a Brookfield synchroelectric viscometer at the shear rates of 23, 46, 115 and 230 sec^{-1}. There was a statistically significant increase in blood viscosity at the shear rates of 23 and 43 sec^{-1} and an increase in blood viscosity was not completely reversible. And plasma viscosity was indifferent to the change in whole blood viscosity. Although the blood gas content was measured just before viscometry, the blood began to lose CO_2 as soon as it is placed in the viscometer. Rand[37] noticed a decrease in viscosity on the majority of the fresh samples during the first few minutes of measurement and it was independent of the shear rate, hematocrit, or temperature gradient. In the present study increased whole blood viscosity after CO_2 insufflation decreased rapidly within 3 minutes (Fig. 13). This phenomenon may be expalined as below. If venous blood is exposed to room air during viscometry, the CO_2 content will be reduced to that of room air and, thus, viscosity will be decreased.

As Burch pointed out, the technical difficulty from this respect was encountered in the study. But in the hypoxic condition or in the areas of the peripheral ischemia, the CO_2 content of blood becomes well beyond the physiologic range. This high CO_2 content produces an increase in whole blood viscosity, and increased whole blood viscosity which is shear-dependent make blood flow velocity slower especially in the periphery, because the diameter of the blood vessel becomes smaller and it results in lower shear rate. Thus increased blood viscosity promotes to produce higher CO_2 content in the circulating blood in the impaired peripheral flow. Through this process it forms a vicious cycle which ultimately leads to stasis or slugging of the blood stream[46].

On the clinical study, the A-V difference of whole blood viscosity was not remarkable. This means that the pump-oxygenator system has been adequately, operated keeping CO_2 content within the normal range.

The reason why exposure of whole blood to CO_2 caused an increase in viscosity is obscure. Carbon dioxide produced in the tissue comes into circulating blood to combine with hemoglobin by the difference of the CO_2 tension. When HCO_3^- in the red blood cell increases, a chloride shift from plasma into the red cell occurs as shown by the Donnan's equation. The swelling of the red blood cell may be found because of the difference of the osmotic pressure. The swelling makes hematocrit rise and then causes increased viscosity. Scribner[47] observed that the rise in the hematocrit values in acidotic dogs is completed within 10 minutes in his experimental study in which respiratory acidosis was induced by inhalation of a gas mixture of 30% CO_2 and 70% O_2 through a tight-fitting intratracheal catheter. The assumption that increased viscosity is caused by a chloride shift was not proved in the study. There was no difference in hematocrit between the oxygenated and venous blood. Burch suggested that irreversible structural changes must have occurred in the red blood cells. Dintenfass[48] concluded that the interior of the red cells is a dynamic system, viscosity of which is not constant but varies depending on the external conditions. The effect of the blood gas content as an external condition may have a great influence on the thixotropic interior of the red cells.

SUMMARY AND CONCLUSION

Basic problems in the hypothermic hemodilution perfusion were considered from the rheological stand point using a cone in cone viscometer.

In experimental study it was well demonstrated the shear rate dependency, that is, non-Newtonian behavior, of whole blood viscosity at all hematocrit levels. The influence of the temperature change was clearly seen when hematocrit was high and the shear rate was low.

The increase of the CO_2 content produced a linear increase in whole blood viscosity, and the O_2 content had a contrary effect on whole blood viscosity. This increased whole blood viscosity after CO_2 insufflation rapidly decreased, within 3 minutes, to a little higher level than that of the untreated samples and then gradually increased.

Viscosity of the plasma was 1.8 cp. at 37°C and showed a Newtonian-like behavior at the same temperature. In the hypothermic condition the plasma demonstrated a shear rate dependency. These findings are very important in speculating the peripheral circulation where the shear rate is low and an axial accumulation of the blood cell occurs.

Except 10% low molecular weight dextran solution (mean molecular weight 30,000), the diluents and the mixed priming solution showed viscosity below that of the plasma. Viscosity of 10% LMWD was more than 2.2 fold as high as that of the plasma.

Clinical data were obtained from 27 patients who underwent hypothermic

hemodilution perfusion for the correction of cardiovascular defects at the Nagoya University Hospital.

In the cyanotic group whole blood was much viscous than whole blood of the non-cyanotic patient.

During hypothermic hemodilution perfusion, the effects of hypothermia and hemodilution on whole blood viscosity are counteracted. The latter influences whole blood viscosity much more than the former, but an inverse relation is present to plasma viscosity. While whole blood viscosity was the lowest during total bypass, plasma viscosity remained the highest, in spite of the diminished serum protein value by dilution. These viscosity changes returned to the subnormal level at the end of bypass.

Whole blood viscosity was more dependent on the dilution rate than the amount of diluent in ml/kg.

As to whole blood, mixed venous blood viscosity was a little higher than that of the oxygenated blood and this tendency was more pronounced at the low shear rates.

Total peripheral resistance during total bypass had no significant effect on whole blood viscosity.

The plasma protein concentration remained the lowest during total bypass and showed no marked difference between the oxygenated blood and the mixed venous blood.

The arterio-venous difference of the CO_2 content, not of the O_2 content, had a little effect on the A-V difference of whole blood viscosity.

Merits and demerits of the cone in cone viscometer as a rotational viscometer were described, comparing with the capillary viscometer. The viscometer used in the present study provids sixteen different grades of shear rate, ranging from 0.05 to 250.2 sec^{-1}. The shear rates within above range were well reproducible on whole blood. However, when plasma or whole blood with very low hematocrit was examined, there was room for further improvement of the equipment.

The relation between the peripheral circulation and blood viscosity was also discussed. Plasma viscosity and blood gas content are important factors in tissue perfusion. When the carbon dioxide in blood increases abnormally, blood viscosity increases and aggravates the impaired peripheral flow, which results in the higher CO_2 content in the circulating blood, subsequently. This vicious cycle ultimately leads to stasis or slugging of the blood circulation.

The results obtained from the present study using the cone in cone viscometer well indicates that the routine with the pump oxygenator at this clinic is proper and adequate.

ACKNOWLEDGEMENT

The author wishes to express his grateful acknowledgement to Professor Dr. Yoshio Hashimoto for his kind guidance and advice. Also expresses his thanks to Assistant Prof. Dr. I. Fukukei and to Lect. Dr. Y. Iyomasa.

REFERENCES

1) DeWall, R. A. and Lillehei, C. W., Simplified total body perfusion, *J.A.M.A.*, **179**, 430, 1962.
2) Gibbon, J. H. Jr., Artificial maintenance of circulation during experimental occlusion of pulmonary artery, *Arch. Surg.*, **34**, 1105, 1937.
3) Dennis, C., Spreng, D. S. Jr., Nelson, G. E., Karlson, K. E., Nelson, R. M., Thomas, J. V., Eder, W. P. and Varco, R. L., Development of a pump oxygenator to replace the heart and lungs; An apparatus applicable to human patients and application to one case, *Ann. Surg.*, **134**, 709, 1951.
4) Gibbon, J. H. Jr., Application of a mechanical heart and lung apparatus to cardiac surgery, *Minnesota Med.*, **37**, 171, 1954.
5) Crafoad, C., Some aspects of the development of intrathoracic surgery, *Surg. Gynec. Obstet.*, **89**, 523, 1955.
6) Lillehei, C. W., Cohen, M., Warden, H. W. and Varco, R. L., The direct vision intracardiac correction of congenital anomalies by controlled circulation, *Surgery*, **38**, 11, 1955.
7) Kirklin, J. W., Dushane, W., Patrick, R. T., Donald, D. E., Hetzel, P. S., Harshberger, H. G. and Wood, E. H., Intracardiac surgery with the aid of a mechnical pump oxygenator system (Gibbon type): Report of eight cases, *Proc. Mayo Clin.*, **30**, 211, 1955.
8) Bigelow, W. G., Callaghan, J. C. and Hopps, J. A., General hypothermia for experimental intracardiac surgery, *Ann. Surg.*, **132**, 531, 1950.
9) Swan, H., Zeavin, I., Holmes, J. H. and Montgomery, V., Cessation of circulation in general hypothermia. I. Physiologic changes and their control, *Ann. Surg.*, **138**, 360, 1953.
10) Lewis, F. J. and Taufic, M., Closure of atrial septal defects with the aid of hypothermia: Experimental accomplishments and the report of one successful case, *Surgery*, **33**, 52, 1953.
11) Ross, D. N., Venous cooling. A new method of cooling the blood stream, *Lancet*, **1**, 1108, 1954.
12) Brock, R. and Ross, D. N., Hypothermia III. The clinical application of hypothermic techniques, *Guy. Hosp. Rep.*, **104**, 99, 1955.
13) Brown, I. W. Jr., Smith, W. W. and Emmons, W. O., An efficient blood heat exchanger for use with extracorporeal circulation, *Surgery*, **44**, 372, 1958.
14) Neptune, W. B., Bougas, J. A. and Panico, F. G., Open-heart surgery without the need for donor blood priming in the pump oxygenator, *New Eng. J. Med.*, **263**, 111, 1960.
15) Zuhdi, N., McCollough, B., Carey, J and Krieger, C., Hypothermic perfusion for open-heart surgical procedures, *J. Int. Coll. Surg.*, **35**, 319, 1961.
16) Long, D. M. Jr., Sanchez, L., Varco, R. L. and Lillehei, C. W., The use of low molecular weight dextran and serum albumin as plasma expanders in extracorporeal circulation, *Surgery*, **50**, 12, 1961.
17) Poiseuille, M., Recherches experimentales sur le mouvement des liquids dans les tubes de tres petits diametres, *Des Seances de L'Academie des Sciences*, **11**, 961, 1841.
18) Haynes, R. H. and Burton, A. C., Role of non-Newtonian behavior of blood in hemodynamics, *Amer. J. Physiol.*, **197**, 943, 1959.

19) Whittaker, S. R. F. and Winton, F. R., The apparent viscosity of blood flowing in the isolated hind limb of the dog and its variation with corpuscular concentration, *J. Physiol.* (*London*), **78**, 339, 1933.
20) Fahraeus, R. and Lindqvist, T., The viscosity of the blood in narrow capillary tubes, *Amer. J. Physiol.*, **96**, 462, 1931.
21) Dix, F. J. and Scott-Blair, G. W., On the flow of suspension through narrow tubes, *J. Appl. Phys.*, **11**, 574, 1940.
22) Wells, R. E. Jr. and Merrill, E. W., Influence of flow properties of blood upon viscosity-hematocrit relationships, *J. Clin. Invest.*, **41**, 1591, 1962.
23) Wells, R. E. Jr., Denton, R. and Merrill, E. W., Measurement of viscosity of biologic fluids by cone plate viscometer, *J. Lab. Clin. Med.*, **57**, 646, 1961.
24) Dintenfass, L., Thixotropy of blood and proneness to thrombus formation, *Circ. Res.*, **11**, 233, 1961.
25) Yamaguchi, T. An experimental study on hypothermic cardiopulmonary bypass with hemodilution technique, *Nagoya J. Med. Sci.*, **30**, 129, 1967.
26) Nakai, T., *J. Nagoya Med. Ass.*, **90**, 285, 1967 (in Japanese).
27) Yamaguchi, T., Rheological studies on peripheral circulation especially on the blood viscosity, *J. Nagoya Med. Ass.*, **90**, 179, 1967 (in Japanese).
28) Iino, S., Experimental and clinical studies on peripheral circulation with rheological consideration, *J. Nagoya Med. Ass.*, **90**, 2, 1967 (in Japanese).
29) Kanai, I., Rinsho-Kensa-Ho-Teiyo. Kanehara-Shoten. Tokyo, 1964. VII-19 (in Japanese).
30) Van Slyke, D. D. and Neill, J. M., The determination of gases in blood and other solutions by vacuum extraction and manometric measurement, *J. Biol. Chem.*, **61**, 523, 1924.
31) Merrill, E. W., Basicproblems in the viscometry of non-Newtonian fluids, *ISA Journal* (*Instrument Soc. of America*), **2**, 462, 1955.
32) Merrill, E. W. and Wells, R. E. Jr., Flow properties of biological fluids, *Appl. Mech. Rev.*, **14**, 663, 1961.
33) Virgilio, R. W., Long, D. M., Mundth, E. D. and McClenathan, J. E., The effect of temperature and hematocrit on the viscosity of blood, *Surgery*, **55**, 825, 1964.
34) Gelin, L. E., Rheology: theoretic background for clinicians, *Medical postgrad.*, **3**, 1, 1965.
35) Dintenfass, L., Viscosity and clotting of blood in venous thrombosis and coronary occlusions, *Circ. Res.*, **14**, 1, 1964.
36) Wells, R. E. and Merrill, E., Shear rate dependence of the viscosity of whole blood and plasma, *Science*, **133**, 763, 1961.
37) Rand, P. W., Lacombe, E., Hunt, H. E. and Austin, W. H., Viscosity of normal human blood under normothermic and hypothermic conditions, *J. Appl. Physiol.*, **19**, 117, 1964.
38) Replogle, R. L., Kundelr, H. and Gross, R. E., Studies on the hemodynamic importance of blood viscosity, *J. Thoracic Cardiovas. Surg.*, **50**, 658, 1965.
39) Wilkinson, W. L., Non-Newtonian fluids: Fluid mechanics, mixing and heat transfer, *New York Pergamon Press.*, p. 34, 1960.
40) Lee, R. E., Anatomical and physiological aspects of the capillary bed in bulbar conjunctiva of man in health and disease, *Angiology*, **6**, 369, 1955.
41) Gibson, J. C., Seligman, A. M., Peacock, W. C., Aub, J. C., Fine, J. and Evans, R. D., Distribution of red cells and plasma in large and minute vessels of normal dog, determined by radioaktive isotopes of iron and iodine, *J. Clin. Invest.*, **25**, 848, 1946.
42) Wright, E. S., Sarkozy, E., Harpur, E. R., Dobell, A. R. C. and Murphy, E. R., Plasma protein denaturation in extracorporealcir culation, *J. Thoracic Cardiovas. Surg.*, **44**, 550, 1962.
43) Lee, W. H. Jr., Krumhaar, D., Fonkalsrud, E. W., Schjeide, O. A. and Maloney, J. V.

Jr., Denaturation of plasma proteins as a cause of morbidity and death after intracardiac operations, *Surgery*, **50**, 29, 1961.
44) Koeppe, H., Der osmotische Druck als Ursache des Stoffaustausches zwischen roten Blutk orperchen und Salzlosungen, *Arch. Ges. Physiol.*, **67**, 189, 1897.
45) Welsh, W. H., Viscosity of the blood, *Heart*, **3**, 118, 1911.
46) Burch, G. E. and DePasquale, N. P., The effect of CO_2 on the viscosity of whole blood, *Archiv Kreislaufforsch.*, **46**, 161, 1964.
47) Scribner, B. H., Smith, K. F. and Burnell, J. M., The effect of acute respiratory acidosis on the internal equilibrium of potassium, *J. Clin. Invest.*, **34**, 1276, 1955.
48) Dintenfass, L., Considerations of the internal viscosity of red cells and its effect on the viscosity of whole blood, *Angiology*, **13**, 333, 1962.