Erythrocyte flexibility and blood rheology

by

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ABSTRACT

Investigations have been undertaken into the interaction between different physical parameters involved in blood rheology. Previous investigations into the role of erythrocyte deformability, in blood rheology, have not always given consistent results. A systematic study has been made, using the centrifuge technique of measuring erythrocyte packing rate as an index of flexibility, to resolve this problem. The rheological parameters of the erythrocyte shape factor, flexibility factor and intrinsic viscosity, measured viscometrically, have been shown to behave in a manner consistent with the erythrocyte flexibility data, confirming the validity of the centrifuge technique.

The effect of altering erythrocyte flexibility on blood viscosity at low shear rates, revealed that as the erythrocyte flexibility is reduced, the viscosity of blood initially falls, during an intermediate phase. As the cells become rigid the blood viscosity increases again, comparable to the steady increase observed at high shear rates. The decrease in flexibility, which should increase viscosity, is initially offset at low shear-rates by a decrease in aggregation. The decrease in viscosity at the intermediate range also involves a change in the shape factor with aggregate size.

Investigations into the effect of tonicity and pH on blood rheology, revealed that hyper and hypo tonicity as well as acidity reduce the erythrocyte flexibility, whereas alkalinity increases it. This is thought to be associated with changes in the erythrocyte membrane. The mechanisms of this effect is not understood.

The blood rheology of man was compared to other species, namely horse, sheep, cattle, camel and goat. The differences in rheological parameters and their interactions in the different species are explained, and are consistent with the rate of oxygen exchange in blood for each specie.

During my investigations it was found that there is a factor in horse plasma which can increase human erythrocyte flexibility by at least five times. Although fractionation of this protein is difficult, commercially available plasma globulins were used to give an indication of the nature of this protein.

A limited survey of human diseases and their effect on erythrocyte flexibility was carried out. Also the effect of **prostacyclin (PGI_2)** on erythocyte flexibility was investigated. Clinical studies of the effect of Dapsone (in the treatment of Dermititis Herpitiformis) and Warfarin (in the treatment of horse Navicular Joint Disease) on blood rheological parameters were carried out.

Finally, an investigation was carried out into the effect of anaesthetics and high-pressure on the erythrocyte flexibility. A small high-pressure chamber was designed and built, which fitted onto the centrifuge. No effect on flexibility was observed for anaesthetics and high-pressure, nor evidence of rheological involvement in the high-pressure reversal of the anesthetic effect. This confirms doubts regarding some theories of anaesthesia, and points to others as a more likely explanation.

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LIST OF ABBREVIATIONS

RBC	Red Blood Cell/s
Φ	Fibrinogen/Fibrinogen Concentration
FLEX.	Flexibility
H	Haematocrit
γ	Shear Rate
τ	Shear Stress
T	Temperature/Taylor's Factor
η_B	Whole Blood Viscosity
η_p	Plasma Viscosity
η_r	Relative Viscosity
ρ_c	Red Cells Density
ρ_p	Plasma Density
K	(Red Cell) Shape Factor
T'_c	(Red Cell) Flexibility Factor
KT'_c	Intrinsic Viscosity
R-L	Ringer-Locke Solution

UNITS

THE UNITS USED IN THIS THESIS ARE cgs . THE CONVERSION TABLE IS GIVEN BELOW

QUANTITY	SI UNITS	cgs UNITS	RATIO cgs/SI
Pressure or Stress	pascal (Pa)	$dyn.cm.^{-2}$	10^{-1}
Force	newton (N)	dyne (dyn)	10^{-5}
Viscosity	$kg.m.^{-1}s^{-1}$	poise (P)	10^{-1}
Density	$kg.m.^{-3}$	$g.cm.^{-3}$	10^{3}
Area	$m.^{2}$	$cm.^{2}$	10^{-4}
Volume	$m.^{3}$	$cm.^{3}$	10^{-6}

CHAPTER ONE

INTRODUCTION

INTRODUCTION

Blood and its circulation in the human body was studied by the Greek, Roman and Arab civilizations many centuries ago. It was known then that the blood circulation was divided into major and minor parts. The major part included the arteries, arterioles and veins, but the minor part was explained to be the link between the arteries (and arteriols) and the veins. It wasn't until the early 16th century that capillaries were implied to exist, from the study of Michael Servitus of the pulmonary circulation. The discovery of capillaries as the 'porosities of the flesh' through which the blood must flow from the arteries to the veins, was made by William Harvey (1628). However, the first direct observation of capillaries was made by Malpighi (1661).

Blood pressure was first measured by Hales (1733), who also estimated the flow and resistance in different parts of the circulation. He realised that the greatest resistance to flow was in the vessels of the circulatory part, now known as the microcirculation, and that resistance increases with length and decreases in diameter of these vessels. However, the first precise measurements of pressure drop in fine glass capillary tubes, used as a model for blood vessels, were obtained by Poiseuille (1840). The equation known by his name was actually formulated by Franz Neumann and Edward Hagenback in 1858, Schiller (1933). The Poiseuille equation relates flow, Q, and viscosity of the flowing fluid, η , to the length, L, diameter, D, and pressure drop, ΔP, across

the capillary tube by the following expression:

$$Q = \Delta P \frac{D^4}{128 \eta L}$$

Although the original intention behind the Poiseuille equation was to describe the flow of blood through a tube, the use of a simple fluid system does not take into account the unusual flow properties of blood, due to the high, particular content of red blood cells. The difference between the bulk viscosity of a simple fluid and blood was first confirmed by Leeuwenhock (1688), but systematic consideration of capillary blood flow as a suspension containing discrete cells was undertaken much later, in the 1960s, by Prothero and Burton (1961; 1962 a and b).

The effect of vessel diameter on the blood viscosity was discovered by Fahraeus and Lindqvist (1931). They found that the apparent viscosity of blood decreases with the tube diameter for vessels below about 300 μm in diameter. This phenomenon has been explained in two ways. The first is, that the layer nearest the vessels wall contains fewer cells than are in the bulk of the blood, and as the vessel diameter decreases, the viscosity of this layer becomes more significant. The second is, as in the case of a colloidal suspension, Dix and Scott-Blair (1940), the cells in small tubes represent a series of discrete velocity steps, the sigma effect, and the representation of the velocity profile as a continuous curve is no longer valid. Overall blood

viscosity is affected by factors such as flow rate, haematocrit, plasma viscosity, protein concentration and tube or vessel bore. Since the diameter of capillaries is less than that of red cells, the red cells have to "squeeze" through the capillaries, and therefore viscosity is also dependent on red cell deformability or flexibility. The extension of the Fahraeus-Linqvist effect to vessel diameters of the order of red blood cell size, has been carried out by several workers in recent years.

Fahraeus (1921 and 1929), using a capillary viscometer, observed that changes in blood viscosity, plasma protein concentration, red cell sedimentation rate and red cell aggregation occur in several diseases. Similar changes, as well as changes in the red cell flexibility, were observed by other workers, Harris et al (1956) and Dintenfass (1971).

With the introduction of rotational viscometers in the 1960s, precise and reproducible measurements of blood viscosity were carried out, Wells (1961). It was found that the viscosity of whole blood or red cell suspensions, and to a lesser degree, plasma, exhibited non-Newtonian characteristics at low shear rates (below $100s^{-1}$). That is, the ratio of shear stress to shear rate, i.e. viscosity, was not constant for a given temperature. Indeed, it was found that as the shear rate was reduced below $100s^{-1}$, the viscosity increased rapidly, in a non-linear fashion. This non-Newtonian characteristic was called 'pseudoplastic' or 'shear thinning'.

The non-Newtonian behaviour of blood at low shear rates is believed to be due to red cell flexibility and aggregation. When red cells are hardened with acetaldehyde, and become inflexible, the blood viscosity is increased from about 4.8 cP, for normal blood, to approximately 10 cP. This high viscosity also becomes independent of shear rate variation, i.e. behaves as a Newtonian fluid, Chien et al (1967a). For normal red cells, the higher viscosity at low shear rates, or slow flow-rates, is due to the energy utilized in flexing the cells and breaking the weak interactive force forming rouleaux and aggregates. Fibrinogen has been suggested to be involved in rouleaux formation, by adsorption onto the red cell membranes, forming a bridge between them, Chein and Jan (1973), more rouleaux being formed at higher concentrations of plasma fibrinogen. Rouleaux do not, however, form with inflexible cells, even at high plasma fibrinogen concentrations, and therefore rouleaux formation depends mainly on the red cell flexibility. For normal red cells, Rampling and Sirs (1972) suggested that fibrinogen acts by increasing the flexibility of normal red cells:

Due to the importance of red cell flexibility, or deformability, in understanding the factors of blood rheology which govern the value of blood viscosity, several methods were devised to measure this parameter. Rand and Burton (1964) measured the erythrocyte membrane stiffness by measuring the pressure needed to force a single red cell through a narrow capillary tube of diameter smaller than that of a red cell (less than 8 μm). Prothero and Burton (1961) devised a method where red cells were filtered

through a Millipore filter and the flow rate of red cell suspension at a given positive pressure was used as a measure of red cell deformability. This technique suffered from the problem of obtaining consistent results due to the lack of uniformity of those types of filters. This problem was overcome by the introduction of polycarbonate Nuclepore filters, Gregersen et al (1967), but recently this procedure has had to be modified because of the large contribution of white blood cells, Kiswetter et al (1981). The third main method was the centrifugation technique of measuring red cell flexibility of Sirs (1970), used in this thesis. This method measures the packing rate of a column of red cells under centrifugal forces of about 200g, equivalent to the force experience by the red cell, during its passage through a capillary in the microcirculation, due to the difference in pressure across the capillary.

Results of investigations, by several workers, using the above-mentioned three main methods of assessing the red cell deformability or flexibility, have shown that blood viscosity depends on several interacting factors. These factors are mainly: plasma viscosity, plasma protein concentration, red cell deformability or flexibility, shear rate, red cell shape and red cell concentration (haematocrit). Other factors such as red cell mean corpuscular volume (MCV), temperature, blood pH and tonicity, were also found to affect blood flow. However, no consistent systematic, quantitative appraisal has been carried out to inter-relate the various factors, mentioned above, which govern the rheology of blood. The various

investigations reported are too often in contradiction or lacking substantiation of their conclusions. Many of the problems behind the contradiction found in the literature are due to the lack of standardisation of experimental techniques and procedures, or appreciation of the physical basis behind the measuring techniques used.

The main intentions behind the investigations I have carried out in this thesis, were to try and link the different factors governing blood rheology, so as to give a clearer picture of their interactions, and explain the overall behaviour. In doing so I have utilized some experimental techniques in preference to others, for reasons explained in the relevant chapters. One factor above all else, permanently kept in mind, throughout my work, was to try and work with blood, in-vitro, keeping it as close as possible to its physiological state. This avoids the problems, in which many previous investigators have found themselves, of having an incomplete data of the inter-relation between the different factors in blood rheology, particularly when suspending red cells in buffer solutions. Results obtained for blood kept close to its physiological state can then be used to compare with in-vivo observation in health and disease.

In the first part of this thesis I have dealt with the inter-relation of the different rheological factors of normal human blood. This was then used to compare the rheology of human blood with the bloods of different species in a following section. I have also investigated the effect

of different human and animal (horse) plasma proteins on the erythrocyte flexibility. The erythrocyte flexibility was measured using a modified form of the centrifuge technique developed by Sirs (1970).

The second part of this thesis deals with clinical studies. The changes in blood rheology due to disease and certain drugs, used on man and horses, are investigated. Lastly, the effect of anaesthetics and high pressure on erythrocyte flexibility, and the phenomenon of reversal of the anaesthetic effect under high pressure, and its effect on erythrocyte flexibility are investigated. A review is made of the historical origins and nature of each aspect of blood rheology at the beginning of each section.

CHAPTER 2

METHODOLOGY

I THE RBC PACKING RATE CENTRIFUGE

(i) Introduction

Two techniques for measuring red blood cell packing rate have so far been used. The stop-start method, Sirs (1968), suffers from the disadvantages that recording was not continuous; and neither the speed nor time of rotation were accurately controlled, although several samples could be processed simultaneously. The automatic recording centrifuge method, Sirs (1970), enabled accurate control of both speed and time of rotation, as well as giving a continuous record of the packing of the red cell column with time. This method however, suffered from one disadvantage, and that is that only one sample could be handled at a time, making a survey or processing of several samples, a time consuming task.

An improvement on the automatic recording technique developed by Sirs, but based on the same principle, is a stroboscopic recording centrifuge, Amin et al (1983). In this technique up to 24 samples can be measured simultaneously and the speed and time of rotation can be accurately controlled.

(ii) Centrifuge Design

In designing the centrifuge, several factors had to be taken into consideration. They were:

1. The centrifugal force had to be in the region of 200g to equal the physiological pressure experienced on the red cell during flow through the capillaries.
2. The size of the centrifuge had to be kept to a minimum.
3. The centrifuge had to 'run-up' to speed in the shortest possible time.
4. The centrifuge driving motor has to have a constant speed and enough power to drive the centrifuge top.
5. Samples placed on the centrifuge should be visible for photographic recording.
6. The centrifuge environment must be thermally controlled.
7. The centrifuge must be synchronized to a stroboscope to give a stationary image for the photographic recording.
8. The cost of the centrifuge should be kept to a minimum so as to be economically viable.
9. The apparatus should be easy to operate and reproducable in results.

Early attempts in designing the centrifuge utilized a standard Hawkesley Microhaematocrit Centrifuge. To reduce the centrifuge rpm to the required g force of about 200g (in the range 1500 to 2000 rpm), the input voltage to the centrifuge was reduced by means of a variac resistor. This reduced the motor speed but it was not possible to maintain it at a constant level for any period of time. Typical values of speed variation with time were:

TIME (sec)	5	25	40	50	65	100	120	160
RPM	1730	1500	1290	2000	1090	1000	1125	1075

Clearly the high speed motor in the Hawksley centrifuge cannot give a constant rotational frequency at voltages other than the mains voltage, for which it was built. This method had to be abandoned.

A second attempt was to reduce the centrifuge rpm using weights attached to a perspex cover of the duraluminium head which will reduce the motor's inertia, while it operated at the mains voltage. This method proved inadequate because again constant speed was not possible to achieve.

Finally it was decided that it would be necessary to build a centrifuge using the duraluminium head from a Hawksley Microhaematocrit Centrifuge driven by a synchronous motor with sufficient horse power to maintain a constant speed.

It was not possible to obtain a reasonably sized commercial synchronous motor of enough power, $\frac{1}{4}$ horse-power, and at a reasonable price. So it was decided to use an available squirrel-cage GEC $\frac{1}{4}$ horse-power motor which provided a constant 1480 rpm, reaching this speed in 3 seconds from switching on while driving the centrifuge top.

To achieve a thermally controlled environment, the motor driven centrifuge top was encased in a wooden box, see Fig (1), and the top was isolated from the motor by a partition to avoid the heat from the motor altering the centrifuge head's environment. Thermal heating of the centrifuge top compartment was achieved by means of a 50 watt blow heater (H.J. Latham Ltd.) whose heating element was connected in series with a variac resistor to give temperature control up to $50^{o} \pm 1^{o}C$. The motor was cooled by an electric fan in the lower motor compartment.

The centrifuge head cover and the box top were made of perspex to enable a photographic record to be made of the samples while being centrifuged.

Underneath the centrifuge head a magnet, M, and a counterweight were attached. The magnet was used to operate the two reed switches, R_S and R_C, placed under the centrifuge head and fixed to the box partition surface. The two switches were positioned 1/3 to 1/2 of a revolution from each other in the path of the magnet. One of these switches, R_S, is used to synchronise a stroboscope (Strobe Automation Ltd.) and the second switch, R_C, is used to synchronise a standard 35 mm reflex camera (Olympus OM10), as shown in Fig (2). The electronic timer controls the camera single shot winding motor and fires it to take one shot every 30 seconds. Plate (1) shows the actual apparatus as used in the Laboratory.

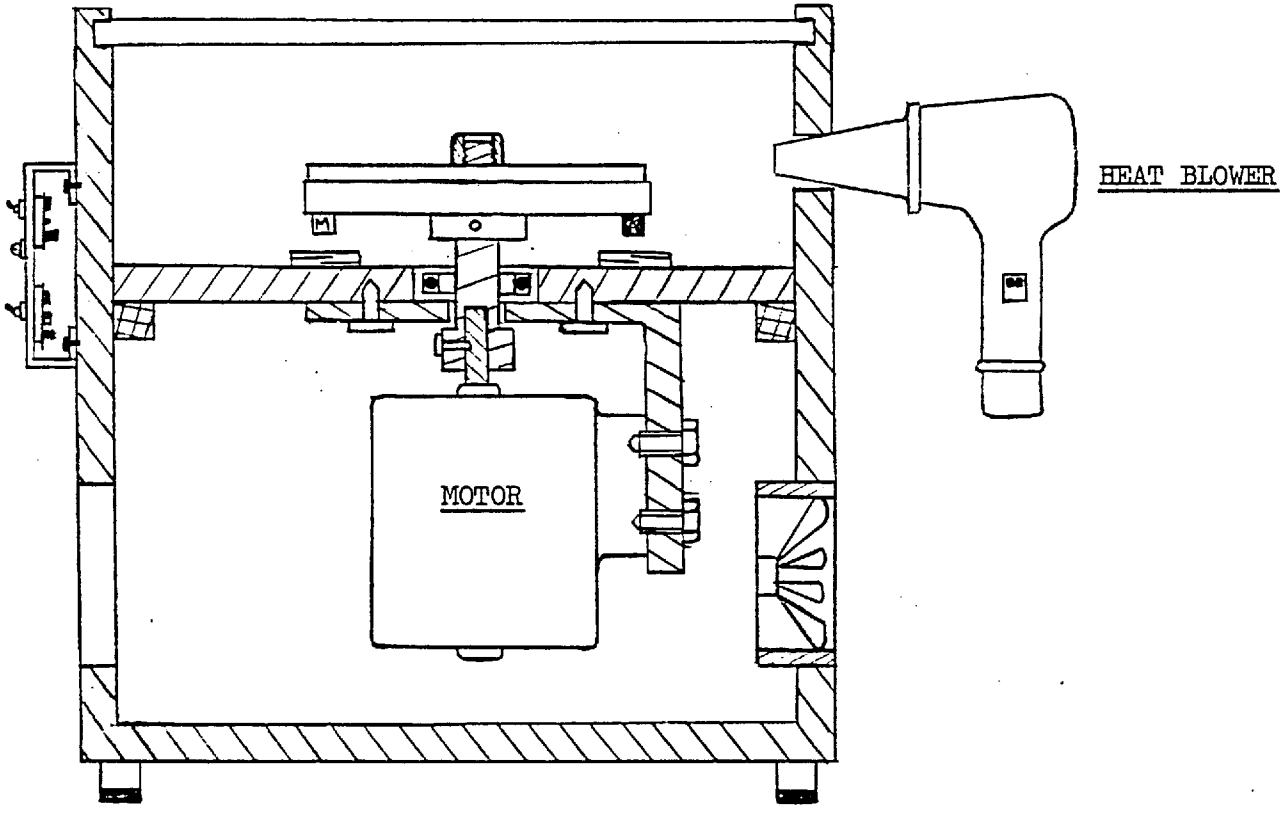


FIG. 1 :- SCHEMATIC DIAGRAM OF CENTRIFUGE BOX

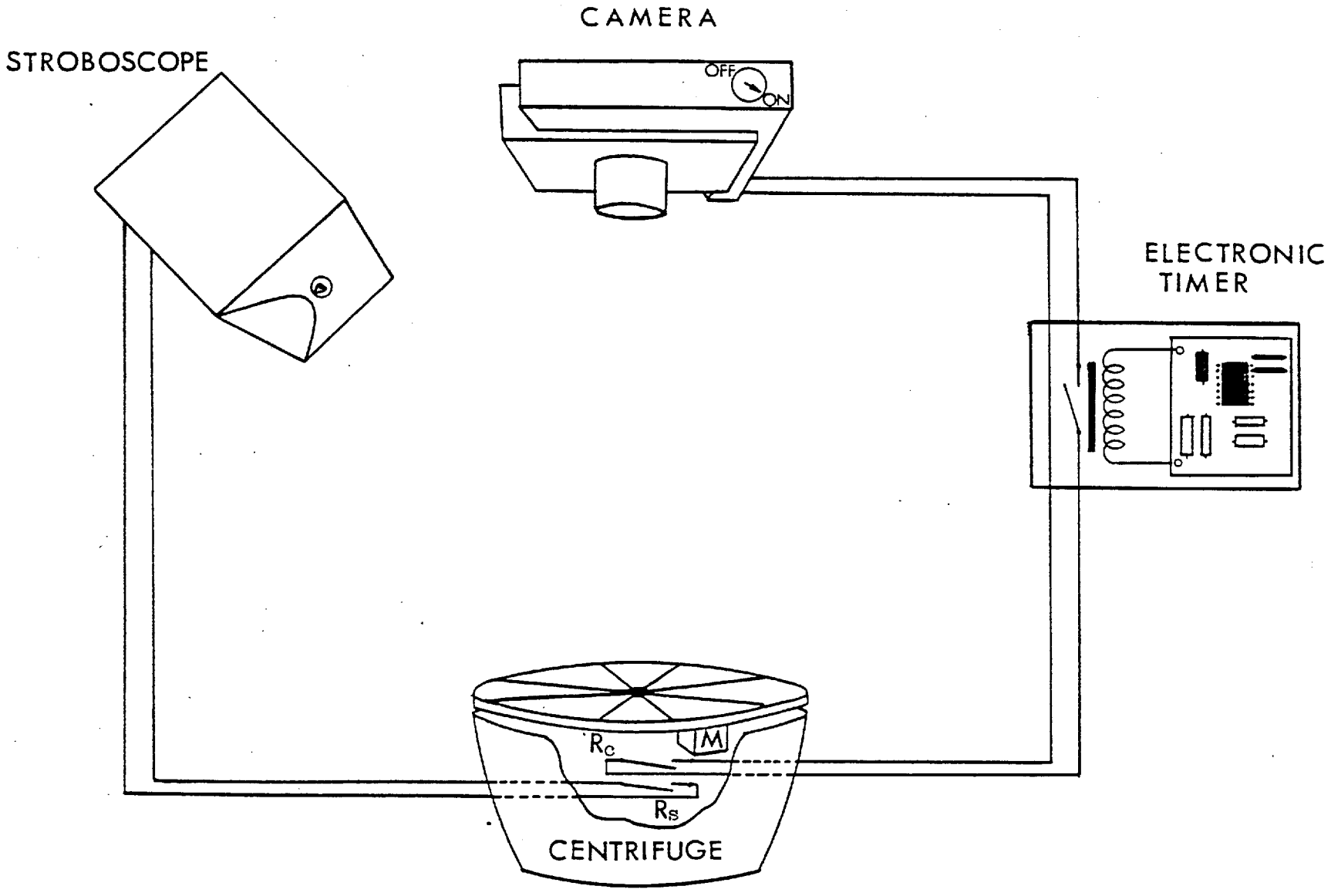


FIG. 2 : ARRANGEMENT OF AUTOMATIC RECORDING CENTRIFUGE APPARATUS

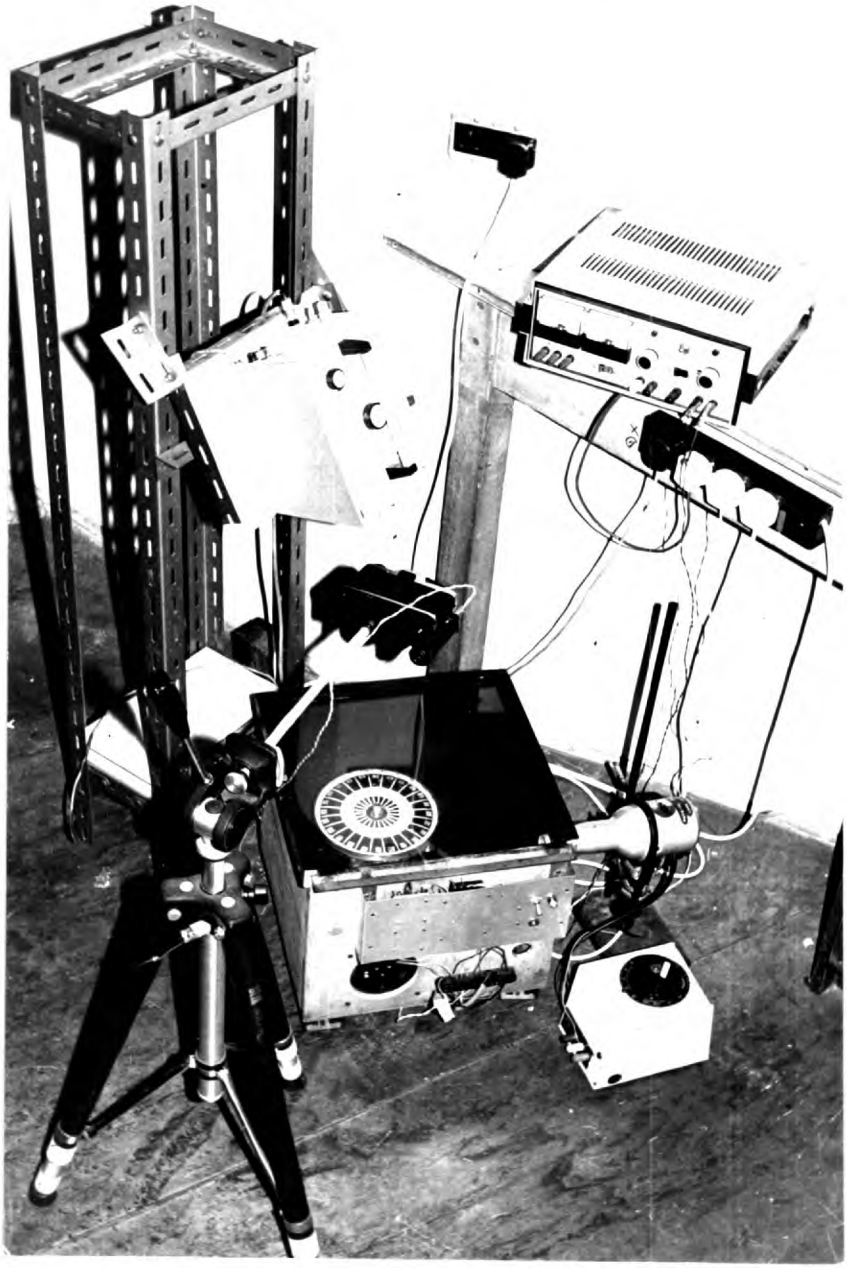


PLATE 1 : APPARATUS USED FOR MEASURING THE RBC PACKING RATE

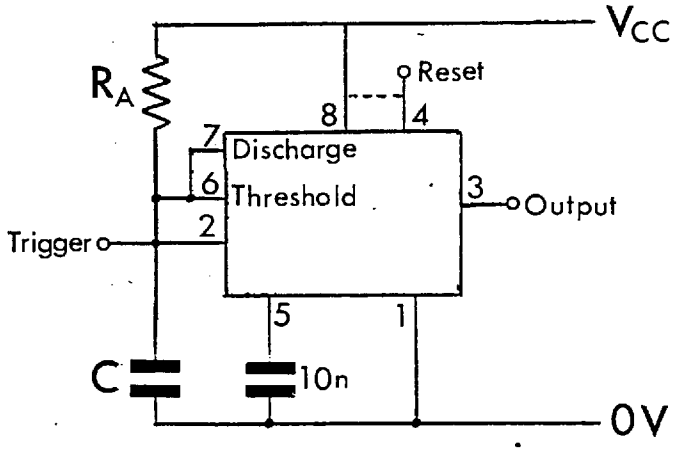
(iii) Electronic Timer Circuit

To measure the packing rate of a column of red cells, capillary tubes containing the blood samples are placed on the centrifuge head and are then centrifuged at about 200g. The position of the top of the red cell column at different intervals of time are recorded by means of photographic records of the centrifuge head. The interval of centrifuging blood samples for the purpose of measuring the packing rate was chosen to be 6 minutes. During this six minutes, photographic records are taken of the centrifuge head every 30 seconds from start of the cycle (6 minute period). To enable us to control accurately the timing of the 30-second camera shots, an electronic timing circuit was used for the duration of the 6 minute experimental cycle.

The electronic timer chosen to operate and control the intervals between camera shots of the centrifuged microhaematocrit tubes, containing the blood samples, is based on the RS (Radio Spares) data sheet no. R/2113, Fig (3). The IC timers used were the single timer RS component type Bipolar 555 and the double timer RS component type Bipolar 556. The 555 timer was used to operate in the monostable mode whereas the 556 timer was operated in the astable and monostable modes. The reasons for choosing these timers were their simplicity of use, reliability and low cost.

BASIC MODES OF OPERATION

Monostable:



Astable:

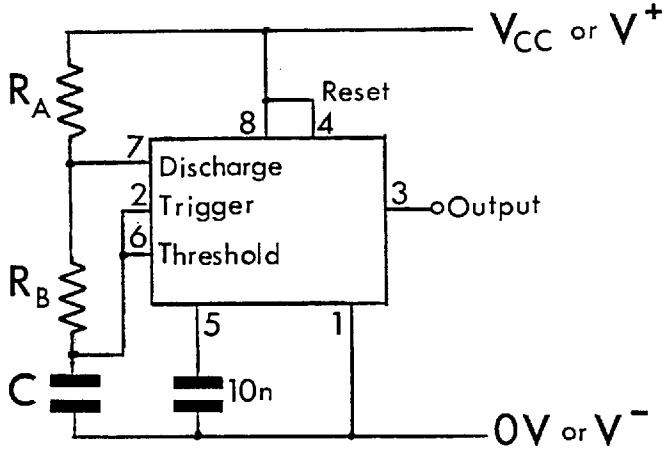


FIG. 3

From the manufacturer's data for these timers, the timing intervals are calculated from the following formulae.

Monostable operation:

$T \approx 1.1\ R_A C$ sec.

Astable operation:

$T_1 \approx 0.7\ (R_A+R_B)C$ sec.

$T_2 \approx 0.7\ R_B C$ sec.

C in F; R_A and R_B in Ω

The resistors R_A and R_B and the capacitor C are components controlling the input levels into the discharge pin of the timers as shown in Fig (3).

The complete timing circuit is shown in Fig (4). The resistors used in the electronic circuit are of the ½ watt RS high stability carbon resistor type. The capacitors are RS electrolytic type. The switches RL_1, RL_2 and RL_3 are fully encapsulated RS component 349-355 type, consisting of a magnetic coil and changeover switches enclosed in a moulded outer case. Rd_c and Rd_m are dry-reed magnetic switches RS component 338-153. M is a RS component 349-838 magnetic coil of 700 Ω resistance.

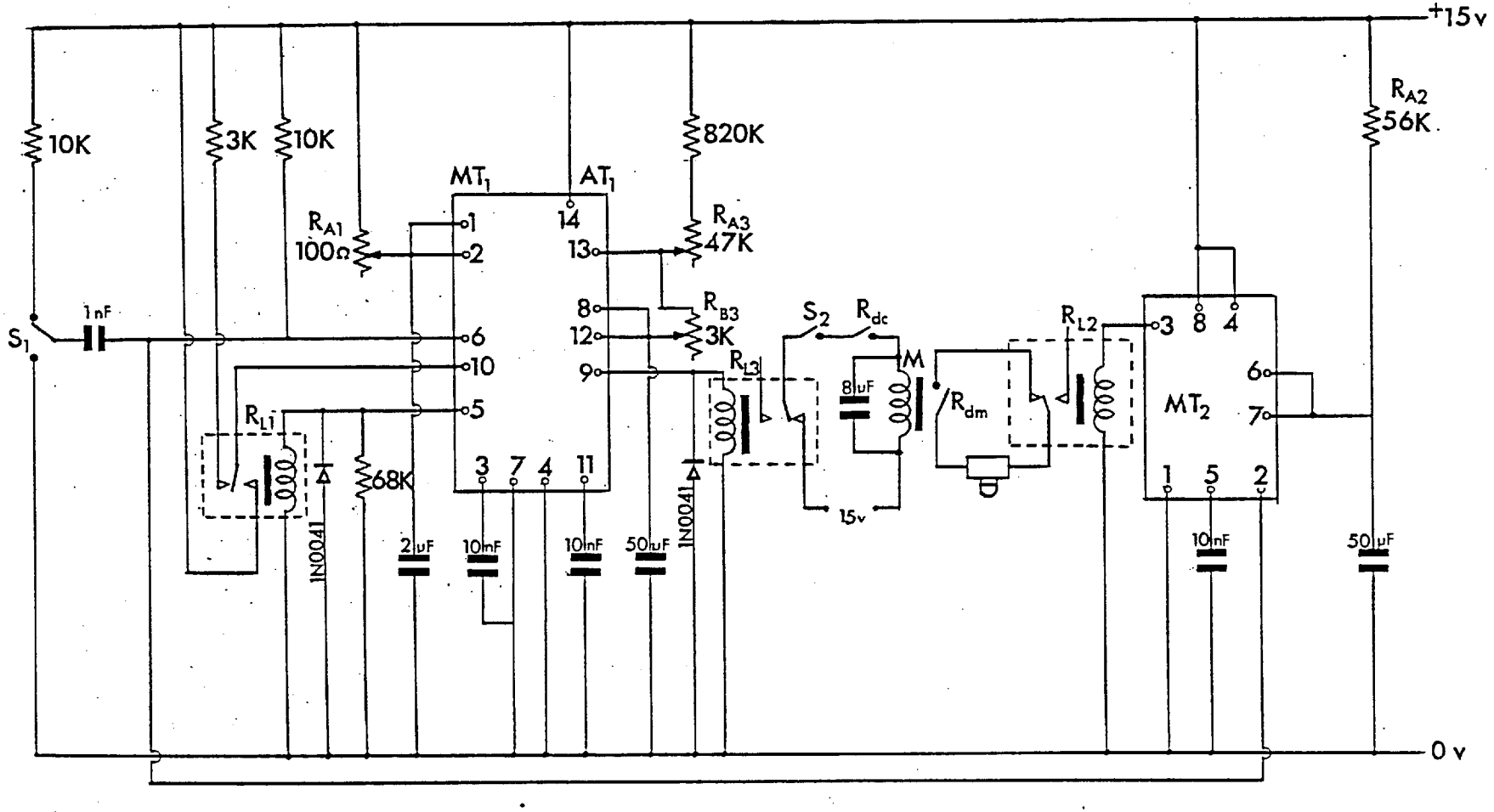


FIG. 4 : ELECTRONIC TIMER CIRCUIT DIAGRAM

To operate the timer circuit, an AC coupled trigger input was used. This triggers the monostable mode timers MT_1 and MT_2 through the 1nF capacitor, and avoids the possibility of false triggering due to external noise.

The first timer circuit stage, MT_1, is one half of the 556 timer operating in a monostable mode. The function of this timer is to force the astable mode timer, AT_1, the second half of the 556 timer, to start at a beginning of an experimental cycle. This is done by using the output pulse of MT_1, of duration 2.2×10^{-4} sec, as shown in Fig (5a), to operate the reed switch RL_1. The operation of this reed switch reduces the reset input level on AT_1 from 14.4 volt to 0.63 volt forcing AT_1 to reset itself to the beginning of a new cycle, Fig (5b).

The astable cycle output is put across the coil of the reed switch RL_3. The contact time in RL_3 is 0.105 sec. and the break is for 29.9 sec., Fig (5c). This is the timing cycle used to control interval between shots on the camera.

R_C is one of the dry-reed switches placed under the centrifuge head and used to synchronise the camera to the centrifuge head rotation and stroboscope, which is synchronised by the second dry-reed switch. The switch S_2 is a switch connected to the manual switch S, and ensures that the camera operates only during the period for which the centrifuge is operated. When S_2, RL_3 and Rd_c are closed, the magnetic coil, M, closes the dry-reed switch Rd_m for a period of 5.6×10^{-3} sec. caused by the discharge

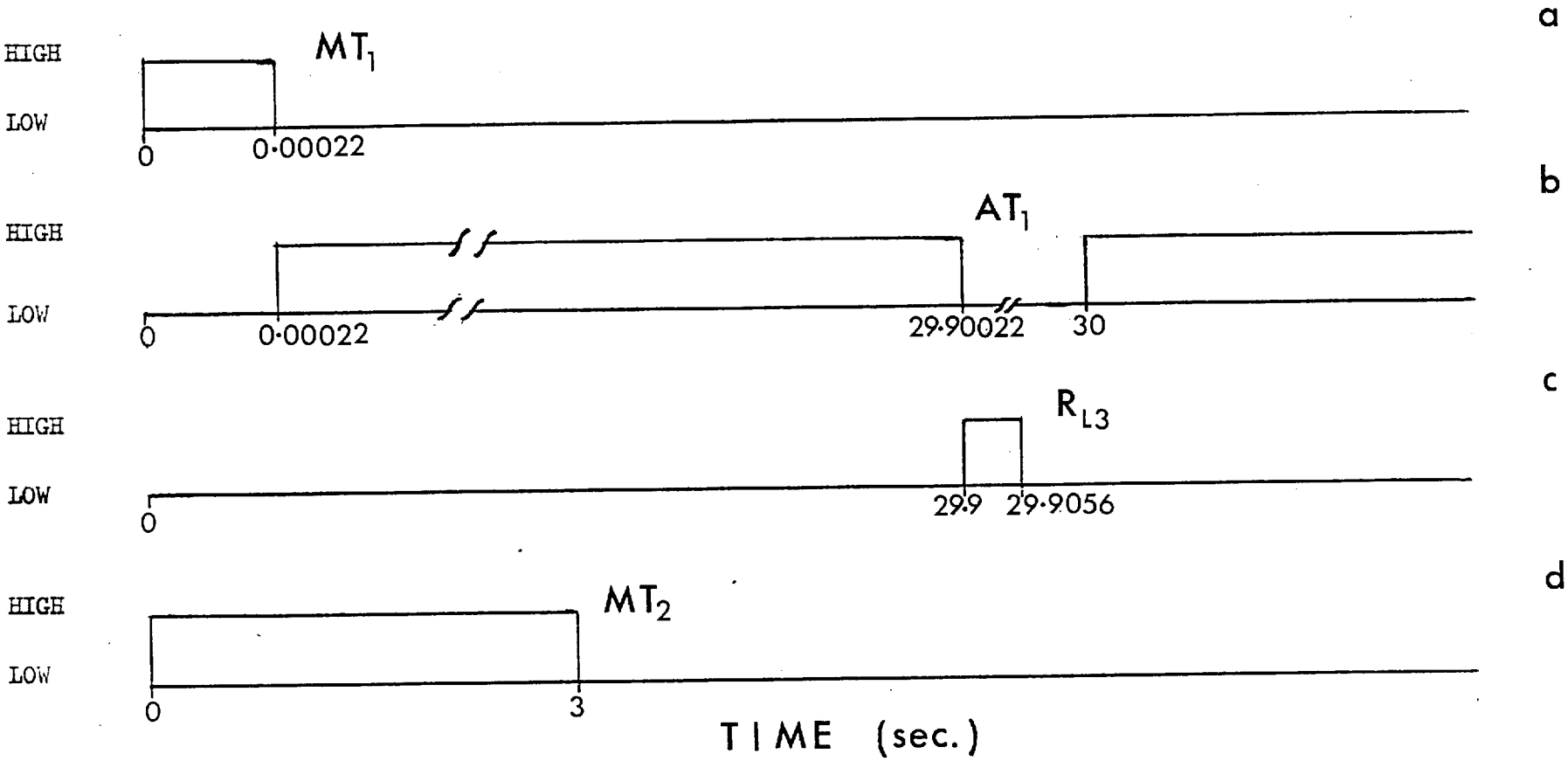


FIG. 5 : OPERATIONAL SEQUENCES FOR MT_1;AT_1;R_{L3};MT_2

from the 8μF capacitor. This delay was found to be the minimum delay time needed to enable the camera winder motor drive to respond to the electronic timer control. The operation time for the camera winder motor is 0.3 sec., therefore it can only respond to one shot during the 5.6×10^{-3} sec. activation period. The timer circuit is connected to the camera winder motor via a relay jack plug.

When the initial trigger is operated to start the monostable pulse of MT_1, which consequently resets AT_1, the astable output is low. This low level will cause the camera to fire at zero time of the cycle. To avoid this occuring, the monostable 555 timers. MT_2, is used to break the camera circuit for 3 sec. from zero time. MT_2 is triggered from the same AC coupled input to MT_1, Fig (5d).

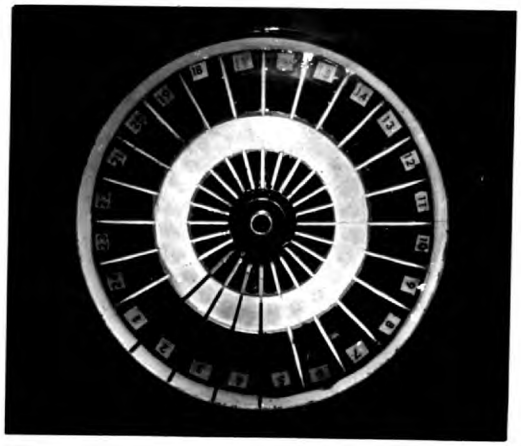
It was found that before triggering the timer circuit, the circuit power, V_{CC}, has to have been switched on for at least 45 sec., otherwise the timer does not respond until after 45 seconds from triggering. This I suspect is due to capacitance in the circuit needing at least 45 seconds to charge up.

The experimental runs are limited to 6 minutes for which the camera is fired for twelve shots starting at 30 seconds from triggering. The 15 v DC circuit power is provided by a Heathkit Regulated HV Power Supply.

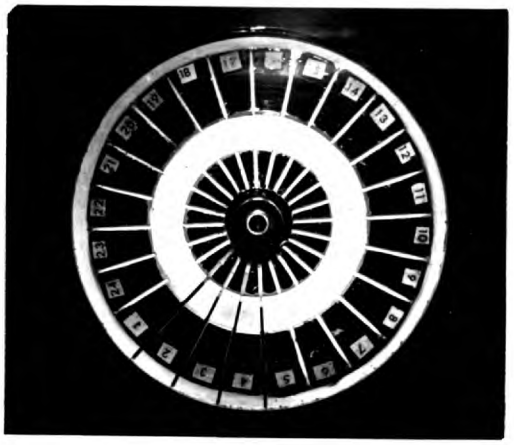
(iv) Measurement of Red Blood Cell Packing Rate

To measure the red cell packing rate of a blood sample, microhaematocrit capillary tubes are filled to a length of 5 cm with the blood sample and are then sealed from the clean end of the tube, by heating in a bunsen flame. The microhaematocrit tubes are then placed onto the centrifuge head and covered with the perspex head cover. The centrifuge is then operated for 6 minutes and photographs of the head are taken every 30 seconds. The photographic film used was a black and white Ilford HP4.

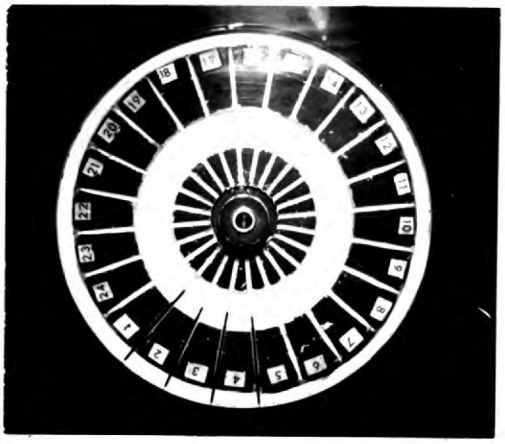
After development, the photographic film was placed in an enlarger to obtain a plot of the results. Using graph paper, with one centimetre along the horizontal axis representing half a minute, the chosen zero line is lined up to the bottom of the red cell column in the image. The top of the red cell column and the plasma column are then marked. The top of the plasma column represents the 100% mark. The same markings are done for the image of the same sample on the following shots, moving the markings 1 cm along the time axis with each new frame. Plate 2 shows the photographs of the centrifuge top during an experimental run. When the plotting is done for each sample, the change in haematocrit with time is measured from the gradient of the plot, Fig (6). The gradient is called the red cell packing rate for that blood sample and is measured in % min^{-1}, for a given temperature, fibrinogen concentration and haematocrit. The error involved in the packing rate measurement is $\pm$ 0.5% min^{-1}.



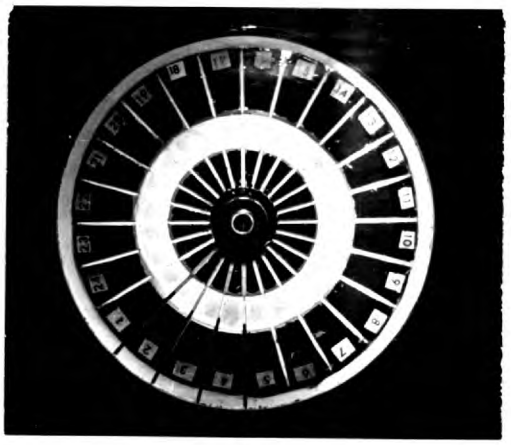
a



b



c



d

PLATE 2 : PHOTOGRAPHS OF RBC PACKING RATE CENTRIFUGE TOP AT TIME INTERVALS AS INDICATED IN FIG. 6.

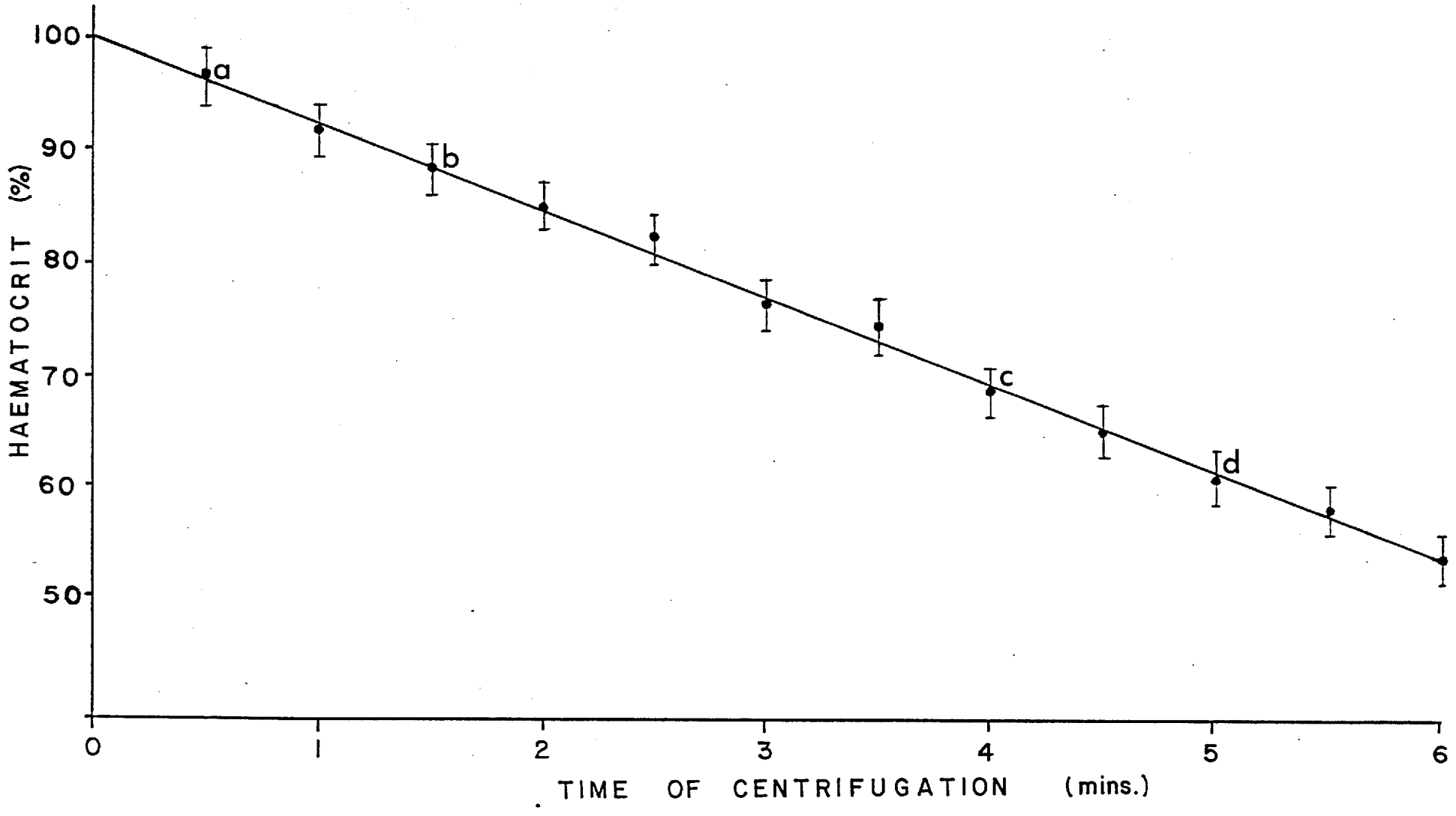


FIG. 6 : RATE OF PACKING OF A RED CELL COLUMN

(v) Blood Mixer

When several microhaematocrit capillary tubes are filled with blood samples for centrifugation, the red blood cells in the first samples prepared can sediment by gravitation. The effect of delaying centrifugation of blood samples was investigated by leaving samples to stand for 0 (control), 5, 10, 15 and 20 minutes before centrifugation at 37.5°C. The results of the packing rates are shown in Table 1. It can be seen that large errors are introduced by delay due to sedimentation and probably aggregation of the red cells.

Since usually several samples are prepared for any experimental run of the packing rate centrifuge, it is necessary to avoid sedimentation of the red cells by mixing the blood samples in the microhaematocrit tubes. For this purpose, a mixer was designed, consisting of a wooden disc rotated in the vertical plane by a small 15 rpm electric motor, Fig (7). The outer edge of the disc was cut at an angle of 45°. The microhaematocrit tubes were fixed to the angled edge by pressing them into a plasticine layer covering the angled edge. The microhaematocrit tubes containing the blood samples are placed onto the mixer as they are prepared and taken off only when all the samples are ready to go onto the centrifuge.

TUBES LEFT STANDING IN VERTICAL POSITION

DELAY TIME (min.)	PACKING RATE (% $min.^{-1}$)	% DIFFERENCE FROM CONTROL
0	9.9	0
5	17.6	77.8
10	20.1	103
15	25.7	159.6
20	34.5	248.5

TUBES LEFT STANDING IN HORIZONTAL POSITION

DELAY TIME (min.)	PACKING RATE (% $min.^{-1}$)	% DIFFERENCE FROM CONTROL
0	9.9	0
15	13.9	140.4
25	23.1	233.3

TEMPERATURE OF MEASUREMENT = 37.5°C

ERROR IN PACKING RATE VALUES = ± 0.5% min^{-1}

TABLE 1

EFFECT OF DELAY OF BLOOD SAMPLES BEFORE CENTRIFUGATION ON RED CELL PACKING RATE MEASUREMENT

MIXING TIME (min.)	0	5	20	40
PACKING RATE % $min.^{-1}$	8.75	8.68	8.65	8.72

TEMPERATURE OF MEASUREMENT = 37.5°C

ERROR IN PACKING RATE VALUES = ± 0.5% min^{-1}

TABLE 2

EFFECT OF MIXING PERIOD PRIOR TO PACKING RATE MEASUREMENT

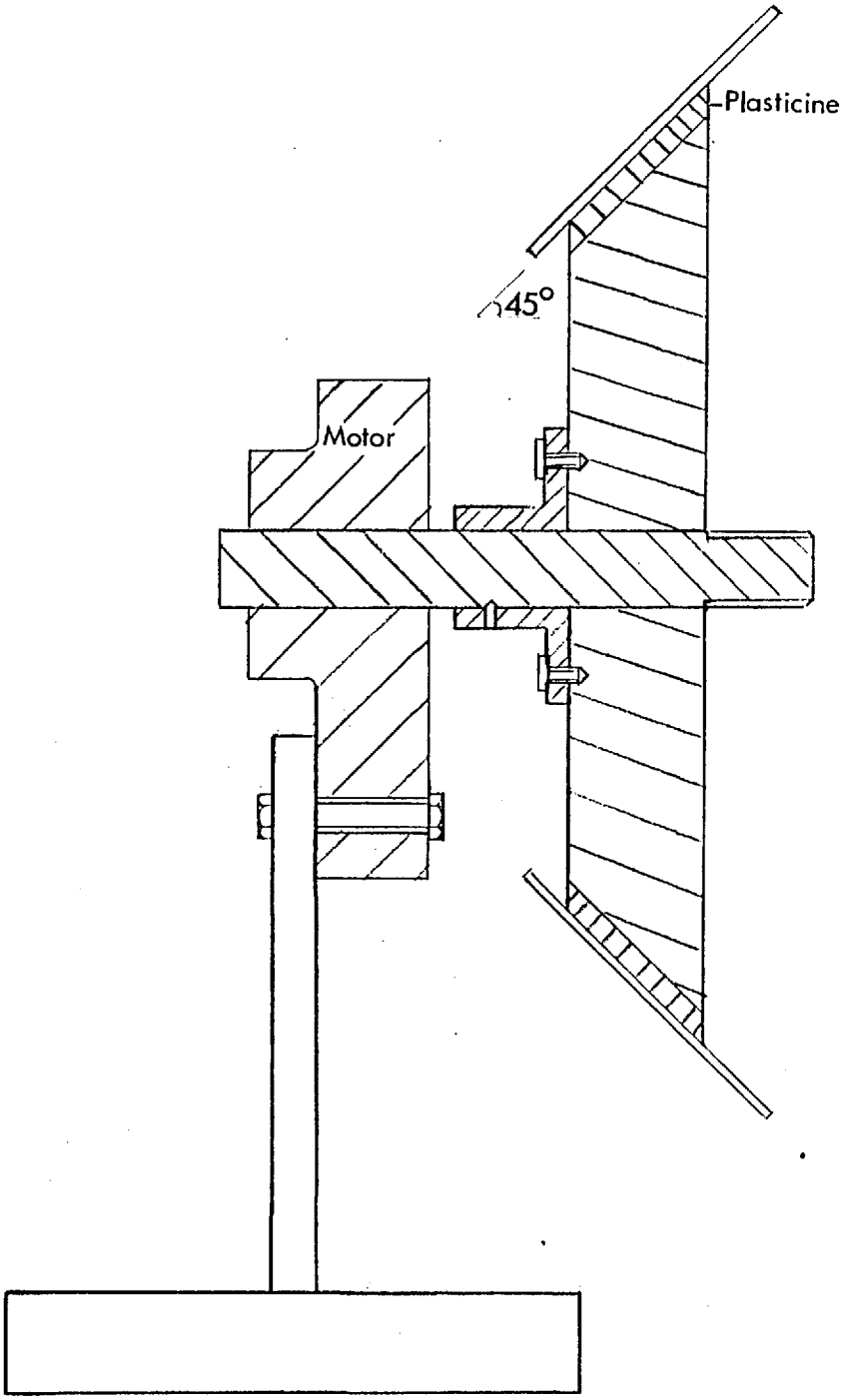


FIG. 7 : ROTARY BLOOD MIXER

The period for which the samples are placed on the mixer and the effectiveness of the mixer was tested by leaving samples rotating for 0 (placed on centrifuge immediately), 5, 20 and 40 minutes before centrifugation. Table 2 shows that the mixing time up to 40 minutes has no effect on the red cell packing rate. Normal experiments involve a delay of less than 5 minutes on the mixer.

(vi) Reproducibility of Results on Packing Rate Centrifuge

To examine the reproducibility of results on the red cell packing rate centrifuge, three separate experimental runs were done on the same blood sample. On each run, four values of the packing rate were obtained. The results are shown in Table 3. It is seen that the variations lie within the error limit of $\pm$ 0.5% min^{-1} of the average value for the twelve measurements.

II MEASUREMENT OF HAEMATOCRIT

To measure the haematocrit of a blood sample, heparinised Hawksley Microhaematocrit glass capillary tubes (Cat. No. 1603) were filled to 5 cm of their length, with the blood sample, and then one end of each capillary tube was sealed by heating in a bunsen flame. The tubes were then placed on a Hawksley Microhaematocrit Centrifuge and the spinning

EXPERIMENTAL RUN NO.	RED CELL PACKING RATE (% min^{-1}) FOR SAMPLE NO. 1	2	3	4
1	8.98	8.7	9.1	8.82
2	8.65	8.85	8.75	9.19
3	9.32	8.9	9.14	8.52

TOTAL AVERAGE: 8.91% min^{-1}

VARIATION FROM AVERAGE: FOR LOWEST VALUE OF 8.52:- 0.39% min^{-1}
FOR HIGHEST VALUE OF 9.32:-0.41% min^{-1}

TEMPERATURE OF MEASUREMENTS: 37.5°C

TABLE 3

REPRODUCIBILITY OF RED CELL PACKING RATE CENTRIFUGE

time set to 4 minutes at a centrifugal force of 13,000g. To read the haematocrit of a spun tube, use is made of the Hawksley Microhaematocrit Reader. The value of the haematocrit of a blood sample is taken as the average of readings of at least two tubes.

The measurement of haematocrit has been the subject of study by several workers. In their reviews, Jackson and Nutt (1951), Chaplin and Mollison (1952) and Vazquez et al (1952) mention several methods used for determining haematocrits. Basically the methods used are dilution of plasma during centrifugation using Evans Blue, the blood's own haemoglobin or isotope labelling of albumin with I^{131} or Na^{24}, to calculate the amount of trapped plasma between the red cells during centrifugation at different g forces. It was found that the correction for trapped plasma varies between 20% for a centrifugation force of 200g and a correction of 2% or less for centrifugation force exceeding 4000g. England et al (1972) and England and Down (1975) have shown that haematocrit correction for trapped plasma is less than 1% when the centrifugation force is of the order of 13,000g as in the case of a Hawksley Microhaematocrit Centrifuge. Sirs (1968), however, argues that the correction to haematocrit obtained by centrifugation is not due to trapped plasma but due to loss of fluid by the red cells because of their centrifugal compression, especially at the bottom of a red cells column, and has shown that values of the haematocrit obtained by the Hawksley centrifuge were consistently less than those calculated by the isotope dilution method by about 10%.

Since high centrifugal forces (13,000g) are involved in a Hawksley Microhaematocrit Centrifuge the correction to haematocrit is most likely due to red cell compression as suggested by Sirs (1968). He finds that the haematocrit measured can be up to 10% below the 'true' haematocrit calculated. In my study, the method of measuring haematocrit by Hawksley centrifuge was used throughout and therefore the error is consistent. Added to this, most of the work involved the effects on the same sample of blood treated in different ways. Therefore any effects shown which directly depend on the haematocrit are only shifted in scale from the effects for the 'true' haematocrit.

III VISCOMETRIC MEASUREMENTS

The viscometric measurements made in our rheological study were obtained using a cone-plate Wells-Brookfield Micro Viscometer (Model LVT C/P), Wells et al (1961). In this type of viscometer, the angle θ between the surface of the cone and the plate into which the sample is placed, Fig (8), is less than 2^{o}, with the cone and plate making contact at the central point. The cone is rotated at a constant speed, ω, and the summation of the torque T on the cone, due to the viscosity of the sample, is related to the cone rotational speed, ω, and the gap width, C, at any radius, r, of the cone from its centre. Since for a given

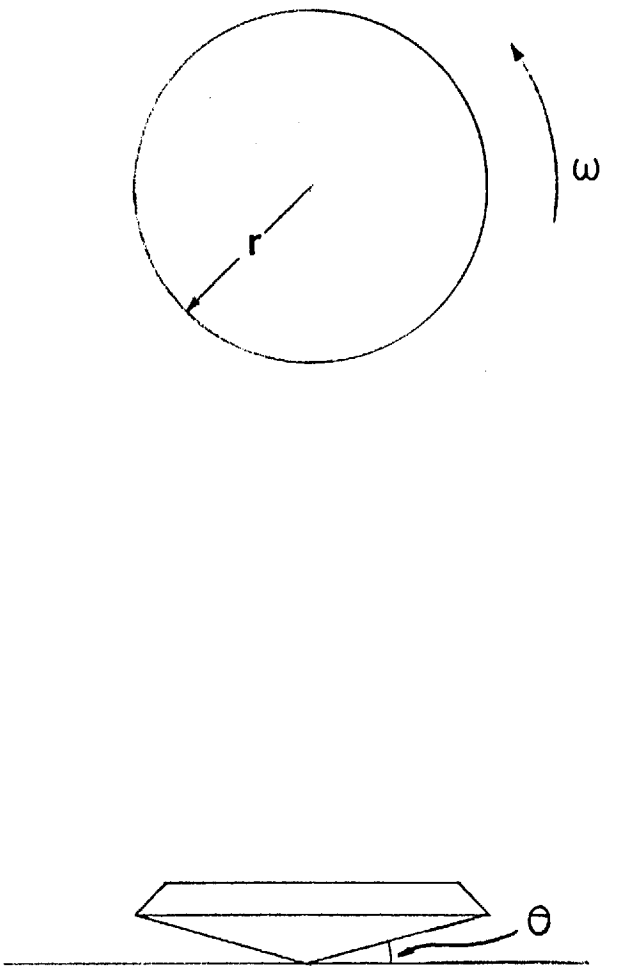


FIG. 8 : SCHEMATIC REPRESENTATION OF THE WELLS-BROOKFIELD VISCOMETER

constant value of ω, the shear rate γ, shear stress τ and sample viscosity η are given as

$$\text{Shear rate, } \gamma \ (s^{-1}) = \frac{\omega}{\sin\theta}$$

$$\text{Shear stress, } \tau \ (\text{dynes cm}^{-2}) = \frac{T}{2/3\ \pi r^3}$$

$$\text{Viscosity } \eta \ (cP) = \frac{\tau}{\gamma} \cdot 100 = \frac{T \sin\theta}{2_3 \pi \omega r^3} \cdot 100$$

where, T = % Full Scale Torque (dyne cm)

r = Cone radius (cm)

ω = Cone angular velocity

θ = Cone angle (1.565^{o})

The advantages of this instrument over other viscometers are:

1) Cone-plate arrangement gives a constant shear rate at all radii r along the cone surface.
2) Rapid analysis of small sample volumes (1 to 2 ml) are possible.
3) Offers wide selection of discrete rates of shear, namely: 230, 115, 46, 23, 11.5, 5.75, 2.3 and $1.15 s^{-1}$.
4) Enables sample temperature to be finely controlled through water bath heating control and water jacket surrounding the sample in the plate of the viscometer.

5) Can be calibrated for volume and viscosity values against standard fluids of known viscosity.

6) Shows presence of microclotts by erratic movements of scale needle.

7) The results are found to be reproducible, Wells et al (1961), with the accuracy being greater for shear rates greater than $11.5s^{-1}$, Zingg et al (1973).

To calibrate the viscometer, standard fluids (Brookfield Engineering Laboratories Inc.) were used. These were Fluid no. 5 of viscosity 5.1 cP at 25°C and Fluid 10 of viscosity 9.1 cP at 25°C. The manufacturer's specification of 1 ml sample volume is needed to measure the viscosity was not found to be correct. To obtain the correct viscosity readings for samples of both standard fluids, equilibrated to 25°C for 4 minutes in the viscometer before readings, it was found necessary to increase the sample volume. Fig (9) shows that the correct viscosity readings for both fluids were obtained for sample volumes between 1.2 and 1.6 ml. The chosen volumes for further measurements was 1.4 ml.

The errors involved in viscosity readings at the different available shear rates are shown in Table 5. It is seen that for errors less than 10%, shear rates greater than $11.5s^{-1}$ should be used. As the temperature control for the heating of the sample in the viscometer cup was to within 0.25°C, no noticeable change in the fluids' viscosity readings were observed for 0.25°C change above or below the measurement temperature of 25°C.

SAMPLE VOLUME ML.	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0
FLUID 5* VISCOSITY (cP)	2.8	3.9	4.9	5.0	5.125	5.15	5.175	5.25
FLUID 10* VISCOSITY (cP)	4.9	6.7	8.5	8.95	9.1	9.2	9.25	9.35

TEMPERATURE OF MEASUREMENTS: 25°C

* FLUIDS 5 AND 10 ARE STANDARDS FROM BROOKFIELD ENGINEERING LABORATORIES INC.
FLUID 5 HAS VISCOSITY OF 5.1 cP AT 25°C
FLUID 10 HAS VISCOSITY OF 9.1 cP AT 25°C

TABLE 4 FOR FIGURE 9

VISCOMETER SAMPLE VOLUME AND SAMPLE VISCOSITY

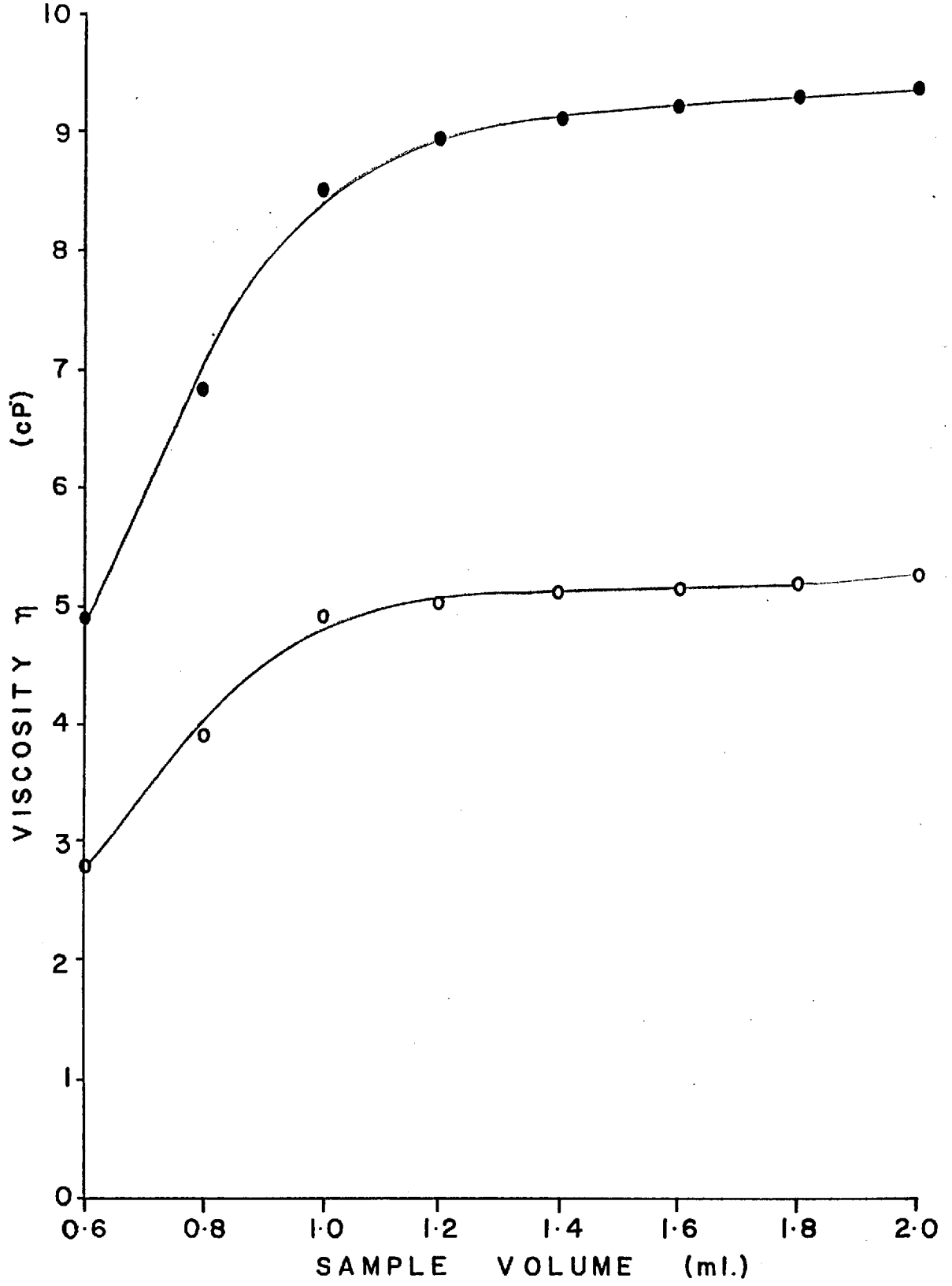


FIG. 9 : VISCOMETER SAMPLE VOLUME AND SAMPLE VISCOSITY

SHEAR RATE (s^{-1})	230	115	46	23	11.5	5.75	2.3	1.15
READING ERROR* %	1	1	1.2	2	4	10	20	100

* BASED ON SAMPLE OF VISCOSITY 5 cP AT SHEAR RATE OF $230s^{-1}$

TABLE 5

READING ERRORS OF VISCOSITY AT DIFFERENT SHEAR RATES
ON WELLS-BROOKFIELD VISCOMETER (LVT-C/P)

The sample was heated by circulating water in a water jacket surrounding the sample in the cup of the viscometer. The water was heated in a water bath connected to the viscometer plate by rubber tubing. The water bath heater (Tecam Tempunit) allowed very accurate temperature control to within 0.25°C accuracy.

When a blood or plasma sample is placed in the viscometer cup, the viscometer is switched on to rotate the cone, and the sample is left to equilibrate in temperature for 2 minutes before the first reading of the viscosity is taken. The viscometer is switched on and rotated for at least 2 minutes between consecutive readings of the sample's viscosity after the initial 2 minutes temperature equilibration time. The viscosity value is taken as the value of two equal consecutive readings. The period between consecutive readings is longer for lower shear rates e.g. 5 minutes for $\gamma = 23s^{-1}$ and 10 minutes for $\gamma = 5.75s^{-1}$.

In the case of blood or plasma samples where readings give erratic movements of the needle due either to microclotts or dirt, the samples were discarded and a fresh sample was used. The cup (plate) and cone were washed with saline or distilled water and dried thoroughly before each placing of a sample into the cup.

IV MEASUREMENT OF FIBRINOGEN CONCENTRATION

The method used to measure the fibrinogen concentration in a red cell suspending medium or plasma, is that described by Rampling and Gaffney (1976) as the Thrombin-Clotting Technique. In this method 0.2 ml of the fibrinogen solution is added to 0.2 ml of saline, and to this solution is added 0.02 ml of a solution of 5000 US (NIH) units of Bovine Thrombin (Thrombostat Parke-Davis) dissolved in 5 ml saline (i.e. 20 units in 0.02 ml). The fibrin clot is left to form for ½ hour at room temperature. The clot is then harvested on a stick and dried on filter paper and is then dissolved in 5 ml solution of 0.1N NaOH and 4M urea by heating in boiling water for ½ hour. The concentration of dissolved fibrinogen is then obtained by the U/V reading using a Unicam SP 1800 Ultraviolet Spectrophotometer set on λ = 280 nm, slit width = 0.3 mm and band width = 1nm, using Pye Unicam quartz Absorption Cells. The reading obtained on the Spectrophotometer is multiplied by 15.3, a factor obtained by Rampling and Gaffney (1976). This value is in $mg.ml^{-1}$. The value used is the average of at least two separate samples. The error involved in measurement of the fibrinogen concentration is $\pm$ 0.1 $mg.ml^{-1}$.

V MEASUREMENT OF pH

The pH of a sample was measured by placing into the sample the glass probe of a digital Corining pH meter (Model 109). The pH meter is set to the temperature of the sample and is calibrated using standard buffer solutions of pH = 4.01 and 7.0. The error in reading of pH values on the digital meter is not more than $\pm$ 0.005 pH unit.

VI MEASUREMENT OF TONICITIES

Tonicity was measured by the freezing point depression method. This was done by the use of an Osmette A Automatic Osmometer (Precision Systems Inc.) which is calibrated before each series of measurements with the manufacturer's own standard fluids of tonicities 100 $mosm.l^{-1}$ and 500 $mosm.l^{-1}$. The volume of sample used was set to 0.2 ml.

The samples used were obtained from red blood cells suspensions centrifuged gently at 500g force to avoid false readings due to shift of fluid out of red cells under centrifugal compression at high g forces. The error in tonicity readings from the osmometer is not greater than $\pm$ 1 $mosm.l^{-1}$.

VII LABORATORY CENTRIFUGES

Three types of centrifuges were used in this study. The first is the standard Hawksley Microhaematocrit Centrifuge which has a spinning speed of 12,000 rpm and a duraluminium top of a radius of 9.1 cm and hence a centrifugal force of about 13000g at the tip. The second type of centrifuge is an MSE Minor Laboratory Preparation Centrifuge which has a radius of 15 cm. and is used on a speed setting of 5 which is a spinning speed of about 1750 rpm and centrifugal force of 500g, to gently spin blood samples, or on a speed setting of 10 which is 2500 rpm and a centrifugal force of 1000g to separate out the suspending medium for analysis. On both speeds the spinning time is not greater than 10 minutes. The third type of centrifuge is the red cell packing rate centrifuge as has already been described.

VIII MEASUREMENT OF MCV (MEAN CORPUSCULAR VOLUME)

The red cell MCV is calculated as the ratio of the sample haematocrit and the number of red blood cells per litre in the sample, i.e.

$$\text{MCV} = \frac{\text{haematocrit (expressed as decimal)}}{\text{count of no. of red cells per litre}}$$

The haematocrit is obtained by centrifugation on a Hawksley Microhaematocrit Centrifuge. The count of red cells per litre of blood sample is obtained by the use of a Coulter Counter Model ZBI (Coulter Electronics Ltd.).

The solution of red blood cells used for counting on the Coulter Counter has to be of 1:50,000 dilution. This is obtained by adding 10 µl of blood sample to 10 ml of a buffer, azide free Isotone II (Coulter Electronics Ltd.), and after mixing, 1 ml. of this solution is added to a further 49 ml. of the isotone II buffer. A count is then obtained on the counter which has been calibrated for human red cells by the following settings

1/(amplification) = 1 1/(aperture current) = $\frac{1}{4}$

Threshold: Lower = 5 Upper = 105

All count readings are corrected for count loss due to coincident passage of cells, which is a function of aperture volume and cell concentration, by the use of the manufacturer's Coincidence Chart. The Coincidence Chart has been derived statistically to correct for readings of counts varying between $1 \times 10^{12}\ 1^{-1}$ to $6.49 \times 10^{12}\ 1^{-1}$ with a maximum error of 15% and standard deviation of 0.4%. After correction from the Chart the error involved in the readings should be better than 1%, as claimed by the manufacturer, especially in the case of high counts as used in my work.

Since the error in counts is 1% or less, the main error in the value of MCV is due to the haematocrit. If we consider the variation of the haematocrit from the 'true' haematocrit due to compression of red cells at high centrifugal forces on the Hawksley Centrifuge, then the error is as much as 10%. However since I am mainly considering the effects on the same blood sample treated under different conditions, the error in the haematocrit is consistent, and the only error arising is in the actual reading of the haematocrit on the Hawksley Microhaematocrit Reader, which is 0.25%. Hence the total error in the MCV value is about 1.25% if 'true' haematocrits are not considered.

IX ANTICOAGULANTS

In my choice of anticoagulant to be used in this work, several criteria had to be taken into consideration. The chosen anticoagulant had to:

1. Be effective in minute quantities.
2. Negligibly dilute the blood sample.
3. Does not alter the morphology of the cellular elements of the blood sample.
4. Can be varied in concentration over a wide range without any detrimental effect on the blood sample.
5. Does not break down or alter the blood sample over at least 24 hours from addition.

Rand et al (1964) found that heparin sodium and EDTA satisfied the first three points above. Galluzzi et al (1964) and Zingg et al (1973) found that heparin or sodium EDTA has no effect on whole blood viscosity, over a period of 8 hours, compared to fresh unaltered whole blood. In addition Zingg et al found that varying the heparin concentration between 14 and 95 IU/ml. had negligible or no effect on whole blood viscosity measured at shear rates between 11.5 and 230s^{-1} at 37^{o}C.

The anticoagulant ACD is not suitable for several reasons. As a fluid it dilutes the blood sample, affecting its viscosity. It also reduces the blood pH, Sirs (1977), and this causes an increase in the red cell volume and/or reduces the red cell flexibility, Rampling and Sirs (1970). In addition ACD has the effect of reducing the efficiency of the cells to exchange oxygen with the internal haemoglobin, Sirs (1969a).

EDTA has been observed to have no effect on whole blood viscosity as mentioned above, but has been observed to reduce red cell flexibility, Rampling and Sirs (1970). This is probably because it prevents clotting by removal of Ca^{++} ions from the blood, Mollison (1967), and this can be detrimental to the red blood cell membrane. Oxalate is hypertonic and dilutes the blood sample and therefore is unsuitable for my work, as it increases η_B, Rand et al (1964).

Heparin, on the other hand, has been found to have least detrimental effect, at normal concentration, on red cell flexibility, Rampling and Sirs (1970), and to have optimal rate of oxygen exchange of the cells, being determined only by the chemical reaction rate of the haemoglobin, Sirs (1967). It has also been found that when injected into a rabbit in concentrations of up to 5000 iu, the in-vivo reaction has no effect on red cell flexibility, Sirs and Macdonald (1974). However, in-vitro, if the concentration of heparin is increased to 250 iu ml^{-1} of blood, the red cell packing rate increases rapidly and can lead to haemolysis at higher concentrations, Sirs (1971).

In conclusion we can say that heparin as an anticoagulant is most suitable and satisfies the five points mentioned above if its concentration is kept to below 100 iu ml^{-1} and the storage time of the blood sample is at most 24 hours.

The heparin used in this work is lithium heparin which is used as anticoagulant covered 5 and 10 ml blood bottles (Anachem Ltd.) with concentrations of 12.5 iu ml^{-1} of blood. When blood samples are transferred from one bottle to another, the concentration of heparin in the sample is kept to at the most 37.5 iu ml^{-1} by the use of non-anticoagulant covered blood bottles if further transfer is needed.

X RED BLOOD CELL HARDENING BY HEATING

When red cells are heated to 45°C or above, the morphology of the cells is altered. Ham et al (1965) have observed that at 45°C the red cells start to become irreversibly rigid and that the surface of the cell membrane starts to show irregularities and the cell osmotic fragility increases. These effects increase with temperature, and between 49°-50°C the cells become spherical in shape. At each of these temperatures, the heating effects are affected by the duration of heating. That is, the longer the red cells are heated at a given temperature, the more rigid the cells become, also more irregularities appear on the membrane. There is also an increase in the osmotic fragility. The sphering of the red cells at 49-50°C has been observed by Teitel (1965), who also found that the filterability of heated cells is decreased.

This affect has been reported by Sirs (1969b) who found that the red cell flexibility, measured by red cell packing rate by centrifugation, is greatly decreased for red cells heated at 48°C for 30 minutes. He also reports that the initial rate of swelling, due to osmotic influx of water, and the onset of haemolysis is unaffected by heating when compared to normal red cells. By his rapid reaction method, it was found that the rate of oxygen exchange with the internal contents of the red cell is lowered by heating.

Since the fibrinogen molecule is denatured by heating at 54°C or above, if red cells are heated in plasma the temperature should be kept below at most 52°C. In my study, the red cells were hardened by heating in either plasma or Ringer-Locke solution. In either case, sphering of the red cells was achieved at 49.5°C, as observed microscopically. Heating was carried out in a temperature controlled water bath.

When red cells are sphered by heating, the rates of uptake of carbon monoxide, nitric oxide and oxygen are not affected, Carlsen and Comroe (1956 and 1958), relative to discoidal cells in isotonic NaCl solution. The blood oxygen dissociation curve is also unaltered. This therefore means that hardening of the red cells has not affected any property of the cell except the rigidity of the membrane structure.

XI RED BLOOD CELL FIXING WITH FORMALDEHYDE

When formaldehyde is added to red blood cells suspensions, the red cells are fixed through the action of formaldehyde on their membranes, and the cells are made relatively inflexible, Sherman (1940). The inflexibility of formaldehyde fixed red cells has been demonstrated by their packing rate as measured by Sirs (1970).

Rampling and Sirs (1972) found that as the concentration of formaldehyde is increased in a blood sample, from 0 to 0.8%, the degree of red cell aggregation decreases to no aggregation.

Although the red cell membrane is fixed by formaldehyde, the osmotic property of the red cell is unchanged, Sirs (1969b), and this shows that the porous sites on the red cell membrane are fixed sites which are unaltered by formaldehyde. It was also found, in the same experiment, that the rate of egress of oxygen from red cell haemoglobin for fixed and normal red cells in isotonic saline are not different, showing that formaldehyde does not affect the intracellular contents during fixing.

To fix red cells with formaldehyde I used concentrations of isotonic 1% formaldehyde solutions, with their pH corrected to 7.4 using isotonic NaOH or HCl solutions, unless the osmolarity or pH are varied. Using this concentration, no aggregation or rouleaux should be present, Rampling and Sirs (1972). The formaldehyde was made into solution either by adding to plasma a small volume of 10% formaldehyde solution or by making up an isotonic 1% formaldehyde in Ringer-Locke solution. This avoids ionic imbalance between the suspension and intracellular contents as well as osmolarity in the case of isotonic solutions.

CHAPTER 3

EFFECTS OF HAEMATOCRIT AND FIBRINOGEN ON RED BLOOD CELL PACKING RATE

I RED BLOOD CELL PACKING RATE AND HAEMATOCRIT

(i) Introduction

Blood samples are placed in microhaematocrit tubes, and are then centrifuged on the packing rate centrifuge. The force experienced by a red cell, with a height h' of red cell column above it, during centrifugation was given by Sirs (1968) as:

$$\text{Centrifugal Force} = h'(\rho_c - \rho_p)g'$$

where ρ_c and ρ_p are the red cells and plasma densities respectively and g' is the centrifugal force, with g being the acceleration due to gravity (981 $cm.s^{-2}$).

With rigid spheres, of the same diameter as a red blood cell, ordered packing is achieved if the haematocrit is 74.5%. Similarly, for discs of the same dimension as a red cell, maximum packing occurs for a haematocrit of 91%. Since a red cell volume is 64% of the volume of a disc of the same diameter and thickness, the maximum packing for red cells should be achieved at a haematocrit of 50.5% and 58.4% when compared to spheres and discs respectively. For randomly packed spheres and discs maximum packing is achieved if the haematocrit is $62 \pm 2\%$ and $54 \pm 2\%$ respectively. Red cell volume is 64-77% of the volume of a disc of the same dimensions, therefore for randomly packed rigid red cells, maximum haematocrit is achieved at 35-42%

respectively, Sirs (1969a). Therefore 35% is taken as the haematocrit limit below which randomly packed red cells are not in contact before centrifugation.

If we assume that at initial centrifugation time the red cells are packed tightly enough to be in contact, further packing of the red cell column will only occur if the red cells are able to distort and bend to enable the red cells to fill in spaces between each other. If temperature, MCV, haematocrit, ρ_c, ρ_p and plasma fibrinogen concentration (ϕ) are kept constant, then for two samples where one packs at a faster rate than the other, the red cells of the faster packing sample have a greater ability to distort. This greater ability to distort and be compressed indicates that the red cells are more flexible than that of the slower packing sample. Therefore, we can say that the red cell packing rate is a measure or index of the red cell flexibility.

One of the main criticisms of the centrifugal measurement of red cell flexibility is the concept that the measurement is of sedimentation rate where rouleaux and aggregate formation is present, and this explains the effect of fibrinogen on packing rate (dealt with in the following section). Sedimentation, however, infers independent movement of cells in a semi-infinite length. This does not apply to the packing of red cells in normal blood. When red cells are randomly packed, we are bound to observe that some cells would form stacked columns as in rouleaux. However, even if all the cells were to form rouleaux and

aggregates, to give maximum packing, the fact that further packing, during centrifugation, occurs for this arrangement shows that the cells have a further ability to distort and compress. This ability depends on their flexibility.

It has been argued by Charm et al (1964) and Rand et al (1964), and observed by Schmid-Schonbein et al (1968) that rouleaux and aggregates of red cells in blood are broken down at shear rates above $46s^{-1}$. Using viscosity data for normal blood at different shear rates, Brooks et al (1967) found that disaggregation of red cells occurred when the shearing force was 5-10 x 10^{-7} dynes and that corresponds to a shear rate of $50-100s^{-1}$, agreeing with results of workers mentioned above.

If in a column of normal blood the red cells sediment to a haematocrit of 60% (100/60 of the column length), then cells must be deformed since maximum packing of rigid cells is achieved at 60% haematocrit, Chien et al (1968). Using this argument, Brooks et al (1967) calculated the deforming force on a red cell at the bottom of the red cell column. This was used to represent the upper limit of force needed to deform a human red cell. The value of the upper limit force was found to be 1.7 x 10^{-6} dyne, corresponding to a shear rate of $170s^{-1}$.

In our measurement of erythrocyte packing rate, the capillary tube is filled to a length of 5 cm and is then placed on a duraluminium Hawksley Microhaematocrit centrifuge head of 9 cm radius. For a rotational speed of

1480 rpm of the centrifuge head, the centrifugal force at the top of the 5 cm blood sample is 106g whereas at the bottom of the column it is 235g. For a red cell lying 1.25 cm down the length of the column, the force experienced by the cell due to centrifugal force and the weight of red cells above it is given as

$$\text{Force, } F = Ah' (\rho_c - \rho_p) g'$$

where A is the cell's cross-sectional area, h' is the length of the cell column above the red cell, ρ_c and ρ_p are the red cells and plasma densities respectively and g' is the centrifugal force acting on the red cell in question.

For a red cell of 4μ radius, A is $50.3 \times 10^{-8} cm^2$, h' is 1.25 cm, $(\rho_c - \rho_p)$ is typically 0.048 $gm.ml^{-1}$ and g' is 138.25g ($g = 981\ cm.s^{-2}$). Putting these values into the above equation gives the value of F as 4.09×10^{-3} dyne*. Comparing this value of F to the value of 1.7×10^{-6} dyne force needed to deform red cells and cause them to disaggregate, it can clearly be seen that the force of adhesion of rouleaux formation or aggregation is negligible when blood is centrifuged in the packing rate centrifuge.

Rampling and Sirs (1972) have also demonstrated that for blood samples equally diluted with either serum, saline or Ringer-Locke solution, although the degree of rouleaux formation in the three samples differed, their packing rate did not. This shows that rouleaux formation is of no consequence on the packing rate value.

* 1 Newton = 10^5 dynes

On the other hand, if sedimentation occurred in the blood samples, there would be a bi-phasic pattern to the packing rate of cells. An initial sedimentation phase, followed by a slower rate when all the cells are in contact. Using the automatic centrifuge technique, it was immediately apparent that no such bi-phasic pattern occurred, Sirs (1968).

Using the centrifugal method of measuring red cell packing rate, I have investigated the effect of varying the haematocrit on the packing rate for normal healthy donors. A calibration curve of the changes of packing rate values for changing haematocrit, at different temperatures, was obtained for normal blood. This was used to define the red cell flexibility index and for correcting the packing rate value at any haematocrit (within stated range) to that index.

(ii) Method

35 ml of blood were obtained from a healthy male donor by vene-puncture, using heparin to prevent coagulation. The measurements were made within four hours to avoid any rheological changes occuring. The blood was divided into sixteen 2 ml samples. The samples were then centrifuged at 500g for 5 minutes. To vary the haematocrit of the samples, different volumes of autologous plasma were removed from samples and added to others, keeping one sample unaltered as our normal blood haematocrit. The

variation of haematocrit was in the range of 30 to 55%. All the samples were then put on a mixer for half an hour to mix well before use and to allow the red cells to regain their normal shape if it was altered by the gentle centrifugation.

Two microhaematocrit tubes were filled from each of the blood samples to a length of 5 cm. The tubes were then placed on the packing rate centrifuge. The variation of haematocrit with time of centrifugation was measured for 6 minutes of centrifugation by photgraphic recording at 30 second intervals. The first experimental run was performed at room temperature, 22^{o}C. The same experiment was repeated for centrifuge temperatures of 30^{o}C and 37.5^{o}C, so as to find out how packing rate varies with temperature. When each experimental run was over, all the tubes were centrifuged for 4 minutes on a Hawksley Microhaemocrit Centrifuge at 13,000g to obtain the haematocrit of each sample.

The red cell packing rate was obtained by measuring the initial linear slope of the plot of change in haematocrit with centrifugation time, obtained from the film negative of the photographic record of the centrifuge head at 30 second intervals.

(iii) Results

For four healthy male donors, S, A, R and C, the variations of red cell packing rate with haematocrit at room temperature (22°C) are shown in Fig (10). Donor S is representative of a small but significant group of 1.5% of normals whose red cell flexibility is more than 3 standard deviations from the total population mean, Rampling and Sirs (1976). Donors A and R have very similar values of packing rate over haematocrit range used, 36-60%. Donor C possesses a lower value of packing rate, probably due to the lower plasma fibrinogen concentration, Φ, whose effect on the packing rate will be investigated in the following section.

Although the value of the packing rate for a given haematocrit, PR_H, differs between the four donors, the actual change in the value relative to the value of the packing rate at 45%, ($PR_{45\%}$), expressed as the ratio $PR_H/PR_{45\%}$, does not vary greatly. Figure (11) shows the value of the ratio $PR_H/PR_{45\%}$ for the four donors. Within the limits of error of $\pm$ 0.5% min^{-1} in the value of the packing rate, we can see that a common curve, the calibration curve, can be drawn for the four donors. This calibration curve can then be used to correct the value of the red cell packing rate at a given haematocrit, for normals only, to the value of the packing rate at a haematocrit of 45%. The value of the red cell packing rate

DONOR – S

HAEMATOCRIT %	36	38	39	39.5	41	42	43	44.5	45	46.5	47.5	48	50	52	53.5	56	58	59
RED CELL PACKING RATE % min^{-1}	12.55 11.5	11.64 11.35	10	10.85	10.25 10.14	9.3	9.7 8.74	8.85 8.5	8.6 8.16	8.4	8.7 8.0	8.0 7.3	7.67 7.13	6.5 6.2	6.65	6.34 6.0	6.16	5.78

DONOR – A

HAEMATOCRIT %	36.75	37.5	38	40	42	44	44.5	45	48	49.5	50.5	52.5	54	57	58.25	59.5	60
RED CELL PACKING RATE % min^{-1}	7.7	7.7 7.3	7.4	6.35	5.55	5.95 5.85	5.2	5.48	5.05	5.0 4.5	5.08 4.8	4.7	4.65 4.55	4.35 4.1	4.35	4.45 3.92	4.3 3.85

DONOR – R

HAEMATOCRIT %	36	37	39.5	40	40.5	41	42.5	43	45	46	46.75	49	51.5	52.5	55.5	56.5	58.5
RED CELL PACKING RATE % min^{-1}	9.5 8.85	8.0	6.72	6.8 6.45	7.15	5.8	6.4 5.85	5.72	5.75	5.7 5.0	5.03	4.75	5.35 4.4	4.7	4.2	4.5 3.95	4.0

DONOR – C

HAEMATOCRIT %	36	37	38	40	43	45	45.5	47	48	50	51	52.5	55.5	57	59.25	60
RED CELL PACKING RATE % min^{-1}	7.15 6.5	6.65	6.6 5.48	5.23	4.5 4.0	3.8	3.25	4.0	4.25	3.4 3.25	2.9	3.15	3.2 2.85	3.35 2.85	2.8	3.25

ERROR IN PACKING RATE MEASUREMENT = $\pm$ 0.5% min^{-1} ERROR IN HAEMATOCRIT MEASUREMENT = $\pm$ 0.125%

TABLE 6 FOR FIGURE 10

VARIATION OF RED CELL PACKING RATE WITH HAEMATOCRIT AT 22°C FOR BLOOD FROM DONORS S, A, R AND C

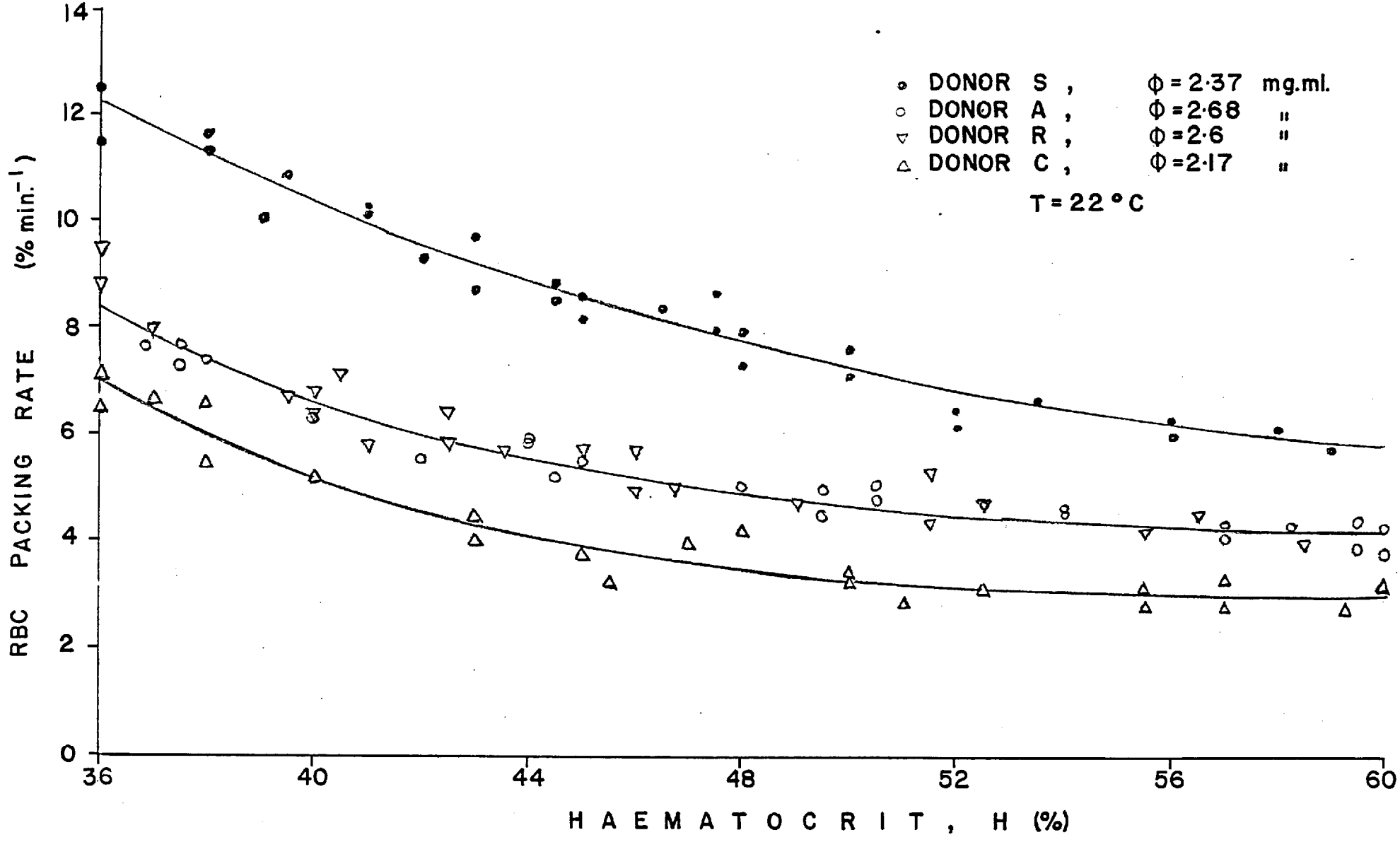


FIG. 10 : VARIATION OF RED CELL PACKING RATE WITH HAEMATOCRIT AT 22°C FOR BLOOD FROM DONORS S,A,R AND C

	HAEMATOCRIT %																								
	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
DONOR S ($PR_H/PR_{45\%}$)	1.42	1.37	1.31	1.26	1.21	1.16	1.12	1.09	1.04	1.0	0.97	0.93	0.90	0.87	0.85	0.83	0.80	0.78	0.76	0.74	0.72	0.71	0.70	0.69	0.68
DONOR A + R ($PR_H/PR_{45\%}$)	1.57	1.48	1.39	1.31	1.24	1.18	1.12	1.08	1.04	1.0	0.96	0.94	0.92	0.90	0.88	0.86	0.84	0.83	0.82	0.81	0.80	0.8	0.79	0.79	0.79
DONOR C ($PR_H/PR_{45\%}$)	1.82	1.68	1.55	1.44	1.33	1.25	1.17	1.12	1.05	1.0	0.96	0.92	0.9	0.87	0.85	0.82	0.81	0.80	0.79	0.79	0.79	0.78	0.78	0.78	0.78

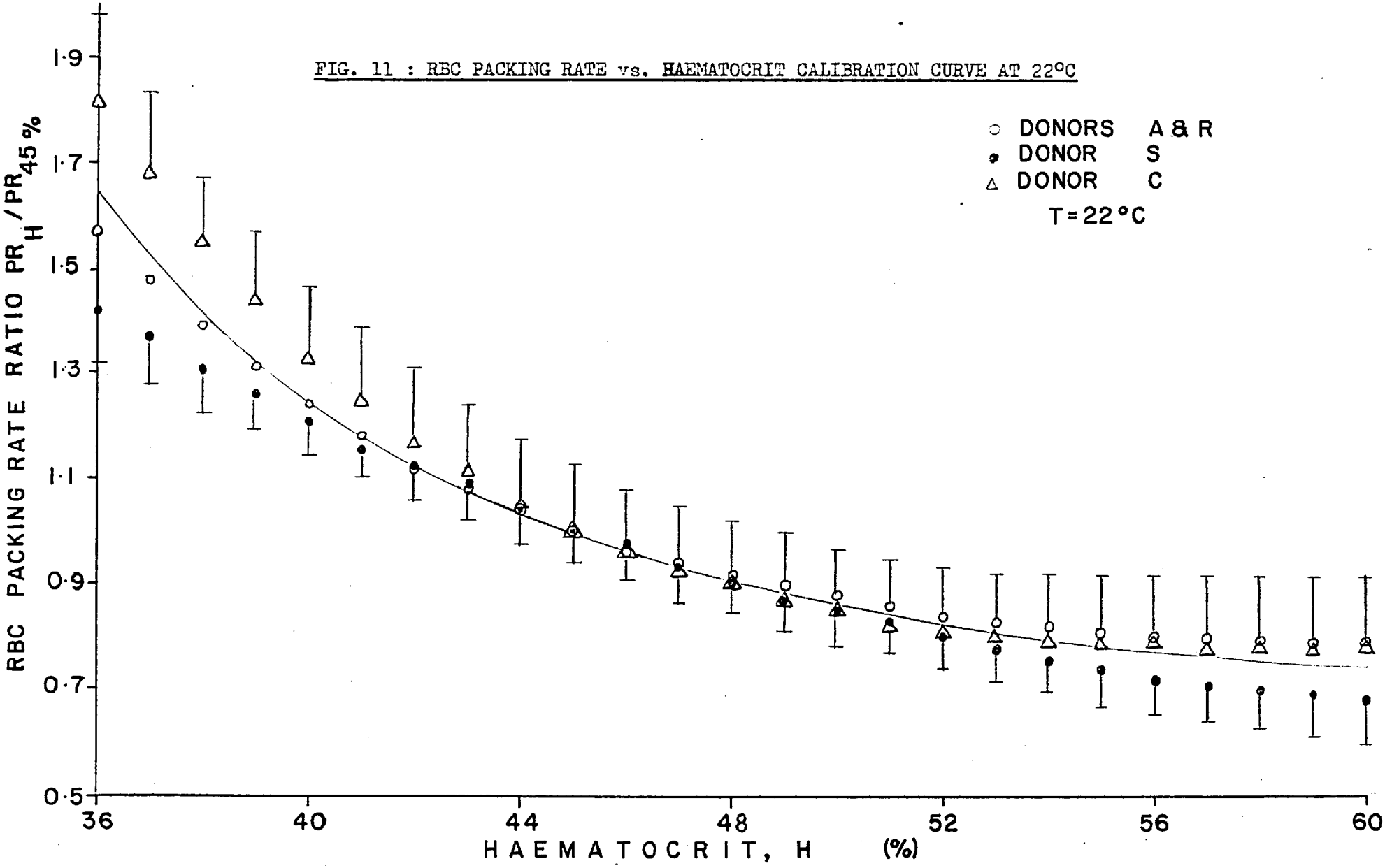
ERROR IN PACKING RATE RATIO = $\pm$ 0.8
ERROR IN HAEMATOCRIT MEASUREMENT = $\pm$ 0.125%

TABLE 7 FOR FIGURE 11

CALIBRATION CURVE AT 22°C

VALUES OF RATIO /(PACKING RATE AT H%)/(PACKING RATE AT 45%)/ AT DIFFERENT HAEMATOCRITS, H, AT 22°C FOR DONORS:- S, A, R AND C.
VALUES OF PR_H AND $PR_{45\%}$ ARE OBTAINED FROM PACKING RATE AGAINST HAEMATOCRIT CURVES.

FIG. 11 : RBC PACKING RATE vs. HAEMATOCRIT CALIBRATION CURVE AT 22°C



at H = 45% is called the red cell flexibility index, and is used as the name suggestes as an index or standard measure of the red cell flexibility.

Since the blood of donor R was found to be representative of the mean of a normal population, Rampling and Sirs (1976), it was not found necessary to carry out a survey of normal donors in our investigation. Since donor A's blood is similar to donor R's blood, A is also taken as a representative of the population mean.

The effect of increasing the temperature on the packing rate at different haematocrits for the same four donor's bloods are shown in Figs (12) and (13) for temperatures of 30°C and 37.5°C respectively. The respective calibration curves are also shown as Figs (14) and (15). Again we can see that the calibration curves can be drawn to be common to the four donors, within the error limits. It is observed that for the calibration curves at the three different temperatures, the deviations in values of $PR_H/PR_{45\%}$ for the four different donors increases towards the lower limit of 36% and upper limit of 60% haematocrit.

From the curves of packing rate against haematocrit for the four donors at different temperatures, the values of the red cell flexibility index at different temperatures are obtained. Figure (16) shows that when the temperature is increased from 22°-37.5°C the red cell flexibility is increased most for donors A and R, to a lesser extent for donor C and least for donor S. The increase is linear for

DONOR – S

HAEMATOCRIT %	36	37	37.5	38.5	39.5	40.5	42	43.5	45	47	48	50	51	52.5	55	57	58.5	59.5
PACKING RATE % min^{-1}	14 13.35	13.25 11.85	12.15	11.7 11.05	10.63	10	10.37 9.25	8.85 9.45	8.55 8.93	7.7	8.3	7 8.15	7.75	7.45 8.2	6.35 7.05	6.45 6.7	6.6	6.45

DONOR – A

HAEMATOCRIT %	37	38	40	40.5	41.5	43	45	46	47	48.5	49.5	53	56	57.5	59	59.5
PACKING RATE % min^{-1}	8.8 9.85	8.84	7.3 8.1	8.1	7.55	7.3	6.85	5.67	5.7 6.7	6.3 6.55	5.15 6.3	5.45 5.6	5.5 5.7	5.2 5.45	5.3	4.8

DONOR – R

HAEMATOCRIT %	36.5	39	41	42	44	46	48	50	52	54	55	56	58.5
PACKING RATE % min^{-1}	9.5	7.75 9.0	7.0 7.25	7.53 7.66	6.75 7.25	6.35 6.55	5.65 6.6	5.55 6.05	5.5 6.1	5.8 6.0	5.25	5.0	4.75 5.45

DONOR – C

HAEMATOCRIT %	36.5	38	40	41	42	43.25	44	45.5	48	48.5	51	54.5	58
PACKING RATE % min^{-1}	7.5 8.0	6.15 7.0	5.9 6.6	5.9	5.4	5.25	5.1	4.75 4.9	4.2	4.3	4.25 4.4	3.25 3.76	3.3 3.45

ERROR IN PACKING RATE MEASUREMENT = $\pm$ 0.5% min^{-1} ERROR IN HAEMATOCRIT MEASUREMENT = $\pm$ 0.125%

TABLE 8 FOR FIGURE 12

VARIATION OF RED BLOOD CELL PACKING RATE WITH HAEMATOCRIT AT 30°C FOR DONORS – S, A, R AND C

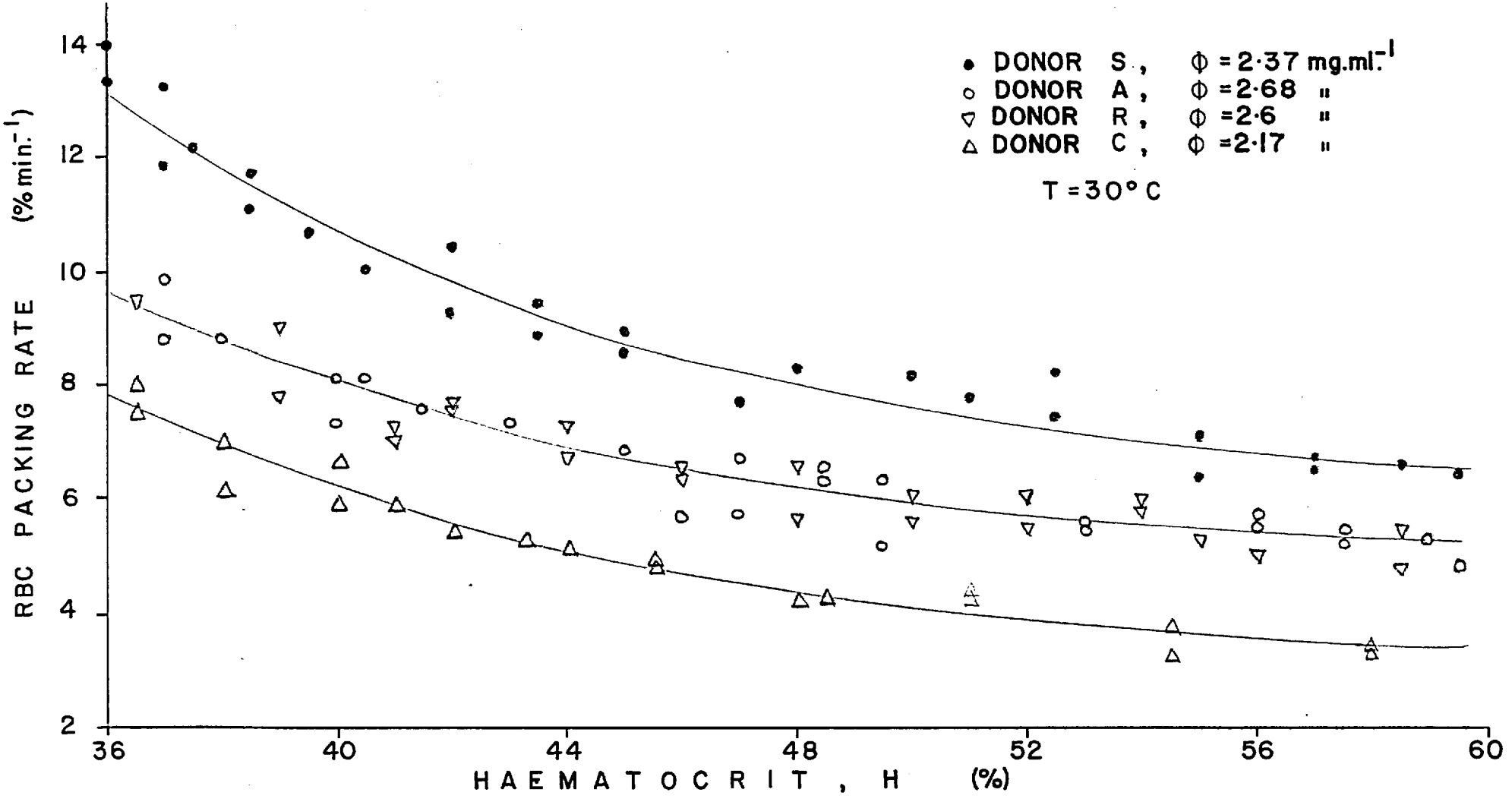


FIG. 12 : RBC PACKING RATE AT DIFFERENT HAEMATOCRITS AT 30°C FOR DONORS S,A,R AND C

DONOR – S

HAEMATOCRIT %	36.5	38.5	39.5	42	44	45	46	48	50	52.5	53	55	56	57	58	59	60
PACKING RATE % min^{-1}	13.2 12.54	11.79 11.36	10.82 10.46	11.13 10.39	9.81 9.3	9.22	9.3 9.1	8.44	7.9 7.35	7.05	7.30 7.04	6.63	6.15	6.7	5.98	6.6 6.38	6.2 6.0

DONOR – A

HAEMATOCRIT %	38	40.5	43	45	48	49	52	53	55.5	57	60
PACKING RATE % min^{-1}	10.6 10.17	9.85	8.9 8.8	8.25 7.85	7.55	7.0	6.0	6.05	5.27	4.7	4.74 4.4

DONOR – R

HAEMATOCRIT %	37	40	42	45	47	47.5	50	53	56.5	59
PACKING RATE % min^{-1}	11.05 10.5	9.8	9.17 9	8	8	7.37	6.72	6.15 5.78	5.17	4.9 4.5

DONOR – C

HAEMATOCRIT %	38	41	43	45	46.75	47.5	48.5	51	53	54	56	57.5
PACKING RATE % min^{-1}	8.5 7.8	6.64	6.65 6.25	6.0	4.65	5.56	5.0	4.65 3.75	4.5	3.05	3.5	2.8

ERROR IN PACKING RATE MEASUREMENT = $\pm$ 0.5% min^{-1} ERROR IN HAEMATOCRIT MEASUREMENT = $\pm$ 0.125%

TABLE 9 FOR FIGURE 13

VARIATION OF RED BLOOD CELL PACKING RATE WITH HAEMATOCRIT AT 37.5°C FOR DONORS – S, A, R AND C

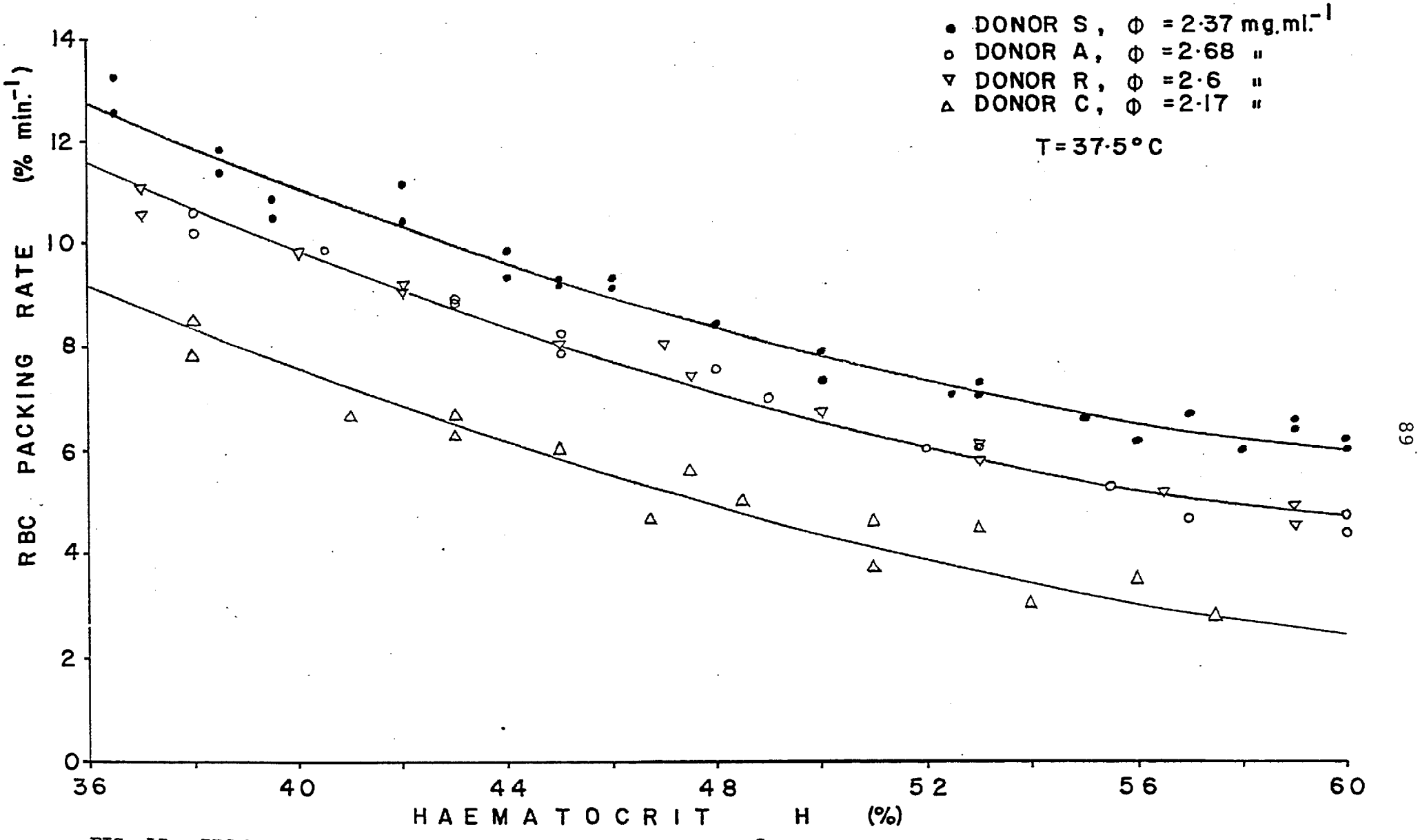


FIG. 13 : RBC PACKING RATE AT DIFFERENT HAEMATOCRITS AT 37.5°C FOR DONORS S,A,R AND C

	HAEMATOCRIT %																								
	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
DONOR S ($PR_H/PR_{45\%}$)	1.497	1.423	1.357	1.29	1.234	1.177	1.123	1.077	1.037	1.0	0.97	0.937	0.914	0.891	0.869	0.846	0.823	0.811	0.8	0.789	0.774	0.766	0.754	0.75	0.75
DONOR A + R ($PR_H/PR_{45\%}$)	1.44	1.373	1.313	1.254	1.209	1.157	1.112	1.075	1.03	1.0	0.97	0.948	0.925	0.896	0.881	0.862	0.851	0.836	0.821	0.806	0.799	0.791	0.791	0.787	0.787
DONOR C ($PR_H/PR_{45\%}$)	1.6	1.512	1.42	1.341	1.27	1.206	1.141	1.087	1.037	1.0	0.96	0.921	0.899	0.86	0.836	0.804	0.785	0.77	0.755	0.74	0.725	0.715	0.705	0.7	0.7

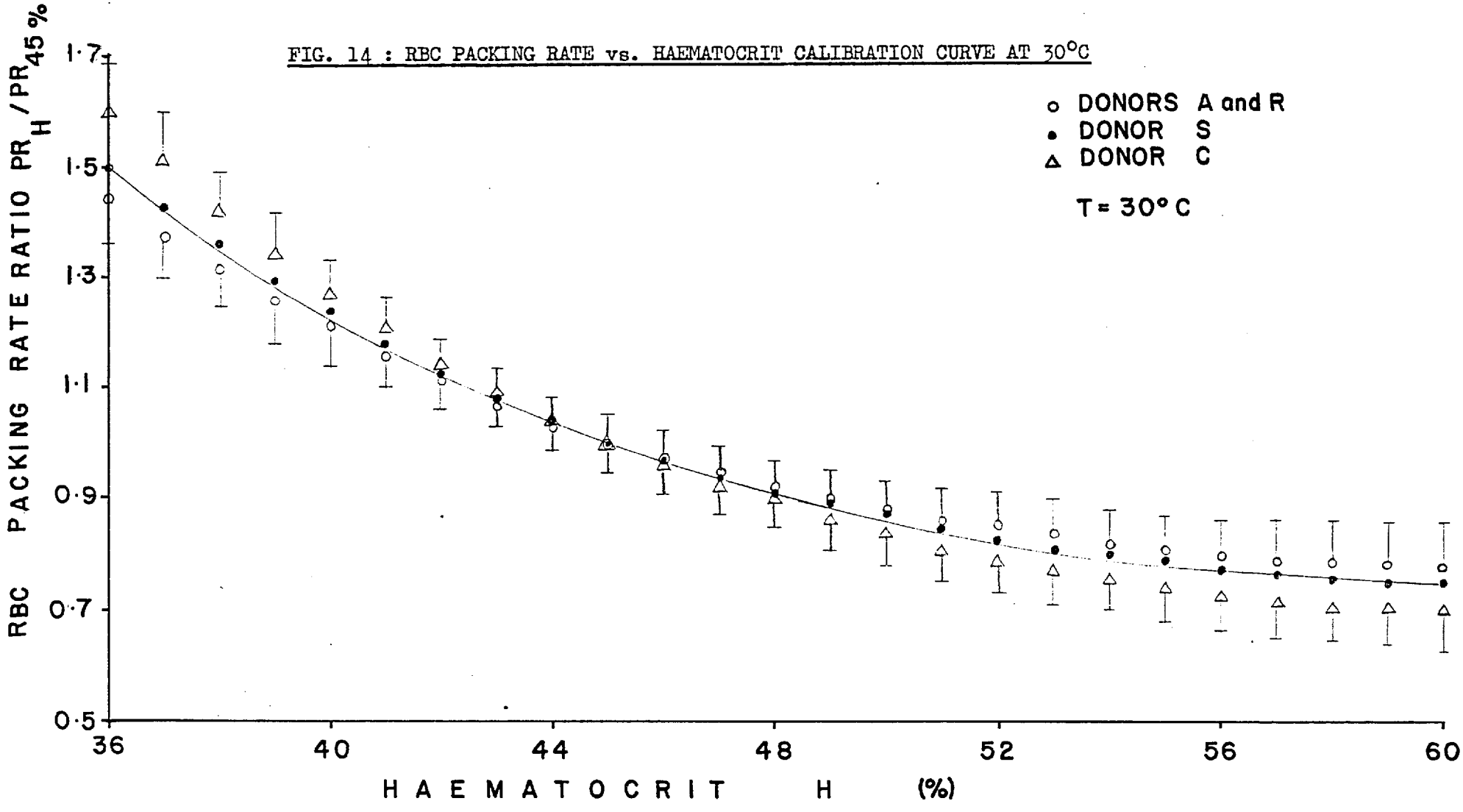
ERROR IN PACKING RATE RATIO = $\pm$ 0.8
ERROR IN HAEMATOCRIT MEASUREMENT = $\pm$ 0.125%

TABLE 10 FOR FIGURE 14

CALIBRATION CURVE AT 30°C

VALUES OF RATIO [(PACKING RATE AT H%)/(PACKING RATE AT 45%)] AT DIFFERENT HAEMATOCRITS, H, AT 30°C FOR DONORS:- S, A, R AND C.
VALUES OF $PR_{H\%}$ AND $PR_{45\%}$ ARE OBTAINED FROM PACKING RATE AGAINST HAEMATOCRIT CURVES.

FIG. 14 : RBC PACKING RATE vs. HAEMATOCRIT CALIBRATION CURVE AT 30°C



	HAEMATOCRIT %																								
	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
DONOR S (PR_H/PR_{45})	1.379	1.33	1.277	1.232	1.188	1.149	1.107	1.071	1.036	1.0	0.964	0.933	0.904	0.875	0.839	0.813	0.79	0.766	0.741	0.723	0.702	0.688	0.67	0.656	0.643
DONOR A + R (PR_H/PR_{45})	1.45	1.388	1.325	1.275	1.225	1.175	1.125	1.088	1.038	1.0	0.963	0.925	0.888	0.856	0.825	0.788	0.75	0.725	0.7	0.675	0.65	0.63	0.613	0.6	0.588
DONOR C (PR_H/PR_{45})	1.586	1.505	1.431	1.362	1.293	1.224	1.172	1.103	1.052	1.0	0.948	0.897	0.845	0.785	0.741	0.698	0.655	0.621	0.586	0.547	0.517	0.491	0.466	0.44	0.422

ERROR IN PACKING RATE RATIO = $\pm$ 0.8
ERROR IN HAEMATOCRIT MEASUREMENT = $\pm$ 0.125%

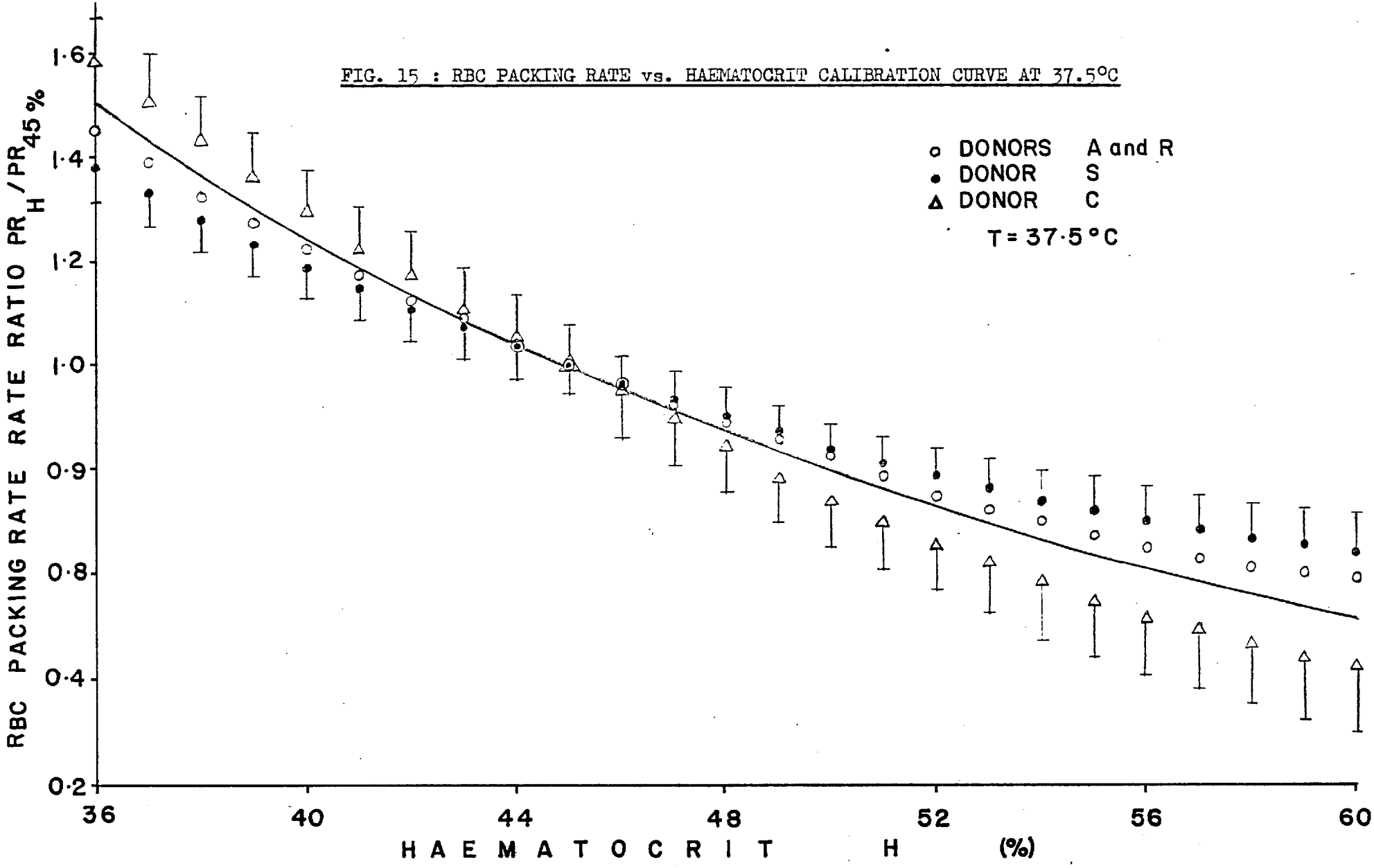
TABLE 11 FOR FIGURE 15

CALIBRATION CURVE AT 37.5°C

VALUES OF (PR_H/PR_{45}) FOR DIFFERENT HAEMATOCRITS AT 37.5°C FOR BLOOD OF DONORS:- S, A, R AND C.

VALUES OF PR_H AND PR_{45} OBTAINED FROM CURVES OF PACKING RATE AGAINST HAEMATOCRIT AT 37.5°C.

FIG. 15 : RBC PACKING RATE vs. HAEMATOCRIT CALIBRATION CURVE AT 37.5°C



	TEMPERATURE (°C)		
	22	30	37.5
DONORS A AND R	5.45	6.7	8.0
DONOR S	8.6	8.7	9.3
DONOR C	3.9	4.86	5.8

ERROR IN FLEXIBILITY INDEX MEASUREMENT = $\pm$ 0.5% min^{-1}
ERROR IN TEMPERATURE MEASUREMENT = $\pm$ 0.05°C

TABLE 12 FOR FIGURE 16

VARIATION OF RED CELL FLEXIBILITY INDEX
WITH TEMPERATURE FOR DONORS A, R, S AND C.

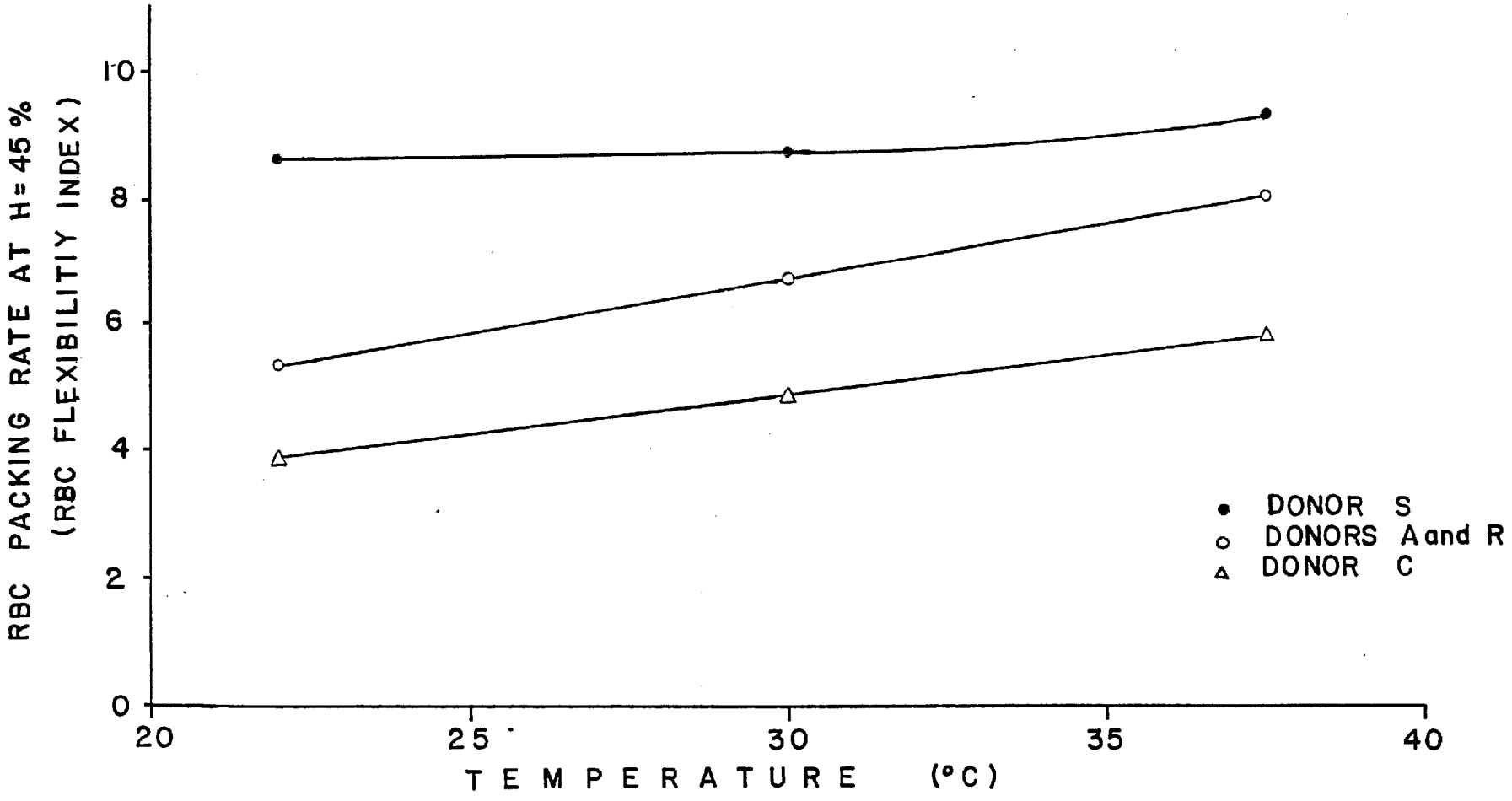


FIG. 16 : EFFECT OF TEMERATURE ON THE RBC FLEXIBILITY INDEX

donors A, R and C, but for donor S, temperature increase seems to have a small effect on the red cell flexibility.

(iv) Discussion

When discs and spheres are randomly packed, maximum haematocrit is achieved at 54 ± 2% and 62 ± 2% respectively. For rigid red cells, the cell volume is 64-77% of a disk of the same dimension. Therefore maximum haematocrit for randomly packed rigid red cells is obtainable at 35-42%, Sirs (1969a). In normal blood, where the red cells are randomly packed, if the haematocrit exceeds 42% then the cells are distorted and packed closely. When blood samples of haematocrits greater than 42% are centrifuged, packing occurs because the weight of red cells acts on cells at the bottom of the red cells column to distort and compress them. If the cells were rigid or inflexible, no packing would occur and no plasma would be separated out from in between the cells.

Since red cell packing rate depends on the centrifugal force, haematocrit, cell to plasma densities and the red cell flexibility, then by keeping the first three factors constant we can compare the red cell flexibility of different blood samples. If the packing rate of the different blood samples differ, then the red cell flexibilities differ, Sirs (1971). Hence the red cell packing rate as measured on our centrifuge method is an index or measure of red cell flexibility. The packing rate

at a haematocrit of 45% is called the red blood cell flexibility index and is used in comparing different bloods.

Although normal donors might possess different red cell flexibilities at a given haematocrit, the general behaviour in variation of packing rate with change in haematocrit is basically the same. This is seen by the closeness of values in the calibration curves to give a common calibration curve at the different temperatures. The deviation in curves for the different donors increase for haematocrits below 38% and above 55%. Below 38% the lower limit of haematocrit at which randomly packed red cells are in contact is reached. Above 55%, the randomly packed red cells are distorted to accommodate the extra cells since the higher limit of haematocrit for random packing is lower, at 42%. This means that for red cells of more flexibility, the distortion is greater than cells of lower flexibility and therefore the packing rate value is less accurate. In general, outside the haematocrit range of 38% to 55%, inaccuracies are introduced into the value of the packing rate because of the number of red cells present.

Williamson et al (1975) observed that red cell membrane fluidity increases significantly as the temperature is increased from 24^{o} to $37^{o}C$. Beside the increased membrane fluidity, the interacellular content viscosity as well as the plasma viscosity decrease with increasing temperature. This trend was also observed for the red cells flexibility index. For donors A and R the red cell flexibility index

increased with temperature in a linear relationship. For donor C the relationship was linear but at a lower gradient showing that although there is a difference in Φ between the plasma of donor C and that of the other donors, temperature affected his cells' flexibility to a lesser degree than donors A and R's cells. For donor S the increase in temperature was observed to increase his red cell flexibility index very slightly compared to the other donors. Donors S and R are of the same blood group A and their MCV was about the same 85-90 fl. as well as their MCHC, 31-32.5 gm dl^{-1}. Hence the higher red cell flexibility of donor S is probably due to a membrane abnormality, Rampling and Sirs (1976). This membrane abnormality is also probably responsible for the lower dependence of the red cell flexibility index on temperature in the case of donor S red cells compared to the other donors.

II FIBRINOGEN EFFECT ON RED CELL FLEXIBILITY AT DIFFERENT TEMPERATURES

(i) Introduction

Fahreaus (1921, 1929) observed that red cell aggregation and ESR of blood varied in disease. These variations were dependent on the plasma proteins, mainly fibrinogen, with the ESR increasing with plasma fibrinogen concentration,

Hardwick and Squire (1952), Wells et al (1964) and Dintenfass and Forbes (1975).

Using the automatic recording centrifuge, Rampling and Sirs (1970) observed that when blood was defibrinated, the red cell flexibility was reduced from normal blood. Similarly, when red cells were repeatedly washed, their flexibility was significantly reduced. The small difference between the residual red cell flexibility in serum and the smaller red cell flexibility in saline suspension, was attributed to plasma proteins other than fibrinogen. By artificially varying the plasma fibrinogen concentration, Rampling and Sirs (1977) observed that the red cell flexibility increased with fibrinogen concentration in a linear relationship.

Natural increases in plasma fibrinogen concentration due to post-surgical trauma, in human patients, Dupont and Sirs (1977), as well as in rabbits, Sirs and Macdonald (1974), were found to maintain the linear relationship to red cell flexibility.

During aggregation, it has been observed, Chien and Jan (1973), that red cells membrane surfaces are parallel to each other and are connected through the monolayer of fibrinogen or macro-molecules (dextran) adsorbed onto the surface of their membranes. In forming parallel surfaces, the cells are deformed when they interact together. Hence

the more flexible the red cells are, the higher is the degree of aggregation. This will be shown to be the case, in the following chapter.

The increased values of the ESR in patients with elevated plasma fibrinogen concentration, Φ, as observed by Fahraeus (1921), can be attributed not only to increased aggregation but also to increased red cell flexibility as a result of increased Φ. This in turn increases the degree of aggregation and the value of the ESR.

Reid et al (1976), using a 'Nucleopore' filter to measure red cell deformability, have observed that there was no correlation between Φ and red cell deformability. This result is in contradiction with results obtained by Schmid-Schonbein et al (1973) using 'Nucleopore' filters and Baar (1976) using a silver membrane sieve, in which they observed that red cell deformability is increased with Φ. Some of the reasons for the contradictions in results obtained with the microfiltration technique are that, due to the size of the filter pores, 3-4 μm, when a red cell flows through the pore, its viscosity would be greatly decreased due to the Fahraeus-Lindqvist effect, Fahraeus and Lindqvist (1931). This would make the interpretation of the microfiltration results rather difficult, Rampling and Sirs (1976). Another problem which plagues the microfiltration technique measurements on whole blood is the problem of the inflexible white blood cells, Bagge et al (1981), which block the micropores, giving false data on the red cell deformability, Barbenel (1981).

Another technique for measuring an index of red cell deformability is the micropipette technique developed by Rand and Burton (1964) for measuring red cell membrane stiffness. Due to the size of the micropipette pore, 3-5 μm, the problem of the Fahraeus-Lindqvist effect is applicable. In whole blood there is the additional complication of the buffy coat on the red cell. When the red cell is sucked into the micropipette the buffy coat layer is sucked in first, and hence the pressure used to suck the red cell into the micropipette is not only dependent on the red cell membrane stiffness but also on the resistance of the buffy coat layer. If plasma proteins are removed by washing and resuspending the red cells in plasma protein-free solution, then when the cells are sucked into the micropipette, contact with the glass surfaces will affect the red cell shape. This effect was observed to transform the cell shape from discs to spheres, Ponder (1929). A change in the shape of the red cell will affect its membrane, and this would affect the measurement of the membrane stiffness.

Using the packing rate centrifuge, which does not suffer from any of the problems mentioned above for the microfiltration and micropipette techniques, I investigated the relationship between Φ and red cell flexibility at different temperatures. I have also investigated the effect of diluting plasma proteins with saline rather than serum, for normal bloods.

(ii) Methods

All blood samples used in these investigations were obtained by vene-puncture from normal healthy male donors. The blood was always heparinized to prevent coagulation using solid lithium heparin at concentration of 12.5 i.u. per ml of blood.

The plasma fibrinogen concentration, ϕ , was varied in two ways. The first method was used to decrease ϕ from normal plasma value, and the second method was used to increase ϕ. In the first method, the plasma was diluted with the sample's autologus serum. The serum was obtained by gently centrifuging the blood sample at 1000g for 5 minutes and then removing the plasma. The plasma was then heated in a water bath at a temperature of 56^{o}C for 10 minutes. At this temperature the fibrinogen molecule is denatured to fibrin and is precipitated out of solution. The fibrin clot is removed from the serum by centrifuging the solution at 1000g for $\frac{1}{2}$ hour to give a clear serum solution. As a check on whether this procedure may have precipitated other proteins, which might affect red cell flexibility, a second serum solution was obtained by clotting out the fibrinogen using thrombin. The thrombin used was Thrombin USP Thrombostat$_{TM}$ (Parke-Davis, NJ, USA) at concentration of 1000 US units per ml. Only 0.5 ml of the thrombin solution was added to each 10 ml of plasma, and the plasma was left overnight at room temperature for the clot to form. The

clot was removed by centrifugation at 1000g for ½ hour. Separate experiments were carried out using the two types of serum solutions to check for any differences.

To increase Φ in a blood sample above the normal plasma value, the second method was employed. In this procedure, human fibrinogen powder (Kabi Grade L, Kabi Pharmaceuticals, Stockholm, Sweden) is dissolved in distilled water to give a maximum obtainable fibrinogen concentration of 10 $mg.ml^{-1}$. Since to each gram of the kabi fibrinogen power, there is 1.2 grams of NaCl and 0.4 grams of sodium citrate added, the tonicity and pH of the solution was corrected. The tonicity is corrected to isotonicity by addition of distilled water, and the pH is corrected to 7.4 by addition of isotonic HCl or NaOH solutions. The final concentration of the fibrinogen solution is reduced to 7 mg ml^{-1}.

Fibrinogen concentrations in blood samples are increased by replacing a minimum volume of plasma with the high concentration fibrinogen solution. The plasma is removed by centrifuging the blood samples at 500g for 5 minutes. To find out whether the sodium citrate in the fibrinogen powder has affected the red cell flexibility values, isotonic solution of pH = 7.4 of sodium citrate, equivalent to the maximum amount added to the samples, was added to a normal blood sample and compared to another normal blood sample diluted with the same volume of saline.

The effect of diluting plasma proteins, other than fibrinogen, on the red cell flexibility index was investigated at different temperatures of 22°, 30° and 37.5°C. 30 ml of blood, of haematocrit corrected to 45%, was divided into eight samples of equal volumes. The samples were gently centrifuged at 500g for 10 minutes. 72.5% of the plasma volume was removed from each sample. To one sample the plasma was replaced by an equal volume of autologus serum, and to another sample, the plasma was replaced by an equal volume of saline. To the other six samples, varying ratios of volumes of serum and saline were used to replace the volume of plasma removed. All the samples were mixed for ½ hour before the red cell packing rate was measured in duplicate for each sample on the packing rate centrifuge, Amin et al (1983).

To measure the plasma fibrinogen concentration, the clot precipitation method of Rampling and Gaffney (1976) was used. This involved precipitation of the fibrin clot using thrombin, collecting and drying the clot, and then dissolving it in 5 ml of 0.1N NaOH/4M urea solution. The protein concentration was then measured by U/V absorption of the solution at λ = 280 nm, in a spectrophotometer as described in section IV of Chapter 2.

(iii) Results

To investigate the effect of diluting plasma proteins (other than fibrinogen) on red blood cell flexibility, the same volume of plasma was removed from all blood samples and replaced by varying ratios of serum and saline. The plasma was removed by centrifuging the samples at 500g for 5 minutes. For zero dilution, the removed plasma was replaced with serum only, and for maximum dilution, the plasma removed was replaced by saline only. Fig (17) shows the obtained results for 24^{o}, 30^{o} and $37.5^{o}C$. It is seen that diluting plasma proteins reduces the red cell flexibility index. However, if the dilution is kept to within 10%, the change in the red cell flexibility index is within the measurement error of $\pm$ 0.5% min^{-1} and 10% of the zero dilution value.

The relationship between plasma fibrinogen concentration and red cell flexibility index was investigated for five normal healthy male donors; A, B, C, R and S. The blood samples were obtained, as in all cases, by vene-puncture, using heparin as anticoagulant. The fibrinogen concentration, ϕ , was reduced from normal blood value using serum prepared in two different ways. The first was prepared by heat precipitating fibrinogen, and the second by clotting fibrinogen with thrombin. To increase ϕ , an isotonic solution of pH = 7.4 of human fibrinogen powder (Kabi Grade L) was made up to a concentration of about 7 mg ml^{-1}. Equal volumes of plasma (10% of total volume) was

TEMPERATURE (°C)	SALINE CONCENTRATION IN PLASMA (%)							
	0	5	12	24	36.5	48.25	60.5	72.5
22	3.25 3.05	3.1 3.025	2.775 2.6	2.45 2.15	2.125 2.0	2.05 1.7	1.925 1.55	1.825 1.52
30	4.65 4.6	4.55 4.375	4.3 4.15	3.55 3.35	2.95 2.85	2.225 2.15	2.325 2.0	2.25 2.075
37.5	5.8 5.325	5.5 5.25	4.925 4.7	4.05 3.775	3.425 3.375	3.1 2.85	2.725 2.6	2.625 2.55

ERROR IN FLEXIBILITY INDEX MEASUREMENT = $\pm$ 0.5% min^{-1}

TABLE 13 FOR FIGURE 17

VALUES OF RED CELL FLEXIBILITY INDEX FOR VARYING DILUTION OF PLASMA PROTEINS (OTHER THAN FIBRINOGEN) WITH SALINE, AT 22°C, 30°C AND 37.5°C

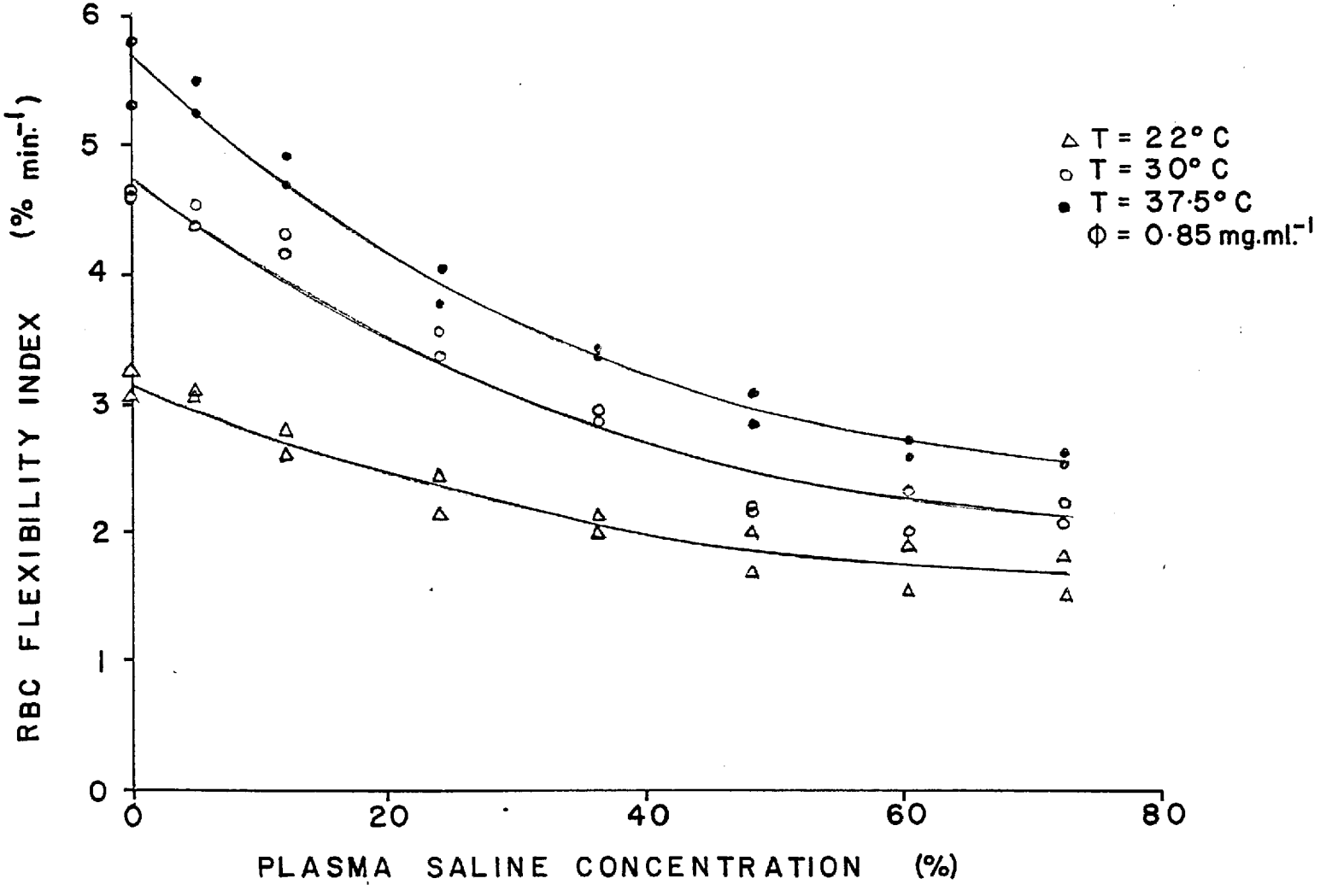


FIG. 17 : EFFECT OF PLASMA DILUTION WITH SALINE ON THE RBC FLEXIBILITY INDEX

removed from the blood samples and replaced with different ratios of serum and fibrinogen solution. Maximum Φ was achieved by adding fibrinogen solution only.

Figs. (18), (19) and (20) show that for subjects A, R and S respectively the relationship between red cell flexibility index and ϕ is linear. Also there is no difference between the two serums used to dilute the plasma and therefore heat precipation of fibrinogen does not affect other plasma proteins which affect red cell flexibility. The values of red cell flexibility for varying ϕ for donors A, B and R are similar, whereas donor S has higher values. This is because S is a donor representative of a small but significant group of 1.5% normals whose red cell flexibility is more than 3 standard deviations from the total population mean, Rampling and Sirs (1976). The dilution of plasma proteins was kept to within 10% of total volume for reasons mentioned above.

When temperature is increased, the values of the red cell flexibility index increases for all values of Φ , but maintains the linear relationship between the two parameters as seen on Figs (21), (22) and (23). Donor C is a normal donor whose red cell flexibility is slightly lower than the average values of donors A, B and R.

To each gram of human fibrinogen powder (Kabi Grade L), 1.2 grams of sodium citrate was added. For a fibrinogen solution of 7 mg ml^{-1} concentration, there is 8.4 mg ml^{-1} of sodium citrate. An isotonic solution made up of 8.4 mg

SERUM DILUTION	FIBRINOGEN CONCENTRATION ϕ (mg ml^{-1})	0	0.44	1.0	1.3	2.02	2.56
THROMBIN CLOTTED SERUM	RED CELL FLEXIBILITY INDEX (% min^{-1})	0.8 0.65	1.325 1.0	2.075 1.75	2.57 2.3	3.15 3.075	4.23 3.58

SERUM DILUTION	ϕ (mg ml^{-1})	0	0.73	1.2	1.68	2.39	2.42	2.76
HEAT PRECIPITATED SERUM	RED CELL FLEXIBILITY INDEX (mg ml^{-1})	0.875 0.75	1.75 1.2	2.41 2.0	2.875 2.53	4.0 3.53	3.75 3.55	4.05 3.94

KABI FIBRINOGEN	ϕ mg ml^{-1}	2.84	2.95	3.01	3.2	3.38	3.8	3.98	4.04
SOLUTION DILUTION	RED CELL FLEXIBILITY INDEX (mg ml^{-1})	4.42 4.2	4.475 4.17	3.83 3.7	4.525 4.25	5.5 4.625	5.425 5.26	5.75 5.45	5.7 5.625

REGRESSION EQUATION:- $Y = 1.211X + 0.713$
CORRELATION COEFFICIENT = 0.987
ERROR IN FLEXIBILITY INDEX MEASUREMENT = $\pm$ 0.5% min^{-1}
ERROR IN ϕ MEASUREMENT = $\pm$ 0.1 mg ml^{-1}

TABLE 14 FOR FIGURE 18

RED CELL FLEXIBILITY INDEX AT DIFFERENT VALUES OF PLASMA FIBRINOGEN CONCENTRATION, ϕ, USING SERUM AND FIBRINOGEN SOLUTION (KABI) DILUTION AT 24°C FOR BLOOD OF DONOR A

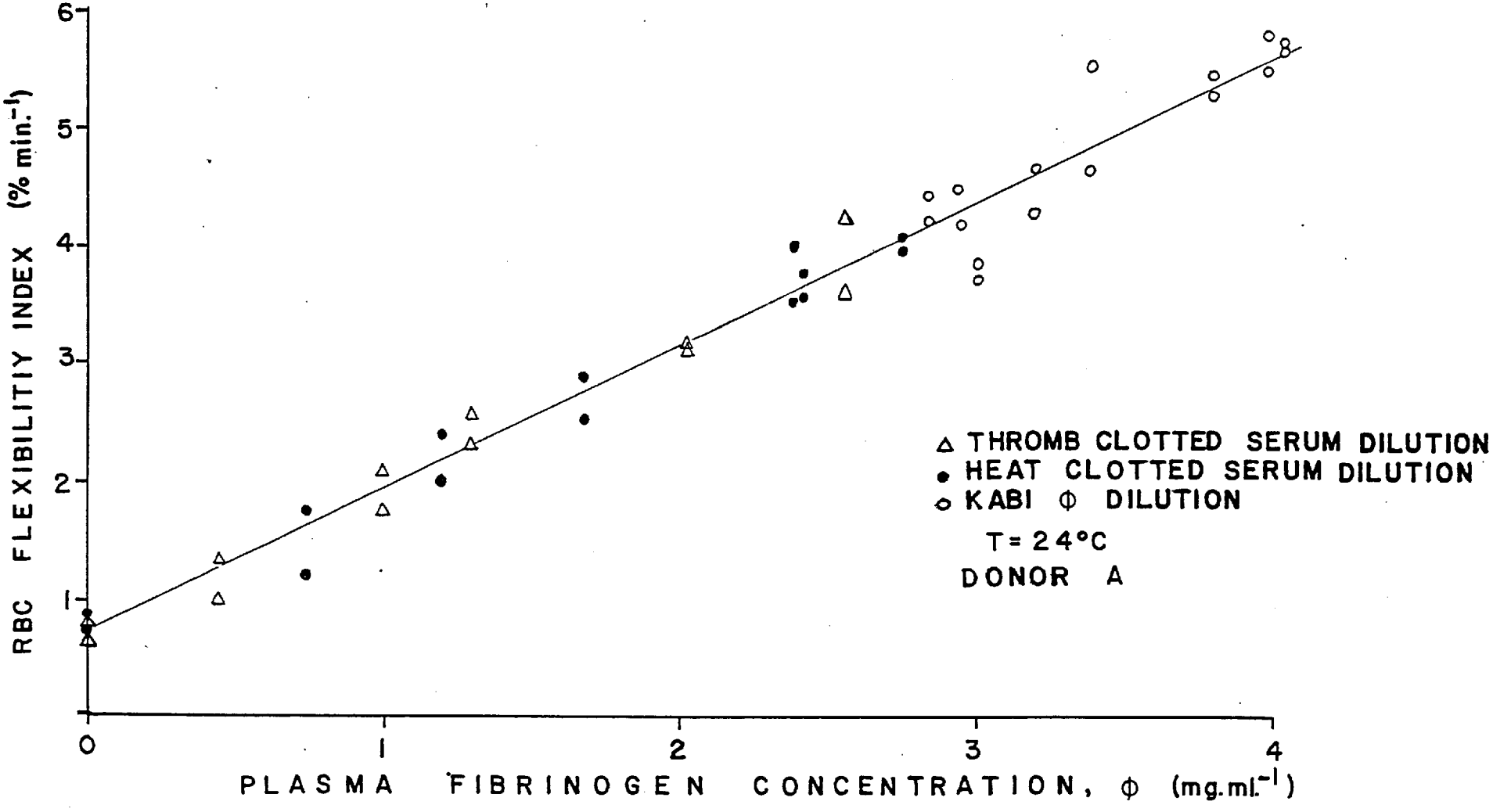


FIG. 18 : EFFECT OF PLASMA DILUTION WITH TWO DIFFERENTLY PREPARED SERUMS ON THE RBC FLEXIBILITY INDEX

SERUM DILUTION

THROMBIN CLOTTED SERUM

ϕ (mg ml^{-1})	0	0.6	1.2	1.85	2.36	2.8
RED CELL FLEXIBILITY INDEX (% min^{-1})	0.75 0.75	1.55 1.3	2.02 2.8	3.17 2.925	3.64 3.47	4.25 4.15

SERUM DILUTION

HEAT PRECIPITATED SERUM

ϕ mg ml^{-1})	0	0.4	1.0	1.42	2.12	2.3	2.38
RED CELL FLEXIBILITY INDEX (% min^{-1})	0.8 0.68	1.4 1.24	2.0 1.65	2.5 2.3	2.875 2.7	3.625 3.4	3.9 3.675

FIBRINOGEN SOLUTION (KABI) DILUTION

ϕ mg ml^{-1})	3.17	3.34	3.43	3.52	3.75	3.86	4.08
RED CELL FLEXIBILITY INDEX (% min^{-1})	4.65 4.575	3.75 3.5	5.1 4.925	5.12 4.575	5.275 5.05	5.0 4.85	6.05 5.55

REGRESSION EQUATION:- $Y = 1.144X + 0.794$
CORRELATION COEFFICIENT = 0.976
ERROR IN FLEXIBILITY INDEX MEASUREMENT = $\pm$ 0.5% min^{-1}
ERROR IN ϕ MEASUREMENT = $\pm$ 0.1 mg ml^{-1}

TABLE 15 FOR FIGURE 19

RED CELL FLEXIBILITY INDEX AT DIFFERENT VALUES OF PLASMA FIBRINOGEN CONCENTRATION, ϕ, USING SERUM AND FIBRINOGEN SOLUTION (KABI) DILUTION AT 24°C FOR BLOOD OF DONOR R

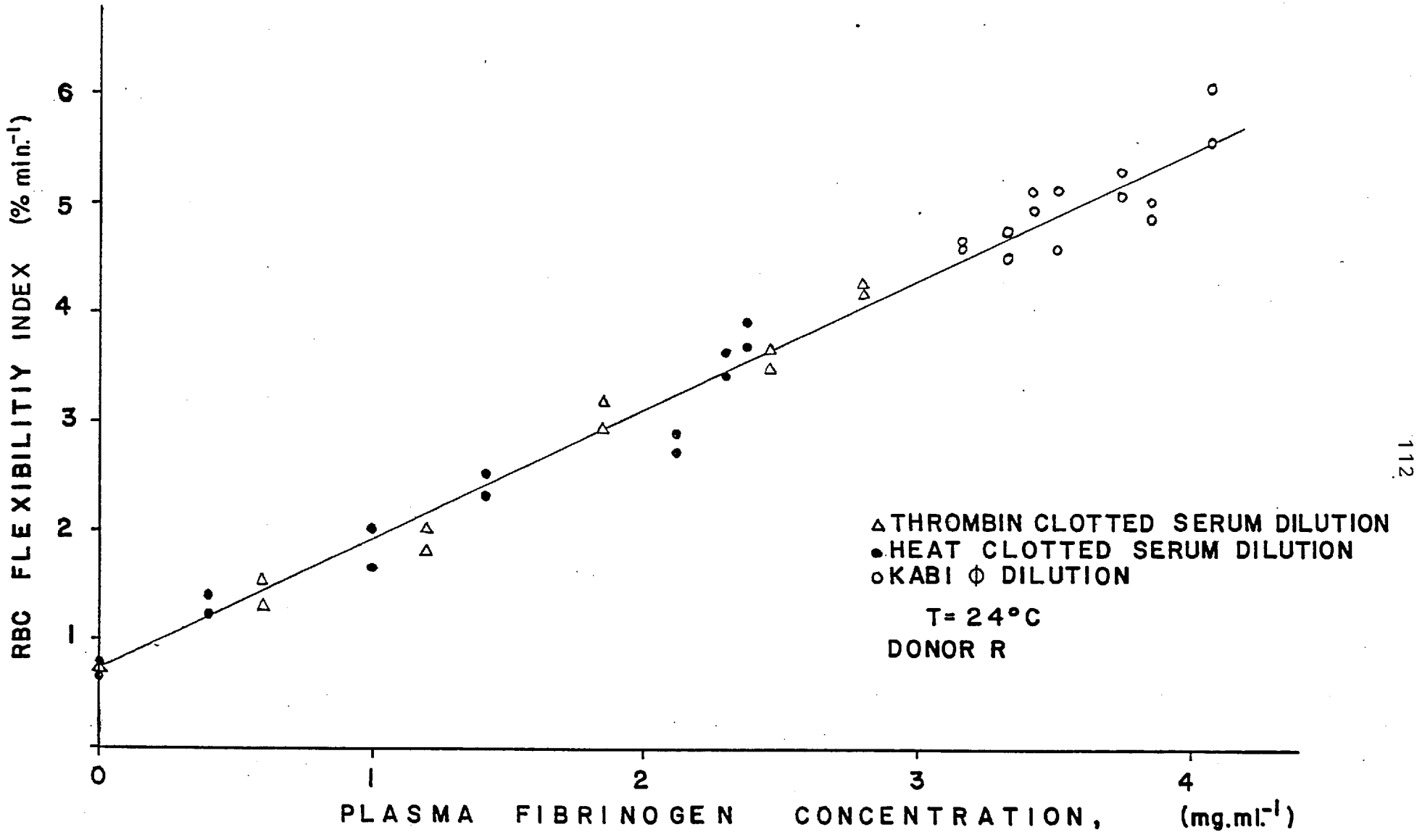


FIG. 19 : EFFECT OF PLASMA DILUTION WITH TWO DIFFERENTLY PREPARED SERUMS ON THE RBC FLEXIBILITY INDEX

SERUM DILUTION THROMBIN CLOTTED SERUM

ϕ (mg ml^{-1})	0.39	0.8	1.4	1.8	2.5
RED CELL FLEXIBILITY INDEX (% min^{-1})	2.83 2.65	3.95 3.06	5.28 4.99	6.95 6.25	8.0 7.75

SERUM DILUTION HEAT PRECIPITATED SERUM

ϕ (mg ml^{-1})	0	0.46	0.87	1.32	1.75	2.2	2.8
RED CELL FLEXIBILITY INDEX (% min^{-1})	1.7	2.85 2.7	4.0 3.0	5.04 4.73	6.64 6.05	8.01 6.55	8.65 8.47

KABI FIBRINOGEN DILUTION

ϕ (mg ml^{-1})	3.4	3.65	3.8	3.95	4.1
RED CELL FLEXIBILITY INDEX (% min^{-1})	10 9.6	10.85 10.43	11.8 10.03	11.66 11.45	11.7 10.98

REGRESSION EQUATION:- $Y = 2.428X + 1.749$
CORRELATION COEFFICIENT = 0.991
ERROR IN FLEXIBILITY INDEX MEASUREMENT = $\pm$ 0.5% min^{-1}
ERROR IN ϕ MEASUREMENT = $\pm$ 0.1 mg ml^{-1}

TABLE 16 FOR FIGURE 20

RED CELL FLEXIBILITY INDEX AT DIFFERENT VALUES OF PLASMA FIBRINOGEN CONCENTRATION, ϕ, USING SERUM AND FIBRINOGEN SOLUTION (KABI) DILUTION AT 24°C FOR BLOOD OF DONOR S

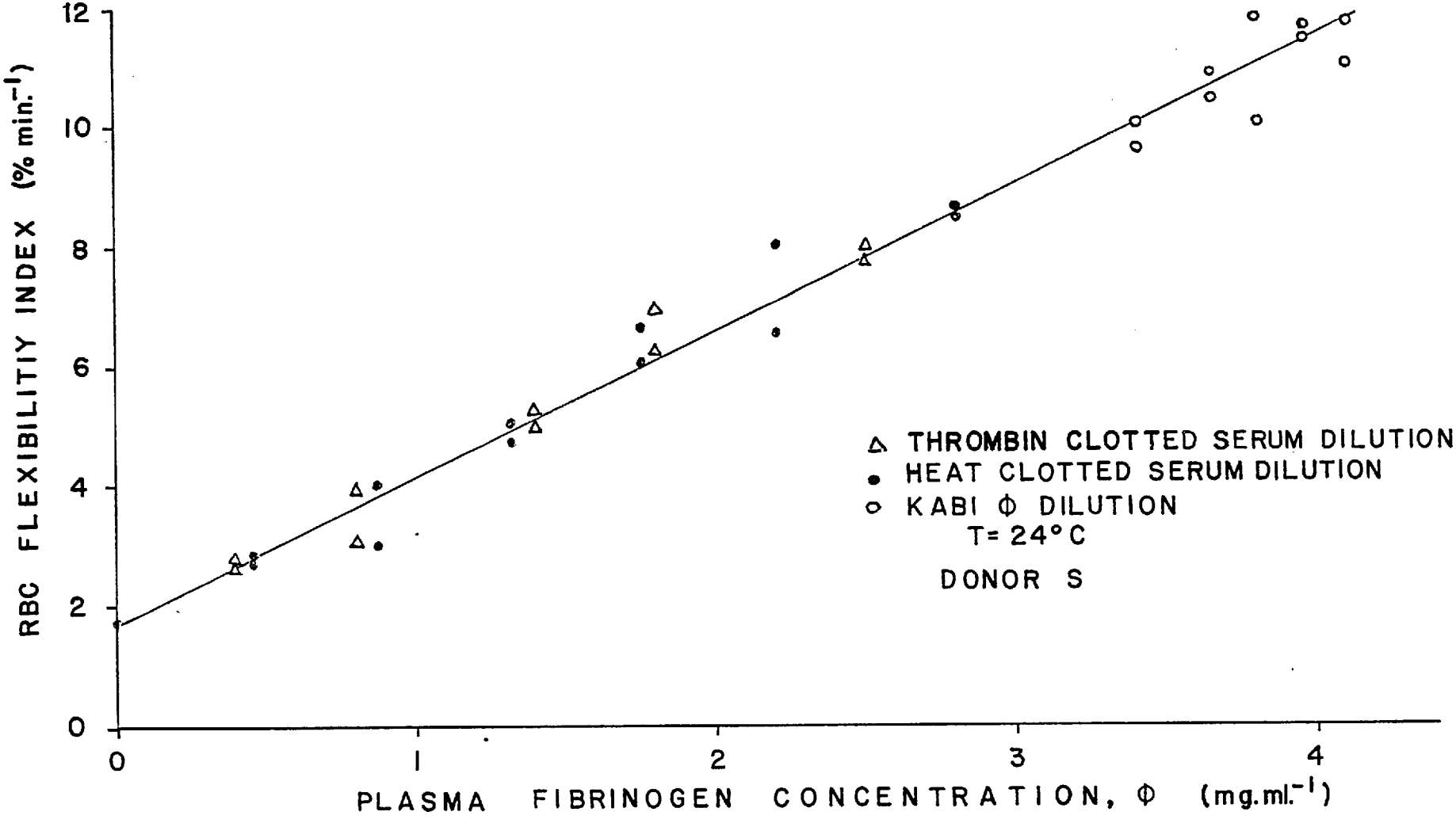


FIG. 20 : EFFECT OF PLASMA DILUTION WITH TWO DIFFERENTLY PREPARED SERUMS ON THE RBC FLEXIBILITY INDEX

EFFECT OF VARYING PLASMA FIBRINOGEN CONCENTRATION, ø, ON RED CELL FLEXIBILITY INDEX FOR BLOOD OF DONORS A, B AND R AT 30° AND 37.5°C

	PLASMA FIBRINOGEN CONCENTRATION, ø (mg ml^{-1})																						
	0 Saline	0 Serum	0.7	1.1	1.2	1.52	1.64	1.68	1.81	1.97	2.1	2.33	2.65	2.68	2.72	2.76	2.96	3.1	3.6	3.91	4.06	4.4	4.6
RED CELL FLEXIBILITY INDEX AT 30°C (% min^{-1})	1.0 1.42	1.56 1.27 1.0	2.7 2.6 2.4	3.86 3.65 3.4 3.26	3.85 3.44 3.3	4.55 4.2 4.0	4.44 3.95	4.25 4.15	4.3 3.85	5.5 5.15	5.5 5.35	5.8 5.53	6.9 6.2	6.43 6.2	7.14 6.7	7.15 6.4 6.25	7.74 7.2 6.5	7.25 7.16	8.13 7.7	8.95 8.5	9.1 8.5 8.16	9.8 9.53	10.24 9.82 9.65

REGRESSION EQUATION:- Y = 1.883X + 1.309
CORRELATION COEFFICIENT = 0.991

	PLASMA FIBRINOGEN CONCENTRATION, ø (mg ml^{-1})																						
	0 Saline	0 Serum	0.73	1.1	1.4	1.5	1.73	1.97	2.07	2.31	2.5	2.68	3.0	3.1	3.43	3.6	3.62	3.84	3.91	4.05	4.1	4.4	4.47
RED CELL FLEXIBILITY INDEX AT 37.5°C (% min^{-1})	3.4	3.75 3.6 3.16	5.15 4.65 4.45 4.28	5.38 5.25 4.64 4.48	6.3 5.75	6.45 6.06 5.75	6.35 6.05 5.95	7.45 6.84 6.1	7.5 6.75 6.25	8.05 7.63 7.5	8.65 7.6 7.4 7.05	8.28 7.8 7.5	8.6 8.25 7.85	8.96 8.85 8.35	9.56 9.45 9.2	9.35 9.0	9.75 9.25	9.64 9.15	10.46 9.76 9.65	9.38 9.04 8.35	10.85 10.75 10.34 9.5	10.95 10.83	10.85 10.68

REGRESSION EQUATION:- Y = 1.615X + 3.514
CORRELATION COEFFICIENT = 0.974

ERROR IN FLEXIBILITY INDEX MEASUREMENT = $\pm$ 0.5% min^{-1}
ERROR IN ø MEASUREMENT = $\pm$ 0.1 mg ml^{-1}

TABLE 17 FOR FIGURE 21

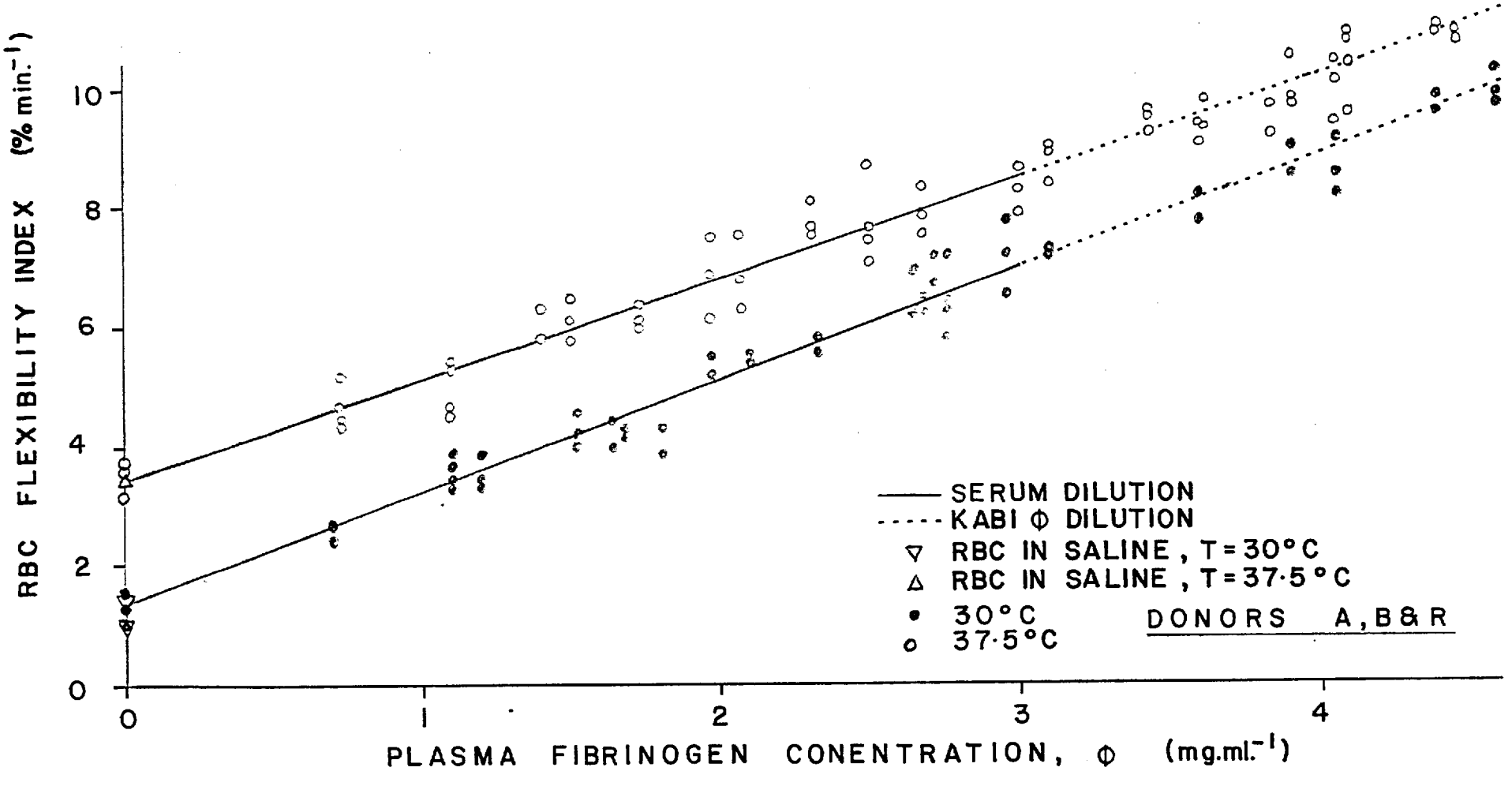


FIG. 21 : VARIATION OF RBC FLEXIBILITY INDEX WITH FIBRINOGEN CONCENTRATION AT 30 AND 37.5°C FOR DONORS A,B AND R

EFFECT OF VARYING PLASMA FIBRINOGEN CONCENTRATION, ø, ON RED CELL FLEXIBILITY INDEX FOR BLOOD OF DONOR C AT 30°C AND 37.5°C

	PLASMA FIBRINOGEN CONCENTRATION, ø ($mg\ ml^{-1}$)							
	0	1.1	1.51	2.17	2.66	2.71	2.85	2.97
RED CELL FLEXIBILITY INDEX AT 30°C ($\%\ min^{-1}$)	0.55 0.45	3.04 2.9 2.48	3.85 3.6 3.15	4.84 4.7 4.54	6.85 6.4 5.7	6.55 6.0 5.88 5.55	6.32 6.2 6.12 5.78	7.0 6.54 6.0
FLEXIBILITY INDEX FOR CELLS IN SALINE AT 30°C ($\%\ min^{-1}$)	0.5 0.3							

REGRESSION EQUATION:- $Y = 2.039X + 0.4662$
CORRELATION COEFFICIENT = 0.987

	PLASMA FIBRINOGEN CONCENTRATION, ø ($mg\ ml^{-1}$)								
	0	0.71	0.81	1.48	2.14	2.23	2.37	2.69	3.4
RED CELL FLEXIBILITY INDEX AT 37.5°C ($\%\ min^{-1}$)	2.5 1.65	3.45 2.84	3.85 3.96	4.85 4.4	5.5 4.7	6.0 5.7	6.72 6.35	6.98 6.35	8.2 7.8
FLEXIBILITY INDEX FOR CELLS IN SALINE AT 37.5°C ($\%\ min^{-1}$)	2.3 2.1								

REGRESSION EQUATION:- $Y = 1.687X + 2.14$
CORRELATION COEFFICIENT = 0.979

ERROR IN FLEXIBILITY INDEX MEASUREMENT = $\pm$ 0.5% min^{-1}
ERROR IN ø MEASUREMENT = $\pm$ 0.1 $mg\ ml^{-1}$

TABLE 18 FOR FIGURE 22

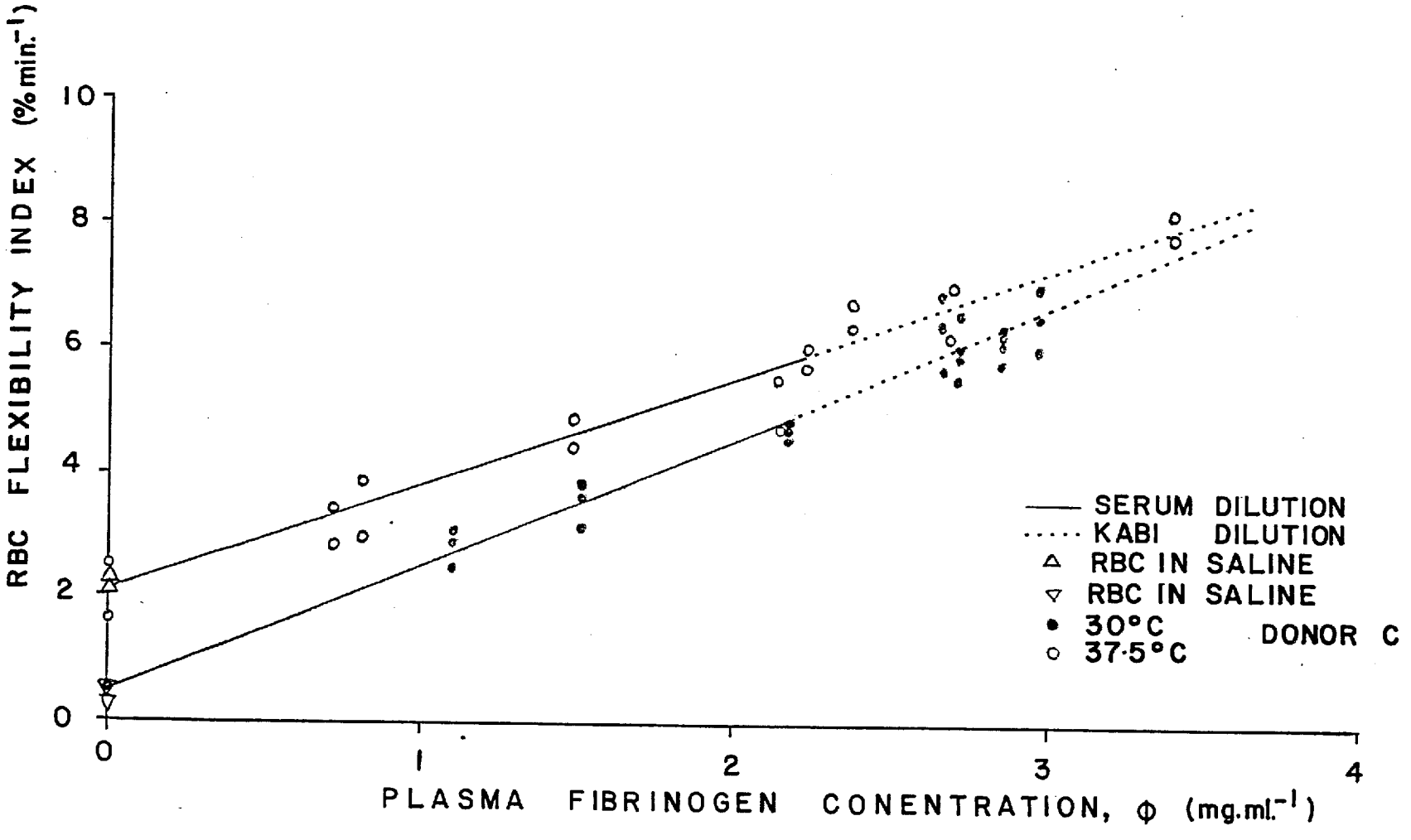


FIG. 22 : VARIATION OF RBC FLEXIBILITY INDEX WITH FIBRINOGEN CONCENTRATION AT 30 AND 37.5°C FOR DONOR C

EFFECT OF VARYING PLASMA FIBRINOGEN CONCENTRATION, ϕ, ON RED CELL FLEXIBILITY INDEX FOR BLOOD OF DONOR S AT 30° AND $37.5^{\circ}C$

	ϕ (mg ml^{-1})												
	0	0.57	1.11	1.63	2.22	2.47	2.53	2.88	3.1	3.23	3.47	3.56	4.36
RED CELL FLEXIBILITY INDEX AT $30^{\circ}C$ (% min^{-1})	2.0 2.0	3.23 2.8	4.64 4.35	6.85 6.15	7.75 7.5	7.5 7.05	8.84 8.7	8.43 8.25	9.66 9.55	9.45 8.6	10.1 9.64	10.4 8.95	13.4 13
RED CELL FLEXIBILITY INDEX AT $37.5^{\circ}C$ (% min^{-1})	4.55 4.2	6.15 5.44	7.3 6.84	8.25 8.14	10.6 8.95	10.45 10	10.75 10.23	12 11.05	12.25 11.74	12.85 12.35	12.65 11.75	12.35 12.05	15.45 15.1
RED CELL FLEXIBILITY INDEX AT $30^{\circ}C$ FOR CELLS IN SALINE (% min^{-1})	2.05												
RED CELL FLEXIBILITY INDEX AT $37.5^{\circ}C$ FOR CELLS IN SALINE (% min^{-1})	4.76 4.35												

For $30^{\circ}C$
REGRESSION EQUATION:- $Y = 2.3728X + 1.967$
CORRELATION COEFFICIENT = 0.981

For $37.5^{\circ}C$
REGRESSION EQUATION:- $Y = 2.3862X + 4.428$
CORRELATION COEFFICIENT = 0.9895

ERROR IN FLEXIBILITY INDEX MEASUREMENT = $\pm$ 0.5% min^{-1}
ERROR IN ϕ MEASUREMENT = $\pm$ 0.1 mg ml^{-1}

TABLE 19 FOR FIGURE 23

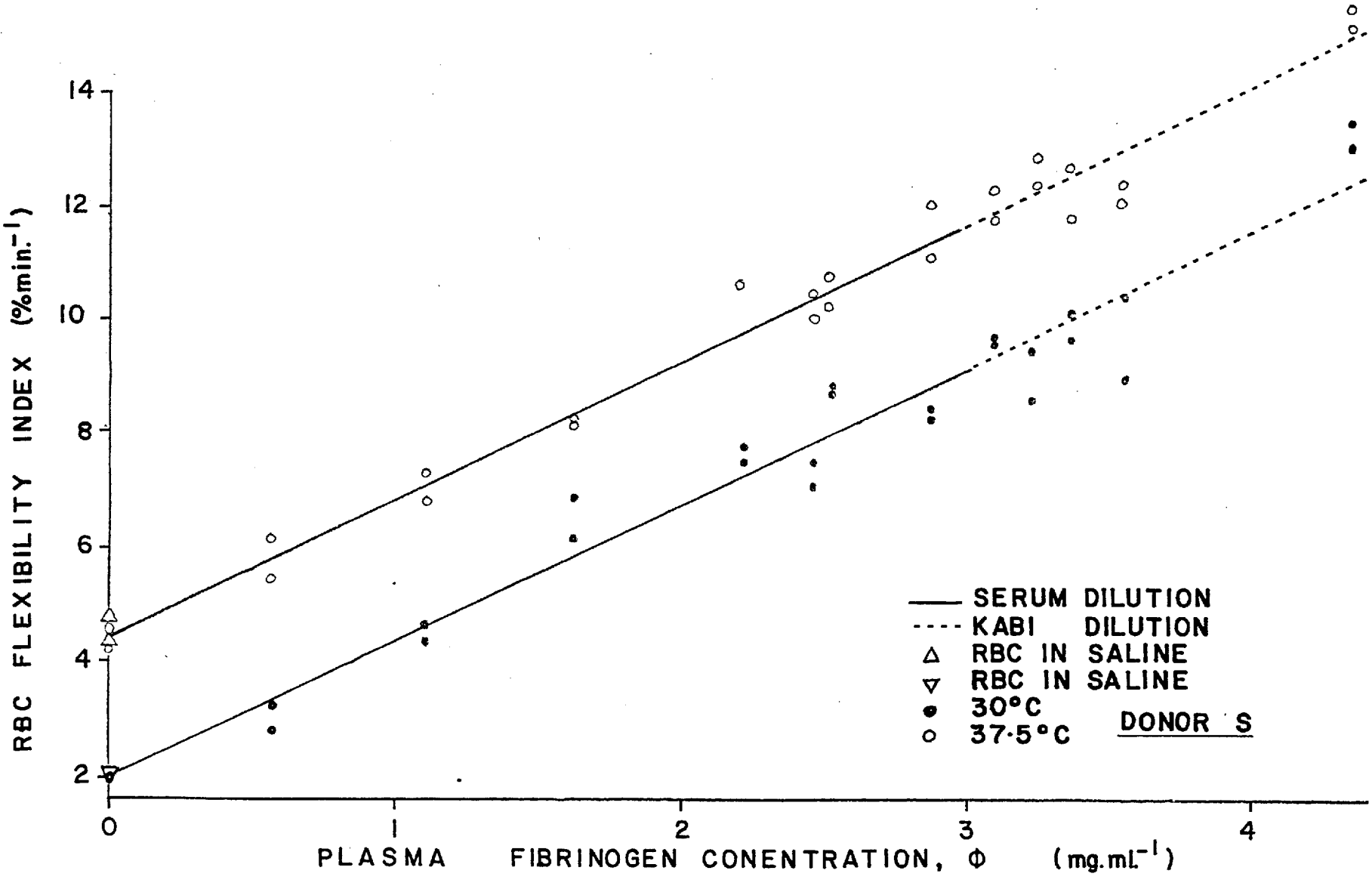


FIG. 23 : VARIATION OF RBC FLEXIBILITY INDEX WITH FIBRINOGEN CONCENTRATION AT 30 AND 37.5°C FOR DONOR S

ml^{-1} concentration of sodium citrate (Analar) and NaCl (Analar) was made up, and its pH was corrected to 7.4 using isotonic NaOH or HCl solutions. 10% of the plasma volume of a blood sample was replaced with an equal volume of the isotonic sodium citrate solution. From another blood sample 10% of the plasma volume was replaced with isotonic saline. The red cell packing rate measurements at 45% haematocrit for both blood samples, i.e. red cell flexibility index, was found to be 7.63 and 7.58 $\pm$ 0.5% min^{-1} for the sodium citrate and saline blood samples respectively. This shows that the concentrations of sodium citrate used in the fibrinogen solution has no effect on the red cell packing rate measurements.

(iv) Discussion

When fibrinogen concentration is increased in whole blood, rouleaux formation and aggregation of red cells increase, Fahraeus (1921). This effect was also observed by Wells et al (1964) and Chein et al (1970) as an increase in low shear rate viscosity. The effect of fibrinogen was, however, less in a suspension of red cells in Ringer-Locke or Ringer-albumin solutions than in plasma, Chein et al (1970). This suggested that plasma proteins, other than fibrinogen, had a stabilizing effect on fibrinogen adsorption on red cells membranes. It was also found that this dependence of viscosity on Φ was broken down when the shear rate was increased to $52s^{-1}$ or greater. Since in the packing rate centrifuge the forces are much greater than

$52s^{-1}$ shear rate, the effect of rouleaux on the packing rate is negligible. This was demonstrated by Rampling and Sirs (1972). They varied the degree of rouleaux formation by plasma dilution with serum, Ringer-Locke and saline, but found that the red cell packing rate was not altered. However, since it is known that increasing ϕ increases the red cell flexibility, Dupont and Sirs (1977), it can therefore be concluded that fibrinogen interacts with the red cell membrane to increase its flexibility.

When plasma proteins, other than fibrinogen, are diluted, the effect of the present fibrinogen concentration on the red cell flexibility is reduced as shown in Fig (17) of my results. This agrees with the suggestion put forward by Chein et al (1970a). Which of the plasma proteins acts to aid and stabilize the fibrinogen interaction with the red cell membrane will be investigated in Chapter 7. Brooks et al (1980) have shown that high molecular weight macro-molecules act to enhance rouleaux formation and have an additive effect on the adsorption of fibrinogen to the red cell membrane. This, therefore, suggests that plasma proteins of lower molecular weight than fibrinogen are adsorbed in such a way to the red cell membrane so as to enable the adsorption of fibrinogen to have maximum effect on the membrane flexibility.

Rampling (1981) reported that the binding of fibrinogen to the red cell membrane is not affected by temperature. From my results I have found that increasing temperature from 22^{o} to $37.5^{o}C$ increases red cell flexibility for a given

value of ϕ. I have also observed that in the absence of fibrinogen, i.e. red cells in serum or saline suspensions, the red cell flexibility is of similar values and increases with increasing temperature. This suggests that increasing temperature alters the red cell membrane flexibility by increasing the membrane fluidity, Williamson et al (1975), and by decreasing the internal viscosity of the red cell. This change in membrane flexibility with changing temperature is possibly due to temperature induced modification of the membrane structure.

CHAPTER 4

BLOOD VISCOSITY

I INTRODUCTION

The viscosity of blood is a measure of the internal resistive and frictional forces encountered between adjacent layers of the fluid during flow. In laminar or streamline flow, the adjacent layers move parallel to each other. In the presence of boundaries to flow, e.g. walls of tube or capillary, a velocity difference will exist between the adjacent layers. This velocity difference is a measure of the shearing within the flowing fluid and its gradient is termed the shear rate, i.e.

$$\text{shear rate, } \gamma = \frac{dv}{dx} \quad (\text{sec}^{-1})$$

where dv is the difference in velocity between two adjacent layers flowing with velocities v_1 and v_2 and dx is the distance between the layers of fluid considered. If F is the force acting per unit area, A, of the fluid during flow, the shear stress τ, expressed in dyn.cm^{-2}, is given as:

$$\tau = \frac{F}{A}$$

Viscosity, η, of a fluid is given as the ratio of τ to γ, measured in Poise, P, or centi Poise, cP, and is expressed as:

$$\eta = \frac{\tau}{\gamma} = \frac{\text{shear stress}}{\text{shear rate}} \quad (P)$$

A Newtonian fluid is defined as a fluid which at a constant temperature possesses a constant viscosity as the ratio of

shear stress to shear rate is constant and any given change in the shear stress applied to the fluid will produce an exactly proportional change in the resulting shear rate.

Blood was found to be a non-Newtonian fluid by Hess (1915), who explained that to define its rheological characteristics, viscosity would have to be measured at a range of constant definable shear rates. Fahraeus and Lindqvist (1931) by measuring viscosity by flow in capillary tubes, and Brundage (1934) by measuring viscosity using an early form of the concentric cylinder viscometers, demonstrated that blood possesses anomalous flow characteristics and does not behave like simple Newtonian fluids. It was not possible to accurately measure blood flow characteristics until the advent of rotational and capillary viscomters in the 1960s.

Wells at el (1961), using a cone and plate viscometer, have shown that blood behaves as a pseudoplastic fluid, i.e. with shear thinning properties. At high shear rates, blood viscosity behaves like a Newtonian fluid, staying constant in value as the shear rate is varied. When the shear rate is decreased below $100s^{-1}$, the viscosity increases rapidly. That is, more shear force is required to move blood slowly than to move it fast. These are the characteristics of blood called 'pseudoplastic' or 'shear thinning'.

The other anomalous property of blood is that of yield stress. Merrill et al (1963, a, b and c, and 1966) as well as Charm and Kurland (1962) reported that normal blood

displays a yield stress, that is, at zero shear rate the shear stress is finite. This means that for static blood, a certain amount of force is needed to overcome the yield stress before flow can begin. This yield stress was attributed to interactions between red blood cells and plasma proteins (mainly fibrinogen), since it was found that the yield stress decreased with plasma fibrinogen concentration, Φ , and absent for red cells suspended in Ringer-Locke solution. Cerny (1963) reported that blood behaves as a Newtonian fluid at high and very low shear rates, separated by a region with non-Newtonian characteristics. This was also pointed out by Chein et al (1966), who could not experimentally obtain a yield stress for blood.

Plasma is a solution of macromolecules of high molecular weights (680,000 for fibrinogen). Although plasma proteins interaction with red cells in blood give rise to many of the non-Newtonian behaviour (e.g. aggregation, as will be shown later), plasma on its own is a Newtonian fluid, as observed by Merrill et al (1963c) and Chein et al (1966), over all range of shear rates.

Cell-cell and cell-protein interactions in normal blood contribute to the non-Newtonian behaviour observed by Chein et al (1967a, b, 1970), Gregersen et al (1967) and Brooks et al (1970). The effect on η_B of parameters such as red cell concentration, MCV, flexibility, shape and interaction with

plasma proteins at different conditions of shear rates and temperatures are investigated in the present study for normal human blood.

II BLOOD VISCOSITY AND HAEMATOCRIT

(i) Introduction

For dilute suspension of rigid particles, less than 1% concentration, Einstein (1906) derived a relation between the relative apparent viscosity, η_r, and particle concentration (haematocrit), H, given as

$$\eta_r = 1 + aH$$

where a is equal to 2.5 for spheres and greater for non-spherical rigid particles.

For blood, the red cells are not spherical in shape and not rigid, with an internal viscosity η_i. Dintenfass (1968) derived an equation to represent red cell suspensions, given as:

$$\eta_r = (1 - TH)^{-a}$$

where T is called Taylor's factor given as

$$T = (p + 0.4)/(p + 1)$$

and p is equal to (η_i/η_p) where η_p is the viscosity of the suspending medium (plasma in the case of whole blood).

These equations can satisfactorily represent the behaviour of whole blood viscosity at low haematocrits but not at high haematocrits where other factors such as cell-cell and cell-protein interaction play a major part as well as shear rate, temperature and cell shape.

The change of viscosity with increasing haematocrit was reported by many investigators. Wells et al (1961) and Rand et al (1964), using a cone-plate viscometer, Chein et al (1966 and 1970), and Brooks et al (1970) using a Couette viscometer and Giombi and Burnard (1970) using a coaxial cylinder viscometer, have all observed similar relationship between viscosity of red cell suspensions and haematocrit. This relationship is basically that, viscosity increases with haematocrit in an almost linear manner up to a haematocrit of 25%, Brooks et al (1970), after which it increases exponentially, tending towards infinity, i.e. behaving like a solid, at haematocrits of about 80% for normal blood, and at a haematocrit of about 50% for hardened red cells. The haematocrit value at which this occurs is decreased by lower shear rate, smaller MCV or lower temperature for reasons explained in later sections.

I have carried out an investigation into the effect of haematocrit on whole blood viscosity of blood obtained from normal healthy male adults used throughout my studies. The investigations are carried out under different conditions of temperatures and shear rates.

(ii) Method

Human blood was obtained from healthy male adults by vene-puncture using solid lithium heparin (12.5 i.u. ml^{-1}) as anticoagulant. To vary the haematocrit, a series of 5 ml samples, from the same blood, were centrifuged at 500g for five minutes. One sample was left unaltered, where as autologus plasma was either removed from or added to the other samples.

Viscosity measurements were carried out using a Wells-Brookfield cone-plate viscometer (Model LVT) as described in section III of chapter 2. Rotational speed control on the viscometer enabled the variation of shear rate, and the heated water bath used to heat the blood sample in the viscometer cup provided temperature control.

Haematocrits of blood samples were measured before samples were placed into the viscometer. The haematocrit was obtained by centrifuging blood-filled microhaematocrit tubes at 13,000g on a Hawksley Microhaematocrit centrifuge.

(iii) Results

Whole blood viscosity, η_B, increases with haematocrit and decreasing shear rate as shown in Fig (24), for a temperature of 37.5°C. When the volume of red cells in a solution is increased, i.e. haematocrit, the viscosity is expected to increase as shown by the Einstein equation for Newtonian fluids at low concentrations:

$$\eta_r = 1 + aH.$$

This behaviour deviates from linearity when the particle concentration H increases. This effect is seen in the relationship between η_B and H in Fig (24) and described for rigid particles by the Dintenfass equation given above. Decreasing the shear rate, at any given haematocrit, increases η_B. The mechanisms of this process is described below in a later section. The effects of shear rate on η_B for a given haematocrit and temperature are shown in Figures 25-28 for temperatures of 20°, 25°, 30° and 37.5°C for haematocrits varying between 38.5 and 60%. The plasma viscosity for each temperature is also given. For the range of shear rates used it was found that the plasma viscosity did not alter, i.e. plasma behaved as a Newtonian fluid.

From the Einstein equation, a logarithmic relationship relating η_B, η_p and H was derived theoretically by Richardson (1950) and later observed by Haynes (1960) and Gregerson et al (1965) and given as

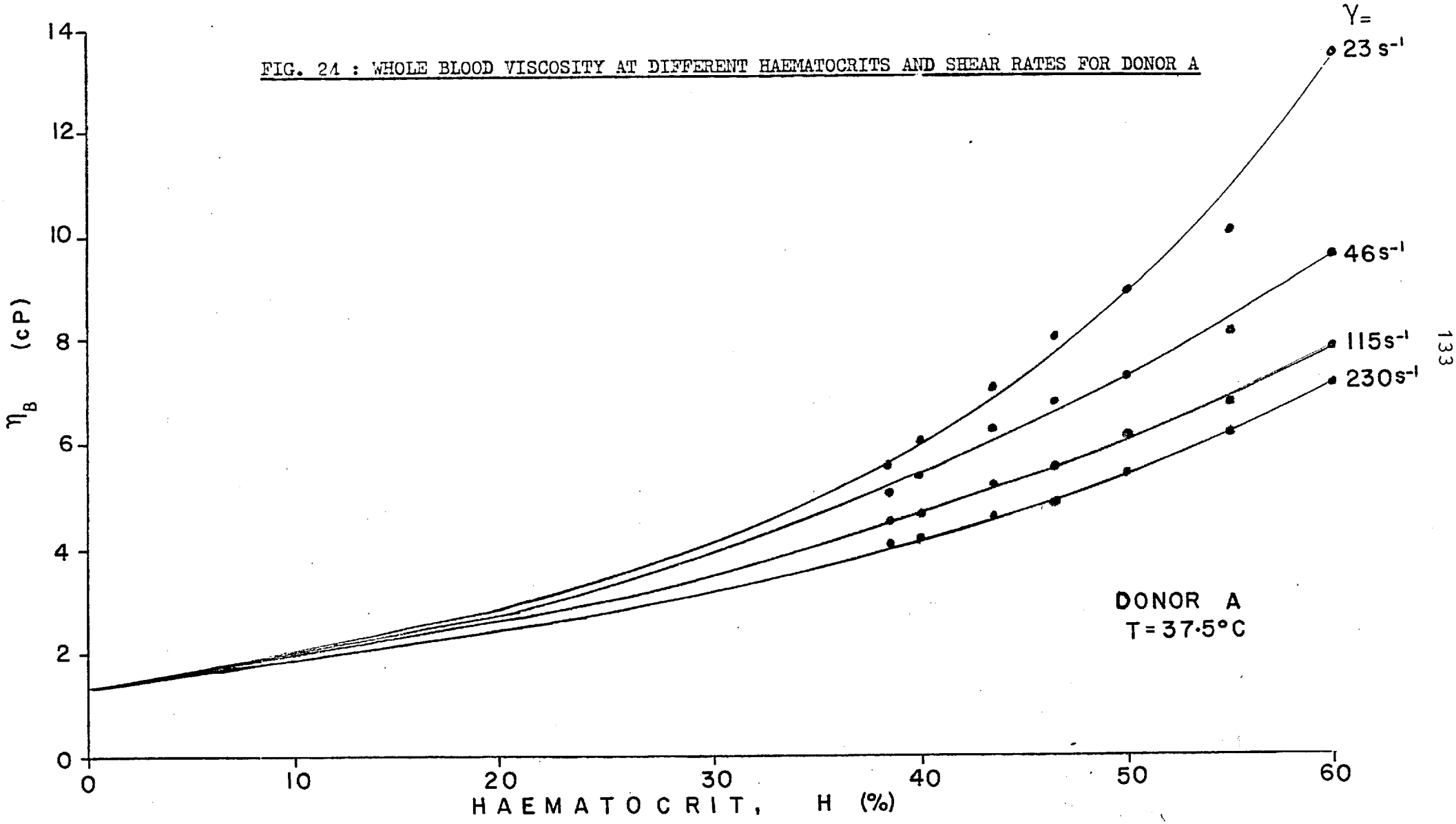
SHEAR RATE s^{-1}	HAEMATOCRIT (%)						
	38.5	40	43.5	46.5	50	55	60
23	5.5 (cP)	6.0	7.1	8.0	8.9	10.0	13.5
46	5.0 (cP)	5.3	6.25	6.75	7.25	8.1	9.5
115	4.5 (cP)	4.6	5.2	5.5	6.1	6.75	7.8
230	4.07(cP)	4.15	4.75	4.95	5.52	6.175	7.1

ERROR IN VISCOSITY MEASUREMENT = ± 0.2 cP
ERROR IN HAEMATOCRIT MEASUREMENT = ± 0.125%

TABLE 20 FOR FIGURE 24

VALUES OF BLOOD VISCOSITY, η_B, FOR VARYING HAEMATOCRITS AT DIFFERENT SHEAR RATES AND 37.5°C

FIG. 24 : WHOLE BLOOD VISCOSITY AT DIFFERENT HAEMATOCRITS AND SHEAR RATES FOR DONOR A



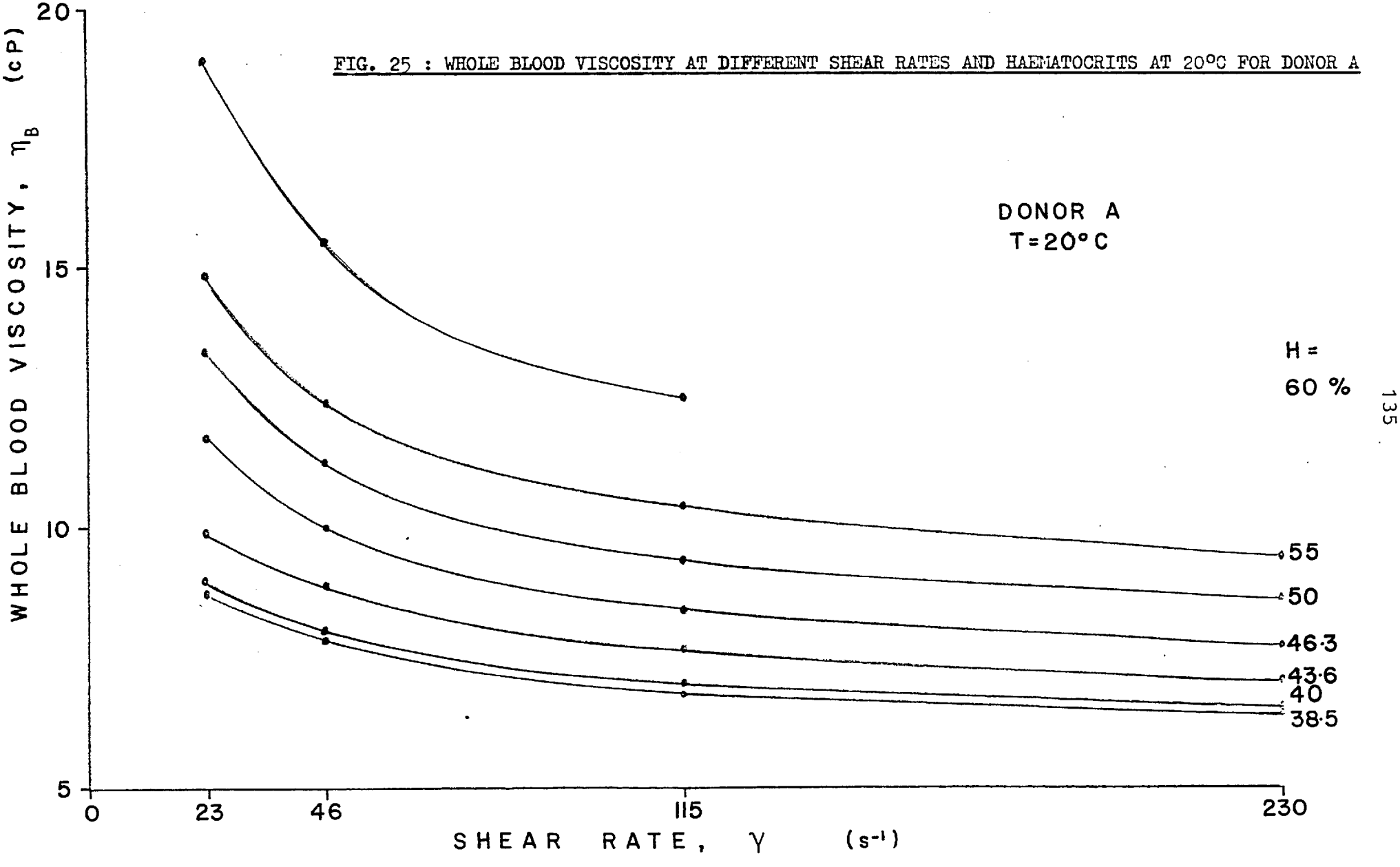
HAEMATOCRIT (%)	SHEAR RATE (s^{-1}) 23	46	115	230
38.5	8.75 (cP)	7.875	6.85	6.43
40	9.0 (cP)	8.0	7.0	6.48
43.5	9.9 (cP)	8.875	7.65	7.0
46.5	11.75 (cP)	10.0	8.44	7.7
50	13.4 (cP)	11.25	9.36	8.6
55	14.85 (cP)	12.375	10.4	9.425
60	19.0 (cP)	15.5	12.5	-

ERROR IN VISCOSITY MEASUREMENT = ± 0.2 cP
ERROR IN HAEMATOCRIT MEASUREMENT = ± 0.125%

TABLE 21 FOR FIGURE 25

VALUES OF BLOOD VISCOSITY, η_B, FOR VARYING SHEAR RATES AT DIFFERENT HAEMATOCRITS AND 20°C

FIG. 25 : WHOLE BLOOD VISCOSITY AT DIFFERENT SHEAR RATES AND HAEMATOCRITS AT 20°C FOR DONOR A



HAEMATOCRIT (%)	SHEAR RATE (s^{-1}) 23	46	115	230
38.5	7.4 (cP)	6.65	5.9	5.5
40	8.0 (cP)	7.125	6.15	5.65
43.5	9.5 (cP)	7.75	7.0	6.225
46.5	10.0 (cP)	8.5	7.24	6.65
50	11.0 (cP)	9.25	7.84	7.25
55	13.0 (cP)	10.75	9.1	8.4
60	14.5 (cP)	12.1	10.3	9.45

ERROR IN VISCOSITY MEASUREMENT = ± 0.2 cP
ERROR IN HAEMATOCRIT MEASUREMENT = ± 0.125%

TABLE 22 FOR FIGURE 26

VALUES OF BLOOD VISCOSITY, η_B, FOR VARYING SHEAR RATES AT DIFFERENT HAEMATOCRITS AND 25°C

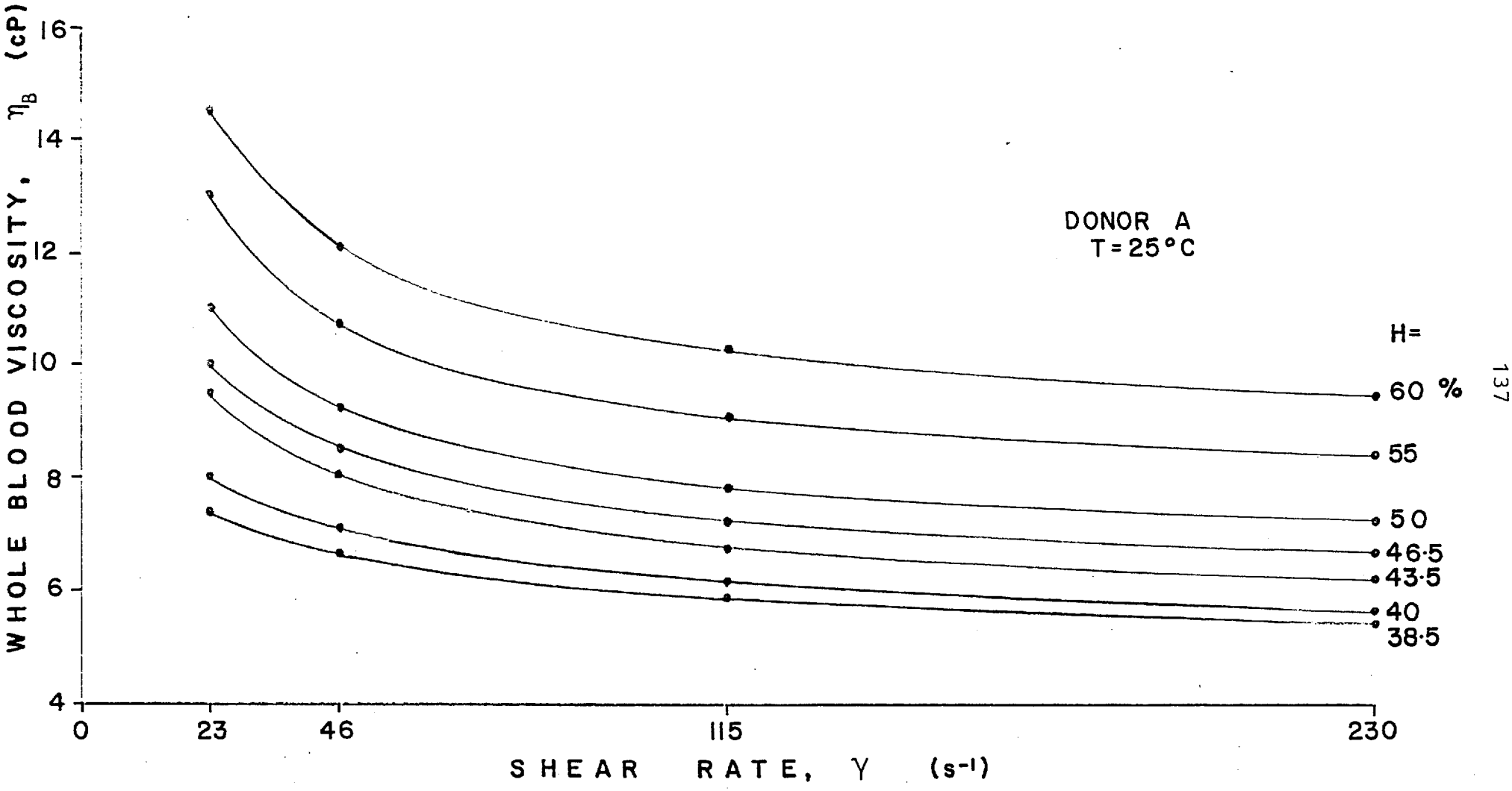


FIG. 26 : WHOLE BLOOD VISCOSITY AT DIFFERENT SHEAR RATES AND HAEMATOCRITS AT 25°C FOR DONOR A

HAEMATOCRIT (%)	SHEAR RATE (s^{-1}) 23	46	115	230
38.5	6.3 (cP)	5.9	5.2	4.78
40	6.8 (cP)	6.1	5.32	4.89
43.5	8.0 (cP)	6.75	5.82	5.35
46.5	8.75(cP)	7.4	6.25	5.75
50	9.85(cP)	8.25	6.9	6.38
55	11.5 (cP)	9.25	7.76	7.175
60	13 (cP)	10.75	9.0	8.3

ERROR IN VISCOSITY MEASUREMENT = ± 0.2 cP
ERROR IN HAEMATOCRIT MEASUREMENT = ± 0.125%

TABLE 23 FOR FIGURE 27

VALUES OF WHOLE BLOOD VISCOSITY, η_B, FOR VARYING SHEAR RATES AT DIFFERENT HAEMATOCRITS AND 30°C

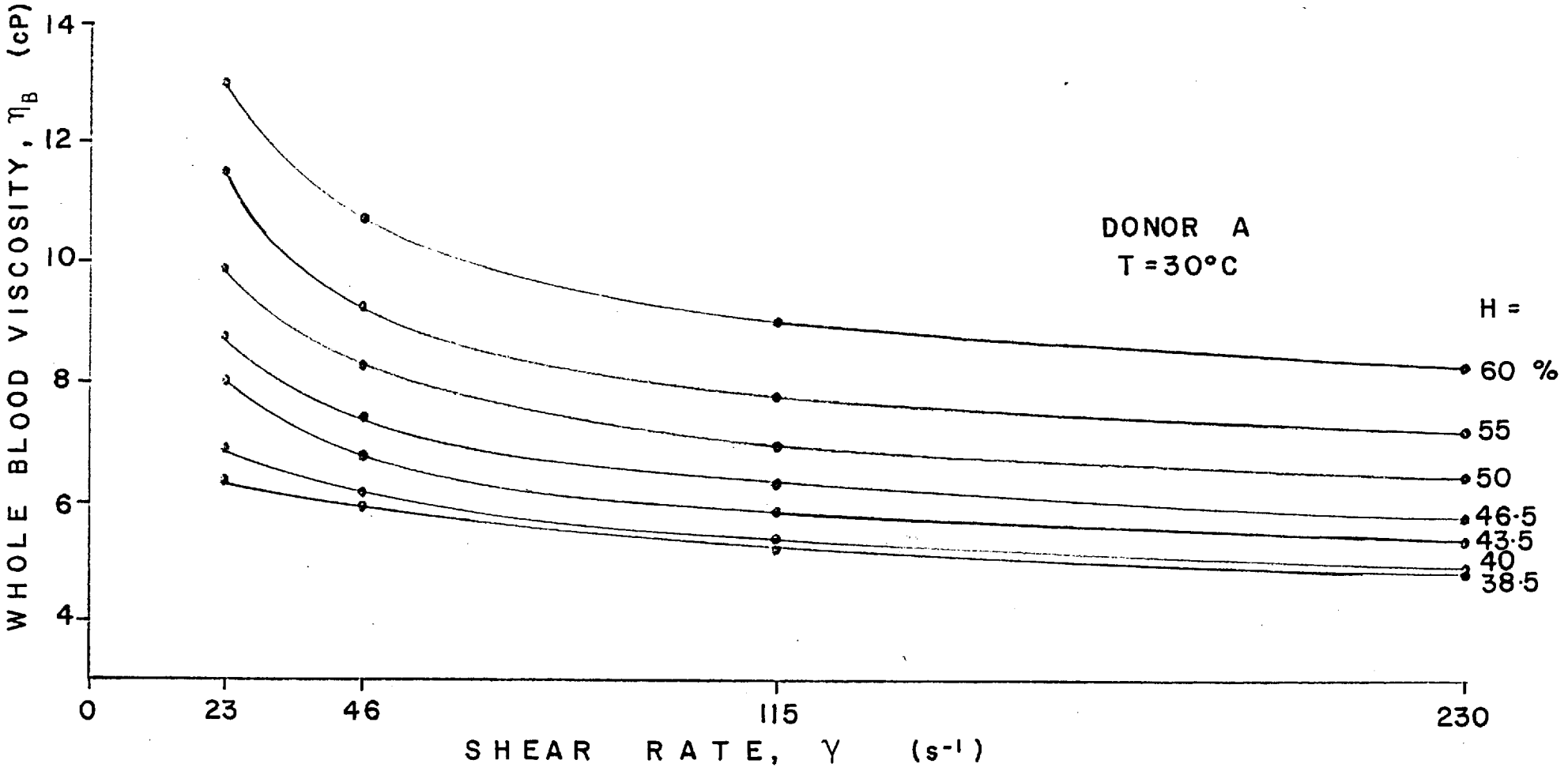


FIG. 27 : WHOLE BLOOD VISCOSITY AT DIFFERENT SHEAR RATES AND HAEMATOCRITS AT 30°C FOR DONOR A

HAEMATOCRIT (%)	SHEAR RATE (s^{-1}) 23	46	115	230
38.5	5.5 (cP)	5.0	4.5	4.07
40	6.0 (cP)	5.3	4.6	4.15
43.5	7.1 (cP)	6.25	5.2	4.75
46.5	8.0 (cP)	6.75	5.5	4.94
50	8.9 (cP)	7.25	6.1	5.52
55	10.0 (cP)	8.1	6.75	6.175
60	13.5 (cP)	9.5	7.8	7.1

ERROR IN VISCOSITY MEASUREMENT = ± 0.2 cP
ERROR IN HAEMATOCRIT MEASUREMENT = ± 0.125%

TABLE 24 FOR FIGURE 28

VALUES OF WHOLE BLOOD VISCOSITY, η_B, FOR VARYING SHEAR RATES AT DIFFERENT HAEMATOCRITS AND 37.5°C

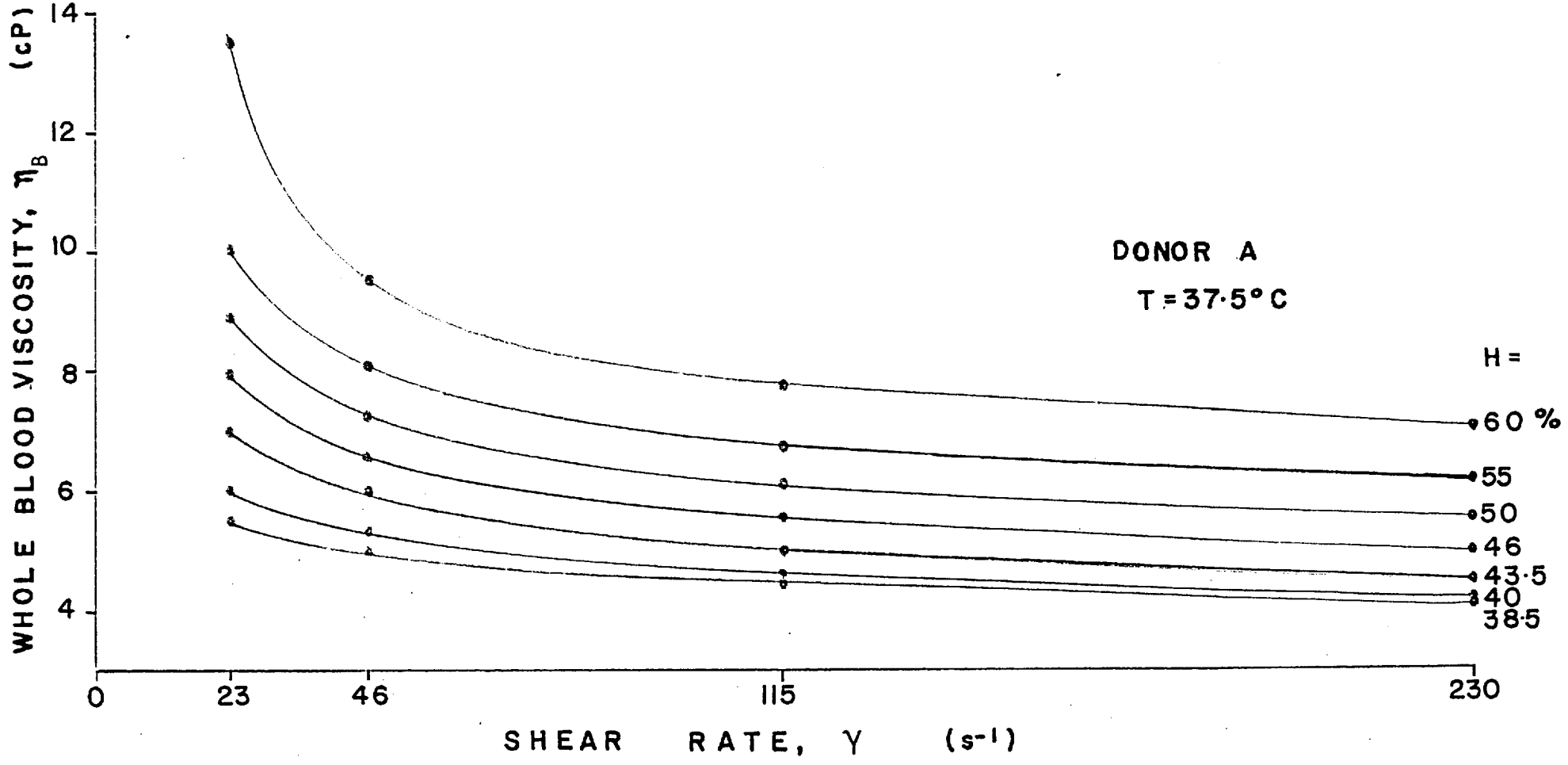


FIG. 28 : WHOLE BLOOD VISCOSITY AT DIFFERENT SHEAR RATES AND HAEMATOCRITS AT 37.5°C FOR DONOR A

$$\log \eta_B = \log \eta_p + aTH$$

where a is a constant, dependent on the red cell shape, and T is the Taylor's factor described earlier in this section. To test the validity of this relationship, the values of η_B at different haematocrits, H, shown in Fig (24) are replotted using the logarithmic equation in Fig (29). Here we see that for high cell concentrations in the range 38.5 to 60%, there still exists a linear behaviour for the four different shear rates used. We can also notice that the gradient, aT, increases with decreasing shear rate. This gradient is called the 'intrinsic viscosity' and is described in a later section in relation to red cell shape.

Decreasing a temperature at any given shear rate increases viscosity, Merrill et al (1963a). The effect of temperature on the logarithmic relationship between η_B, η_p and H is seen in Fig (30). Here again, the gradient aT is increased with decreasing temperature for measurements at a shear rate of $230s^{-1}$.

(iv) Discussion

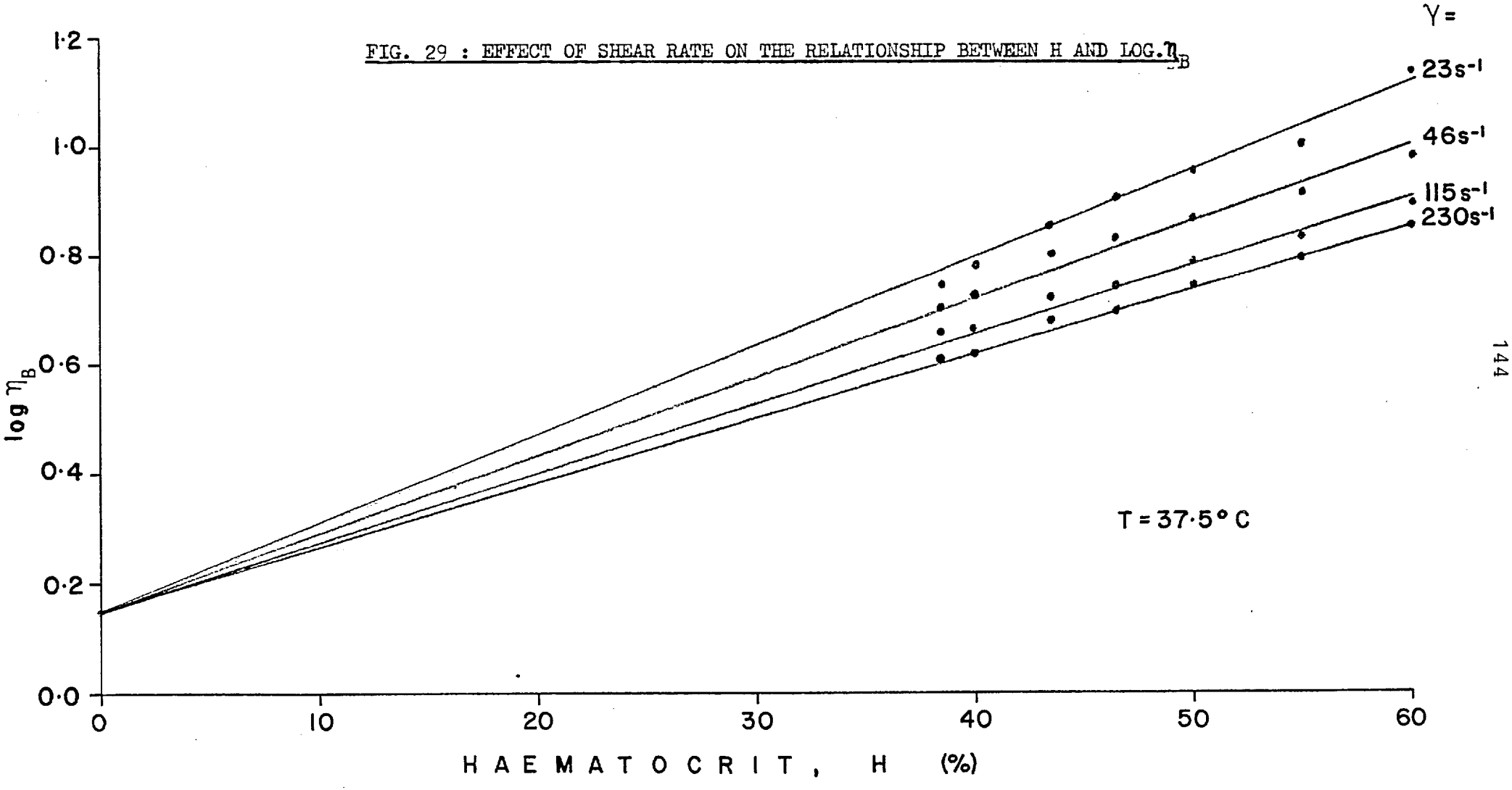
At high shear rates, blood, even with high concentration of red cells, behaves similar to a Newtonian fluid, with its viscosity tending to a constant value as the shear rate is increased further. When the shear rate is decreased below about $100s^{-1}$ (or higher for higher haematocrits and lower temperatures) the deviation from Newtonian characteristics

SHEAR RATE, γ (s^{-1})	HAEMATOCRIT, H (%)						
	38.5	40	43.5	46.5	50	55	60
23	0.7404	0.7782	0.8513	0.9031	0.9494	1.0000	1.1303
46	0.6990	0.7243	0.7959	0.8293	0.8603	0.9085	0.9777
115	0.6532	0.6628	0.7160	0.7404	0.7853	0.8293	0.8921
230	0.6096	0.6181	0.6767	0.6946	0.7419	0.7906	0.8513

TABLE 25 FOR FIGURE 29

VALUES OF LOG. η_B FOR DIFFERENT VALUES OF H AND γ AT T= 37.5°C

FIG. 29 : EFFECT OF SHEAR RATE ON THE RELATIONSHIP BETWEEN H AND LOG. η_B

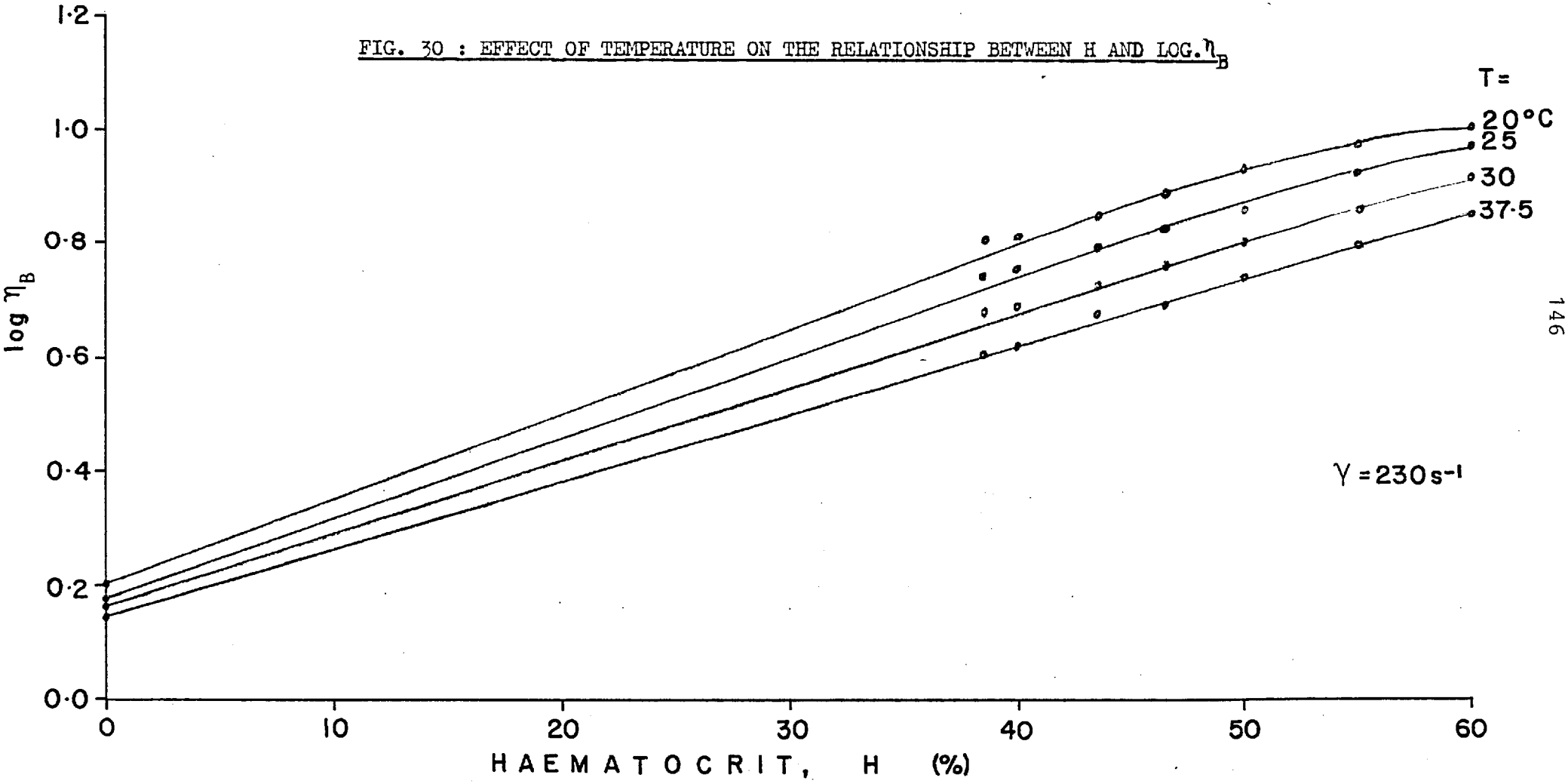


TEMPERATURE, T (°C)	HAEMATOCRIT, H (%)						
	38.5	40	43.5	46.5	50	55	65
20	0.8082	0.8116	0.8451	0.8865	0.9345	0.9743	1.0050
25	0.7404	0.7521	0.7941	0.8228	0.8603	0.9243	0.9754
30	0.6794	0.6893	0.7284	0.7597	0.8048	0.8558	0.9191
37.5	0.6096	0.6181	0.6767	0.6937	0.7419	0.7906	0.8513

TABLE 26 FOR FIGURE 30

VALUES OF LOG. η_B FOR DIFFERENT VALUES OF H AND T AT $\gamma = 230\ s^{-1}$

FIG. 30 : EFFECT OF TEMPERATURE ON THE RELATIONSHIP BETWEEN H AND LOG. η_B



is observed. In this region the viscosity increases rapidly with decreasing shear rate giving blood the 'pseudoplastic' or 'shear thinning' characteristics.

To relate the viscosity of blood to its haematocrit, the Einstein equation can be used only for haematocrits of less than 1%. The Dintenfass equation gives a better relationship for a non-Newtonian fluid like blood. But it too is also limited to low haematocrits and gives a better linear relationship (in its logarithmic form) at high shear rates than low shear rates as seen in Fig (29) and shown in a later section.

The deviation of blood from Newtonian behaviour for increasing haematocrit is a result of interaction between cells, and cells and plasma proteins. This interaction is not accounted for in the original Einstein equation for rigid particles, or fully accounted for in the Dintenfass equation. The mechanism of these interactions are very complex and as yet have not been quantitatively described. It is however seen that decreasing shear rate and temperature increase the degree of these interactions and result in increasing the value of η_B. The nature of these interactions known as red cell aggregation and rouleaux formation will be discussed fully in a later section as well as the effect of other rheological parameters on blood viscosity.

III BLOOD VISCOSITY AND RED CELL FLEXIBILITY

(i) Introduction

Normal, deformable red blood cells can be packed by centrifugation to nearly 100% cells, whereas hardened red cells can only be packed to a maximum of 60%, Chien et al (1968). The red cell deformability is therefore responsible for the flow of normal red cells above a haematocrit of 60%. This will obviously affect the viscosity of whole blood which besides being dependent on red cell deformability, has been shown above to depend on red cell concentration, temperature and shear rate.

The role of red cell deformability, or flexibility, in viscometric measurements can be demonstrated by comparing a suspension of normal red cells and hardened red cells. It was found that the viscosity of hardened red cells suspension is much greater than that of normal cells, and that for hardened cells it is independent of shear rate, Chein et al (1970). This means that hardened red cells suspensions behave like a Newtonian fluid whereas the shear dependent normal cells suspension does not, as shown earlier. Therefore it can be said that red cell flexibility is one of the factors responsible for non-Newtonian behaviour of blood. This is readily seen when suspensions of normal red cells in plasma, serum and saline or Ringer-Locke solutions are compared at low shear rates, Merrill et al (1963c), Chein et al (1966) and Brooks et al

(1970). It was observed by these workers that for a given haematocrit, at low shear rates, the suspension viscosity is greater in the presence of plasma proteins, in particular fibrinogen, than in its absence, as for saline or Ringer-Locke solutions. In addition to the cell-cell interaction present in all the suspensions at the same haematocrit, the greater viscosity in the presence of fibrinogen was attributed to cell-protein interaction. Rampling and Sirs (1972) observed that red cell flexibility is increased with fibrinogen concentration whereas red cells are inflexible in Ringer-Locke solution without fibrinogen.

Chein et al (1970) have shown that shear rate dependence of red cell suspensions in plasma at low shear rates is greater than that in serum, which in turn is greater than that in Ringer-Locke solution. This means that red cells in plasma behave more as a non-Newtonian fluid than red cells in serum, which behave more as a non-Newtonian fluid than red cells in Ringer-Locke solution. Since suspensions of the same haematocrit were compared, the cell-cell interaction was similar for the three different suspensions. However, serum contains plasma proteins, less fibrinogen, which are present in plasma. So it was concluded that the presence of plasma proteins in serum (less fibrinogen) gives rise to cell-protein interactions and that fibrinogen increases this interaction even further, as in plasma. At low shear rates, Chein et al (1967a) have shown that blood viscosity increases due to red cell aggregation, which increased in degree as the fibrinogen concentration was elevated. Since red cell flexibility is increased by increasing the

fibrinogen concentration, Rampling and Sirs (1972), it was concluded that increasing fibrinogen concentration increased the red cell flexibility which in turn increased red cell aggregation and rouleaux formation, and this led to increased blood viscosity, Dupont and Sirs (1977). This interdependence is present at low shear rates where the low shear stress permits cell-cell contact, and the action of fibrinogen causes aggregation and rouleaux formation through its adsorption onto the surfaces of the aggregating cells.

At high shear rates, the high shear stress causes a break down of aggregates at shear rates of $50s^{-1}$ and above, Chein et al (1970), and the red cell suspension approaches the Newtonian fluid characteristics, i.e. independence of shear rate. Wells et al (1961) observed that as the shear rate is increased from $12.4s^{-1}$ to $123.6s^{-1}$ in a cone-plate viscometer, whole blood viscosity decreased and levelled-off in value at about $100s^{-1}$, where it became independent of shear rate. Merrill et al (1963a), using a Couette type viscometer, observed the same behaviour. Chein et al (1967a) observed that as shear rate was increased the viscosity of normal red cells decreased further than that of hardened cells at the same haematocrit. This was explained as being due to normal and hardened cells, at low shear rate, having similar flow properties because of minimum deformation of normal cells. As the shear rate is increased, the normal cells become more deformed and the suspension viscosity decreases. However since hardened cells cannot deform, their suspension viscosity remains unchanged at a high value. As the shear rate increases

further, the normal cells become maximally deformed, and their suspension enters a Newtonian region with a constant low viscosity.

At the Newtonian region of blood viscosity, at high shear rates, changes in fibrinogen concentration does not affect the relative viscosity, η_r , Chein et al (1970), and the dominant interaction is not cell-protein interactions, as at low shear rate, but cell-cell interaction, Chein et al (1966), since only changes in haematocrit affects the viscosity. The role of increasing plasma viscosity with fibrinogen concentration was found to have no effect on whole blood viscosity at high shear rates by Brundage (1934), which confirms the importance of cell-cell interaction in that region.

Although Chein and his co-workers, as well as other investigators not using the centrifugal method of Sirs (1970) for measuring the red cell flexibility, have shown the difference in viscosity in the absence and presence of fibrinogen, the affect of fibrinogen on red cell flexibility was not measured and demonstrated to be an important factor in viscosity, especially at low shear rates. Rampling and Sirs (1972) have shown the effect of fibrinogen on red cell flexibility and Dupont and Sirs (1977) have shown this effect on blood viscosity of a patient suffering from post-surgical trauma. Hence it is not possible to obtain a consistent systematic picture of the role of red cell flexibility and fibrinogen effect on viscosity at low and high shear rates.

In the present study, I have tried to show a systematic picture of the interplay between red cell flexibility, fibrinogen concentration, plasma viscosity, red cell aggregation and shear rates on whole blood viscosity.

(ii) Methods

Blood was obtained from normal healthy male adults by vene-puncture, using solid lithium heparin as anticoagulants in collection bottles at concentrations of 12.5 i.u. ml^{-1}. Viscometric measurements were obtained using a cone-plate Wells Brookfield viscometer as described in section III of Chapter 2.

Red cell flexibility was measured by the automatic recording centrifuge method, in duplicate, described in section I of Chapter 2 and in Amin et al (1983). Fibrinogen concentration was measured by the thrombin clotting technique of Rampling and Gaffney (1976), in duplicate, using the procedure described in section IV of Chapter 2.

Autologus serum was obtained by heating plasma at $56^{o}C$ for 10 minutes then centrifuging at 1500g for an hour to remove precipitated clotted fibrinogen. To reduce plasma fibrinogen concentration, plasma was diluted with its own serum. To increase plasma fibrinogen concentration, an isotonic solution of Kabi (Grade L) human fibrinogen powder in Ringer-Locke of pH = 7.4 was used. The solution

fibrinogen concentration was made up to its maximum of 10 $mg.ml^{-1}$, so as minimum volumes of it could be used to dilute plasma.

For red cells suspended in serum, the blood sample was centrifuged at 500g for 10 minutes and as much plasma as possible was removed. The cells were then washed in autologus serum three times before resuspending in serum. For red cells suspensions in Ringer-Locke solutions, after removing the plasma by centrifugation at 500g, the cells were washed in large volumes of isotonic Ringer-Locke solutions of pH = 7.4 and then resuspended in a fresh volume of the same Ringer-Locke solution.

Heat hardening of red cells was carried out by washing the red cells in isotonic Ringer-Locke solution as for Ringer-Locke suspensions, and then the cells suspension was heated in a water bath at 48.5°C for 30 minutes. For hardened cells suspension in plasma, the suspending Ringer-Locke solution was removed by centrifugation at 500g for 10 minutes and the cells were then washed in autologus plasma three times before resuspending in plasma again. The same procedure was carried out for red cells suspension in autologus serum. This procedure was followed so as to avoid any change in plasma proteins, in particular fibrinogen, i.e. clotting, if the red cells were heated in the presence of plasma proteins.

Heat hardening was chosen instead of formaldehyde fixing because my investigation was partly into the aggregation at

low shear rates of normal and hardened cells. By fixing the membrane proteins and plasma protein adsorption sites, formaldehyde may alter the aggregability of the red cells. The other complication is of the formaldehyde fixing plasma proteins in solution, and in particular the fibrinogen molecules, hence altering the adsorption of the proteins onto the red cell membrane.

(iii) Results

Figure (31) shows the variation of whole blood viscosity with plasma fibrinogen concentration for normal blood samples from two subjects, A and S, at the same haematocrits of 45%, 37.5°C and shear rate of $230s^{-1}$. Subject S is representative of a small but significant group of 1.5% of normals whose red cell flexibility is greater by more than three standard deviations from the total population mean, Rampling and Sirs (1976 and 1977). Subject A is representative of the mean of the normal population. The more flexible red cells of subject S show a slightly lower whole blood viscosity than subject A's normal cells, and is unaltered by changes in plasma fibrinogen concentration.

The change in plasma viscosity with fibrinogen concentration at shear rate of $230s^{-1}$ and 37.5°C is shown in Figure (32). Here we see that subject S plasma viscosity, η_p, is lower than that of subject A for all fibrinogen concentration,

SUBJECT A

FIBRINOGEN CONCENTRATION, Φ (mg. ml.$^{-1}$)	0	0.683	1.375	1.875	2.325	2.9	3.25	4.16	4.85
WHOLE BLOOD VISCOSITY η_B (cP)	4.6	4.65	4.5	4.525	4.5	4.5	4.625	4.525	4.55

SUBJECT S

FIBRINOGEN CONCENTRATION, Φ (mg. ml.$^{-1}$)	0	0.75	1.25	1.75	2.3	2.685	3.5	4.25	4.95
WHOLE BLOOD VISCOSITY η_B (cP)	4.4	4.4	4.4	4.4	4.3	4.25	4.5	4.5	4.5

ERROR IN VISCOSITY MEASUREMENT = ± 0.5 cP
ERROR IN Φ MEASUREMENT = ± 0.1 mg. ml.$^{-1}$

TABLE 27 FOR FIGURE 31

VARIATION OF WHOLE BLOOD VISCOSITY, η_B, WITH PLASMA FIBRINOGEN CONCENTRATION, Φ, FOR NORMAL AND HEAT HARDENED RED CELLS AT 45% HAEMATOCRIT AND 37.5°C FOR SHEAR RATE OF 230s^{-1}

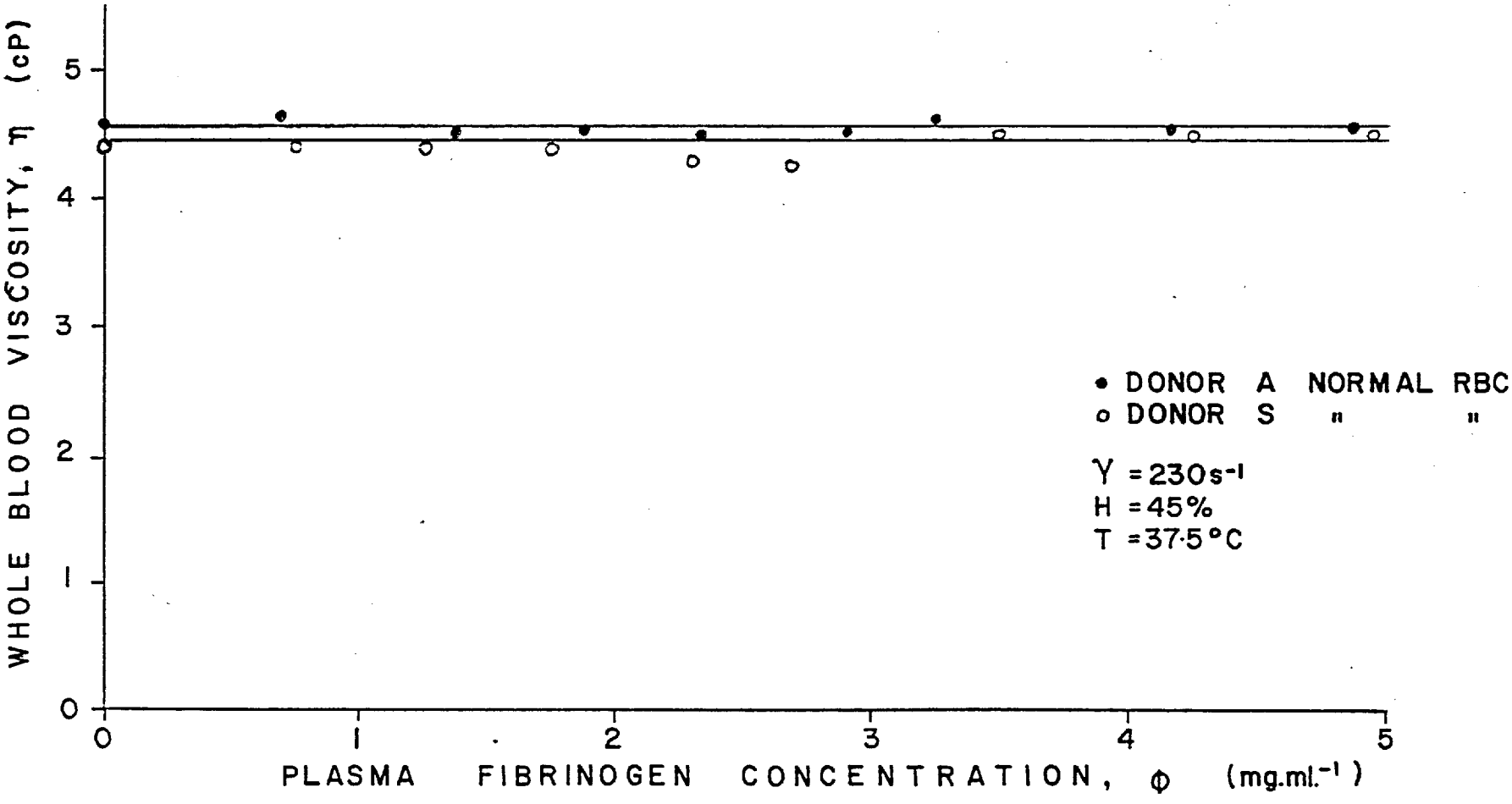


FIG. 31 : VARIATION OF DONORS A AND S WHOLE BLOOD VISCOSITY WITH PLASMA FIBRINOGEN CONCENTRATION

DONOR A

FIBRINOGEN CONCENTRATION, (mg. ml.$^{-1}$)	0	0.7	1.75	1.875	2.125	2.375	2.875	3.25	4.04	4.8
PLASMA VISCOSITY η_p (cP)	1.35	1.365	1.4	1.425	1.45	1.475	1.475	1.5	1.525	1.56

REGRESSION EQUATION:- Y = 0.463 X + 1.34 CORRELATION COEFFICIENT = 0.983

DONOR R

FIBRINOGEN CONCENTRATION, (mg. ml.$^{-1}$)	0	0.75	1.25	1.75	2.28	2.67	3.375	4.125	4.975
PLASMA VISCOSITY η_p (cP)	1.343	1.38	1.4	1.44	1.45	1.45	1.525	1.525	1.57

REGRESSION EQUATION:- Y = 0.0455 X + 1.35 CORRELATION COEFFICIENT = 0.983

DONOR S

FIBRINOGEN CONCENTRATION, (mg. ml.$^{-1}$)	0	0.725	1.25	1.75	2.285	2.55	3.375	4.125
PLASMA VISCOSITY η_p (cP)	1.225	1.25	1.2675	1.305	1.35	1.35	1.365	1.4

REGRESSION EQUATION:- Y = 0.0445 X + 0.1225 CORRELATION COEFFICIENT = 0.98

ERROR IN MEASUREMENT OF Φ = ± 0.1 mg. ml.$^{-1}$

ERROR IN MEASUREMENT OF η_p = ± 0.1 cP

TABLE 28 FOR FIGURE 32

VARIATION AND PLASMA VISCOSITY WITH FIBRINOGEN CONCENTRATION AT 37.5°C and SHEAR RATE OF 230s^{-1}

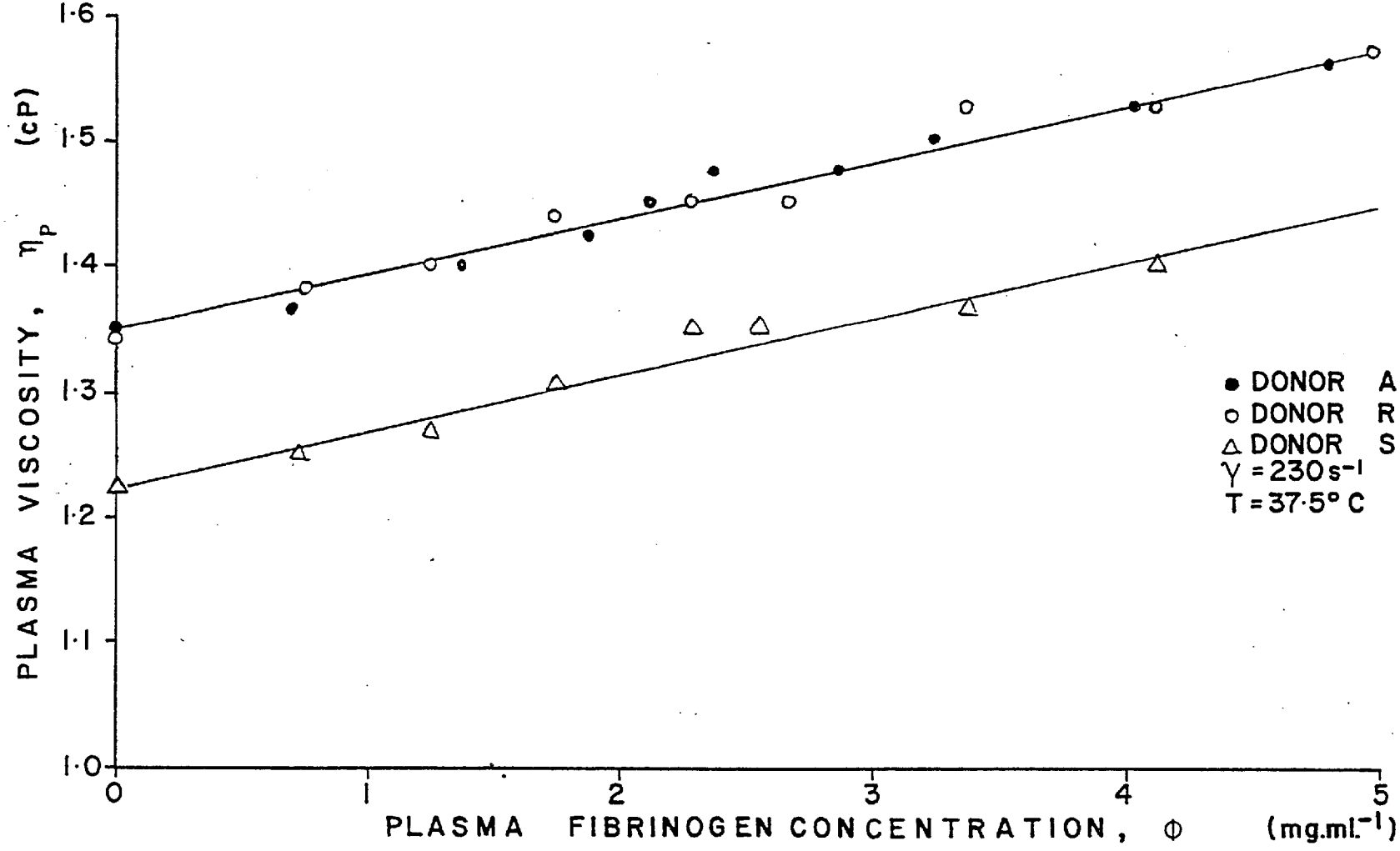


FIG. 32 : VARIATION OF PLASMA VISCOSITY WITH FIBRINOGEN CONCENTRATION FOR DONORS A,R, AND S

possibly due to lower concentration of other plasma proteins. However the increase in plasma viscosity with fibrinogen concentration is linear in both cases with a gradient of 0.44 cP per 1 mg.ml^{-1} change in fibrinogen concentration.

The effects of varying plasma fibrinogen concentration, Φ, in whole blood, as well as removing all plasma proteins, on the suspension viscosity, η_B, are shown in Figure (33), for varying shear rates. At high shear rates, η_B, for whole blood, defibrinated blood (serum suspension) and Ringer-Locke suspension of red cells, with and without human fibrinogen powder (Kabi Grade L) are similar in value. As the shear rate is decreased below 46s^{-1}, η_B for whole blood increases. The increase in η_B is however less for the Ringer-Locke plus fibrinogen solution although the fibrinogen concentration is greater than that of whole blood. The value of η_B is decreased further for the serum suspension and is lowest for the Ringer-Locke suspension, where η_B seems to be less shear dependent than the other suspensions, i.e. closer to Newtonian fluid behaviour.

The reversal in value of η_B at shear rates below 11.5s^{-1} for the Ringer-Locke suspension is probably due to the larger error in the reading at that range of $\dot{\gamma}$ as well as the possibility of cells settling in the viscometer cup.

	SHEAR RATE, (s^{-1})					
	230	115	46	23	11.5	5.75
RED CELLS IN PLASMA Φ = 2.68 mg. ml.$^{-1}$	4.38	5.46	6.76	8.5	10.3	12.0
RED CELLS IN RINGER-LOCKE Φ = 3.0 mg. ml.$^{-1}$	4.41	5.38	6.69	8.19	9.96	10.375
RED CELLS IN SERUM Φ = 0	4.38	4.7	5.7	6.8	8.0	8.60
RED CELLS IN RINGER-LOCKE Φ = 0	4.37	4.78	5.25	5.408	5.50	5.25

HAEMATOCRIT = 45% TEMPERATURE OF MEASUREMENTS = 37.5°C

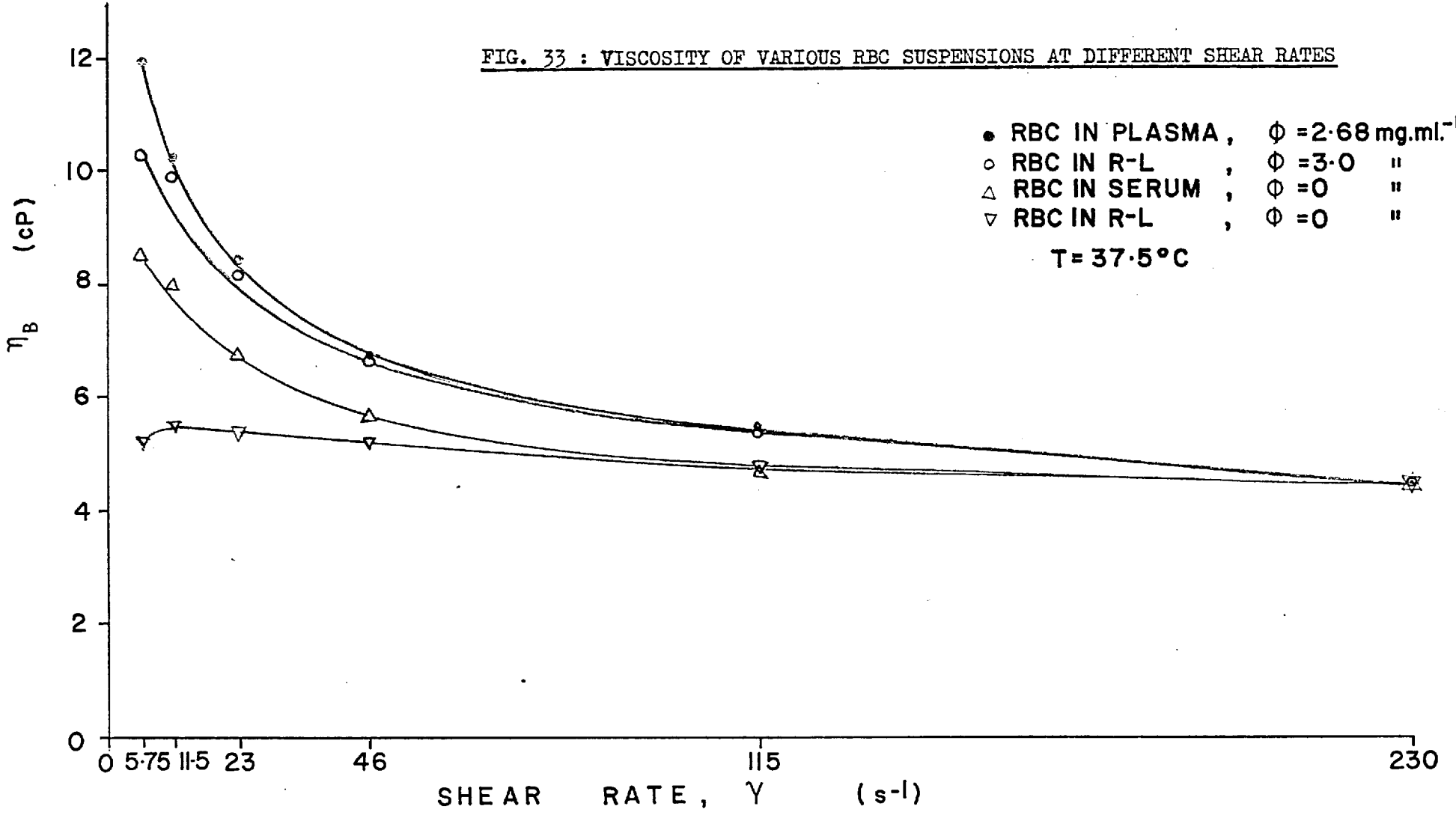
η_p = 1.4 ± 0.1 cP; $\eta_{RL+\Phi}$ = 1.4 ± 0.1 cP; η_s = 1.25 ± 0.1 cP; η_{RL} = 1.05 ± 0.1 cP FOR ALL γ USED.

ERROR IN VISCOSITY MEASUREMENT = ± 0.2 cP
ERROR IN Φ MEASUREMENT = 0.1 mg. ml.$^{-1}$

TABLE 29 FOR FIGURE 33

VALUES OF SUSPENSION VISCOSITY, η_B, FOR RED CELLS SUSPENSION IN PLASMA, SERUM AND RINGER-LOCKE SOLUTION AT VARYING SHEAR RATES

FIG. 33 : VISCOSITY OF VARIOUS RBC SUSPENSIONS AT DIFFERENT SHEAR RATES



The viscosity of the red cell suspending mediums for all the samples was found to be unaltered by shear rate, and thus behaves as a Newtonian fluid.

The whole blood viscosity, η_B, at varying shear rates, γ, for normal and heat-hardened red cells in plasma, and at the same haematocrit of 44% and 37.5°C, is shown in Figure (34). At high shear rates, η_B for hardened cells is greater in value than for normal cells. When the shear rate, γ, is decreased, η_B for normal cells increases more rapidly than for hardened cells.

As was shown in section II of Chapter 2, when plasma fibrinogen concentration, ϕ, in whole blood is reduced, the red cell flexibility is also reduced in a linear relationship. In addition, plasma viscosity, η_p, decreases in value, Figure (32). The change in η_p does not affect η_B at high shear rates, as shown in Figure (33). From Figure (31), by gradual change in plasma fibrinogen concentration, the red cell flexibility was gradually altered. This has no effect on η_B at high shear rates ($\gamma = 230s^{-1}$) because the effect of changing the red cell flexibility is masked by the change in η_p. Figure (34) shows the change in η_B with for normal and hardened red cells at the same plasma fibrinogen concentration. However, to find the changes in η_B for varying degrees of red cell flexibility at the same ϕ, the red cells were washed three times and resuspended in isotonic Ringer-Locke solution of pH = 7.4. The suspension was divided into five equal samples. These samples were then heated at 48.5°C for varying intervals of 0 (unheated),

	SHEAR RATE, γ (s^{-1})				
	230	115	46	23	11.5
NORMAL RED CELLS IN PLASMA	4.3 (cP)	5.15	7.4	11.1	18.4
HARDENED RED CELLS IN PLASMA	5.75(cP)	6.4	7.81	8.84	9.7

HAEMATOCRIT = 44% TEMPERATURE OF MEASUREMENTS = 37.5°C

η_p = 1.4 cP FOR ALL VALUES OF γ USED

RED CELL FLEXIBILITY FOR NORMAL CELLS = 8.85 ± 0.5 % min^{-1}

RED CELL FLEXIBILITY FOR HARDENED CELLS = 0.93 ± 0.5% min^{-1}

ERROR IN VISCOSITY MEASUREMENT = 0.2 cP

TABLE 30 FOR FIGURE 34

VALUES OF WHOLE BLOOD VISCOSITY, η_B, FOR NORMAL AND HARDENED RED CELLS IN PLASMA

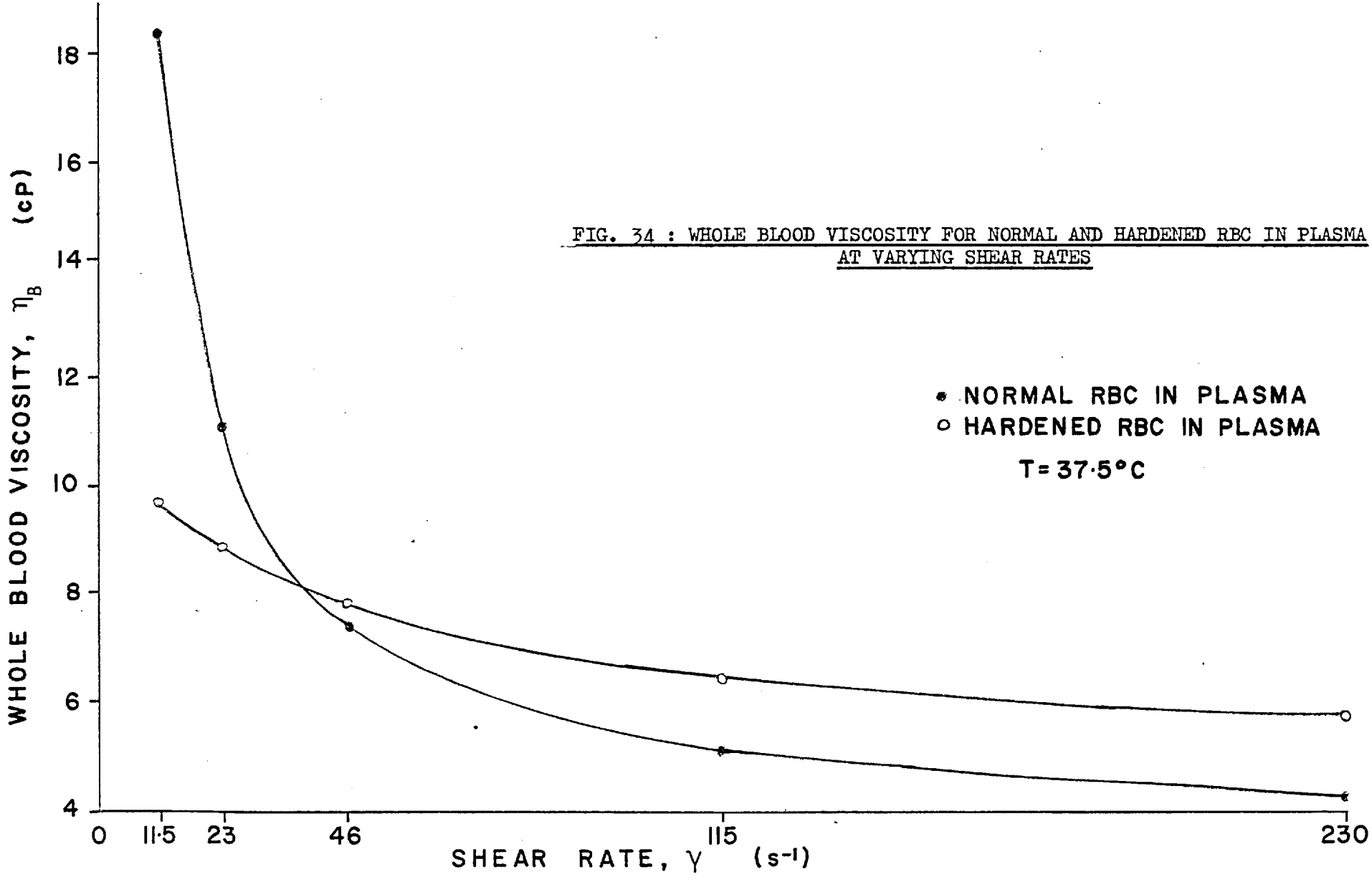


FIG. 34 : WHOLE BLOOD VISCOSITY FOR NORMAL AND HARDENED RBC IN PLASMA AT VARYING SHEAR RATES

5, 10, 20 and 30 minutes. Each of these samples were then divided into a further three samples. The red cells of one sample was resuspended in plasma, the second resuspended in serum and the third in a fresh volume of Ringer-Locke solution. The end products are suspensions of red cells of varying rigidities suspended in plasma, serum and Ringer-Locke. The haematocrits were corrected to be equal in all the samples.

By keeping the ϕ in the red cell suspension unaltered, altering the red cell flexibility by altering the membrane rigidity, will affect the suspension viscosity η_B and relative viscosity η_r. The more rigid the red cell membrane becomes, without altering red cell shape, the greater is the value of η_B and η_r. For high shear rates between $230s^{-1}$ and $46s^{-1}$, where rouleaux or aggregates do not exist, Schmidt-Schonbein et al (1969), the value of η_r increases with decreasing red cell flexibility as well as with decreasing shear rate, as shown in Figures (35), (36) and (37) for the three different suspensions. However, when the shear rate is decreased to $23s^{-1}$ and $11.5s^{-1}$, figures (38)and (39) respectively, it was observed that the value of η_r decreased initially as the red cell flexibility was decreased before increasing again when the red cell flexibility was lower than 3.5% min^{-1}. The initial decrease in η_r was more noticeable at $\gamma = 11.5s^{-1}$, Figure (39), than for $\gamma = 23s^{-1}$, Figure (38). This behaviour of η_r occurred only in the plasma suspension and not in the serum or

RED CELLS IN PLASMA

RED CELL FLEXIBILITY INDEX % min^{-1}	SHEAR RATES, γ (s^{-1}) 230	115	46	23	11.5
2.17	3.97 (cP)	4.41	5.93	7.9	11.04
2.8	3.75	4.3	5.67	6.6	9.0
4.39	3.66	4.14	5.86	7.17	9.66
5.57	3.32	3.67	4.83	7.27	10.13
6.71	3.17	3.79	5.18	8.21	12.43

RED CELLS IN SERUM

RED CELL FLEXIBILITY INDEX % min^{-1}	SHEAR RATES, γ (s^{-1}) 230	115	46	23	11.5
1.4	4.79 (cP)	5.5	7.81	10.79	16.5
2.05	3.98	4.45	5.97	7.81	11.48
2.3	3.45	4.0	5.32	6.77	9.68

HAEMATOCRITS = 45%

η_p = 1.4 ± 0.1 cP; η_s = 1.25 ± 0.1 cP; η_{RL} = 1.05 ± 0.1 cP FOR ALL γ USED

TEMPERATURE OF MEASUREMENTS = 37.5°C

TABLE 31 FOR FIGURES 35-39

. VALUES OF RELATIVE VISCOSITY, η_r, FOR DIFFERENT RED CELL FLEXIBILITY INDEX FOR RED CELL SUSPENSIONS IN PLASMA, SERUM AND RINGER-LOCKE AT DIFFERENT SHEAR RATES, γ, AND 37.5°C

Table 31 continued overleaf

RED CELL FLEXIBILITY INDEX % min^{-1}	SHEAR RATE, γ (s^{-1})				
	230	115	46	23	115
1.67	4.86 (cP)	5.36	6.87	9.78	15.3
1.9	4.6	5.05	6.45	9.0	13.65
2.22	4.34	4.84	6.32	8.13	11.65
2.45	4.05	4.6	6.0	7.57	10.4
2.67	3.85	4.4	5.77	7.03	9.33

ERROR IN FLEXIBILITY INDEX MEASUREMENT = ± 0.5% min^{-1}

ERROR IN RELATIVE VISCOSITY MEASUREMENT:-

AT γ = 230s^{-1}, ERROR = ± 0.15

AT γ = 115s^{-1}, ERROR = ± 0.25

AT γ = 46s^{-1}, ERROR = ± 0.5

AT γ = 23s^{-1}, ERROR = ± 0.75

AT γ = 11.5s^{-1}, ERROR = $\pm$ 1.5

TABLE 31 (continued)

● RBC IN PLASMA
○ RBC IN SERUM
△ RBC IN RINGER-LOCKE
H = 45 %
T = 37·5 °C

FIG. 35

$\dot{\gamma} = 230\ s^{-1}$

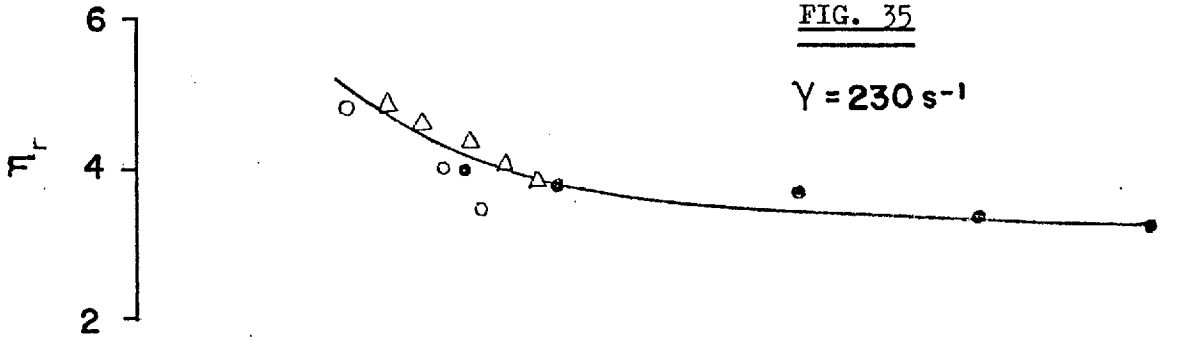


FIG. 36

$\dot{\gamma} = 115\ s^{-1}$

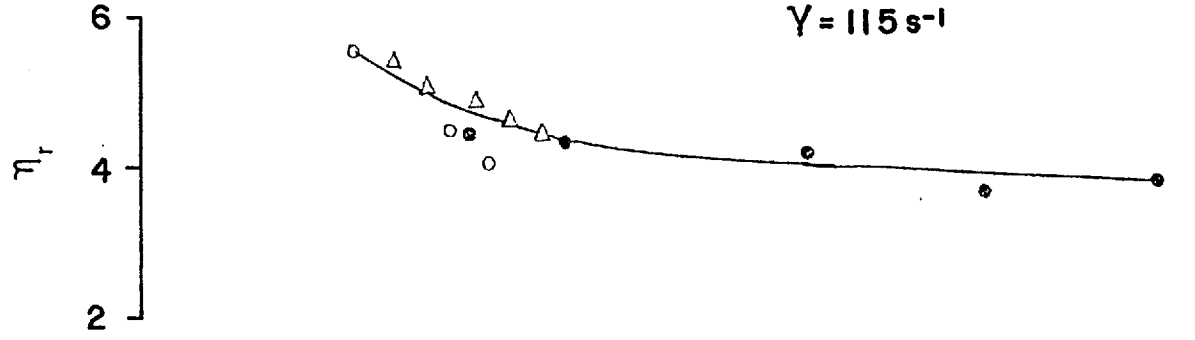
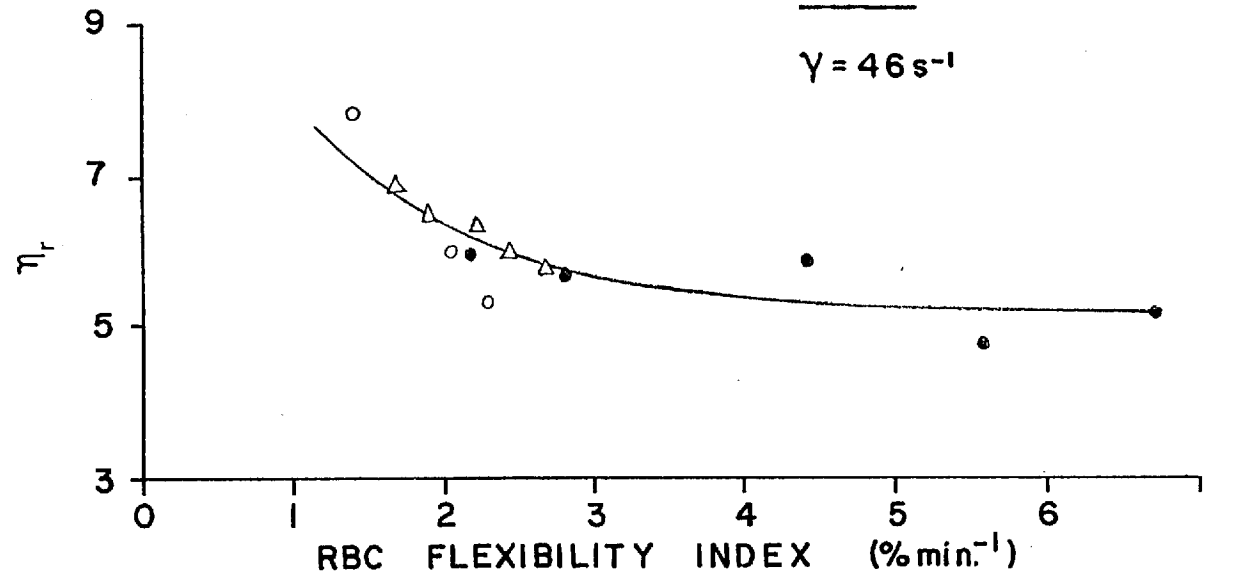


FIG. 37

$\dot{\gamma} = 46\ s^{-1}$



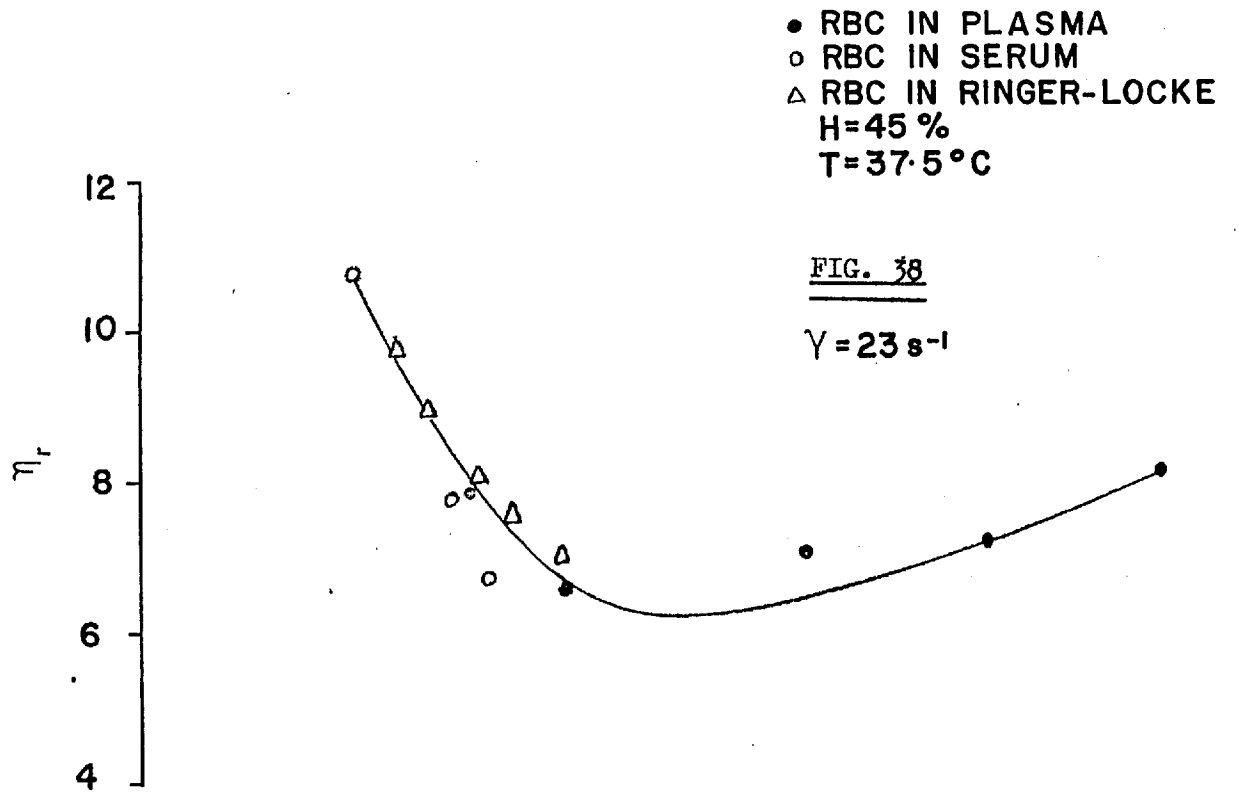

• RBC IN PLASMA
o RBC IN SERUM
△ RBC IN RINGER-LOCKE
H=45 %
T=37·5 °C
γ = 23 s⁻¹
η_r
12
10
8
6
4

FIG. 38

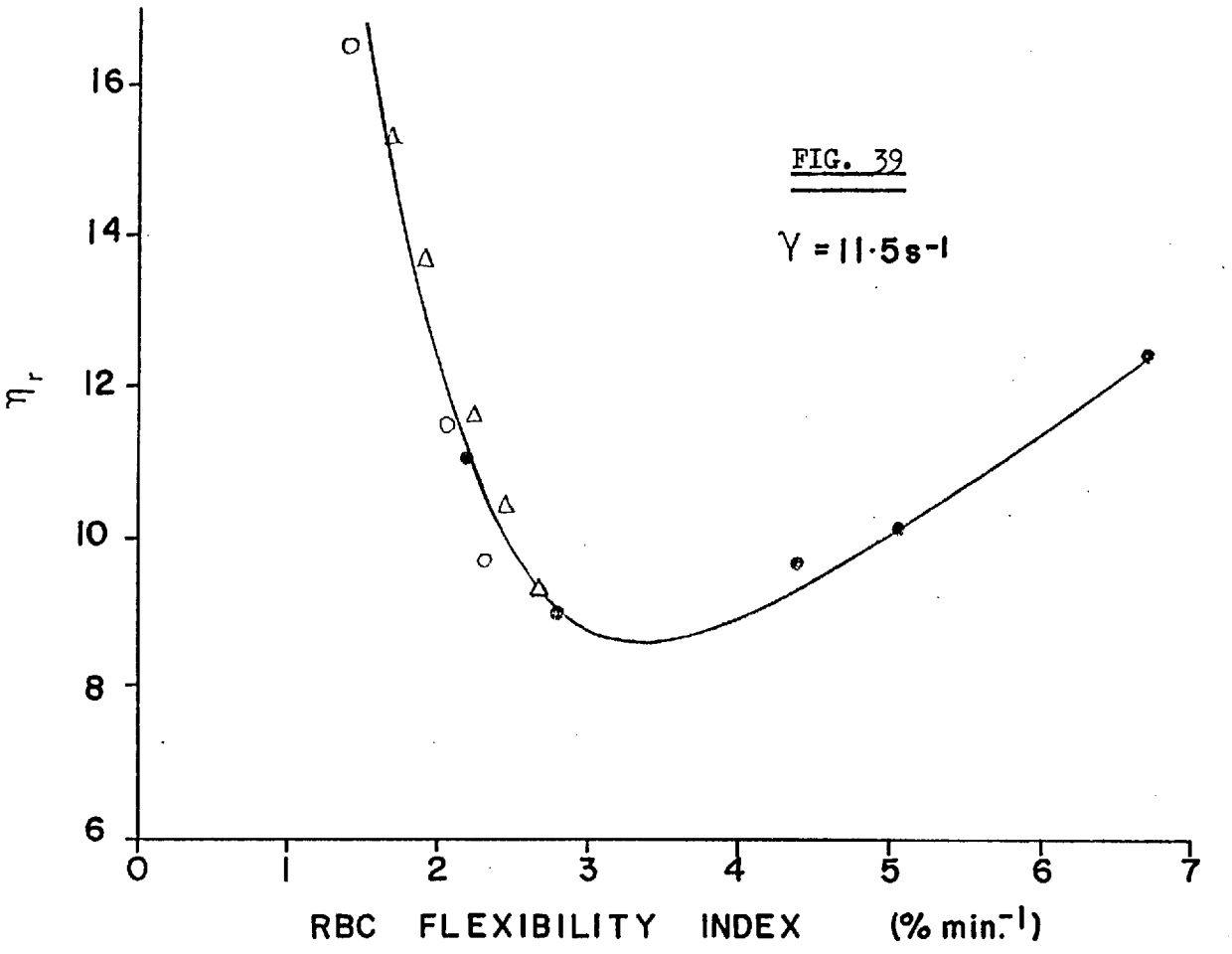

γ = 11·5 s⁻¹
η_r
16
14
12
10
8
6
0
1
2
3
4
5
6
7
RBC FLEXIBILITY INDEX (% min.⁻¹)

FIG. 39

Ringer-Locke suspension because of the higher range of red cell flexibilities achievable in plasma as a result of the presence of fibrinogen.

(iv) Discussion

When red blood cells are hardened, the value of the suspension viscosity, η_B, at all shear rates, is greater than the viscosity of a similar suspension of normal red cells, Chein et al (1967b). If suspensions of normal cells in plasma, serum and Ringer-Locke are compared, it was found by Chein et al (1966), that at low shear rates and 45% haematocrit, η_B increases for suspensions of higher plasma protein concentration. However at high shear rates the values of η_B for the three suspensions become equivalent. The reason for that is that for normal cells at high shear rates, the high shear stress aligns the red cells to the direction of flow, Goldsmith (1971), by deforming the cell shape into prolate ellipsoids, Schmid-Schonbein and Wells (1969). If cells are hardened, high shear stress does not deform the cells and the viscous resistive forces of the cells to flow are greater, increasing the value of η_B.

When plasma fibrinogen concentration is increased, we have already seen that this increases the red cell flexibility. Therefore we would expect that η_B will also be reduced at high shear rates. However, as ϕ is increased, η_p also increases slightly, Figure (32). This would be expected to have a counter effect of increasing η_B. In fact what is

found to occur is that when Φ is increased, the effect of increased red cell flexibility on η_B is counter-balanced by the effect of η_p on η_B. The result is that at high shear rates, η_B is little changed, Figure (31). Applied to blood flow in the capillaries, where high shear rates exist, a change in the plasma fibrinogen concentration, Φ, should not affect the value of η_B or blood flow.

Chein et al (1967b) have demonstrated that for varying Φ, the red cell suspension viscosity, η_B, increased less as the shear rate was increased from $0.1s^{-1}$ to $5s^{-1}$. Hence we would expect that this trend for higher shear rates would agree with our results. The interpretation of the effect of Φ on η_B at high shear rate has not been given satisfactorily by investigators who use techniques to measure red cell deformability other than the centrifuge technique of Sirs (1970). The reason has been the difficulty of measuring red cell flexibility, as pointed out by Kiesewetter et al (1981) for artifacts in the filtration technique, micropipette technique, and the single red cell passage time through single-pore membrane technique. An additional problem with these three techniques is that the size of pore through which the red cells are made to pass, between 2 and 6 μm, gives a situation where the Fahraeus-Lindqvist effect is applicable. This means that the apparent viscosity and hence flow rate measurement (filtration measurement for red cell deformability) is far more complicated than is realised.

Chein et al (1967b), observed that increasing ϕ increases the degree of rouleaux formation in normal red cell suspensions at low γ. It has also been shown by Chein and Jan (1973) that rouleaux formation involves deformation of the red cell shape. Hence it is reasonable to deduce that rouleaux formation increases with red cell flexibility for any given ϕ. Using the filtration technique to measure red cell deformability, Reid et al (1976), found that there was no correlation between red cell deformability and plasma fibrinogen. This is clearly in contradiction to viscometry and optical results of Chein and several other workers mentioned in this thesis.

At high shear rates, $\gamma = 230s^{-1}$, and same haematocrit, the viscosity, η_B, of red cells suspended in plasma, serum, Ringer-Locke or Ringer-Locke plus human fibrinogen (Kabi Grade L) is nearly equal. The reason is that the difference in fibrinogen and hence red cell flexibility and its effect on η_B is counterbalanced by the difference in η_p, the suspending medium's viscosity. Hence at this high sh'ear rate, cell-protein interaction is not the dominant factor influencing the value of η_B. As we have seen at $\gamma = 230s^{-1}$, η_B increases, in a logarithmic relationship with the haematocrit and therefore at this high shear rate it is cell-cell interaction which is a more dominant factor and is equal in all the four samples due to the equal haematocrit.

When the shear rate is decreased, the value of η_B for red cells suspension in plasma protein-free Ringer-Locke does not change appreciably. This shear independent behaviour is

characteristic of Newtonian fluids but in this case the high cell concentration and decreasing shear rate means that the frequency of cell-cell collision, and hence interaction, is increased, increasing η_B slightly, Brooks et al (1970). In the presence of plasma proteins other than fibrinogen, i.e. serum suspension, the value of η_B at low shear rates is increased much more than for Ringer-Locke solution, particularly when $\dot{\gamma}$ is less than $46s^{-1}$. Since red cell aggregation is absent in serum, Schmidt-Schonbein et al (1968), the presence of plasma proteins other than fibrinogen (globulins) gives rise to the non-Newtonian behaviour of blood at low $\dot{\gamma}$ because of increased cell-cell interaction, as well as a smaller degree of cell-protein interaction. This is compared to red cells in saline, where the non-Newtonian behaviour at low $\dot{\gamma}$ is due to cell-cell interaction only.

In the presence of fibrinogen, without other plasma proteins, at low shear rates, the red cell suspension viscosity η_B is increased further in value than for the case of serum. The reason for the increase in η_B is due to red cell aggregation and rouleaux formation caused by fibrinogen adsorption onto red cell membranes, Chein et al (1967b). If we compare the value of η_B for Ringer-Locke plus fibrinogen suspension, and the plasma suspension we notice that η_B for the plasma suspension is greater, although it has the lowest value of Φ in the two suspensions. Fahraeus (1921 and 1958), Merrill et al (1963c) and Chein et al (1966) have shown that increasing the degree of red cell aggregation increased η_B at low

γ, and that the degree of aggregation increased with ϕ. The lower value of η_B for a higher ϕ in the Ringer-Locke plus fibrinogen solution than whole blood, suggests that other plasma proteins are necessary for the degree of cell-protein interaction, and may act to stabilize this interaction, as suggested by Begg and Hearns (1966) and demonstrated by Chein et al (1970).

At high shear rates, the suspension viscosity η_B is dependent on the ability of the red cells to deform and align with the flow direction, Goldsmith (1970). The more the cells are able to deform in shape as γ is increased, the less is the viscous force offered by the cells to the flow, and hence the less is the value of η_B. The shape transition under shear results in rotation of the cell membrane around the cell contents at high γ, Schmid-Schonbein and Wells (1969). Hence the shape transition depends on the fluidity of the cell membrane as well as the internal viscosity of the red cell. The dependence of η_B on the membrane fluidity or rigidity is shown in Figure (34).

At high shear rates, hardened red cells show a higher value of η_B due to the rigidity of the red cell membrane. Therefore at high γ, beside cell-cell interaction, membrane flexibility, independent of plasma protein concentration, is another dominant factor in determining η_B. The increased membrane rigidity, however, does not affect the cell-protein interaction at low shear rates. The increase in η_B with decreasing γ for hardened red cells is comparable to the

case of normal cells over the range of low γ used. However, at the lowest value of γ used ($5.75s^{-1}$), η_B for hardened cells is less than for normal cells possibly due to a lesser ability to form rouleaux and aggregates, which is decreased by the rigidity of the red cell membrane.

If the red cell flexibility is decreased by increasing the membrane rigidity and keeping the protein concentration unaltered, η_B is increased. Figures (35) to (39) show the effect of varying the membrane rigidity by heating cells for different intervals at 48.5°C, on the relative viscosity, η_r, for red cells suspended in plasma, serum and Ringer-Locke, under different conditions of shear rate, γ. At high shear rate, γ ($230s^{-1}$), it is observed (Figure (35)) that the increase in η_r, with increasing membrane rigidity (or decreasing red cell flexibility), occurs in the three suspending mediums. The same trend is observed for γ = $115s^{-1}$ (Figure (36)), but for slightly elevated values of η_r. When γ is reduced to $46s^{-1}$, Figure (37), where aggregations start to appear, Schmid-Schonbein and Wells (1969), it was observed that there was a slight initial fall in the value of η_r, at high flexibility between 6.75% min^{-1} and 4% min^{-1}, before η_r increased again, as the red cell flexibility was reduced below 4% min^{-1}. This initial fall in η_r is more pronounced as γ is reduced to $23s^{-1}$ (Figure (38)), and again to $11.5s^{-1}$ (Figure (39)).

For a dilute suspension of rigid particles, the Einstein equation, relating viscosity to cell concentration, Einstein (1906), was related to whole blood by Sirs (1981) and given

in the form

$$\eta_r = 1 + KCT$$

where η_r is the relative viscosity (= η_B / η_p), C is the red cell concentration (haematocrit), K is the shape factor and is 2.5 for spheres and greater for unsymmetrical shapes. T is Taylor's factor, Taylor (1932), and given as

$$T = (p + 0.4)/(p + 1)$$

where p is equal to the ratio (η_i / η_p) and η_i is the suspended particle's internal viscosity.

When the red cell is hardened by heating or formaldehyde fixing, the red cell membrane becomes very rigid, but the internal viscosity is not necessarily altered. For a dilute emulsion of one incompressible viscous liquid in another, and an interfacial film separating the two liquids (somewhat similar to the red cell membrane), Oldroyde (1955) derived an equation for η_r given as

$$\eta_r = 1 + \frac{[\,1 + 2{\cdot}5(\eta_i + \eta_m)/\eta_o\,]}{[\,1 + (\eta_i + \eta_m)/\eta_o\,]}\, C_c$$

where η_o is suspending medium viscosity, C_c is the cell concentration and η_m is a function of the rheological properties of the interfacial film and diameter. Applied to a dilute suspension of red cells, η_m is a rheological property of the membrane and cell diameter.

In a similar way to Oldroyd's equation, a simplistic modification to Taylor's factor, T, could be made to take into account the membrane rigidity. This modification is to add a factor η_m to η_i in p to give p' as

$$p' = (\eta_i + \eta_m)/\eta_p$$

where η_m is a factor dependent on red cell membrane rigidity in the case of dilute suspension of red cells. Due to the complexity of trying to obtain an equation relating the Einstein, Dintenfass or Sirs equations to blood viscosity and properties of the red cell membrane, no expression produced so far can express the behaviour of dilute suspensions of red cells satisfactorily. Hence my modification of p' is an attempt to shed light on the importance of membrane rigidity on η_B.

By substituting p' for p in the expression for T, the new expression for T is given as

$$T'_c = (p' + 0.4)/(p' + 1)$$

T'_c is now called the flexibility factor and used in Sirs equation.

From Figure 39, at low γ ($11.5s^{-1}$) red cell aggregation and rouleaux formation is prevalent in whole blood. The presence of aggregation increases η_r by increasing η_B as has already been demonstrated. When the red cell is at its maximum flexibility index value, T'_c is least in value. The

high flexibility of the red cell means that it can deform to its maximum extent, under the given conditions, forming aggregates with a greater ease. This means that the cell's flexibility enables it to deform its shape easily, and hence we expect the shape factor K to have its greatest value due to unsymmetry in shape caused by deformation.

When the red cell becomes more rigid, the value of T'_c approaches 1 and K decreases in value towards the value of 2.5, its least value. Hence with decreasing red cell flexibility due to increasing membrane rigidity, there is an opposite change in the values of K and T'_c.

As was shown in Figure (34), when red cells are hardened, the degree of aggregation at low shear rates is greatly reduced, and is indicated by the lower value of η_B for hardened cells compared to normal cells. The changing rigidity of the red cell membrane will affect the shape of the cell participating in the aggregates, as well as the size of the aggregates. The less flexible the cell, the smaller is the size of aggregates formed, Chein et al (1981), because increasing the rigidity of the cell demands a greater energy of deformation needed to deform the cell to form aggregates.

From Figure (39), at low shear rate, $11.5s^{-1}$, as the red cell flexibility is reduced from normal value, the relative viscosity decreases first, reaching a minimum at 3.5% min^{-1}, before increasing at lower flexibility values. At low shear rates, cell-cell interactions and aggregations are the

dominant factors in determining the value of viscosity and blood flow. The aggregates are continuously and reversibly breaking down into cells and reforming into aggregates again. When the red cell flexibility is reduced, the degree of aggregation and size of aggregates are decreased, reducing the total viscous energy needed for blood flow, Chein et al (1981), and hence decreasing the value of η_r. The decrease in aggregate size increases the value of the shape factor K because larger aggregates are more likely to be symmetrical, hence having lower value of K than smaller aggregates or single cells. Hence, reducing the red cell flexibility increases both the values of T'_c and K in the expression

$$\eta_r = 1 + KT'_cC$$

increasing the value of the relative viscosity η_r. Therefore, a balance exists between the decrease in η_r due to decreasing flexibility and viscous energy, and an increase in the value of η_r due to increasing value of K and T'_c as the flexibility is decreased. Initially as the red cell flexibility is decreased, the balance is in favour of decreasing η_r until a point is reached when the effect of increasing K and T'_c become dominant and the balance is changed to increase η_r. Beside the increasing value of KT'_c, as the cells become inflexible, the value of η_r is increased further because a situation of η_r being the relative viscosity of a suspension of rigid cells is reached.

IV CONCLUSIONS

In a suspension of red cells, several rheological factors interact to determine the suspension viscosity η_B. Such factors are:

i) Concentration of red cells (H)
ii) Concentration of plasma proteins (fibrinogen, ϕ)
iii) Red cell flexibility and cell membrane rigidity (T'_c)
iv) Red cell shape (K)
v) Shear rate used to measure η_B ($\dot{\gamma}$)
vi) Temperature of measurement

Two other factors, suspension pH and osmolarity, and their effect on η_B will be shown in the following chapter of this thesis.

As was shown, the haematocrit, H, is related in a linear relationship to the logarithm of the suspension viscosity η_B or the relative viscosity η_r, with $\log \eta_B$ or $\log \eta_r$ increasing as H is increased. The value of η_B depends on the shear rate and temperature of measurement; η_B decreases with temperature increase due to decrease in suspending medium viscosity and increase in red cell flexibility. When η_B is measured at high shear rate, the presence or absence of plasma proteins make no difference to the value. In the absence of plasma proteins, when the shear rate is decreased, the red cell suspension behaves nearly as a Newtonian fluid, with the value of η_B being

almost shear independent and the only small increase in η_B as γ is decreased is due to cell-cell interaction. This cell-cell interaction, decreases with decreasing haematocrit.

In the presence of plasma proteins, red cell suspensions show non-Newtonian behaviour. The viscosity η_B becomes shear dependent, increasing with decreasing γ. The increase at low γ is enhanced by increase in suspension fibrinogen concentration, ϕ. This is a result of the effect of increasing ϕ on red cell flexibility, which acts to increase the flexibility. Increasing the flexibility of the red cell increases the degree of aggregation and rouleaux formation at γ lower than $46s^{-1}$ and this in turn increases η_B.

The interaction between the red cell shape and its flexibility will be dealt with in greater detail in the following chapter. However, the effect of K and T'_c on η_r, at low shear rates, was demonstrated to decrease η_r before increasing it as the red cell flexibility and degree of aggregation are decreased from the normal state, by increasing the red cell membrane rigidity. This behaviour was explained in terms of interactions between the shape factor K, the flexibility factor T'_c, red cell aggregation and viscous energy.

The effect of each rheological factor on η_B is demonstrated in Table 32.

RHEOLOGICAL FACTOR	CHANGE IN FACTOR VALUE (INCREASE OR DECREASE)		AT SHEAR RATE γ		EFFECT ON SUSPENSION VISCOSITY η_B
			LOW ($<46 s^{-1}$)	HIGH	
HAEMATOCRIT, H	INCREASE		✓		INCREASE
				✓	INCREASE
		DECREASE	✓		DECREASE
				✓	DECREASE
FIBRINOGEN CONC., Φ (IN PRESENCE OF OTHER PLASMA PROTEINS)	INCREASE		✓		INCREASE
				✓	NONE
		DECREASE	✓		DECREASE
				✓	NONE
FLEXIBILITY FACTOR, T	INCREASE		✓		DECREASE
				✓	INCREASE
		DECREASE	✓		INCREASE
				✓	DECREASE
SHAPE FACTOR, K	INCREASE		✓		DECREASE
				✓	DECREASE
		DECREASE	✓		INCREASE
				✓	INCREASE
SHEAR RATE, γ	INCREASE		✓		DECREASE
				✓	DECREASE
		DECREASE	✓		INCREASE
				✓	INCREASE
TEMPERATURE, t	INCREASE		✓		DECREASE
				✓	DECREASE
		DECREASE	✓		INCREASE
				✓	INCREASE

TABLE 32

GROSS EFFECTS OF VARIOUS RHEOLOGICAL FACTORS ON VISCOSITY OF RED CELLS SUSPENSION AT LOW AND HIGH SHEAR RATES

CHAPTER 5

EFFECTS OF TONICITY AND pH ON BLOOD RHEOLOGY

I INTRODUCTION

The variation of plasma or suspending medium's tonicity affects the red blood cell in several ways. The red blood cell volume, i.e. its MCV, is altered by water exchange into and out of the cell (i.e. hypotonicity shifts water into the cell and hypertonicity out of the cell), to maintain osmotic equilibrium. These changes affect other factors such as the red blood cell shape and membrane rigidity, as well as the plasma and red cell suspension viscosity. Corresponding changes occur 'in vivo' following renal failure and severe burns, where there is a change in plasma tonicity due to change either in ionic concentration or protein concentration by loss of water (in case of burns). Most experimental investigations were carried out 'in vitro' for obvious reasons.

Ponder (1948) made an extensive study of the effect of varying tonicity on the red blood cell MCV and shape. He measured the cell diameter with a microscope and directly observed the cell shape. The change in MCV with tonicity of the suspending medium was found to depart from the ideal osmometer model where the red blood cell would behave as a balloon - like body with no internal structure and a very thin wall separating two ideal solutions. The relationship between the red blood cell volume, V, and the suspension tonicity, T, is given as:

$$V = W \left(\frac{1}{T} - 1\right) + 100$$

where W, equal to 0.7, is the fraction of 'osmotically active' cell water, and the initial volume of the cell in an isotomic medium is denoted by 100. Experimental investgiations revealed that the volume increase in hypotonicity and hypertonicity were less than calculated using this 'ideal' equation. To reconcile the experimental results and theory a constant R was introduced so that

$$V = RW\left(\frac{1}{T} - 1\right) + 100$$

The value of R depends on the conditions under which measurements are made, varying from one under ideal conditions, to less than one in normal experimental circumstances.

Savitz et al (1964) proposed a similar theoretical explanation. Their equation describing an ideal osmometer was given as:

$$\pi V_w = \text{constant} = \phi RT(n) \quad \text{(van't Hoff's Law)}$$

where π is the osmotic pressure, R is the gas constant, T is the absolute temperature, ϕ is a constant representing the mean osmotic coefficient for all intracellular solutes, n is the total amount of intracellular solute and V_W is the volume of cell water.

To allow for the red blood cell not behaving as an ideal osmometer, Savitz et al suggested the equation:

$$\pi_o (V_o - b) = \pi_1 (V_1 - b)$$

where b is the non-water volume. They proposed that b consists of the non-water space measured by desication, b_{des}, and the osmotic dead space, b_{calc}. Using these two parameters, they found that the ratio

$$[(V_{co} - b_{calc})/(V_{co} - b_{des})]$$

is always equal to 0.80 and hence only 80% of the cell water participates in the osmotic processes whereas the other 20% is osmotically 'non-active' water, called 'bound water'. This 'bound water' is used to explain the departure from ideal osmometer behaviour. Gary-Bobo and Solomon (1968) have also proposed the concept of the 'bound water' to account for the non-ideal osmometer behaviour of the red blood cell.

The effect of red blood cell volume, MCV, change with tonicity on the rheological properties of blood has been investigated by Welsh (1911), Murphy (1966), Meiselman et al (1967), Ham et al (1968), Rand et al (1968), Giombi and Burnard (1970) and Meiselman (1978). It was found that at the same haematocrit, the viscosity of red blood cells suspension is decreased with hypotonicity and increased with hypertonicity compared with an isotonic suspension. The increased viscosity with hypertonicity is believed to be due to the increased MCHC. Altering the tonicity of the haemoglobin solution has no effect on its viscosity, as reported by Ham et al (1968). However, when the

haemoglobin concentration is increased, above 36 $gm.dl^{-1}$, its viscosity increases rapidly and exponentially, as found by Ponder (1948), Teitel-Bernard (1932), Erslev and Atwater (1963), Ham et al (1968) and Cokelet and Meiselman (1968). This exponential increase in viscosity of haemoglobin solution is due to the gelling of the solution, which becomes paracrystallised or crystallised, as the concentration is increased further. The increase in the internal viscosity of the red blood cell, due to the haemoglobin solution, leads to an increase in the viscosity of the red blood cells suspension.

Red blood cell deformability was found to decrease with hypertonicity by Ham et al (1968) and Meiselman (1978), when measured by the method of packing efficiency. Murphy (1966) found that red blood cell filtrability decreases for 10% change in MCV caused by hypertonicity or hypotonicity. The decreased red blood cell deformability at hypertonicity is associated with the increased concentration and viscosity of the haemoglobin solution inside the red cell, i.e. increased internal viscosity, η_i. For hypotonicity, the decreased deformability is associated with increased membrane tension, and hence rigidity, as the cell increases its volume and eventually its surface area at higher hypotonicity, after which the red blood cell membrane can rupture at the point of haemolysis, Hamburger (1895).

When the red blood cell is suspended in a hypotonic solution, its volume increases before its surface area. In hypertonic situations the cell volume decreases but it is

not established whether the surface area changes or not. This change in the red blood cell [membrane surface area/volume] ratio is associated with a shape change. The shape change has been used to explain the departure of the red blood cell from ideal osmometer behaviour, Ponder (1948), and to account for changes in the rheological parameters such as the whole blood viscosity, η_B, Wells et al (1963) and Giombi and Burnard (1970). Scheraga (1955) calculated the shape factor, α, for oblate and prolate ellipsoids from the velocity gradient of a viscous fluid. The value of α for a perfect sphere is 2.5 and is equivalent to the Einstein shape factor, as used by Taylor (1932) in his equation for the viscosity of a suspension of spheres, of immiscible fluids at concentrations of less than 1%. Although the red blood cell shape is given as a possible cause for several discrepencies in the rheological behaviour of red blood cells suspensions at different conditions, none of the authors attempted to measure it or show how it can account for any of the rheological changes.

From as early as 1877, Ewald (1877) noted that when the pH of a red blood cells suspension is altered from the physiological range of 7.35-7.45, rheological changes occur. Hampson and Maizels (1926) found that when the pH is made more acidic, the red blood cell volume, MCV, increases, up to pH = 5.4, after which it decreases reaching a minimum at pH = 3. When the pH is made more alkaline, the MCV decreases up to a pH just above 8, after which the MCV starts to increase again and haemolysis occuring at pH = 11. If the initial tonicity of the cell

suspension at a given pH is altered, the change in MCV as a result of change in pH is altered but the turning points around pH = 5.4 and pH = 8 remain unaltered.

The increase in MCV with acidity was suggested by Murphy (1969) to be caused by the low pH affecting the 'osmotically active' cell water, resulting in osmotic swelling of the red blood cell. Gary-Bobo and Solomon (1968), however, have suggested that the osmotic properties of the red blood cell water at different pH levels result from changes in the effective charge on the haemoglobin molecule with dilution.

Dintenfass and Burnard (1966), Murphy (1967) and Giombi and Burnard (1970) have reported that at low pH the viscosity of red blood cells suspensions, η_B, increases and the red blood cell deformability decreases. For the same change in volume due to tonicity or low pH it was found that η_B is greater in the case of low pH. This suggests that there is an additional effect of pH on the cell membrane other than that due to swelling, resulting in a more rigid cell membrane. The effect of pH on the intracellular content was ruled out as a contributory factor to the additional effect by Murphy (1967), who found that pH change did not alter the viscosity of haemolysates of normal haemoglobin. No explanation as to how the membrane rigidity is increased by low pH has been offered.

When the pH of the red blood cells suspension is increased to pH = 8, Murphy (1966) observed that the suspension

viscosity, η_B, increased slightly and the red blood cell filtrability decreased slightly. The effect of increasing pH on the red blood cell has not been investigated further than the work done by Murphy (1966).

Rampling (1981) has observed that fibrinogen binding to the red blood cell membrane was increased by acidity and hypertonicity, but was unaltered by alkalinity and slightly decreased by hypotonicity. Within the experimental problems of the procedure used to measure the binding on a packed red cell column used in this investigation, the result obtained is contradictory to that obtained by the investigators mentioned above. Increased fibrinogen binding or adsorption is associated with increased red blood cell deformability as observed by Chien et al (1970), Wells et al (1964) and Rampling and Sirs (1977). Therefore if the fibrinogen adsorption on the red blood cell membrane surface is increased by low pH values, we would expect the membrane to be less rigid than at pH = 7.4. The results of Murphy (1967), Dintenfass and Burnard (1966) and Giombi and Burnard (1970) suggest that the membrane becomes more rigid at lower pH. This contradiction of results leads to the question as to whether the fibrinogen adsorption onto the red cell membrane, or the membrane itself is the most influential factor influencing red cell deformability?

Parpat et al (1946) observed that increasing temperature increased the osmotic resistance of the red blood cell, i.e. hemolysis occured at a faster rate at higher temperatures. Murphy (1969) reported that temperature did

not alter red blood cell osmotic properties but altered the maximal membrane surface area at time of lysis. He also suggested that temperature alters the static and elastic components of the red blood cell membrane surface area.

From this review of literature of investigations into the effect of tonicity and pH on blood rheology, several points arise. The idea of 'bound water' was used by Savitz et al (1964) to account for the departure of the red blood cell from an ideal osmometer during tonicity changes. The departure of the red blood cell from an ideal osmometer during changes in pH of the suspension was accounted for by Gary-Bobo and Solomon (1968) as being due to changes in the effective charge on the haemoglobin molecule. However, in both investigations, changes in parameters such as membrane rigidity and red cell shape were not taken into consideration. The suggestions that changes in volume or membrane surface area are the only factors involved are difficult to sustain when the methods for measuring red cell volume or surface area involve errors of at least 15%. Indirect methods to measure changes in red cell shape have not been attempted and therefore the influence of this parameter is not known. Although the data presented by Rampling (1981) on the role of fibrinogen adsorption at varying tonicities and pH in blood rheology is contradictory to other workers' data, it highlights the question of the role of plasma proteins on factors such as red blood cell deformability, neglected by workers in the field.

The discrepencies and contradictions existing in the literature on the effect of tonicity and pH on blood rheology have prompted the following further investigations. I have tried to be systematic in measuring all possible parameters, using available techniques and sometimes different methods from other workers, for extremes in tonicity and pH. This approach will hopefully help us to build a more complete picture of both effects on the red blood cell and of their similarities and differences.

II METHODS

(i) Varying Tonicity of Red Blood Cells Suspension

a) Plasma dilution

To vary the tonicity of red blood cells suspending medium, while keeping the samples as close as possible to their original physiological state, the red cells are kept in plasma. The tonicity was varied by diluting the plasma with minimum volumes of hypotonic or hypertonic solutions. The hypotonic solution used was a Ringer-Locke solution, to which no NaCl was added. Into this solution, human fibrinogen powder (Kabi Grade L) was dissolved to give an equal fibrinogen concentration to that of the original plasma. After the fibrinogen was dissolved, the solution

pH was corrected to 7.4 by addition of either isotonic NaOH or HCl solution (0.154N). The final solution tonicity, measured by freezing point depression, was found to be around 80 mosm. l^{-1}.

Blood obtained from a normal healthy male donor bv vene-puncture, using lithium heparin as anticoagulant, was divided into samples of equal volume. The samples were centrifuged at 500g for 5 minutes, and equal volumes of plasma removed from all the samples. To vary the tonicity, the removed plasma was replaced with the Ringer-Locke/fibrinogen solution, with varying amounts of NaCl. This method minimises changes in plasma ionic content as well as fibrinogen concentration.

In order to obtain the range of tonicities used, 43% of the plasma volume was diluted with the Ringer-Locke/fibrinogen solution. It was shown in Fig. 17 of Chapter 3 that diluting plasma proteins, other than fibrinogen, reduced the red cell flexibility index value. To find out to what extent the red cell flexibility index value was reduced by dilution, the value of the red cell flexibility index was obtained for different plasma dilutions with the Ringer-Locke/fibrinogen solution. This was compared to normal, undiluted whole blood.

b) Formaldehyde fixing of red cells in suspensions of varying tonicities

In order to measure the shape factor, K, of red cells, the cells were made inflexible. This was done by washing the red cells three times and resuspending in a Ringer-Locke solution of chosen tonicity and pH = 7.4, to which human albumin (Kabi Lyophilized Powder, Kabi, Stockholm, Sweden) was added to a concentration of 2 $g.l^{-1}$. The reason for the removal of plasma proteins such as fibrinogen and globulins (Chapter 6) was to remove the proteins which increased the red cell flexibility. Albumin was not removed because in the absence of albumin, normal red cells change their shape from biconcave to cup-shape, Jay (1975). After suspending the red cells in the Ringer-Locke/Albumin solution, the cells were fixed in their shape with formaldehyde.

The tonicity of a solution is increased by about 100 mosm. l^{-1} for each 1% increase in formaldehyde concentration. Hence to fix the red cells suspended in a solution of a chosen tonicity, addition of pure formaldehyde would alter its tonicity. Formaldehyde fixes the red cell membrane very rapidly (in about 3 msec.), whereas the time needed by the red cell to equilibrate to a tonicity change is much slower (up to 15 sec.), Sirs (1969). This difference in times means that addition of pure formaldehyde to a red cell suspension could fix the red cell shape at any stage of the alteration due to tonicity change. To avoid this problem, a solution (pH = 7.4) of 2 $g.l^{-1}$ albumin,

0.42g.l^{-1} KCl, 0.24 g.l^{-1} $CaCl_2$, 0.2 g.l^{-1} $NaHCO_3$ and 1 g.l^{-1} glucose was made up. To this solution formaldehyde was added to a concentration which gives the same tonicity as the red cell suspension, after correcting the pH to 7.4. To a known, gently stirred, volume of red cell suspension, a calculated volume of the albumin/formaldehyde/Ringer-Locke solution was added to give a red cell suspension of 1% formaldehyde concentration. Thus by using this procedure, no change in tonicity, red cell shape or pH occurs during fixing with formaldehyde.

The inflexibility of the fixed red cells was checked by measuring its red cell flexibility index. This was found to be less than 0.25% min^{-1}. for all the samples used.

(ii) Varying pH of Red Blood Cells Suspension

a) Bubbling CO_2 OR O_2 through blood

To lower the pH of a blood sample, CO_2 gas was bubbled through the sample. The CO_2 was bubbled through water at room temperature before being bubbled through the blood, so as to have the same temperature and water vapour pressure as the blood sample. The gas was introduced into the sample through a Pasteur pipette. The pH was monitored by a digital pH meter probe placed in the blood sample. The same procedure was used for O_2 gas bubbled through a blood sample to increase its pH.

To maintain the sample pH during viscosity measurements, the Wells-Brookfield viscometer was enclosed in a clear plastic bag into which CO_2 or O_2 was flushed through a rubber tubing. This maintains an atmosphere of CO_2 or O_2 around the blood sample when the pH is lowererd or increased respectively.

The advantage of this method is that the blood is not diluted in any way and is close to its physiological state. The disadvantages are that the pH range attainable is limited and is unstable, especially on the acidic side, because the dissolved CO_2 in the blood escapes into the environment due to the CO_2 concentration being greater in the blood sample. This problem is avoided to a certain extent when the sample is kept in a CO_2 environment, as in the viscosity measurement. However, it is not possible to be absolutely sure of the pH value inside the viscometer when measurements are made.

b) Plasma dilution

Direct alteration of the plasma pH was made by dilution with isotonic NaOH or HCl. The total volume of added acid or base was kept equal in all samples so as to have equal plasma dilution. When the required pH was reached, acid or base volume, especially in the intermediate values, was

made up to the total volume by isotonic Ringer-Locke solution. The plasma dilution was kept to a minimum of 18.2% in all the samples. It is assumed that no difference exists between the red cell response to added acid (HCl) and bubbled CO_2.

c) Formaldehyde fixing of red cells in suspensions of varying pH

Whole blood red cells are washed three times and resuspended in an isotonic Ringer-Locke/albumin ($2g.l^{-1}$) solution, in which the pH was altered to the chosen value by addition of isotonic solutions of NaOH or HCl. To a Ringer-Locke solution without NaCl, albumin was added to a concentration of 2 $g.l^{-1}$ and formaldehyde was added to give a 3% concentration. The solution tonicity was found to be isotonic. The pH of this solution was corrected to equal that of the red cell suspension. To a known, gently stirred, volume of the red cell suspension, a calculated volume of the formaldehyde solution was added to give a red cell suspension of 1% formaldehyde concentration.

The inflexibillity of the fixed red cells was checked by measuring their flexibility index. This was found to be less than 0.25% min^{-1}.

III RESULTS

(i) Osmotic Effect

The effect of dilution, with Ringer-Locke/fibrinogen solution, of the plasma of a blood sample on the red cell flexibility index is shown in Fig. 40. Here, varying volumes of plasma were removed from a series of blood samples, but one (normal sample), of equal volume. The plasma was replaced with the same volume of Ringer-Locke/fibrinogen solution, of the same fibrinogen concentration as the original plasma. The unaltered blood sample, i.e. normal blood sample, represents the zero dilution. The dilution of plasma proteins other than fibrinogen results in a decrease of the flexibility index. Dilution in the order of 45%, as was used in the following experiment, resulted in a 25% reduction in the value of the red cell flexibility index, compared to normal blood.

When the red blood cells suspending medium is made hypotonic , the red cells swell, increasing their MCV and the sample's haematocrit. In hypertonic medium, the red cells shrink in volume, MCV, decreasing the sample's haematocrit. These changes affect the red cell flexibility as well as the whole blood viscosity, η_B. The effects of tonicity on the MCV, haematocrit, relative viscosity, η_r, and flexibility, with the same number of cells in each sample, are shown in Fig. 41. The red cell packing rate (flexibility) is faster in hypertonic solutions, and slower in hypotonic solutions. This could be due to the

PLASMA DILUTION %	0	10	20	30	40	50	60
RED CELL FLEXIBILITY INDEX (% min^{-1})	5.84 5.81	5.30 5.43	5.32 5.10	5.19 5.30	4.25 4.65	4.12 4.25	3.99 4.26

PLASMA OSMOLARITY = 283 mosm. l^{-1}

TEMPERATURE OF FLEXIBILITY INDEX MEASUREMENTS = 37.5°C

EXPERIMENTAL DILUTION USED = 43%

REDUCTION IN RED CELL FLEXIBILITY INDEX AT 43% DILUTION = 25.9%

ERROR IN FLEXIBILITY MEASUREMENT = ± 0.5%

TABLE 33 FOR FIGURE 40

EFFECT OF PLASMA DILUTION ON RED CELL FLEXIBILITY INDEX.
PLASMA DILUTED WITH RINGER-LOCKE/FIBRINOGEN SOLUTION OF SAME FIBRINOGEN CONCENTRATION AS PLASMA

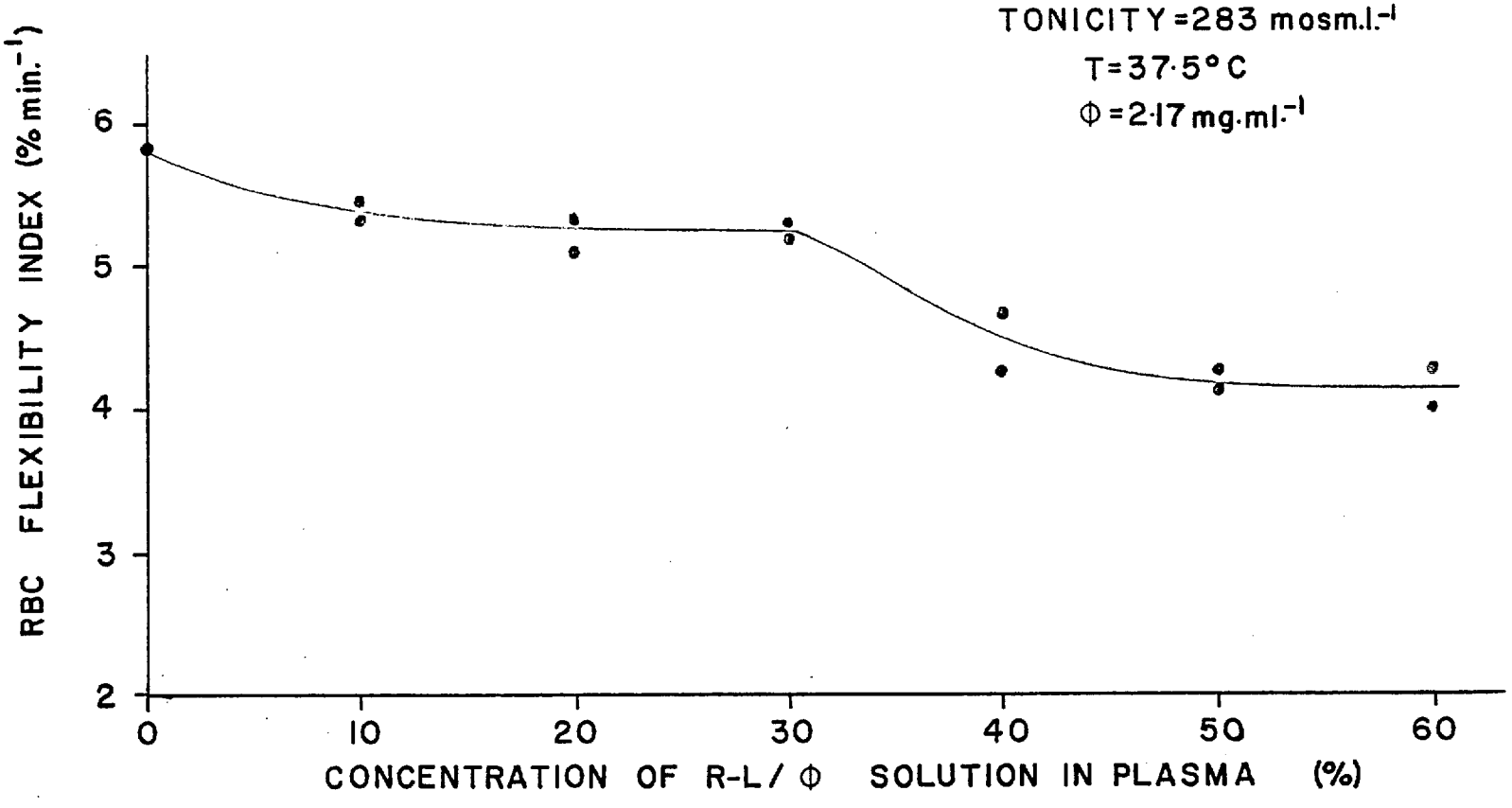


FIG. 40 : EFFECT OF DILUTING PLASMA WITH RINGER-LOCKE/FIBRINOGEN (KABI) SOLUTION ON THE RBC FLEXIBILITY INDEX

SUSPENDING MEDIUM TONICITY MOSM.M^{-1}	179	208	251	283	360	427	482	ERROR
RED CELL MCV (fl.)	114	106	95	86	73	67	59	±2.5
HAEMATOCRIT %	58.5	53.5	47.5	43.5	39	35.5	33.5	±0.125
RED CELL PACKING RATE (% min^{-1}) (AVERAGE OF TWO VALUES)	2.45	3.46	5.55	7.39	8.13	9.05	9.88	±0.5
RELATIVE VISCOSITY η_r	4.82	3.43	3.14	3.21	3.57	3.71	3.79	±0.2

TEMPERATURE OF MEASUREMENTS: 37.5°C

PLASMA FIBRINOGEN CONCENTRATION: 2.67 $mg.ml^{-1}$

PLASMA VISCOSITY: 1.4 cP

SHEAR RATE FOR VISCOSITY MEASUREMENTS: $230s^{-1}$

TABLE 34 FOR FIGURE 41

EFFECT OF VARYING TONICITY ON HEMORHEOLOGICAL PARAMETERS

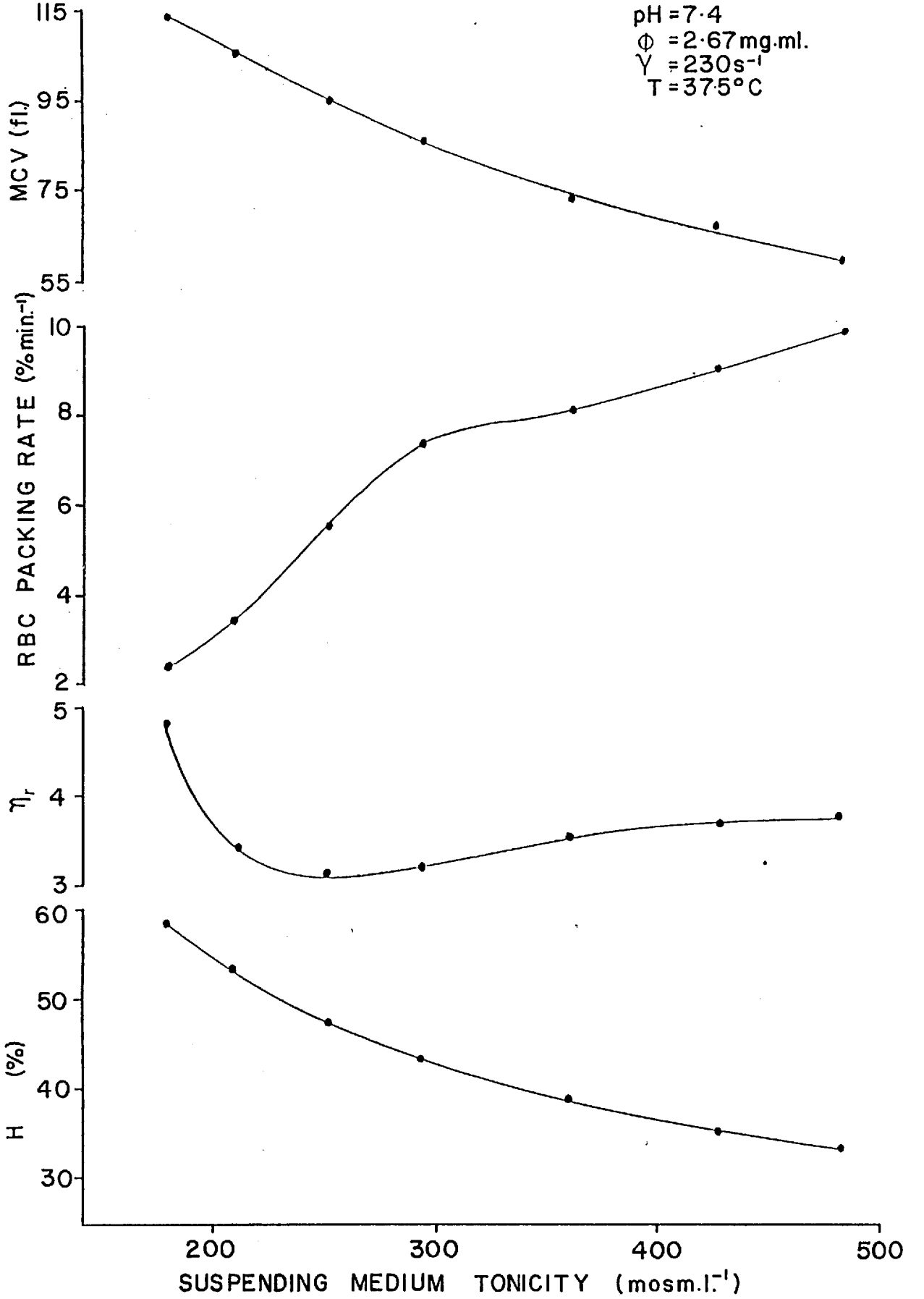


FIG. 41 : VARIATION OF RHEOLOGICAL PARAMETERS WITH CHANGES IN SUSPENSION TONICITY

difference in haematocrits. The relative viscosity, η_r, measured at a shear rate of $230s^{-1}$ on the Wells-Brookfield viscometer, is higher in both hypertonic and hypotonic solutions, compared to isotonic conditions. This suggests that the increased red cell flexibility in hypertonic solutions is in contradiction to the value η_r.

The rate of increase in η_r is rapid below 200 $mosm.l^{-1}$ and can be accounted for partly by the increased cell rigidity (reduced flexibility) and partly by the increase in haematocrit due to swelling of the red cells. Other factors such as shape changes, caused by the cell swelling, affect the value of η_r, Meiselman et al (1967).

The calibration curves of red cell packing rate at different haematocrits, Fig. 42, show that variations of tonicities do not greatly affect the general shape of the calibration curve, over the haematocrit range 36-68%. However, the value of the red cell packing rate decreases with increasing tonicity for the same haematocrit.

When red cells are suspended in hypotonic or hypertonic solutions, the density of the cells changes. In the measurement of the red cell packing rate, the centrifugal force is dependent on the difference between the density of the suspending medium and the density of the cells. Allowing by Archimedes principle, for the buoyancy of the cells in the suspending medium, the pressure at the bottom of a cell column is given by Sirs (1968) as

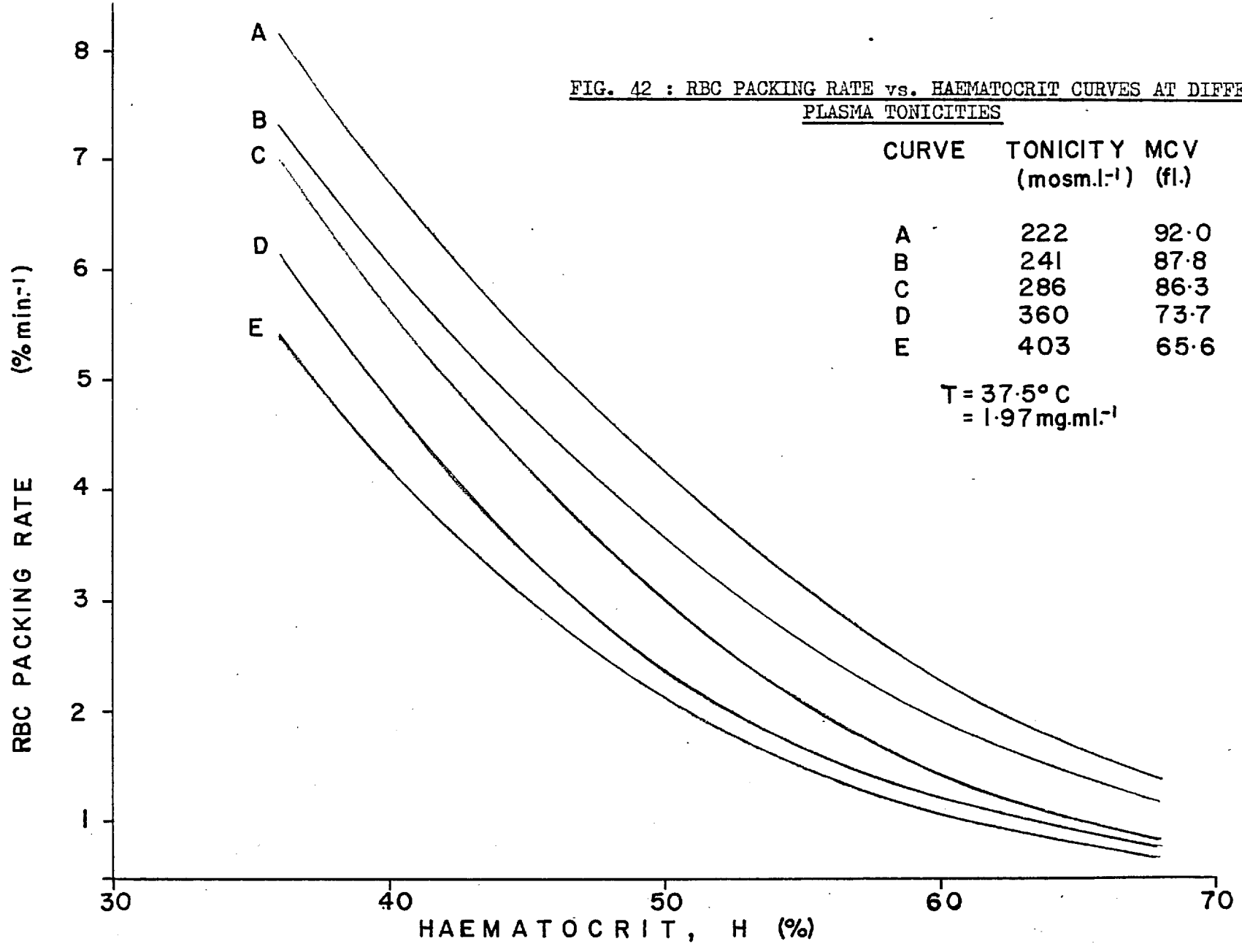


FIG. 42 : RBC PACKING RATE vs. HAEMATOCRIT CURVES AT DIFFERENT PLASMA TONICITIES

CURVE	TONICITY (mosm.l⁻¹)	MCV (fl.)
A	222	92·0
B	241	87·8
C	286	86·3
D	360	73·7
E	403	65·6

T = 37·5° C
= 1·97 mg.ml⁻¹

$$h' \ (\rho_c - \rho_p) \ g' \times 10^{-1} \ Pa$$

where ρ_c is the density of the cells, ρ_p the density of suspending medium, h' is the cell column length taken as the fractional haematocrit multiplied by the total length of the cells plus plasma, or suspending medium, and g' is the centrifugal acceleration. Hence, in the measurement of the red cell packing rate at different tonicities, we need to find the conditions under which the force on the red cell column, from all different samples, remains constant.

The densities ρ_p and ρ_c were measured at varying tonicities, with (a) the same number of cells and (b) the same volume (haematocrit) of cells. The results are shown on Table 35. It is seen that for hypotonicity $(\rho_c - \rho_p)h'$ is constant, within 5% difference, for the case of same number of cells, whereas for hypertonicity $(\rho_c - \rho_p)h'$ is constant, within 5% difference, for the case of same volume of red cells (i.e. haematocrit). When the red cell swells due to hypotonicity, water enters the cell and dilutes the haemoglobin content reducing its density, and hence ρ_c. By keeping the same number of cells but decreasing the tonicity, the cell density, ρ_c, at the new increased haematocrit, h', will be less than the cell density at isotonicity. This is due to water shifting into the cells, diluting their contents and swelling them. There will be a small decrease in ρ_c due to loss of chloride ions shifting out of the cells, and a decrease in the value of ρ_p due to hypotonicity. However, since the length of the blood volume of 5 cm, and the centrifugal force are the

same for all the samples, the product $(\rho_c - \rho_p)h'$ is what concerns us here. From Table 35 it is seen that $(\rho_c - \rho_p)h'$ remains unchanged, within 5% difference, for the same number of cells.

When the red cells suspension is made hypertonic, the MCV decreases, resulting in a concentration of intracellular contents. For the case of the same number of cells, the value of ρ_c is increased due to increased MCHC by loss of fluid. The value of ρ_p is also increased due to increased ionic concentration at hypertonicity. The increase in ρ_c is not as much as expected because the percentage of cell membrane in a volume of red blood cells is increased at hypertonicity, and the density of the red cell membrane, composed largely of lipids, is less than the density of water. This effect can be seen when the values of ρ_c for tonicities of 357 $mosm.l^{-1}$ and 548 $mosm.l^{-1}$ are compared to the isotonic value, for the same number of cells, in Table 35. The value of ρ_c first increases then decreases at the high tonicity to a comparable value with the isotonic value. This is due to the increase in percentage of membrane in a volume of red cells. To compensate for this effect in $(\rho_c - \rho_p)$, it was necessary, in the case of hypertonicity, to maintain the same volume of red cells (i.e. haematocrit) rather than the same number of cells, making the product $(\rho_c - \rho_p)h'$ equivalent in the samples of different tonicities.

By correcting for $(\rho_c - \rho_p)h'$ at hypertonicity, by comparing equal haematocrits, but keeping the same number

TONICITY (l^{-1}) (mosm. l^{-1})	303	245	245	213	213	302	357	357	548	548
Hcl, h' (%)	44	52	44	60	44	47	42.5	47	34	47
RED CELL COUNT ($X10^{12}$ l^{-1})	5.3	5.3	4.49	5.3	3.89	5.38	5.38	6.26	5.38	7.4
MCV (fl)	83	98.1	98.1	113.2	113.2	87.3	75.1	75.1	63.5	63.5
ρ_p $g.ml^{-1}$	1.1009	1.0071	1.0071	1.0127	1.0127	1.0042	1.0136	1.0136	1.0253	1.0253
ρ_c at h' ($g.ml^{-1}$)	1.0449	1.0544	1.0519	1.0538	1.0355	1.0531	1.0594	1.0639	1.0536	1.0751
$(\rho_c - \rho_p)$ ($g.ml^{-1}$)	0.056	0.0473	0.0448	0.0411	0.0228	0.0489	0.0458	0.0503	0.0283	0.0498
$(\rho_c - \rho_p)h'$ ($g.ml^{-1}$)	2.464	2.46	1.971	2.466	1.003	2.298	1.947	2.364	0.962	2.341
% CHANGE FROM ISOTONIC $(\rho_c - \rho_p)$ h'	0	-0.16	-20.1	+0.08	-59.3	0	-15.3	+2.9	-58.1	+1.9

TABLE 35

EFFECT OF TONICITY ON RHEOLOGICAL PARAMETERS

of red cells for hypotonicity, we can now correct for the effect of tonicity on η_r and red cell flexibility index. Figure 43 shows the variation of η_r with tonicity after correction for haematocrit . The value of η_r is lowest at isotonicity and increases for both hypo and hypertonicity. The rate of increase for hypotonicity is greater than that for hypertonicity.

From the value of η_r and haematocrit for each of the blood samples, the value of the intrinsic viscosity, KT'_c, was obtained from Fig. 43, using the Einstein equation, Einstein (1906), for viscosity of a dilute suspension (1% or less concentration) of rigid particles. This was modified by Taylor (1932) and given as

$$\eta_{rl} = 1 + HKT'_c \quad \text{and} \quad \eta_r = (\eta_{rl})^H$$

where η_r is the suspension relative viscosity and η_{rl} is the relative viscosity of 1% concentration of particles (red cells), H is the concentration (0.01), K is the shape factor, equal to 2.5 for spheres and greater of unsymmetrical particles, and T'_c is the red cell flexibility factor as described in the previous chapter.

The variation of KT'_c together with the value of the red cell packing rate (flexibility) corrected for hypertonicity, are shown in Fig. 44. The red cell flexibility is highest at isotonicity and decreases for both hypo and hypertonicity, as was observed by Murphy (1966) measuring red cell filterability. The rate of

SUSPENDING MEDIUM TONICITY MOSM. L^{-1}	179	208	251	283	318	403	487	556
SUSPENSION RELATIVE VISCOSITY η_r	4.82	3.43	3.14	3.21	3.65	5.25	7.71	9.95

TEMPERATURE OF MEASUREMENTS: 37.5°C

SHEAR RATE FOR VISCOSITY MEASUREMENTS: $230s^{-1}$

TABLE 36 FOR FIGURE 43

THE EFFECT OF TONICITY ON RED CELLS SUSPENSION RELATIVE VISCOSITY, η_r, CORRECTED FOR ($\rho_c - \rho_p$) AT HYPERTONICITY

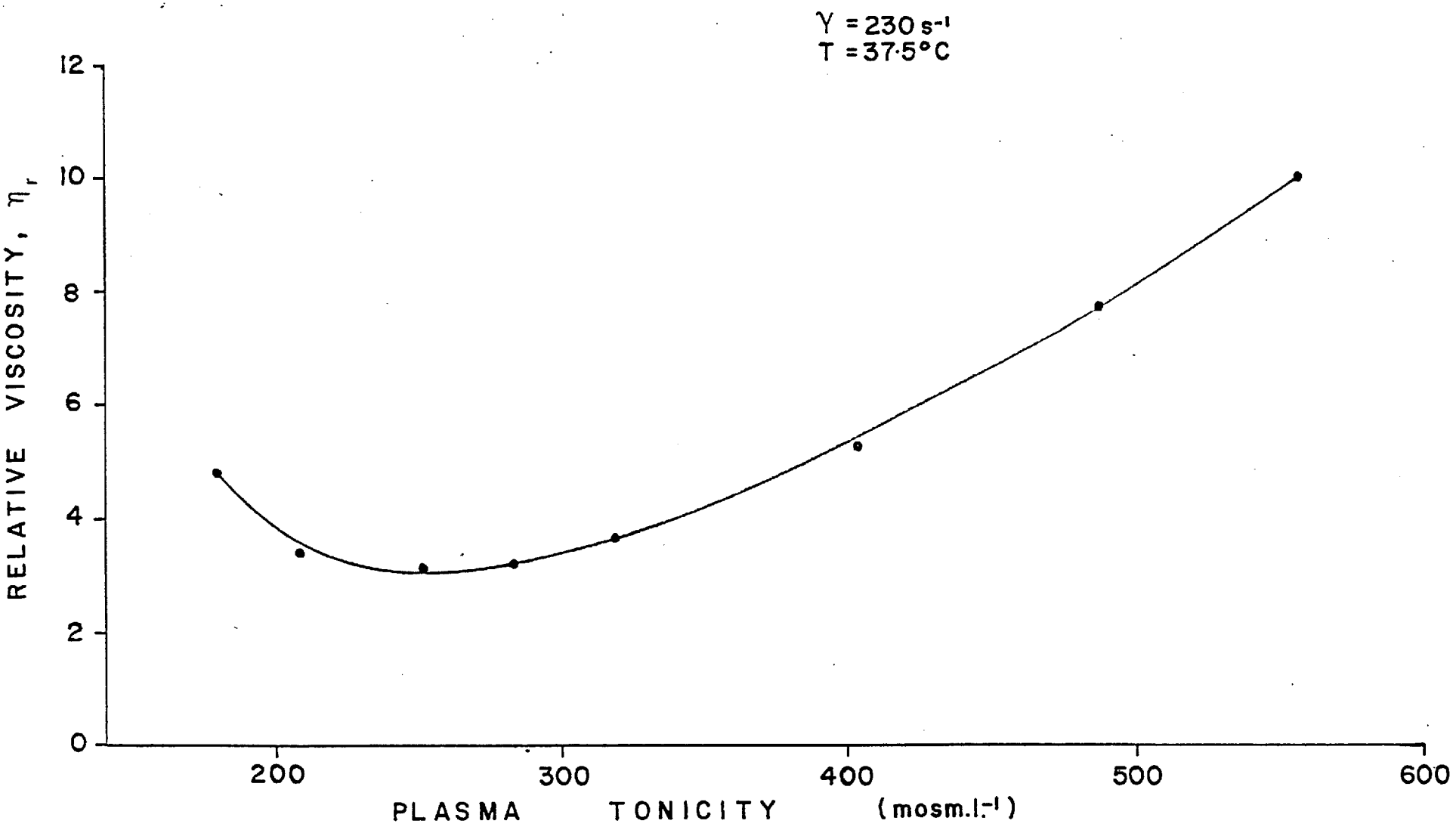


FIG. 43 : RELATIVE VISCOSITY AT DIFFERENT PLASMA TONICITIES (DONOR A)

SUSPENDING MEDIUM TONICITY MOSM.L^{-1}	179	208	251	283	318	336	403	487	556	641
RED CELL PACKING RATE (% min^{-1})	2.45	3.46	5.55	7.0 7.2	6.53 6.78	6.53 6.6	5.23 5.62	3.23 3.28	1.8 2.1	1.78 1.9

SUSPENDING MEDIUM TONICITY MOSM.L^{-1}	179	208	251	283	318	403	487	556
INTRINSIC VISCOSITY, KT'$_c$	2.73	2.33	2.44	2.72	3.02	3.89	4.81	5.42

SHEAR RATE FOR VISCOSITY MEASUREMENTS = 230s^{-1}
PLASMA FIBRINOGEN CONCENTRATION, Φ = 2.67 mg.ml.$^{-1}$
TEMPERATURE OF MEASUREMENT = 37.5°C

ERROR IN MEASUREMENT OF TONICITY = ± 1 mosm. l^{-1}
ERROR IN MEASUREMENT OF FLEX. INDEX = ± 0.5% min^{-1}
ERROR IN MEASUREMENT OF KT'$_c$ ± 0.2

TABLE 37 FOR FIGURE 44

THE EFFECT OF TONICITY ON THE RED CELL PACKING RATE AND INTRINSIC VISCOSITY, KT'$_c$.
RED CELL FLEXIBILITY INDEX CORRECTED FOR ($\rho_c - \rho_p$) AT HYPERTONICITY

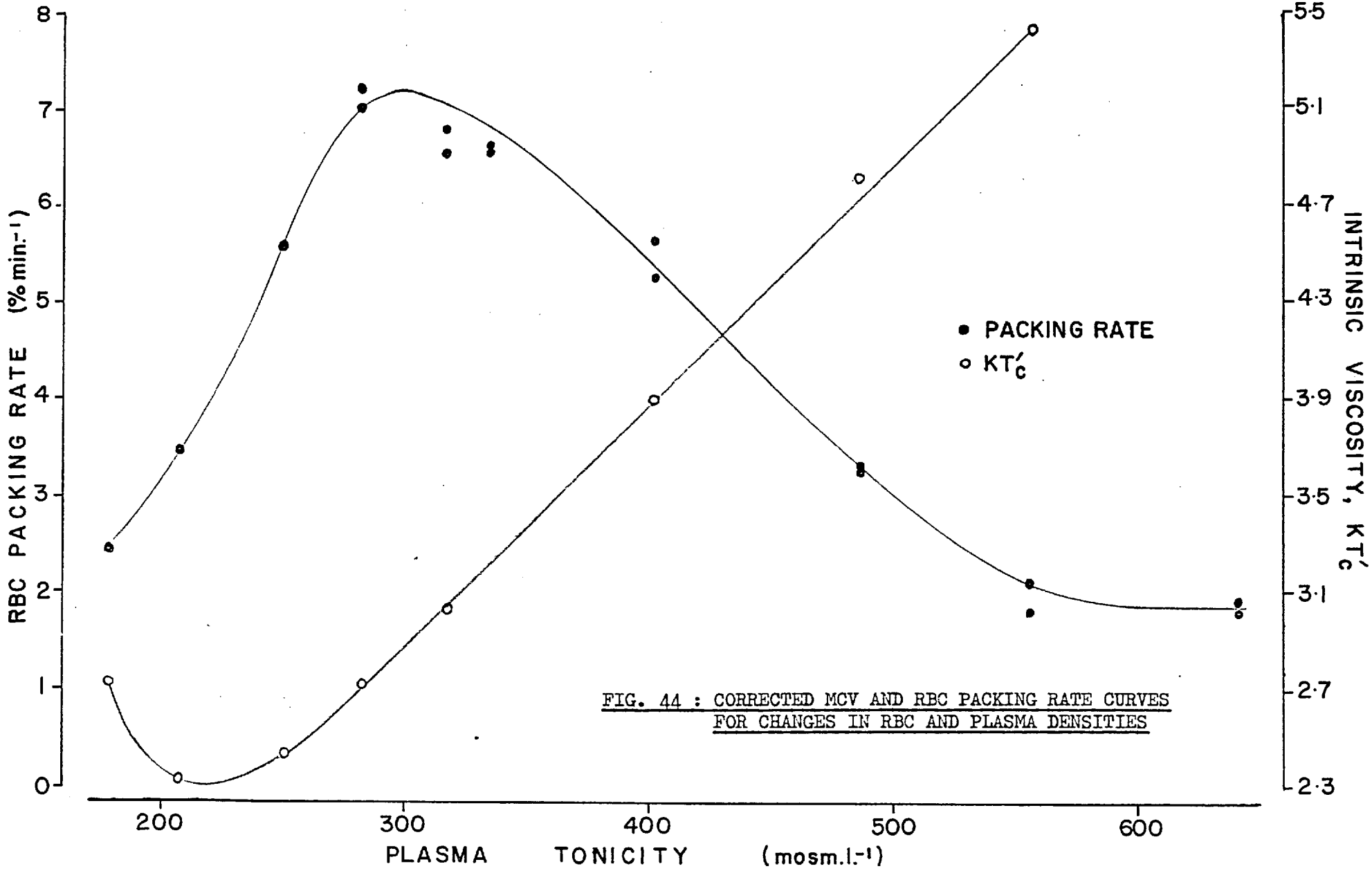


FIG. 44 : CORRECTED MCV AND RBC PACKING RATE CURVES FOR CHANGES IN RBC AND PLASMA DENSITIES

decrease being greater for hypotonicity than for hypertonicity. This behaviour in red cell flexibility with tonicity corresponds to the behaviour of η_r in Fig. 43. The highest red cell flexibility, at isotonicity, gives the lowest value of η_r. The rapid decrease in red cell flexibility at hypotonicity gives the rapid increase in η_r for the same region of tonicities, and the slower decrease of red cell flexibility at hypertonicity also gives the slower increase in η_r, for the same region of tonicities. The value of KT'_c, however, is lowest around 210 $mosm.l^{-1}$ and increases for lower and higher tonicities.

When the red cell MCV is altered by variations in tonicity, the cell membrane surface area to cell volume ratio is altered. That is the cell shape is altered. To find what changes occur in the cell shape, the value of the shape factor K at different tonicities was obtained. When the cells are made inflexible, the red cell flexibility factor T'_c becomes equal to 1, and the value of the intrinsic viscosity, KT'_c, becomes equal to K. By suspending the red cells in an albumin/Ringer-Locke solution at different tonicities, the cell shape is altered. To fix the cell shape and make the cells inflexible, the cells are fixed in a 1% formaldehyde/albumin/Ringer-Locke solution of the same tonicity. The values of the red cell shape factor, K, at different tonicities are shown in Fig. 45. As tonicity is decreased, from isotonicity, K decreases, whereas as the tonicity is increased, K increases up to a tonicity of 450 $mosm.l^{-1}$, where K reaches an optimum value before starting to decrease slightly as tonicity is increased further.

SUSPENDING MEDIUM TONICITY MOSM. L^{-1}	210	252	373	438	512
SHAPE FACTOR, K	3.3	3.495	4.05	4.19	4.12

TEMPERATURE OF MEASUREMENTS: 37.5°C

SHEAR RATE FOR VISCOSITY MEASUREMENTS: $230s^{-1}$

ERROR IN MEASUREMENT OF K ± 0.15

TABLE 38 FOR FIGURE 45

EFFECT OF TONICITY ON RED CELL SHAPE FACTOR K.
RED CELLS FIXED IN 1% FORMALDEHYDE RINGER-LOCKE SOLUTION OF VARYING TONICITIES

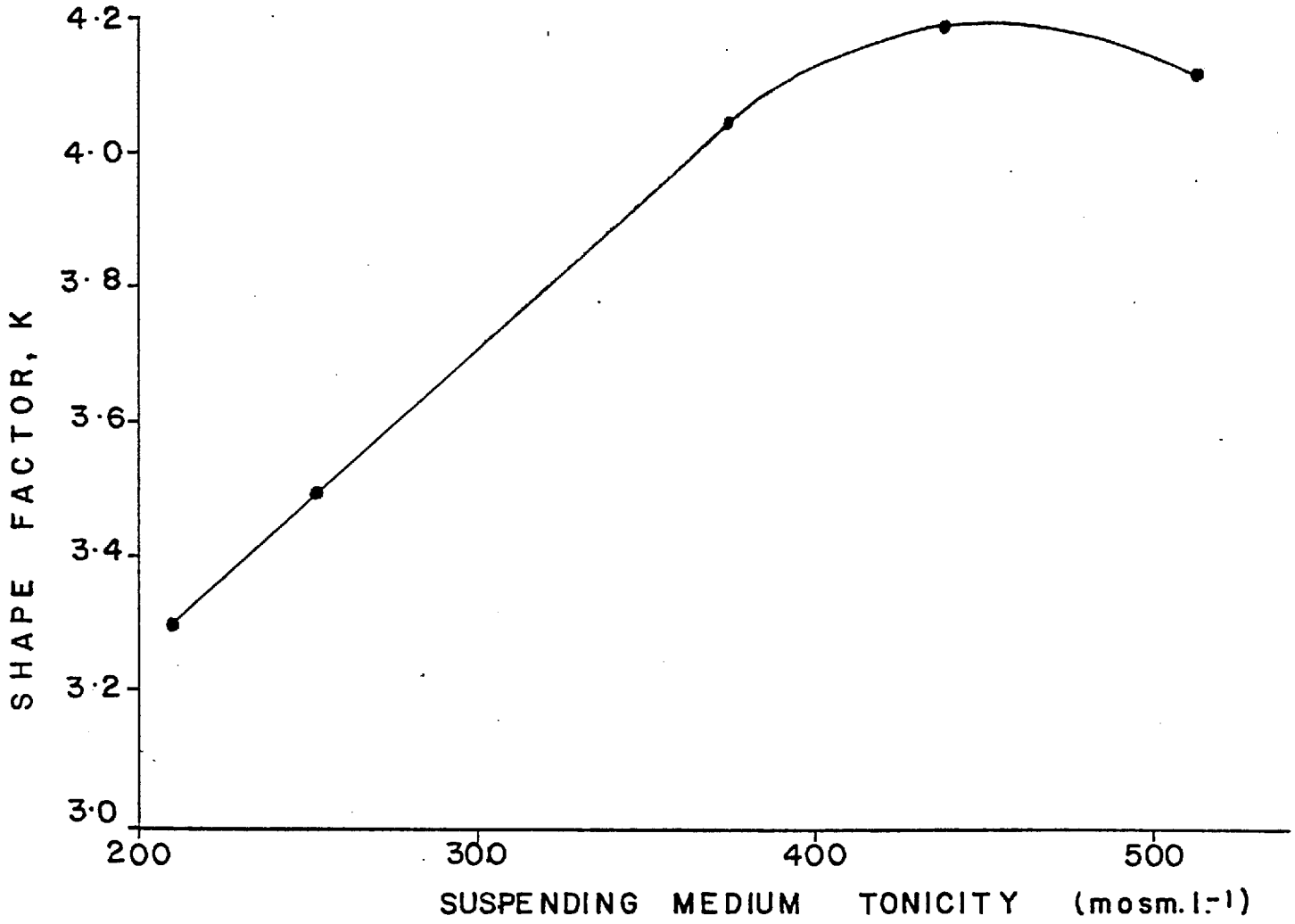


FIG. 45 : SHAPE FACTOR,K, FOR FORMALDEHYDE FIXED RBC IN RINGER-LOCKE SOLUTION AT SHEAR RATE OF $230s^{-1}$ AND 37.5°C

(ii) pH Effect

By bubbling CO_2 gas through blood, the pH is reduced, whereas by bubbling O_2, the pH is increased. The effects of varying the blood pH on the rheological parameters are shown in Fig. 46. Acidity causes the red cell to swell in volume, increasing the MCV, and subsequently the haematocrit for the same number of red cells. The red cell packing rate is also decreased and the relative viscosity, η_r, as a result is increased. However, when the blood is made alkaline, the MCV decreases up to pH = 8 before it starts to increase again, as observed by Hampson and Maizels (1926). The red cell packing rate increases with alkalinity up to the same region of pH = 8 before it starts to decrease, as the MCV increases.

When the pH is reduced below the physiological range of 7.35 to 7.45, η_r remains unaltered until the pH is reduced below 6.8, where η_r begins to increase. Similarly, when the pH is increased above 7.45, η_r remains unaltered until pH reached 8, above which η_r increases. The contradiction between the unaltered value of η_r up to pH = 8 and the increasing red cell packing rate over the same range of pH, lies in the fact that as in the case of hypertonicity, it is necessary to correct for $(\rho_c - \rho_p)h'$, since the MCV is decreasing, by correcting for the same volume of cells.

pH OF SUSPENDING MEDIUM ERROR ± 0.01	6.5	6.53	6.67	7.1	7.39	7.6	7.7	7.8	7.95	8.2
RED CELL MCV (fl.)	126.3	117	95.5	86.5	84.2	81.7	79.5	79.3	74.8	78.6
HAEMATOCRIT %	58.5	56	50	47.5	47.25	45.75	45	45	44.25	44.75
RED CELL PACKING RATE (% min^{-1}) (AVERAGE OF TWO VALUES)	6.1	6.12	6.15	6.63	7.7	8.85	9.85	10.2	10.1	9.5
RELATIVE VISCOSITY η_r	3.8	3.73	3.42	3.27	3.27	3.27	3.23	3.3	3.23	3.53

TEMPERATURE OF MEASUREMENTS = 37.5°C

PLASMA FIBRINOGEN CONCENTRATION = 2.86 $mg.ml.^{-1}$

PLASMA VISCOSITY = 1.4 c P

SHEAR RATE FOR VISCOSITY MEASUREMENTS = $230s^{-1}$

TABLE 39 FOR FIGURE 46

EFFECT OF VARYING pH ON HEMORHEOLOGICAL PARAMETERS

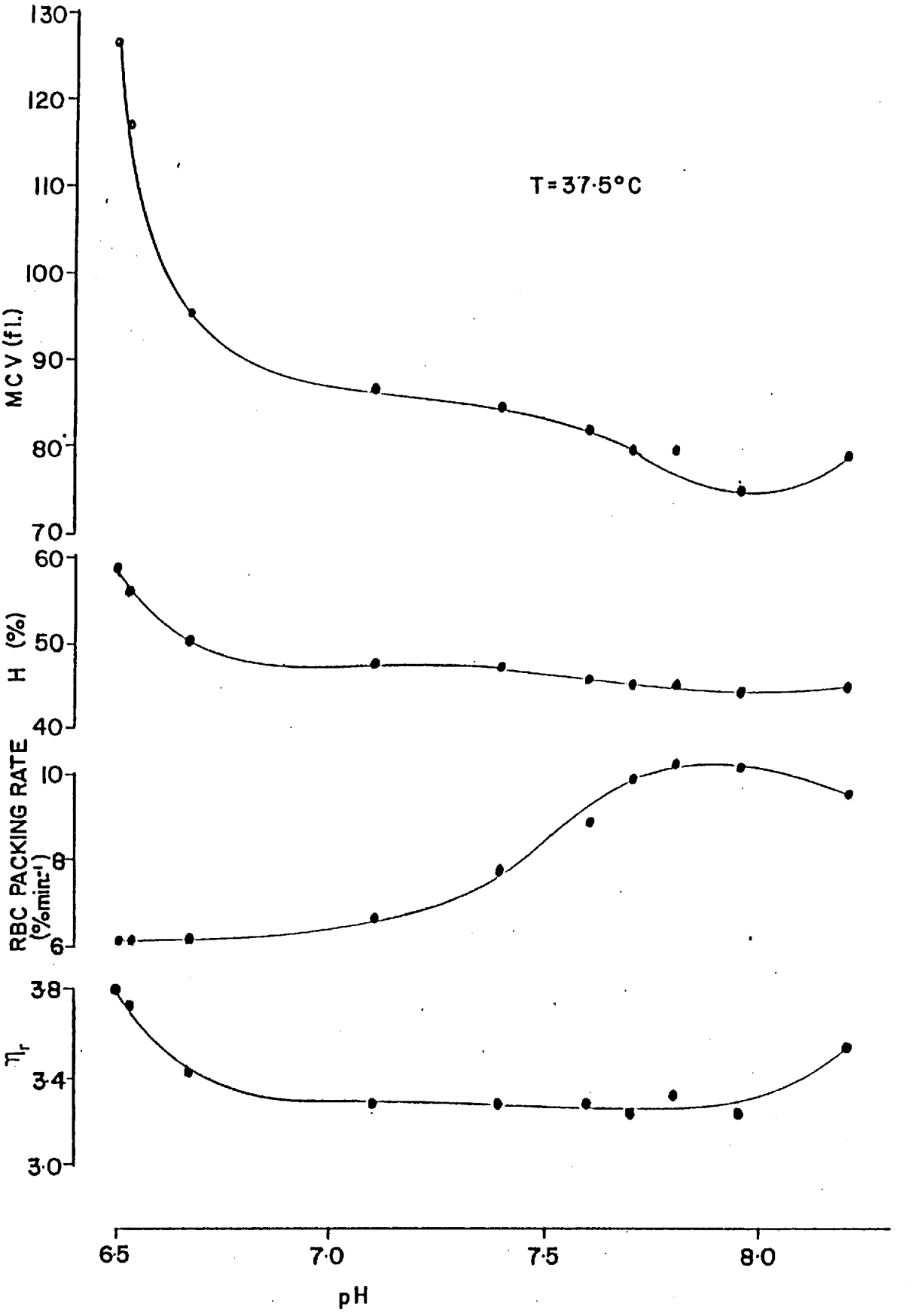


FIG. 46 : VARIATION OF RHEOLOGICAL PARAMETERS WITH pH AT 37.5°C

The effect of pH on the calibration curve of red cell packing rate at different haematocrits, Fig. 47, does not only alter the general shape of the curve, but also shows that above 40% haematocrit, the red cell packing rate is increased by acidity as well as alkalinity for a given haematocrit.

Giombi and Burnard (1970) observed that as the plasma pH is altered, so does its tonicity, as a result of shift of fluid into and out of the red cells during MCV changes. They gave a figure of 1/3 of the haematocrit change in blood samples where the pH was lowered, by isotonic acid, was due to accompanying changes in plasma tonicity. By measuring the tonicity of plasma samples where the pH was changed, by plasma dilution using isotonic acid or base, it was found that there was indeed a change in plasma tonicity, as shown in Fig. 48. To correct the red cell MCV at different pH, and eliminate the effect of tonicity changes on its values, the percentage change in MCV for a given change in tonicity was calculated from the MCV' against tonicity curve in Fig. 46. The MCV values at varying pH was then corrected to give a new curve, Fig. 48.

From Fig. 46, for a change in MCV of 10 fl (from 95 to 105 fl), there is a 6% change in haematocrit. Therefore a 6.2 fl. decrease in MCV at pH = 6.6 (Fig. 48), as a correction due to tonicity change, the haematocrit should change by 3.72%. The actual change in haematocrit for a change in pH from 7.4 to 6.6 is 5%. Therefore 3.72%, or 0.744 of actual change, should be due to tonicity change. This is clearly

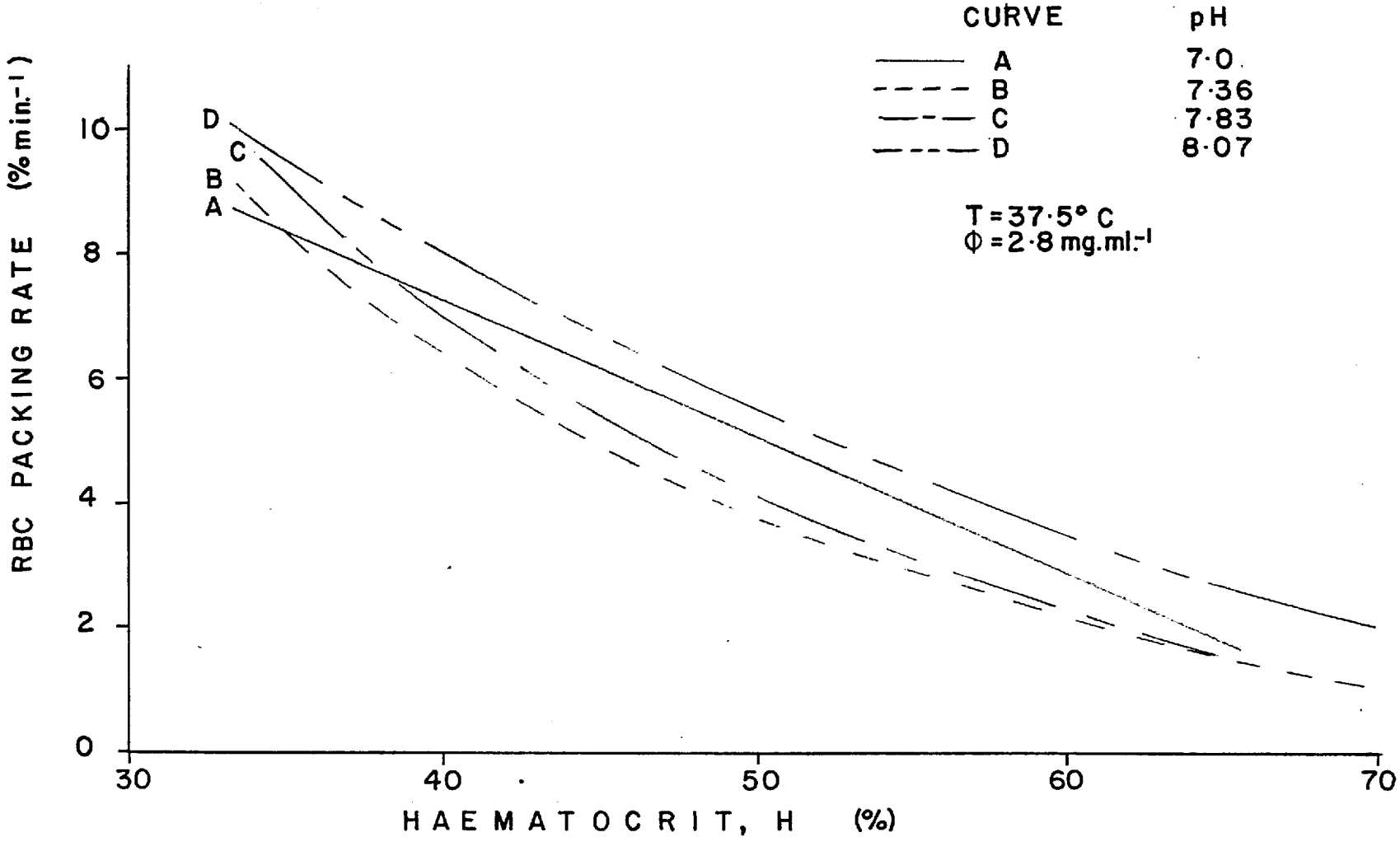


FIG. 47 : RBC PACKING RATE vs. HAEMATOCRIT CURVES AT DIFFERENT pH VALUES (DONOR A)

PLASMA pH	MCV fl.	PLASMA TONICITY MOSM.L^{-1}	MCV vs pH CURVE CORRECTED FOR TONICITY CHANGE (fl.)	PLASMA pH	SALT CONCENTRATION TO TONICITY gm.1^{-1}	% CHANGE IN SALT CONCENTRATION PER 0.1 pH UNITS FROM pH = 7.4
6.6	118.6	259	112.3	6.6	8.158 ± 0.03	0.014 ± 0.002
6.8	100	266	101.7	6.8	8.362	0.01
7.1	96.75	276	90.5	7.0	8.573	0.01
7.4	86	285	86	7.2	8.787	0.01
7.58	80	291	80.5	7.4	8.987	-
7.9	75	302	76.75	7.6	9.189	0.01
8.12	82.5	300	83.5	7.8	9.416	0.011

COLUMN 4 VALUES ARE VALUES FROM CORRECTED CURVE (BROKEN CURVE ON FIGURE 12)

TABLE 40 FOR FIGURE 48

EFFECT OF VARYING pH ON TONICITY OF RED CELLS SUSPENSION

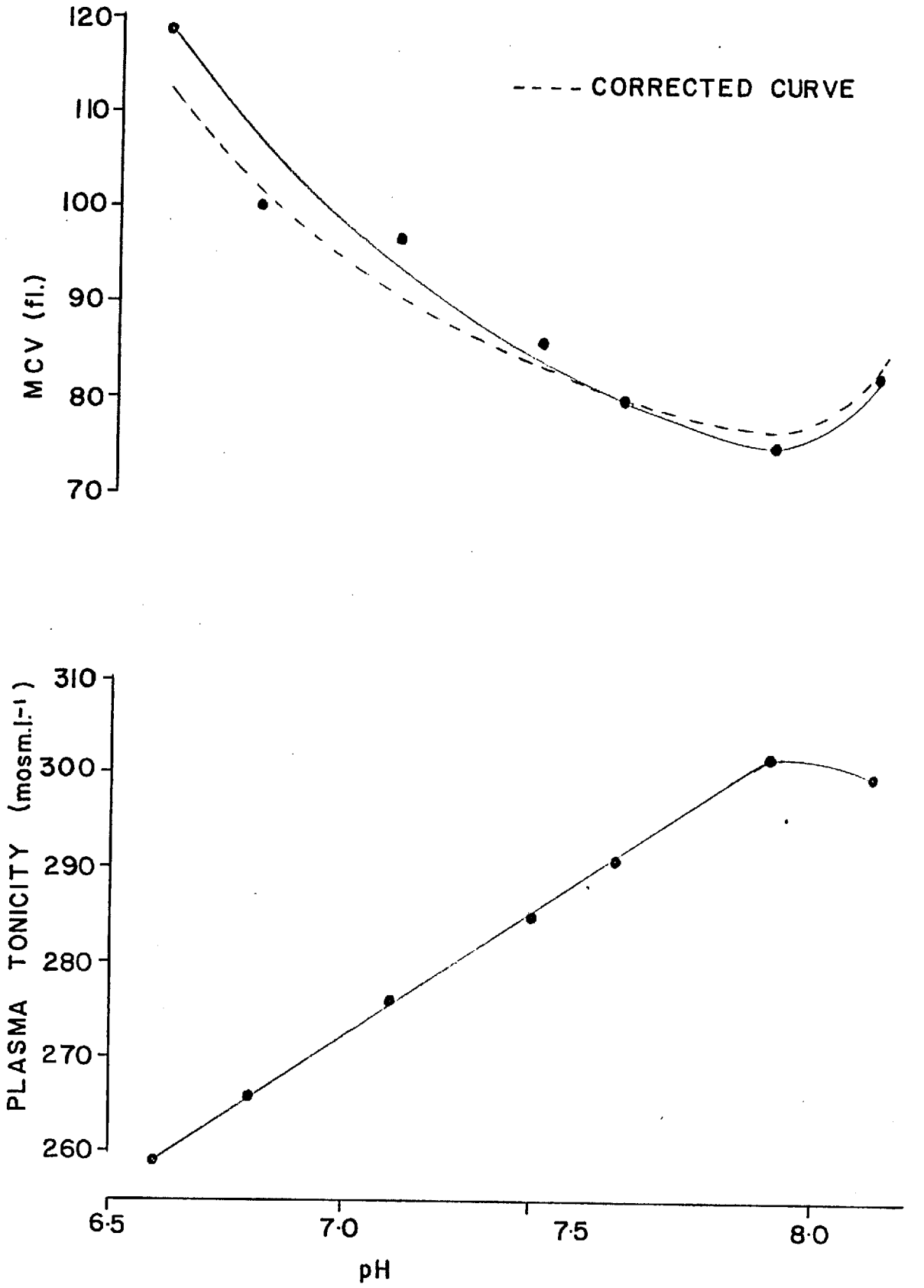


FIG. 48 : CORRECTION OF MCV vs pH CURVE FOR PLASMA TONICITY CHANGES DUE TO pH

much larger than the figure of 1/3 of the haematocrit given by Giombi and Burnard (1970). The difference might lie in the fact that the foetal red cell, used in their experiments, is of larger MCV than the normal adult red cell, and perhaps there is a difference in response to pH or tonicity change, or both.

Parpart et al (1947) observed that a change of 0.1 units of pH alters the plasma tonicity by an equivalent of 0.01% salt concentration. From Table 40, of Fig. 48, it is seen that my results agree with these values, within experimental errors.

When the effect of alkalinity is corrected for in $(\rho_c - \rho_p)h'$, as for hypertonicity, by comparing samples of equal volumes (haematocrit) of red cells. The variation of η_r with pH, corrected for alkalinity, is shown in Fig. 49. The value of η_r over the pH range 6.95 to 7.65, is unchanged. Below pH = 6.95, η_r increases rapidly, whereas above pH = 7.65, η_r decreases rapidly. The variations of red cell packing rate (flexibility) with pH, corrected for $(\rho_c - \rho_p)h'$ are shown in Fig. 50. The red cell flexibility between pH = 7.55 and 7.25 is unaltered, whereas for pH values below 7.25 the flexibility decreases more rapidly as the pH is lowered to 7, than for lower values of pH. Above pH = 7.55, the red cell flexibility increases with pH. The increase in flexibility above pH = 7.55 corresponds with the decrease in η_r for the same pH range. Below pH = 7, the red cell flexibility does not change appreciably whereas η_r increases rapidly. This change in red cell

PLASMA pH	6.53	6.6	6.95	7.39	7.55	7.825	8.07
RELATIVE VISCOSITY η_r	4.3	4.02	3.77	3.77	3.73	3.6	3.3

TEMPERATURE OF MEASUREMENTS: 37.5°C

PLASMA FIBRINOGEN CONCENTRATION: 2.23 mg. $ml.^{-1}$

PLASMA VISCOSITY: 1.2 c P

SHEAR RATE FOR VISCOSITY MEASUREMENTS: $230s^{-1}$

TABLE 41 FOR FIGURE 49

RELATIVE VISCOSITY, η_r, AT DIFFERENT pH VALUES CORRECTED FOR ($\rho_c - \rho_p$) AT ALKALINITY

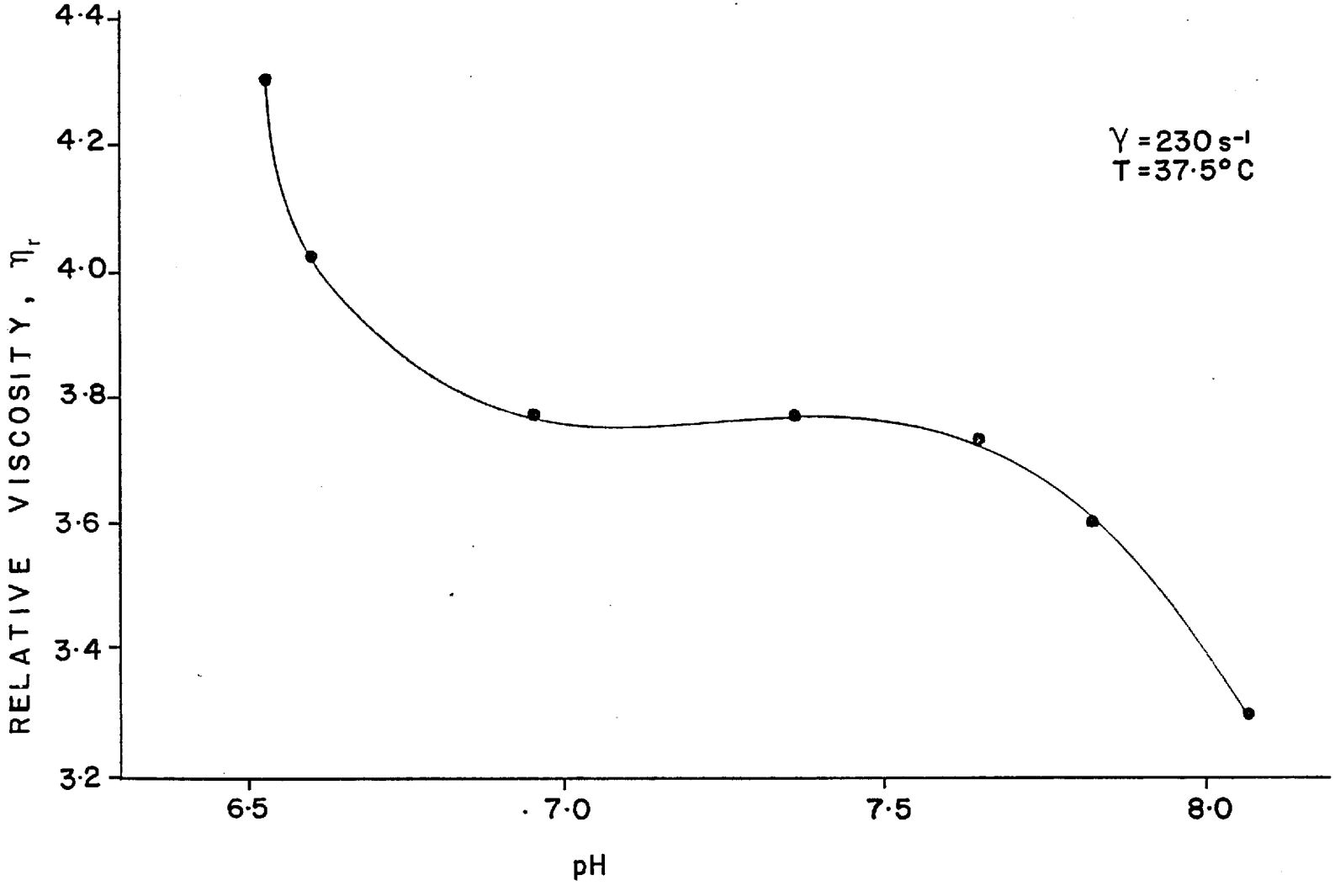


FIG. 49 : RELATIVE VISCOSITY AT DIFFERENT pH VALUES CORRECTED FOR CHANGES IN RBC AND PLASMA DENSITIES

flexibility can also be seen in the change in the value of the intrinsic viscosity KT'_c, also shown in Fig. 50. The values of KT'_c were obtained from the values of η_r corrected for $(\rho_c - \rho_p)h'$, in Fig. 49. For the pH range of 7.25 to 7.55, KT'_c is at its optimum value and is not changed appreciably. For pH values below 7.25 and above 7.55, the value of KT'_c decreases. The decrease being greater for pH values above 7.55, corresponding to the greater increase in red cell flexibility over the same pH range.

Compared with the effect of hypertonicity, alkalinity decreases the MCV in a similar way up to pH = 8. However, hypertonicity decreases the red cell flexibility whereas alkalinity increases it. This suggests that pH increase affects either the intracellular content, decreasing the internal viscosity, the cell shape, or the red cell membrane, making it more flexible. To investigate the effect of pH on the haemoglobin solution, normal haemoglobin solutions were prepared by freezing washed red blood cells, in isotonic Ringer-Locke solution, to fracture the membranes. The haemoglobin-red cell membranes solution was diluted four times its volume with isotonic Ringer-Locke solution to enable the removal of cell ghosts by centifugation. The haemoglobin solution was then dialized in a solution of isotonic Ringer-Locke or saline and Polyethylene Glycol 6000 (BDH for biochemistry) to concentrate the haemoglobin. Another haemoglobin solution was obtained by adding 1 $mg.ml^{-1}$ of Saponin to the washed red cells, mixing the solution for 2 hours then

PLASMA pH	6.53	6.6	6.95	7.39	7.55	7.825	8.07
RED CELL PACKING RATE ($\% \ min^{-1}$)	6.1	6.1	6.375	7.7	7.8	8.9	10.6

PLASMA pH	6.53	6.6	6.95	7.39	7.55	7.825	8.07
INTRINSIC VISCOSITY KT'_c	2.64	2.69	2.8	2.85	2.83	2.75	2.56

TABLE 42 FOR FIGURE 50

RED CELL PACKING RATE, CORRECTED FOR $(\rho_c - \rho_p)h'$ AT ALKALINITY, AND INTRINSIC VISCOSITY, KT'_c, FOR VARYING PLASMA pH

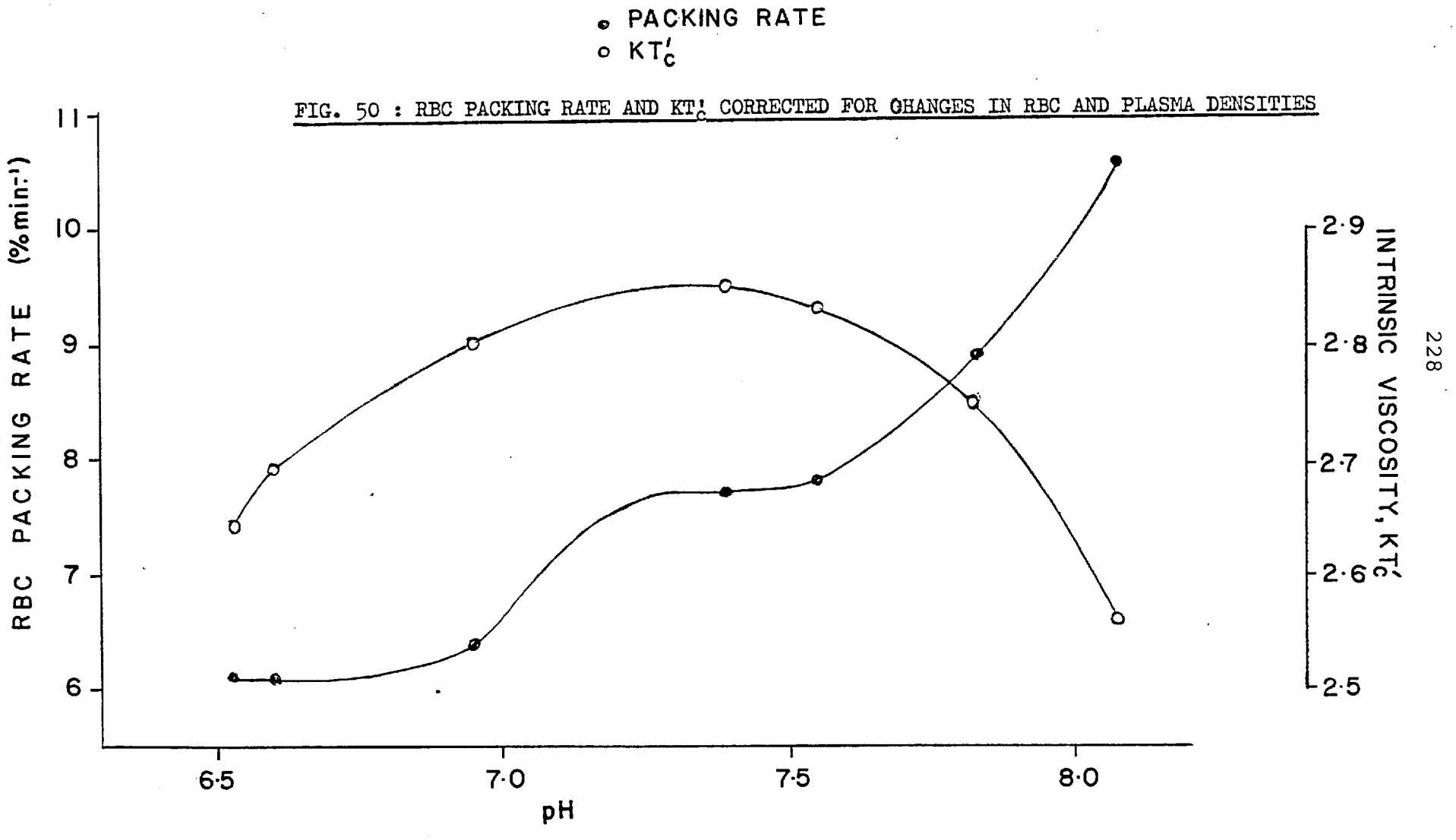


FIG. 50 : RBC PACKING RATE AND KT'_c CORRECTED FOR CHANGES IN RBC AND PLASMA DENSITIES

centrifuging the ghosts and stroma out of solution. For haemoglobin solutions varying in concentrations between 13.4 and 23 $g.dl^{-1}$, it was not possible to change the pH of the solution by isotonic acid or base. This is only possible with relatively dilute haemoglobin solutions, more dilute than found in swollen cells. This demonstrates that the haemoglobin has a great buffering power and is not easily affected by pH. Additionally, its viscosity does not alter even if the pH is altered, as observed by Murphy (1967).

The red cell shape factor, K, obtained by measuring the intrinsic viscosity of inflexible red cells suspension, fixed in 1% formaldehyde/Ringer-Locke/albumin solution at different pH is shown in Fig. 51. The value of K decreases with acidity and increases with alkalinity.

Over the range of tonicities and pH values no echinocytes were seen by microscopic observations. However, at 600 $mosm.l^{-1}$, irregularities (bumps) on the cell membranes surfaces were observed as a stage before echinocyte formation.

IV DISCUSSION

When the tonicity of blood samples is altered, we have seen that not only does the haematocrit change, due to cells

SUSPENDING MEDIUM pH	6.5	6.9	7.4	7.75	8.1
SHAPE FACTOR K	2.5	2.91	4.07	4.45	4.56
RED CELL MCV (fl.)	126.5	98.5	86.5	78.5	85.75

TEMPERATURE FOR MEASUREMENTS: 37.5°C

SHEAR RATE FOR VISCOSITY MEASUREMENTS: $230s^{-1}$

TABLE 43 FOR FIGURE 51

RED CELL SHAPE FACTOR, K, AND MCV AT DIFFERENT pH VALUES.
RED CELL FIXED IN ISOTONIC 1% FORMALDEHYDE/RINGER-LOCKE/ALBUMIN SOLUTION

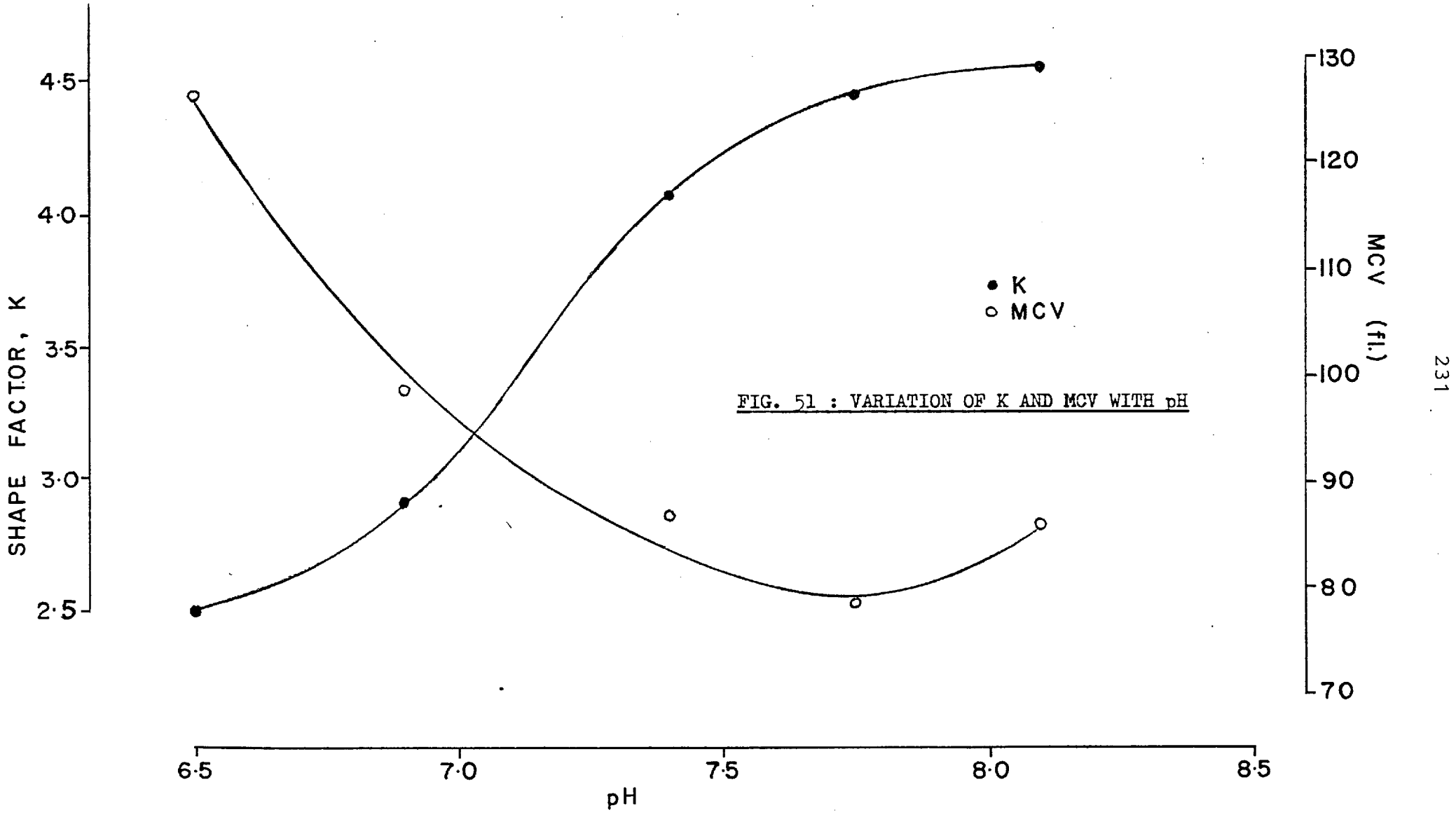


FIG. 51 : VARIATION OF K AND MCV WITH pH

swelling and shrinking (change in MCV), but also the relative viscosity, η_r, and the red cell packing rate. Additionally, the swelling and shrinking of the red cells alters both the cell density, ρ_c, and the plasma density, ρ_p. Hence to ensure equivalent conditions, I have chosen to compare the samples under conditions of equal forces experienced by the red cells during centrifugation, in the measurement of packing rate. This was done by correcting for the product $(\rho_c - \rho_p)h'$ in the different samples. It was found that correcting for haematocrit was necessary for hypertonicity but not for hypotonicity.

Comparing blood samples, after correcting for $(\rho_c - \rho_p)h'$ at hypertonicity, has shown that optimum red cell flexibility (packing rate) and least relative viscosity, η_r, occured at isotonicity. For both, hypo and hypertonicity, the red cell flexibility decreases and η_r increases, Figs. 43 and 44. This seems to be a reasonable result since by changing tonicity the red cell membrane surface area to cell volume ratio, the cell internal viscosity and membrane tension, or rigidity, are altered. The decrease in red cell flexibility (packing rate) for hypo and hypertonicity agrees with the observation of Murphy (1966) who found that red cell filterability decreases for hypo and hypertonicity. However, the same author as well as Giombi and Burnard (1970) obtained a lower value of η_B at hypotonicity compared to isotonicity because they corrected for haematocrit. This contradicted their result of decreased red cell deformability at hypotonicity.

Tonicity changes were found to alter the intrinsic viscosity, KT'_c, due to the change in cell shape and flexibility, Fig. 44. As the tonicity is decreased, from isotonicity, the red cell swells and becomes more spherical in shape. This decreases the value of K, the shape factor, towards its miminum value of 2.5 for spheres. However, while K is decreasing with hypotonicity, lowering the value of KT'_c, the red cell flexibility is also decreasing, increasing the value of T'_c, towards its maximum value of 1 at cell zero flexibility. However, initially, as the tonicity is decreased, the decrease in K offsets the increase in T'_c until the tonicity is lowered to approximately 220 $mosm.l^{-1}$. At this region of tonicity, the value of KT'_c reaches a minimum before starting to increase, as the tonicity is lowered further. This upturn in value is probably due to a point being reached where the rapid decrease in red cell flexibility, and hence rapid increase in the value of T'_c, prevents K offsetting T'_c in KT'_c. This results in KT'_c increasing in value as T'_c is still increasing with decrease in cell flexibility.

The tonicity changes in the blood samples were obtained by diluting plasma by 43% of its volume with a Ringer-Locke/fibrinogen solution. This dilution reduced the red cell flexibility index by 25% from normal blood. If no such reduction in flexibility index occured, the value of the red cell packing rate in Fig. 44 would be higher. This would mean that the value of T'_c would be lower for the hypotonic values and hence the change in T'_c

as the tonicity is lowered would not be sufficient to prevent K offsetting the change of T'_c in KT'_c. That is, the value of KT'_c would decrease continually as the tonicity is lowered. This would then agree closer with the change in the shape factor K with tonicity for inflexible (formaldehyde fixed) red cells, Fig. 45. Hypotonicity decreases K whereas hypertonicity increases it.

For hypertonicity, the red cell flexibility (packing rate) decreases and, therefore, T'_c increases towards its maximum value of 1. In the same time hypertonicity shrinks the red cells causing its shape to become irregular, increasing its shape factor, K, as seen in Fig. 45. The increase in K and T'_c with hypertonicity increases the value of KT'_c, as observed. Above a tonicity of 450 $mosm.l^{-1}$ the shape factor, K, reaches an optimum value and either remains unchanged or is decreased slightly as the tonicity is increased further. Although this could cause the value of KT'_c to 'level off', the continual decrease of red cell flexibility, and hence increase in T'_c, over the same tonicity range, results in KT'_c increasing further.

For the effect of tonicity on blood rheology, it can be seen that the effects on the red cell flexibility and relative viscosity, η_r, corrected at hypertonicity, as well as the effects on the intrinsic viscosity KT'_c and shape factor K, are now all consistent with each other as was argued in my explanation above.

Turning to the effects of altering pH in blood samples, to maintain a constant centrifugal force, during measurement of red cell packing rate, it was found necessary to correct for $(\rho_c - \rho_p)h'$ in the case of alkalinity but not for acidity. The result of the corrections on the red cell flexibility (packing rate) and relative viscosity η_r are shown in Figs. 49 and 50. Acidity in blood causes the red cell to swell (increasing MCV) in a similar way to hypotonicity. The swelling of the red cell increases the tension and rigidity of the cell membrane, decreasing the red cell flexibility and increasing η_r. As the pH is lowered from the physiological range of 7.35 to 7.45, the value of η_r remains unchanged until the pH is lowered below 6.95, where η_r increases rapidly. The red cell flexibility, however, begins to decrease for pH values below 7.25 and continues to decrease until pH = 6.7, where it 'levels off' down to pH = 6.5. The shape factor, K, obtained by measuring the intrinsic viscosity of inflexible (formaldehyde fixed) red cells, Fig. 51, decreases with acidity as the cell swells and becomes spherical at pH = 6.5, where K = 2.5. As the pH is lowered from 7.4, the cell flexibility begins to decrease, increasing the value of T'_c while K decreases. The changes in K and T'_c result in a decrease in the initial value of KT'_c as the pH is lowered below 7.4. Between pH = 7.4 and 7.25, the red cell flexibility, and hence T'_c, is unaltered. Therefore, the fall in KT'_c is due to the fall in the value of K. Below pH = 7.25, T'_c begins to increase in value as the red cell flexibility decreases. However, the decrease in K is dominant and offsets the changes in T'_c, decreasing the

value of KT'_c. When the value of T'_c again reaches a region where it changes very little, between pH = 6.6 and 6.5, the decrease in KT'_c decreases at a faster rate over the same pH range, due to the continual decrease in K.

The effect of alkalinity on blood is to increase the red cell flexibility (packing rate), Fig. 50, and decrease η_r, Fig. 49. The red cell shape factor, K, is also increased by alkalinity, Fig. 51. The increase in MCV as the pH is increased to 8, causes the value of K to reach a plateau, before possibly decreasing at pH values greater than 8.1. The rapid increase in red cell flexibility due to alkalinity will decrease the value of T'_c. This rapid decrease in T'_c will offset the increase in K, to result in the value of KT'_c decreasing with alkalinity. The dominance of T'_c over K in determining the value of KT'_c can be seen from the observation that the change in the value of K, as pH approaches 8, 'levels off' into a plateau, whereas the value of KT'_c continues to decrease more rapidly than in the case of acidity. This can only be accounted for by the rapid decrease in the value of T'_c as a result of rapid increase in red cell flexibility.

Therefore, when pH is altered in a blood sample, as the blood becomes more acidic, the changes in the shape factor, K, are more dominant than the changes in the flexibility factor, T'_c, in determining the intrinsic viscosity. At alkalinity, the reverse becomes true, i.e. changes in T'_c

are more important than changes in K for determining the changes in KT'_c. This of course is only true for the pH range between 6.5 and 8.1.

The effects of changes in K and T'_c on η_r are that in the acidic region, the decreasing red cell flexibility as well as the increasingly spherical shape transformations of the red cell, lead to a situation where the viscosity of a suspension of rigid spherical particles is being measured. This viscosity will increase compared to normal red cells. Alkalinity on the other hand causes the red cells to become more flexible and hence, although the red cell shape is becoming more irregular, the increased red cell flexibility will decrease the suspension viscosity (at high shear rates). These effects of K and T'_c on η_r agree with the observed changes in η_r with pH, Fig. 49.

The change in the shape factor, K, due to alkalinity is similar to that due to hypertonicity. Similarly, the change in MCV for both effects are similar. Therefore, the increase in red cell flexibility due to alkalinity, compared to the decrease in red cell flexibility at hypertonicity, is not an effect due to red cell shape change, MCV or red cell internal viscosity, η_i, but is due to the effect of alkalinity on the red cell membrane structure. The process by which alkalinity increases red cell membrane flexibility is not understood and should be the subject of further study.

The increase in η_r with acidity was observed by Murphy (1966) and Dintenfass and Burnard (1966).

Rampling (1981) reported that the fibrinogen binding on the red blood cell membrane increases with acidity but is not altered by alkalinity. Since fibrinogen adsorption on the red cell membrane surface increases its flexibility, Rampling and Sirs (1977), the data given by Rampling (1981) is in contradiction to my results. This raises the question as to whether the red cell membrane flexibility is increased as a result of the effect of fibrinogen adsorption, or whether it is a property of the membrane itself, affected by other factors such as pH and structural changes which may influence the determination of the amount of fibrinogen adsorbed?

CHAPTER 6

COMPARATIVE BLOOD RHEOLOGY OF MAN AND OTHER SPECIES

I INTRODUCTION

Differences between the blood rheology of man and other species have been observed by many workers. Fahraeus (1921 and 1929) observed that when human blood is defibrinated, the ESR is decreased appreciably, whereas for horse blood, the ESR is decreased by defibrination but remains significantly higher in value than human whole blood. He also observed that the degree of aggregation of human red cells decreases by defibrination whereas in horse blood it remains high even by defibrination. The relative aggregation of erythrocytes from man and other species, in whole blood and solutions of dextrans of different molecular weights, was observed by Richter (1966). Richter's data shows that erythrocyte ESR and aggregability are high in horse, moderate in man and low in sheep and cattle.

Besides measurements of erythrocyte ESR and aggregation of species other than man, very few other rheological measurements have been carried out by investigators in the field.

As was shown in previous chapters, the rheological behaviour of blood depends on parameters such as red cell flexibility, plasma fibrinogen concentration, haematocrit, MCV, viscosity, shear rate and red cell shape. All these parameters are interdependent and their interactions give blood its observed characteristics.

For a dilute suspension of rigid particles, Einstein (1906) related the suspension viscosity to the particle concentration and shape in an equation which was modified by Dintenfass (1968) to apply to red cell suspensions. The equation was given in the form

$$\eta_r = (1 - HT)^{2.5}$$

where η_r is the relative blood viscosity (η_B/η_p), H is the haematocrit ($\leqslant$ 1%), the 2.5 is the value of the shape factor for spheres, and T is Taylor's factor given as

$$T = \frac{(p + 0.4)}{(p + 1)}$$

$$p = \frac{\eta_i}{\eta_p}$$

When the values of η_i, the red cell internal viscosity, were calculated using the Dintenfass equation, for a given η_p and H, it was found that the values were unacceptable, Sirs (1981). An alternative equation derived by Taylor (1932) for dilute suspensions, in the form

$$\eta_r = 1 + KTC \qquad (6.1)$$

was used by Sirs (1981), and shown to agree more closely to experimental values than the Dintenfass equation. K is the shape factor, equal to 2.5 for spheres and greater for unsymmetrical shapes, T is the Taylor's

factor and C is the volume fraction of the disperse phase (haemotocrit).

For deformable erythrocytes, in the absence of aggregation (at high shear rates), Sirs (1981) suggested the use of a red cell flexibility factor, T_c, instead of the Taylor's factor T. Although there is no theoretical analysis available inter-relating erythrocyte deformability and η_r in terms of T_c for immiscible fluid drops, as in Taylor's derivation, T_c can be used as a first approximation. Since erythrocyte flexibility is dependent not only on the erythrocyte internal viscosity but also on the erythrocyte membrane rigidity, I suggest that T_c can be approximated to be in the form

$$T'_c = \frac{(p' + 0.4)}{(p' + 1)}$$

$$p' = \frac{\eta_i + \eta_m}{\eta_p}$$

where η_m is a viscous term dependent on membrane mechanical properties such as rigidity and elasticity as well as the effect of interaction of plasma proteins with the membrane. This expression for T'_c is used to replace Taylor's factor, T. For rigid particles T'_c equals 1 and decreased below 1 as the particles (red cells) become more deformable, in the same way as T and T_c.

From the experimental observations of Haynes (1960), Peric (1964) and Gregersen et al (1965), as well as the theoretical calculations of Richardson (1950), Taylor's equation (6.1) can be expressed in the terms of a logarithmic relationship

$$\log \eta_B = \log \eta_p + aH \qquad (6.2)$$

i.e.

$$\log \eta_r = aH \qquad (6.3)$$

H being the haematocrit and a is the slope of the line. Since Taylor's equation was derived for particle concentrations of 1% or less, the value of η_r at 1% concentration, η_{r1} is obtained from

$$\eta_r = (\eta_{r1})^H$$

and is substituted in equation (6.1) to obtain the value of KT'_c. As the measurement of η_{r1} is not very accurate experimentally, the value of η_r at H = 1% is obtained from a plot of equation (6.2) for low haematocrits, as long as the range of haematocrits used produces a linear plot.

The interactions between parameters such as shape factor, K, intrinsic viscosity, KT'_c, red cell flexibility, plasma fibrinogen concentration, Φ, and membrane rigidity were investigated for man, horse,

sheep, cattle, goat and camel. An attempt was also made to explain some of the similarities and differences in the rheological behaviour of blood from the various species.

II METHODS

Human blood was obtained by puncture of the ante-cubital vein whereas blood from other species was obtained by puncture of the jugular vein. In all cases, solid lithium heparin was used as anticoagulant. The experimental work was carried out within six hours from bleeding. The horse, sheep and cattle bloods were obtained from the Wellcome Laboratories (Beckenham, Kent) whereas the goat (Golden Gurnsey) and camel (Camelus Bactriens) bloods were obtained from the London Zoo Animal Hospital (Regents Park, London). In the case of the goat and camel, the bloods were obtained from the same healthy animal, whereas the horse, sheep and cattle bloods were obtained from various animals of each specie. The horse blood provided was from non-racing farm breeds (no record of specific breed was supplied).

To measure the variation of red cell packing rate at different haematocrits for each specie, the specie blood sample (20 ml) was divided into a series of 2 ml samples. To vary the haematocrit, all the 2 ml samples

were centrifuged at 500 g for five minutes and then different volumes of plasma were removed from some samples and added to others. One sample was left unaltered as the normal blood sample. All samples were then mixed on a rotating mixer for 20 minutes. The packing rate measurement on the automatic recording centrifuge was carried out in duplicate for each sample, at a chosen temperature.

To investigate the effect of varying the plasma fibrinogen concentration, ϕ , on the red cell packing rate at a chosen haematocrit (red cell flexibility), serum was used to dilute the plasma. The serum was prepared by heating plasma at 56^{o}C for 10 minutes, and then centrifuging off the precipitated fibrin clot at 1000g for ½ hour to give a clear serum solution. The animal blood sample (20 ml.) was divided into 2 ml. samples. The samples were then centrifuged at 500g for 10 minutes. One sample was left unaltered (normal blood) whereas different volumes of plasma were removed from the other samples and replaced with the same volume of autologus serum. For zero ϕ , as much plasma as possible is removed from the sample and the red cells were then washed twice in serum, centrifuging at 500g for 5 minutes each time and mixing the sample for 15 minutes in between washings. The cells are then resuspended in serum. The red cell packing rate is measured in duplicate for each sample.

To measure the red cell MCV for each specie, 10 μl of a blood sample at a given haematocrit was added to 10 ml of Isotone II buffer solution (Coulter Electronics Ltd). 1 ml. of this solution was then added to 49 ml. of Isotone II buffer solution. This gives a 50,000 dilution factor. This final solution was used to count the number of red cells per litre of blood, at the given haematocrit, on the Coulter Counter, as described in section VIII of Chapter 2. The MCV is calculated by dividing the haematocrit by the cell count per litre.

Viscosity measurements of η_B and η_p were carried out on a Wells-Brookfield cone-plate viscometer, as described in section III of Chapter 2.

To harden red cells to different degrees of inflexibility without altering their shape, two methods of hardening were employed. The first was to heat blood samples for intervals of 5, 10, 15 and 20 minutes, at 48.5^{o}C in a water bath. The second was to add isotonic (pH = 7.4) formaldehyde solution to the blood samples to give 0.25, 0.5, 0.75 and 1% formaldehyde concentration. The heat treated cells were observed microscopically to ensure no shape transformation to spheriods has occured. This was also apparent from the packing rate measurements, because spherical hardened cells have near zero packing rate at the haematocrit used.

III RESULTS

(i) Red Cell Packing Rate vs. Haematocrit and Φ

Horse red cells are very flexible compared to human red cells, at any haematocrit. Fig. (52) shows the variation of red cell packing rate with haematocrit for horse blood at 30°C. The packing rate at H = 45% is about 42.5% min^{-1} compared to 6.75% min^{-1} for man at the same temperature.

The red cell flexibility index (H = 45%) for horse is 21.75% min^{-1} in serum ($\Phi = 0$) and increases rapidly in the presence of small amounts of fibrinogen to 42.5% min^{-1}. For Φ greater than 0.5 $mg.ml.^{-1}$ the red cell flexibility index remains unchanged at 42.5% min^{-1} at 30°C, Fig. (53).

Sheep red cells are relatively inflexible and possess a low packing rate at all haematocrits compared to human or horse red cells, Fig. (54). Variation of plasma fibrinogen concentration, Φ , has no effect on the sheep red cell flexibility index, 0.7% min^{-1} at 30°C, as shown in Fig. (55).

Similar to sheep, cattle red cells are also relatively inflexible, possessing low packing rates at all haematocrits, Fig. (56). Also fibrinogen has no effect on the red cell flexibility index, which is 0.88% min^{-1} at 30°C for all values of Φ , Fig. (57).

VARIATION OF RED CELL PACKING RATE WITH HAEMATOCRIT FOR HORSE BLOOD AT 30°C

HAEMATOCRIT (%)	36.5	41	42	42.5	43	46	48	50	52	54	56	58.5	62	64	66	67	68	69	69.5	70.5	71	72	73.5
RED CELL PACKING RATE (% min^{-1})	69.5 66.5	56.75	51 48.5	59 58	47.5	39 37.5	42.5 40	33.5 30	25.75 20	25 20.5	23.75 17.5	17 15.5	11.5 10.5	11.25 9.5	7.5	7.25	10.5	8.0	12 8.5	8.0	7.5	8.5 7.25	8.0

MAXIMUM ERROR IN PACKING RATE MEASUREMENTS = $\pm$ 5% min^{-1}

ERROR IN HAEMATOCRIT MEASUREMENTS = $\pm$ 0.125%

TABLE 44 FOR FIGURE 52

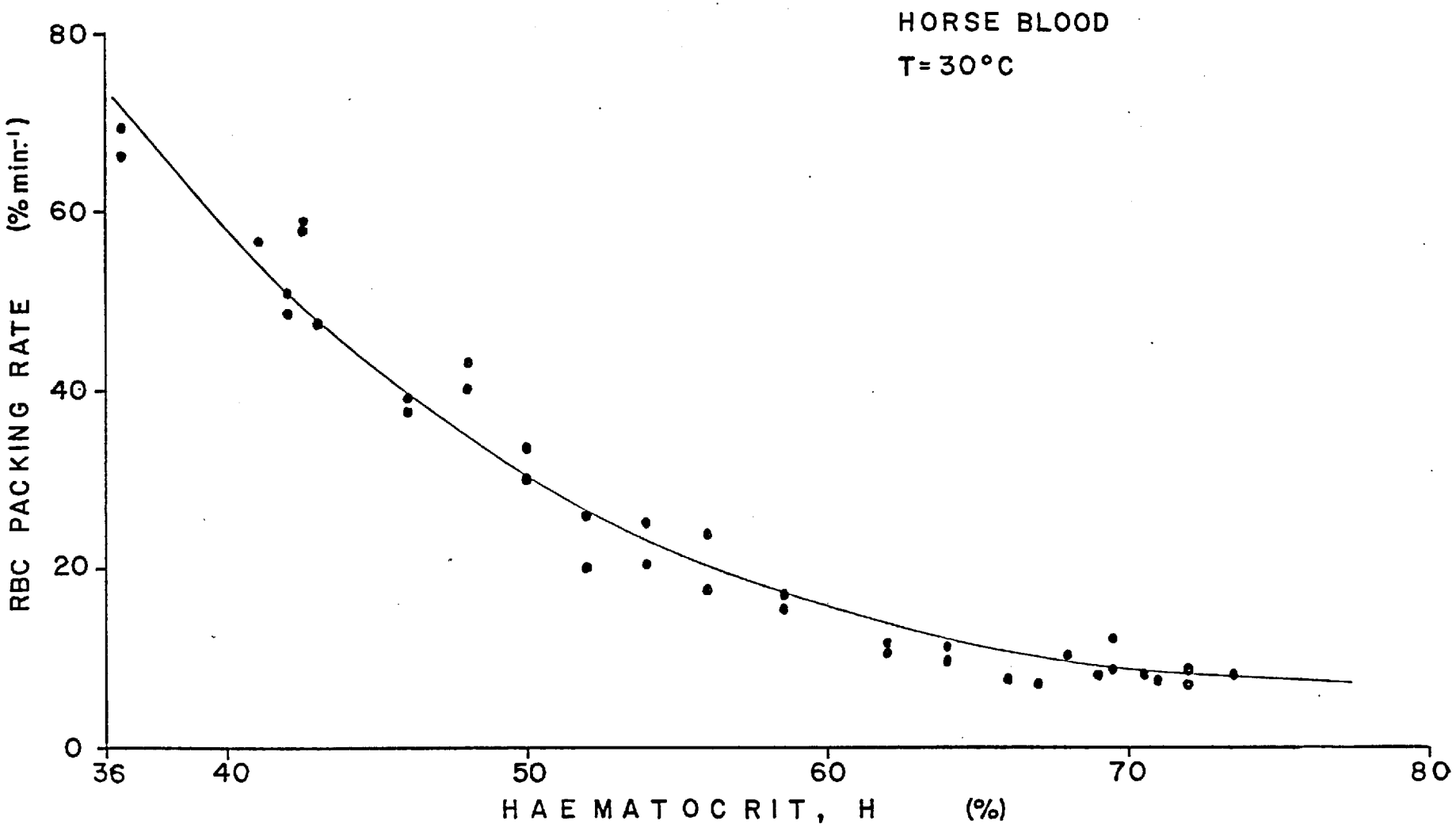


FIG. 52 : VARIATION OF HORSE RBC PACKING RATE WITH HAEMATOCRIT AT 30°C

PLASMA FIBRINOGEN CONCENTRATION, $mg.ml.^{-1}$	0 (SERUM)	0.225	0.53	1.37	1.75	2.15	3.25	4.15	4.75	5.175	6.5	0 (SALINE)
RED CELL FLEXIBILITY INDEX % $min.^{-1}$	22.4 21.1	42.5 41.7	42.7 42.3	43.4 42	42.8 42.6	42.8 41.7	42.8 41.9	41.6 41.1	43.1 42.5	43.5 42	42.76 42.43	4.08 3.93

MAXIMUM ERROR IN PACKING RATE MEASUREMENTS = ± 1.5% min^{-1}

ERROR IN Φ MEASUREMENTS = ± 0.1 $mg.ml.^{-1}$

TABLE 45 FOR FIGURE 53

EFFECT OF VARYING Φ ON THE FLEXIBILITY INDEX OF HORSE RED CELLS AT 30°C

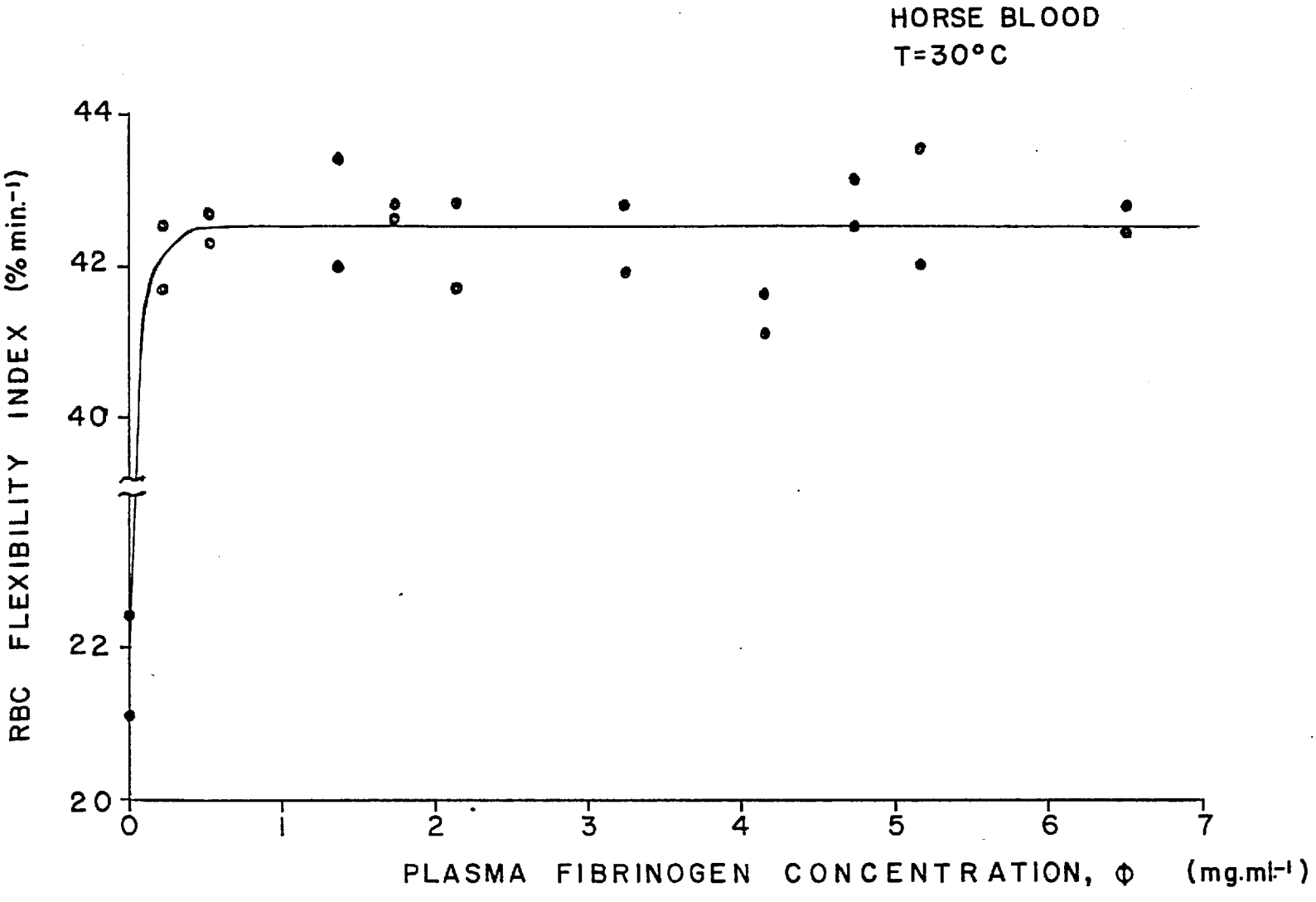


FIG. 53 : VARIATION OF HORSE RBC FLEXIBILITY INDEX WITH PLASMA FIBRINOGEN CONCENTRATION

VARIATION OF SHEEP RED CELL PACKING RATE WITH HAEMATOCRIT AT 30°C

HAEMATOCRIT	30.75	32	33.5	34.5	35	36.75	37	37.5	39	40	42	43	46	46.5	52	52.5	55	62	64
RED CELL PACKING RATE % min^{-1}	1.8	1.7 1.6	1.55	1.09	1.24	1.235 1.13	1.19	0.82	1.07	0.7	0.83	0.8 0.75	0.69	0.69 0.73	0.58	0.36	0.71 0.42	0.43 0.38	0.55 0.53

TABLE 46 FOR FIGURE 54

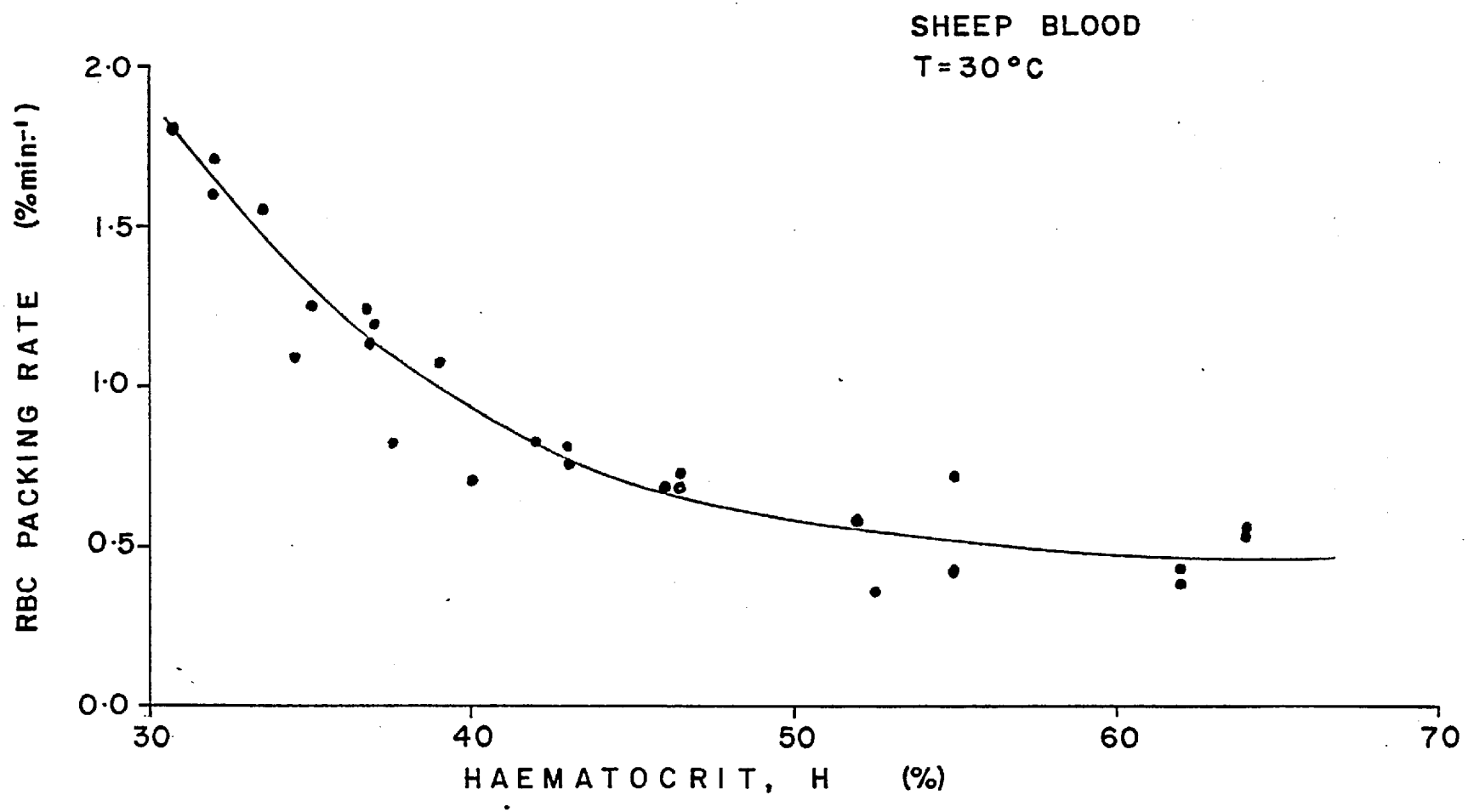


FIG. 54 : VARIATION OF SHEEP RBC PACKING RATE WITH HAEMATOCRIT AT 30°C

PLASMA FIB. CONC., Φ ($mg.ml.^{-1}$)	0 (SERUM)	0.5	0.75	1.35	2.075	3.4	3.625	4.375	5.075	0 (SALINE)
RED CELL FLEXIBILITY INDEX (% $min.^{-1}$)	0.675 0.73	0.675 0.7	0.74 0.765	0.635 0.7	0.67 0.855	0.57 0.76	0.63 0.77	0.75 0.787	0.62 0.8	0.62 0.59

TABLE 47 FOR FIGURE 55

VARIATION OF SHEEP RED CELL FLEXIBILITY INDEX WITH PLASMA FIBRINOGEN CONCENTRATION AT 30°C

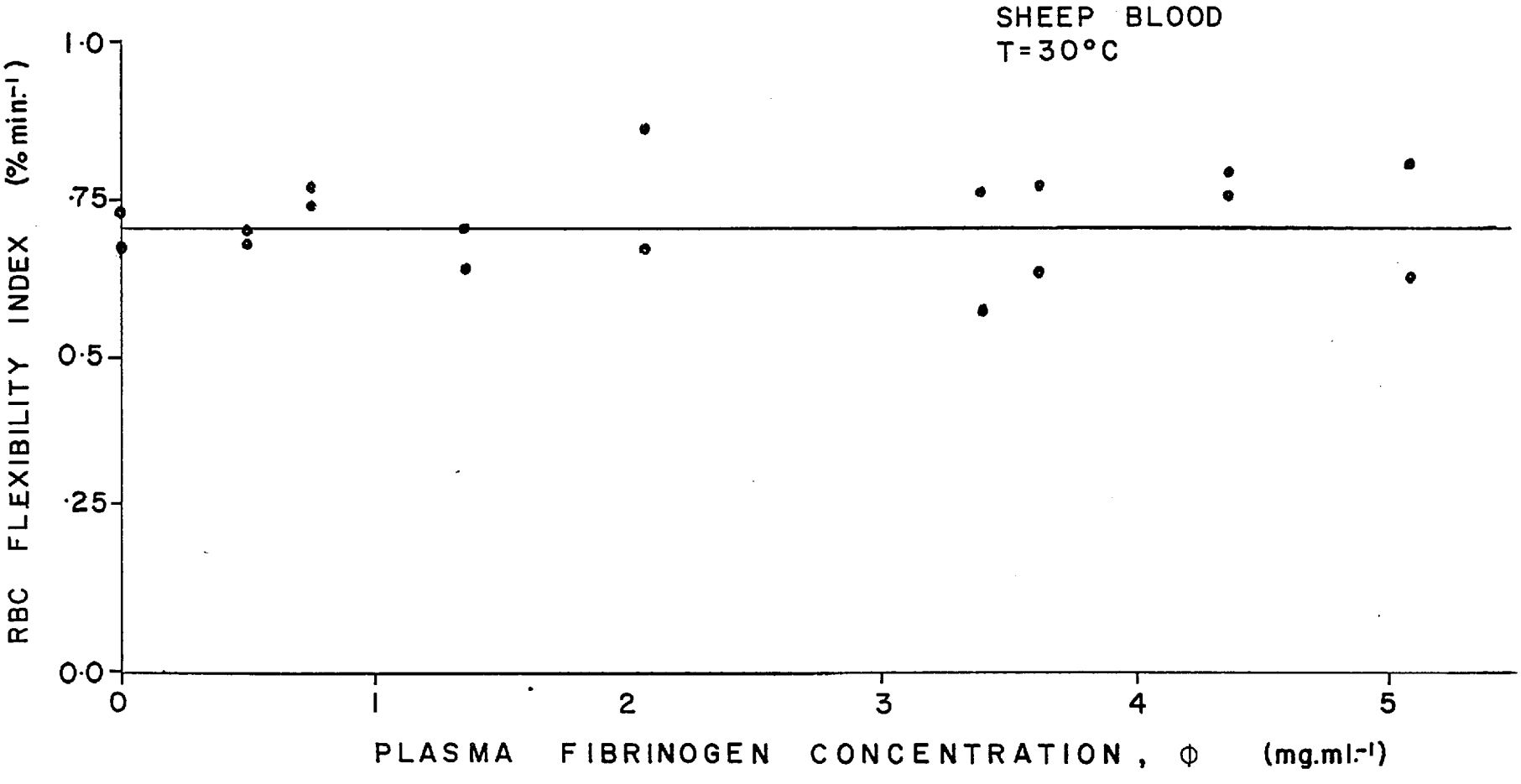


FIG. 55 : VARIATION OF SHEEP RBC FLEXIBILITY INDEX WITH PLASMA FIBRINOGEN CONCENTRATION

VARIATION OF CATTLE RED CELL PACKING RATE WITH HAEMATOCRIT AT 30°C

HAEMATOCRIT, H (% min $^{-1}$)	28.5	30	31	32	32.5	33.5	36	37	38.5	38.9	42.5	42.9	45	45.5	47.5	52	56	58
RED CELL PACKING RATE (% min $^{-1}$)	2.02 1.99 1.86	1.87 1.71	1.64	1.64	1.62	1.5	1.25	1.09	1.14	1.11	1.09	1.07	0.79	0.94	0.92 0.77	0.74 0.72	0.62 0.57	0.65 0.63

ERROR IN MEASUREMENT OF H = $\pm$ 0.125%

ERROR IN MEASUREMENT OF RBC PACKING RATE = $\pm$ 0.5% min^{-1}

TABLE 48 FOR FIGURE 56

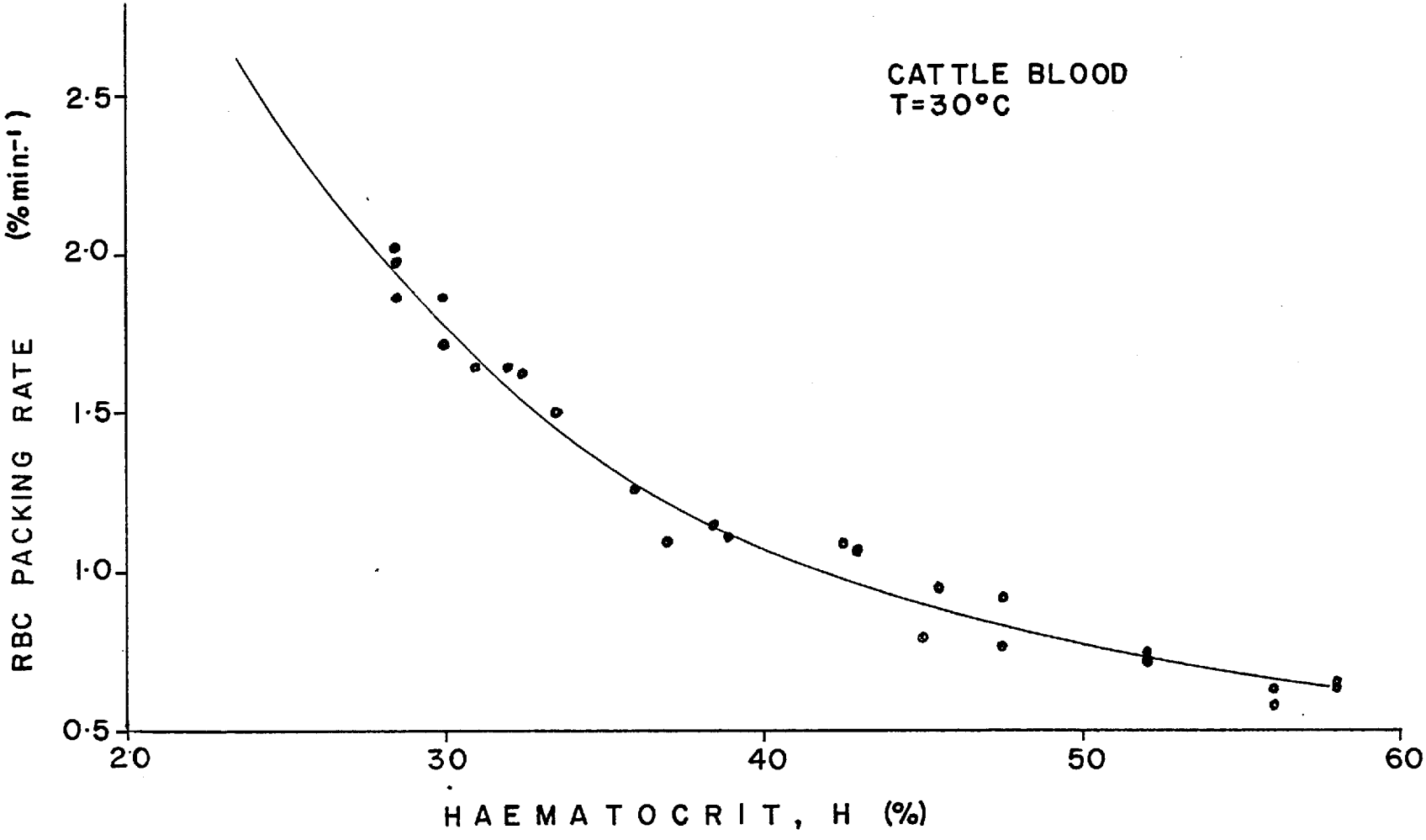


FIG. 56 : VARIATION OF CATTLE RBC PACKING RATE WITH HAEMATOCRIT AT 30°C

VARIATION OF CATTLE RED CELL FLEXIBILITY INDEX WITH PLASMA FIBRINOGEN CONCENTRATION AT 30°C

PLASMA FIB. CONC. ϕ (mg ml^{-1})	0 (SERUM)	0.2	0.53	0.8	1.525	2.0	2.2	2.35	3.0	3.275	3.4	3.625	3.9	4.275	4.42	4.6	5.1	5.25	5.65	7.02	7.125	0 (SALINE)
RED CELL FLEX. INDEX (% min^{-1})	0.96 0.88 0.78	0.83	0.78	0.82	0.84 0.99	0.93	0.89 0.73	0.92	0.84	0.92	0.82	0.8	0.86	0.88 0.86	0.86	0.86	0.99	0.897	0.965 0.94	0.795	0.92	0.87 0.91 0.79

TABLE 49 FOR FIGURE 57

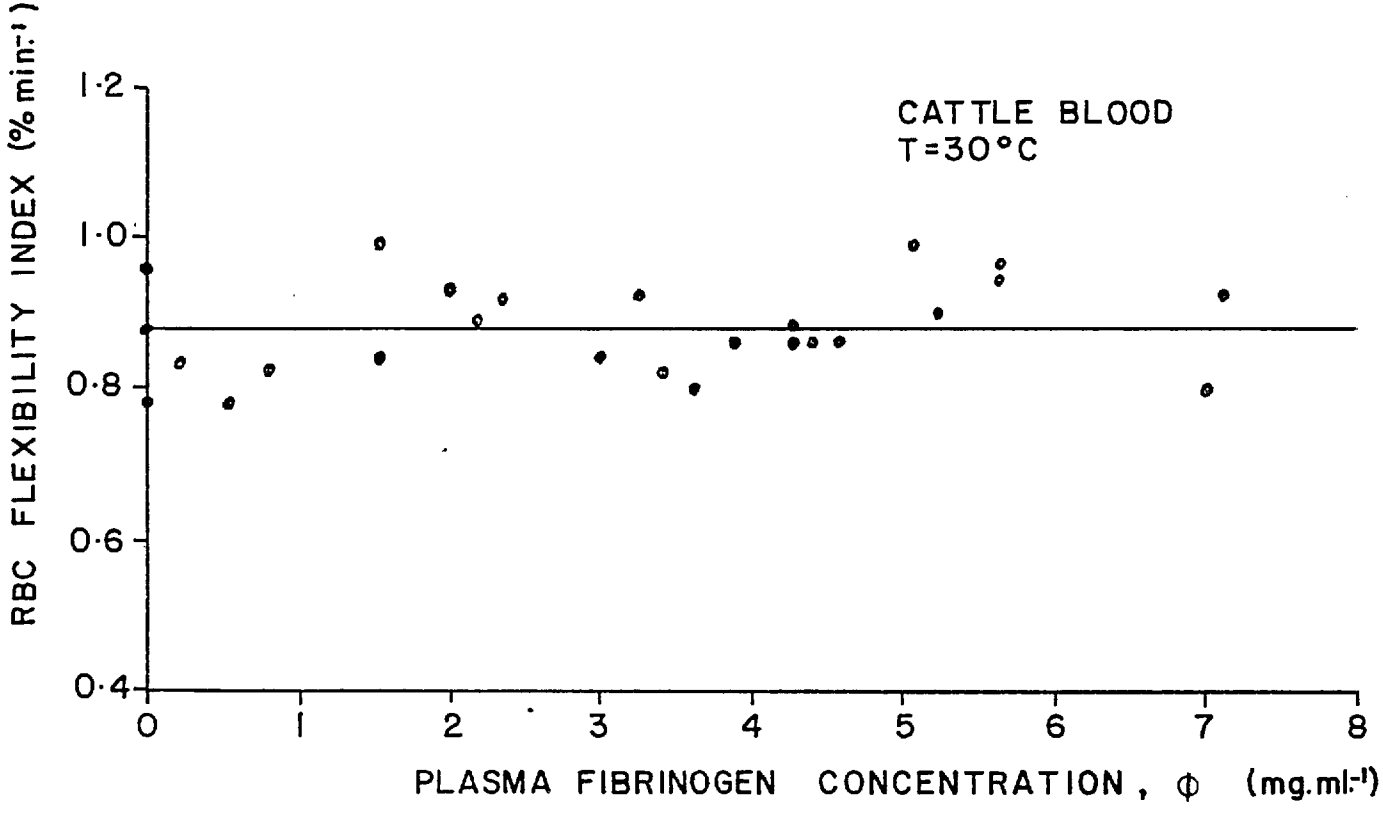


FIG. 57 : VARIATION OF CATTLE RBC FLEXIBILITY INDEX WITH PLASMA FIBRINOGEN CONCENTRATION

Goat red cells are also inflexible, with low packing rates at all haematocrits, Fig. (58), and again fibrinogen does not affect the red cell flexibility index, equal to 0.75% min^{-1} for all values of Φ at 30°C, Fig. (59).

Camel red cells are not as inflexible as those of sheep, cattle and goat, but are less flexible than human red cells at all haematocrits, as the values of its packing rates show in Fig. (60). However similar to sheep, cattle and goat, fibrinogen does not alter its red cell flexibility index at 30°C, as shown in Fig. (61), with the value of the flexibility index maintained at about 3.75% min^{-1} for all values of Φ in normal blood.

(ii) Viscometric Measurements

Due to the absence of fibrinogen effect on the red cell flexibility index, for the blood of the species, other than man, investigated, it was expected that the whole blood viscosity, η_B, would increase with Φ. For blood at 45% haematocrit and 30°C, Figs. (62) to (66) for horse, sheep, cattle, goat and camel respectively, show that as Φ is increased, the plasma viscosity, η_p, and whole blood viscosity, η_B, will increase.

From measurements of η_B at varying shear rates, γ, as Fig. (67) shows, at an equivalent haematocrit of 30%,

HAEMATOCRIT, H (%)	27	31.25	34.5	38	42	43	45.5	47.5	48.75	50	54	55.5	59
RED CELL PACKING RATE (% min. ^{1})	1.65 1.55	1.33 1.2	1.35 0.8	1.075 0.95	0.8 0.7	0.8 0.575	0.7 0.6	0.67 0.55	0.525 0.45	0.6 0.55	0.49 0.46	0.47 0.42	0.47 0.45

ERROR IN MEASUREMENT OF H = ± 0.125%

ERROR IN MEASUREMENT OF PACKING RATE = ± 0.5% min^{-1}

TABLE 50 FOR FIGURE 58

VARIATION OF GOAT RED CELL PACKING RATE WITH HAEMATOCRIT, H, AT 30°C

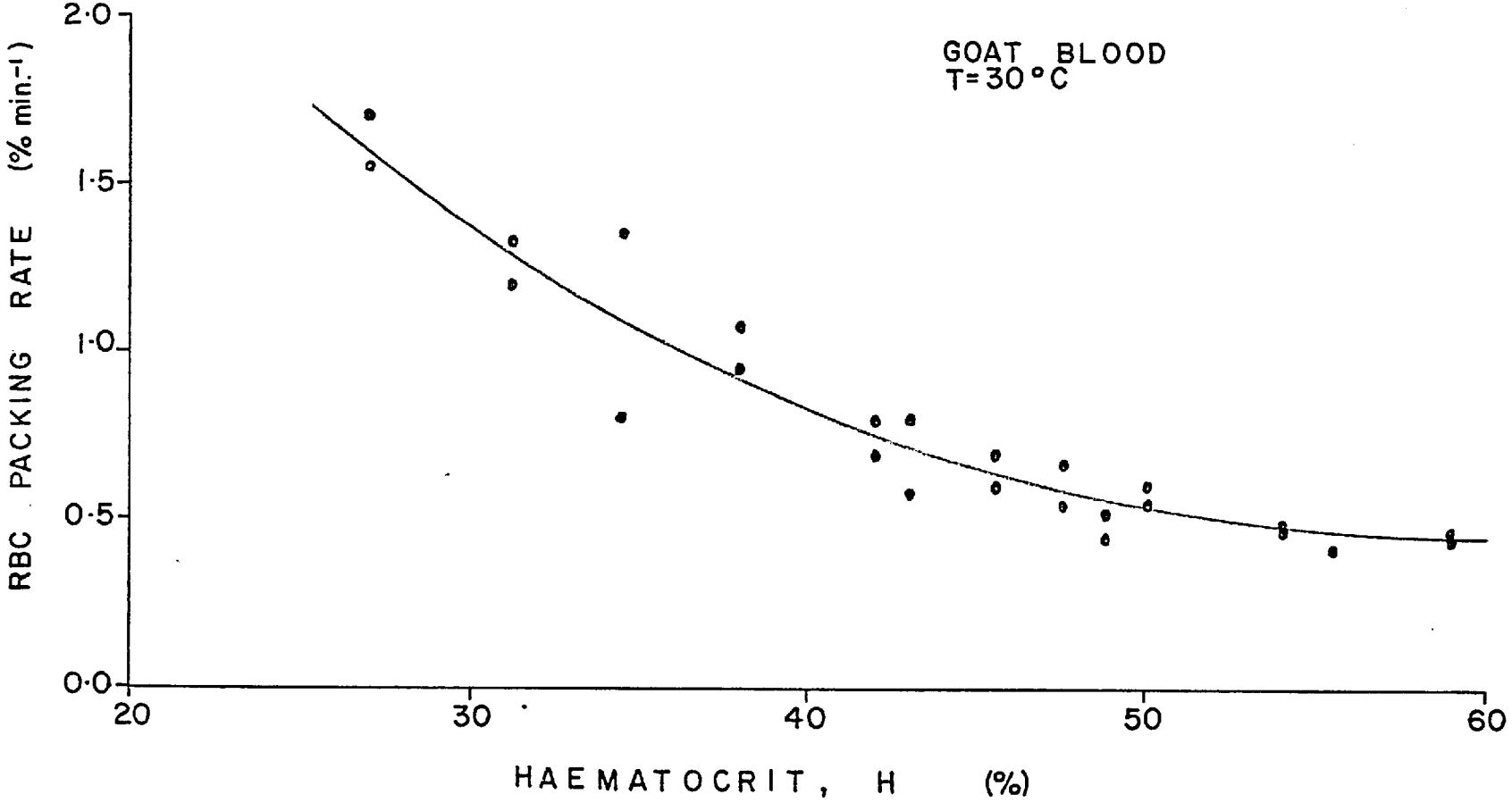


FIG. 58 : VARIATION OF GOAT RBC PACKING RATE WITH HAEMATOCRIT AT 30°C

VARIATION OF GOAT RED CELL FLEXIBILITY INDEX WITH PLASMA FIBRINOGEN CONCENTRATION, ϕ, AT 30°C

PLASMA FIB. CONC. ϕ (mg. ml.$^{-1}$)	0 (Serum)	0.25	0.415	0.75	1.25	1.875	2.125	2.375	2.625	3.0	3.65	3.75	3.85	4.55	0 (Saline)
RED CELL FLEXIBILITY INDEX (% min^{-1})	0.757 0.748	0.78 0.725	0.735 0.7	0.75 0.74	0.76 0.73	0.775 0.75	0.817 0.777	0.725 0.7	0.75 0.725	0.77 0.765	0.808 0.665	0.767 0.66	0.785 0.685	0.775 0.71	0.73 0.65

ERROR IN MEASUREMENT OF $\phi = \pm$ 0.1 mg. ml.$^{-1}$

ERROR IN MEASUREMENT OF FLEXIBILITY INDEX = $\pm$ 0.5% min^{-1}

TABLE 51 FOR FIGURE 59

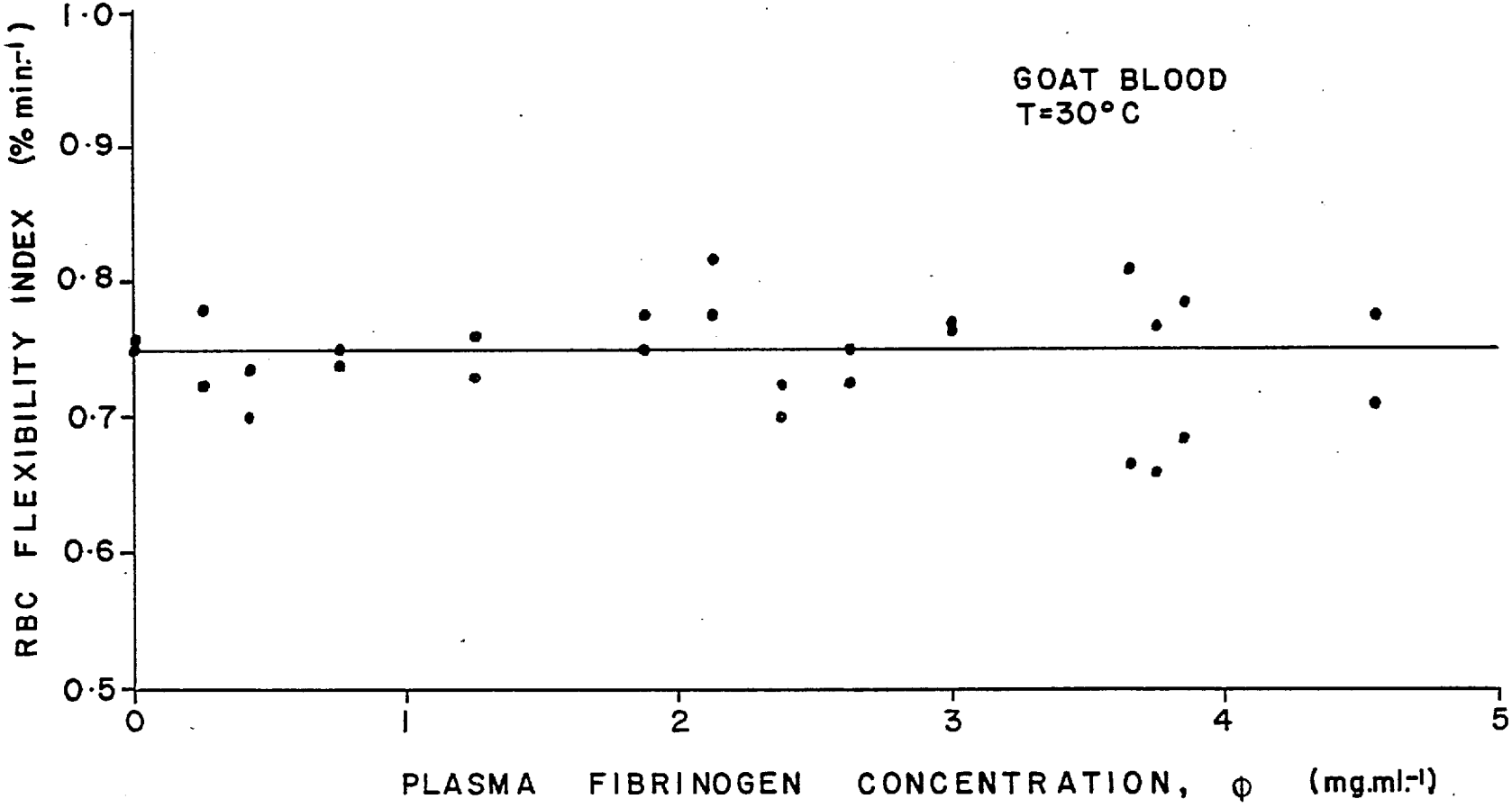


FIG. 59 : VARIATION OF RBC FLEXIBILITY INDEX WITH PLASMA FIBRINOGEN CONCENTRATION FOR GOAT BLOOD

HAEMATOCRIT, H %	27.5	30	31.5	35.35	36	38	40	42.5	45.5	47	48.75	52	53.25	54.5	55.5
RED CELL PACKING RATE % min^{-1}	7.45 6.75	7.0 6.65	6.55 6.15	6.15 5.45	5.45 5.15	5.0 4.75	4.85 4.0	4.15 3.65	3.85 3.14	4.3 3.65	3.45 3.0	3.0 2.3	3.0 2.7	3.24 2.25	2.65 2.15

ERROR IN MEASUREMENT OF H = $\pm$ 0.125%
ERROR IN MEASUREMENT OF PACKING RATE = $\pm$ 0.5% min^{-1}

TABLE 52 FOR FIGURE 60

VARIATION OF CAMEL RED CELL PACKING RATE WITH HAEMATOCRIT AT 30°C

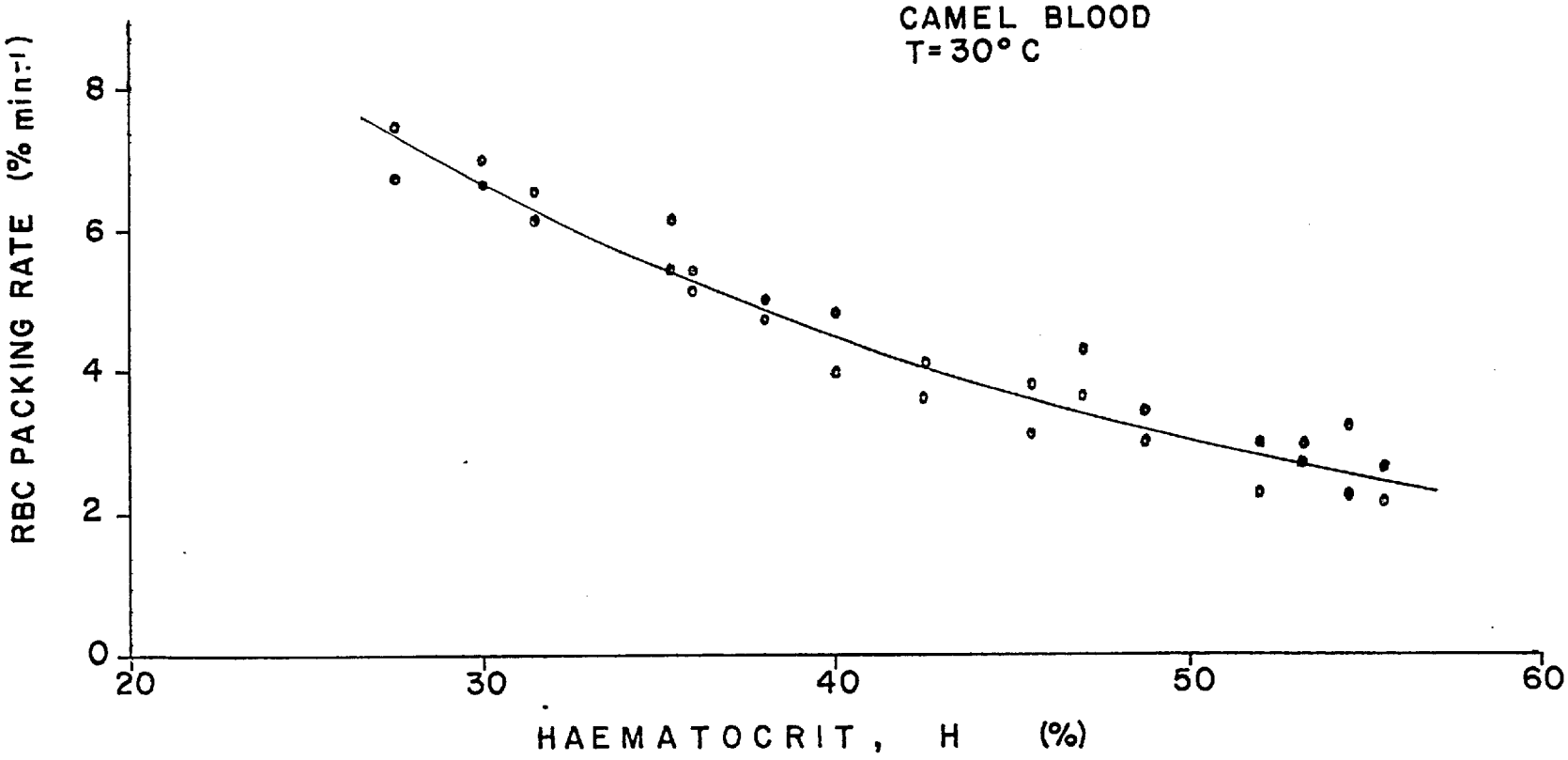


FIG. 60 : VARIATION OF CAMEL RBC PACKING RATE WITH HAEMATOCRIT AT 30°C

PLASMA FIB. CONC. Φ (mg.ml.$^{-1}$)	0 (SERUM)	0.5	1.07	1.38	2.08	2.64	2.87	3.09	0 (SALINE)
RED CELL FLEXIBILITY INDEX (% min.$^{-1}$)	3.69 3.82	3.89 3.76	3.77 3.59	3.81 3.75	3.75 3.67	3.76 3.61	3.78 3.69	3.78 3.65	2.0 1.89

ERROR IN MEASUREMENT OF Φ = $\pm$ 0.1 mg.ml.$^{-1}$

ERROR IN MEASUREMENT OF FLEX. INDEX = $\pm$ 0.5 % min^{-1}

TABLE 53 FOR FIGURE 61

VARIATION OF CAMEL RED CELL FLEXIBILITY INDEX WITH PLASMA FIBRINOGEN CONCENTRATION, Φ , AT 30°C

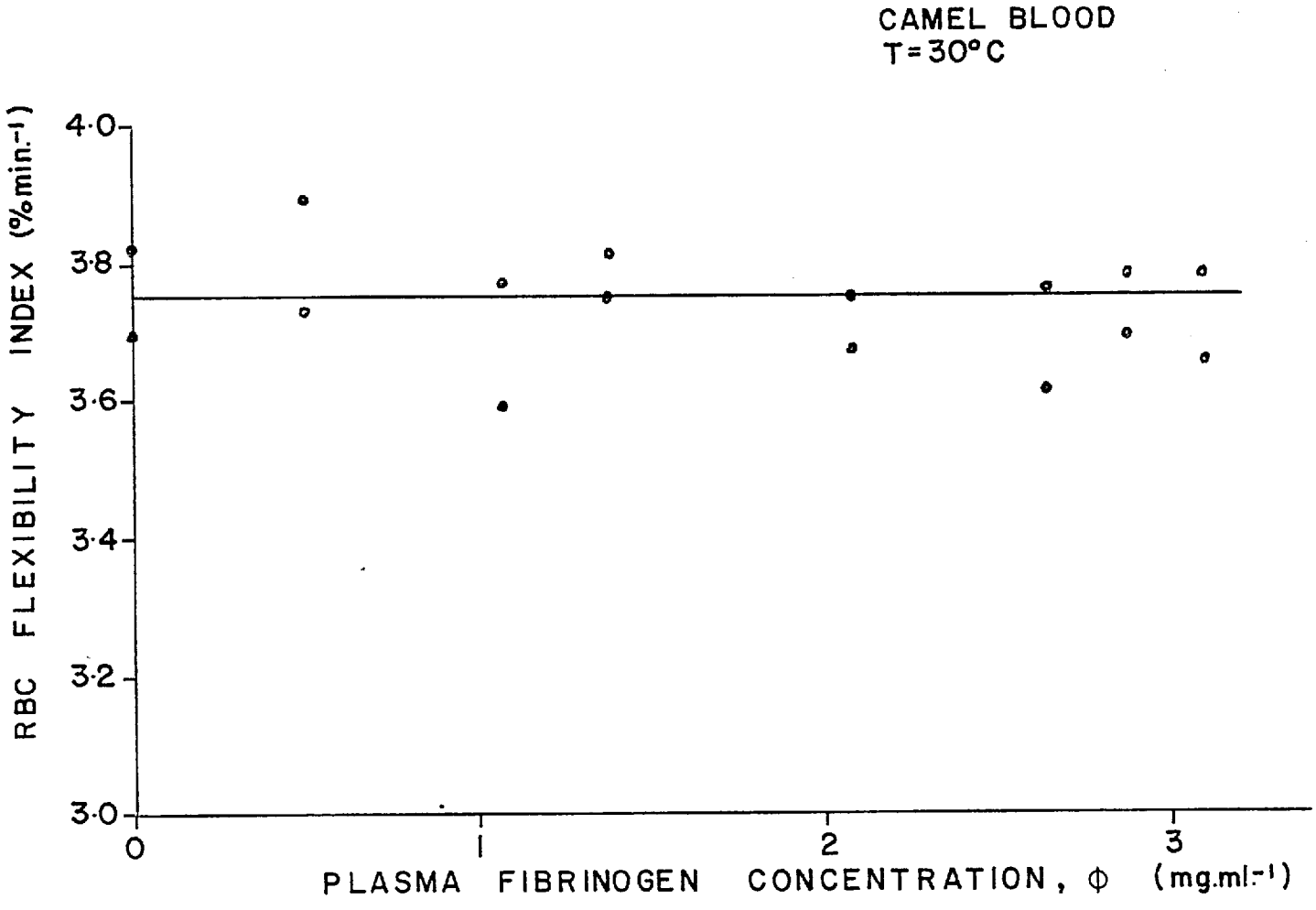


FIG. 61 : VARIATION OF CAMEL RBC FLEXIBILITY INDEX WITH PLASMA FIBRINOGEN CONCENTRATION

PLASMA FIBRINOGEN CONCENTRATION, ϕ (mg ml^{-1})	0	0.7	1.4	1.8	2.6	2.7	3.075	3.6	3.7	4.3	4.4	5.25	6.0
PLASMA VISCOSITY, η_p (cP)	1.8	1.85	1.85	1.95	1.975	2.0	2.1	2.1	2.0	2.1	2.1	2.19	2.25

REGRESSION EQUATION:- Y = 0.0744X + 1.794
CORRELATION COEFFICIENT = 0.963

PLASMA FIBRINOGEN CONCENTRATION, ϕ (mg ml^{-1})	0	0	1	1.5	2	2.15	2.75	3.25	3.7	3.7	4.3	4.45	4.45	5.25	5.75	6	6.22
WHOLE BLOOD VISCOSITY η_B (cP)	7	6.8	7.1	7.2	7.35	6.78	7.45	7.5	7.9	8	7.43	7.53	7.97	8.05	8.1	8.25	8.01

REGRESSION EQUATION:- Y = 0.2167X + 6.85
CORRELATION COEFFICIENT = 0.898

ERROR IN ϕ MEASUREMENTS = $\pm$ 0.1 mg ml^{-1}
ERROR IN VISCOSITY MEASUREMENTS: FOR $\eta_B = \pm$ 0.2 cP FOR $\eta_p = \pm$ 0.05 cP

TABLE 54 FOR FIGURE 62

VARIATION OF HORSE WHOLE BLOOD AND PLASMA VISCOSITIES WITH PLASMA FIBRINOGEN CONCENTRATION AT 30°C, 230s^{-1} AND 45% HAEMATOCRIT

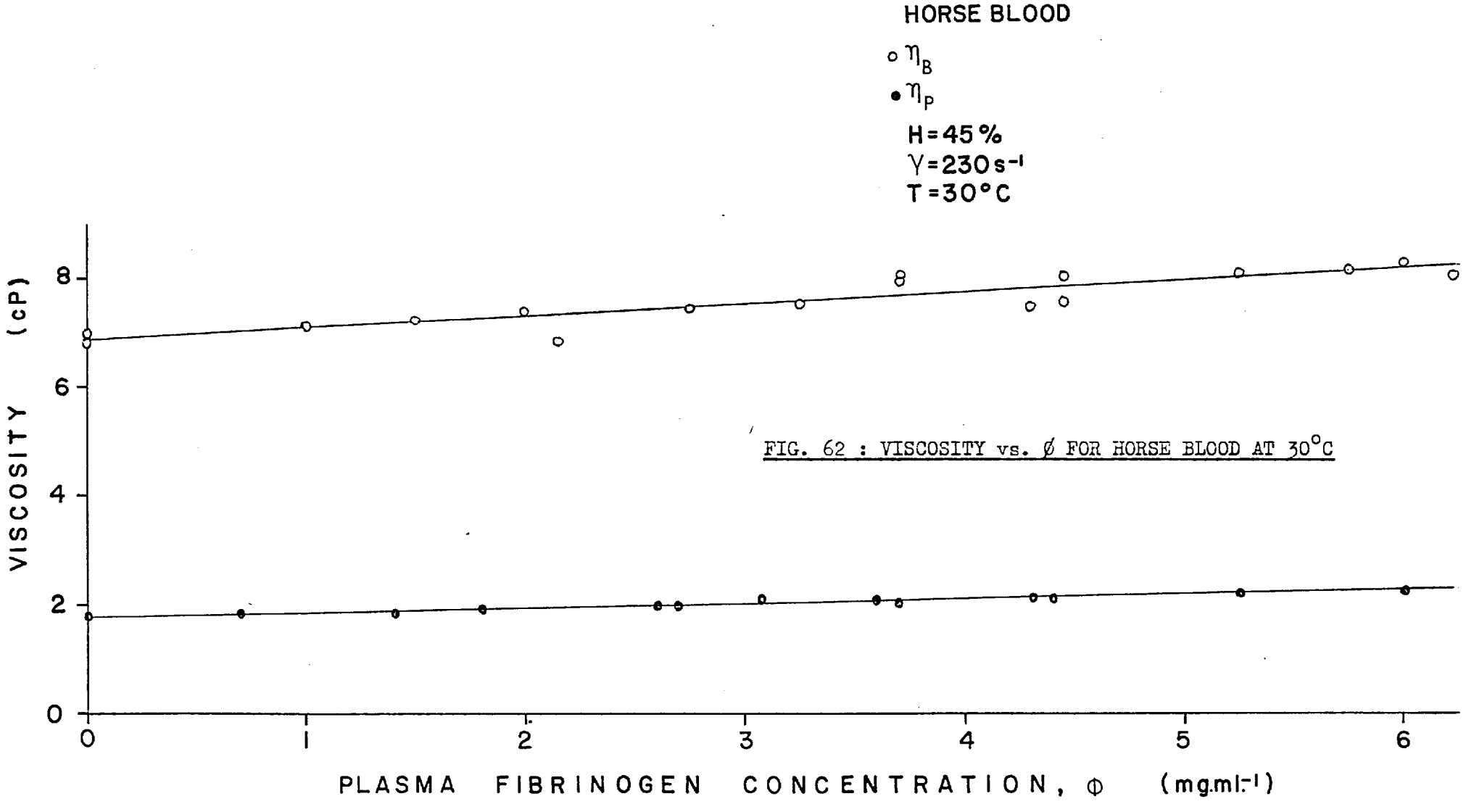


FIG. 62 : VISCOSITY vs. Ø FOR HORSE BLOOD AT 30°C

PLASMA FIBRINOGEN CONCENTRATION, ϕ ($mg.ml.^{-1}$)	0	0.54	0.77	1.36	1.6	1.76	2.89	3.04	3.9
WHOLE BLOOD VISCOSITY η_B (cP)	4.43	4.5	4.4	4.81	5.2	4.6	5.25	4.75	5.2
PLASMA VISCOSITY η_p (cP)	1.4	1.425	1.45	1.525	1.625	1.6	1.75	1.75	1.875

FOR η_B:- REGRESSION EQUATION:- $Y = 0.1997X + 4.44$
CORRELATION COEFFICIENT = 0.745

FOR η_p:- REGRESSION EQUATION:- $Y = 0.1276X + 1.375$
CORRELATION COEFFICIENT = 0.99

ERROR IN ϕ MEASUREMENTS = $\pm$ 0.1 $mg.ml.^{-1}$
ERROR IN VISCOSITY MEASUREMENTS: FOR $\eta_B = \pm$ 0.2 cP FOR $\eta_p = \pm$ 0.05 cP

TABLE 55 FOR FIGURE 63

VARIATION OF SHEEP WHOLE BLOOD AND PLASMA VISCOSITIES WITH PLASMA FIBRINOGEN CONCENTRATION AT 30°C, $\dot{\gamma} = 230s^{-1}$ AND HAEMATOCRIT OF 45%

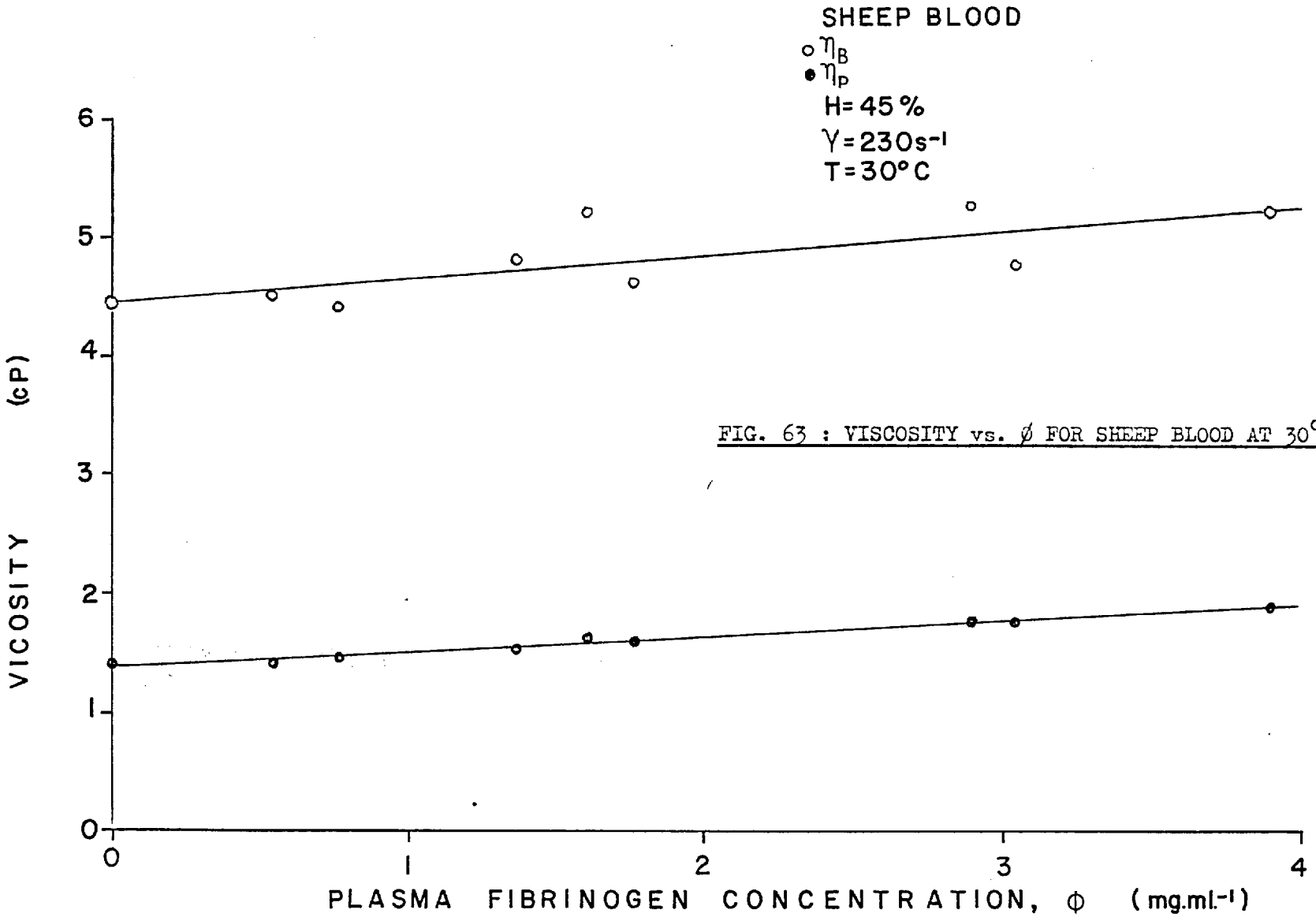


FIG. 63 : VISCOSITY vs. ϕ FOR SHEEP BLOOD AT 30°C

PLASMA FIB. CONC., Φ ($mg.ml.^{-1}$)	0	1.354	2.81	2.86	3.31	3.63	4.57	4.64	7.12
WHOLE BLOOD VISCOSITY η_B (CP) H=45%	4.51	4.5	4.85	4.9	4.55	4.65	4.85	4.86	4.9
PLASMA VISCOSITY η_p (CP)	1.6	1.59	1.55	1.71	1.53	1.75	1.73	1.83	1.85

FOR η_B: REGRESSION EQUATION: $Y = 0.0599\ X + 4.499$
CORRELATION COEFFICIENT = 0.7

FOR η_p: REGRESSION EQUATION: $Y = 0.0463\ X + 1.53$
CORRELATION COEFFICIENT = 0.734

ERROR IN MEASUREMENT OF VISCOSITY = $\pm$ 0.2 cP

ERROR IN MEASUREMENT OF Φ = $\pm$ 0.1 $mg.ml.^{-1}$

TABLE 56 FOR FIGURE 64

VARIATION OF CATTLE WHOLE BLOOD VISCISOTY, η_B, AND PLASMA VISCOSITY, η_p, WITH PLASMA FIBRINOGEN CONCENTRATION, Φ, AT T = 30°C, γ = $230s^{-1}$ AND 45% HAEMATOCRIT

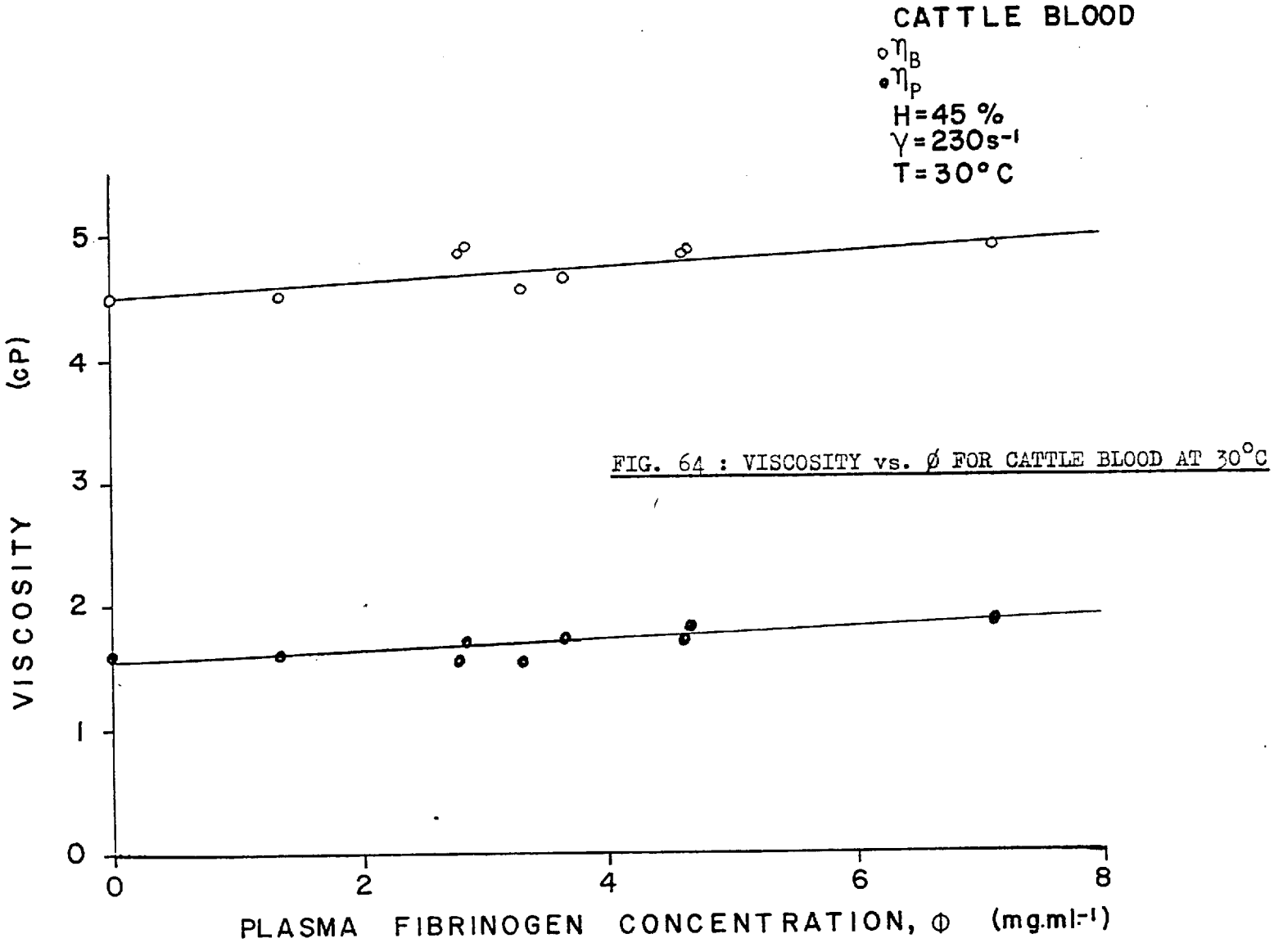


FIG. 64 : VISCOSITY vs. Ø FOR CATTLE BLOOD AT 30°C

PLASMA FIB. CONC., Φ ($mg.ml.^{-1}$)	0	1.375	2.6	3.25	3.32	3.35	3.4	3.9	4.23
WHOLE BLOOD VISCOSITY η_B (cP) H=45%	4.6	4.7	4.9	4.9	5.0	5.05	4.925	5.1	5.05
PLASMA VISCOSITY η_p (cP)	1.1	1.1	1.25	1.22	1.45	1.34	1.3	1.3	1.3

FOR η_B: REGRESSION EQUATION:- $Y = 0.1203\ X + 4.59$
CORRELATION COEFFICIENT = 0.96

FOR η_p: REGRESSION EQUATION:- $Y = 0.0649\ X + 1.079$
CORRELATION COEFFICIENT = 0.78

ERROR IN MEASUREMENT OF VISCOSITY = $\pm$ 0.2 cP

ERROR IN MEASUREMENT OF Φ = $\pm$ 0.1 $mg.ml.^{-1}$

TABLE 57 FOR FIGURE 65

VARIATION OF GOAT WHOLE BLOOD VISCOSITY, η_B, AND PLASMA VISCOSITY, η_p,
WITH PLASMA FIBRINOGEN CONCENTRATION, Φ, AT T = 30°C, γ = $230s^{-1}$
AND 45% HAEMATOCRIT

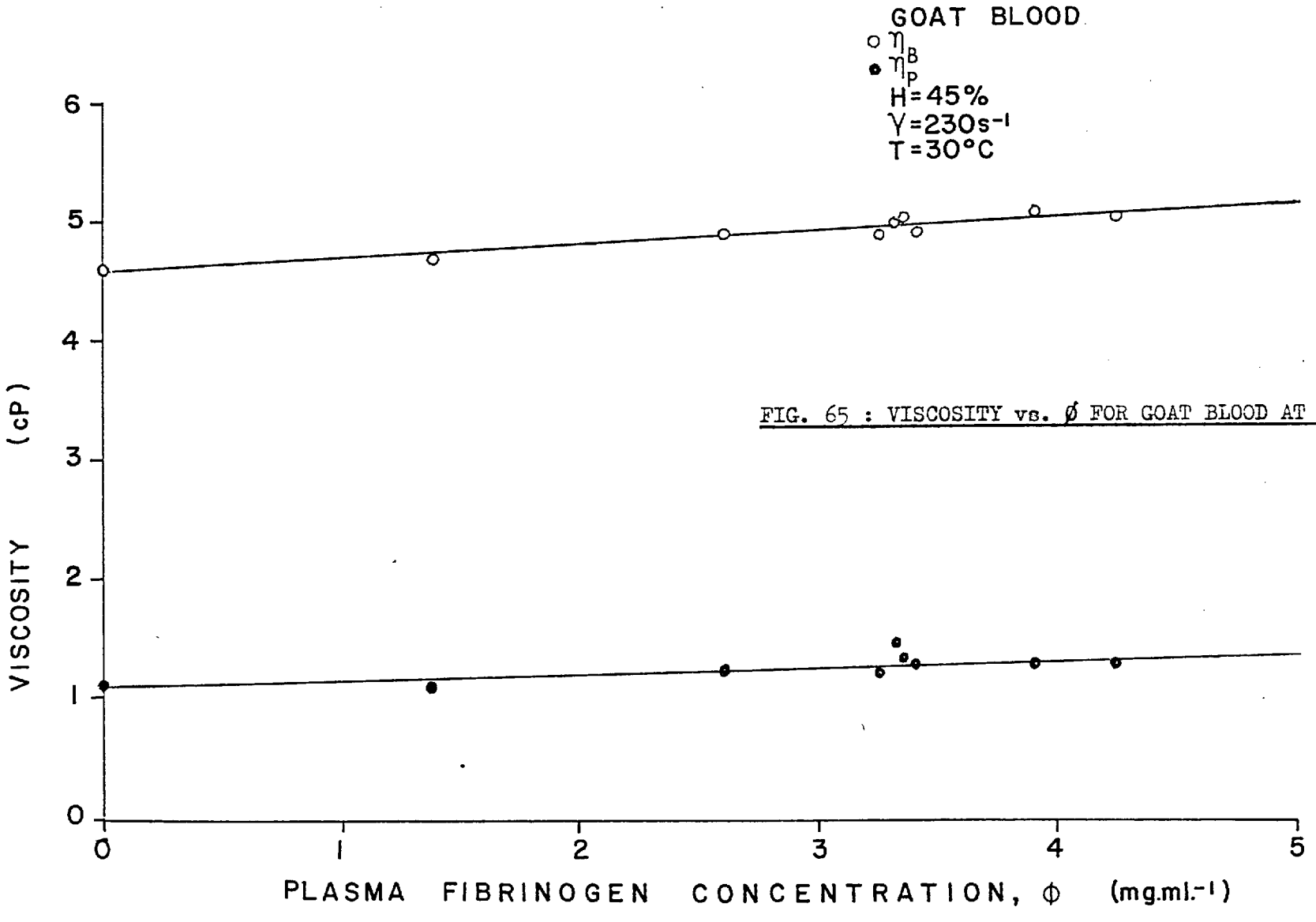


FIG. 65 : VISCOSITY vs. Ø FOR GOAT BLOOD AT 30°C

PLASMA FIB. CONC., Φ ($mg.ml.^{-1}$)	0	0.5	1.07	1.38	2.08	2.64	2.87	3.09
WHOLE BLOOD VISCOSITY, η_B (cP) H=45%	6.1	6.1	6.22	6.28	6.37	6.65	6.63	6.75
PLASMA VISCOSITY, η_p (cP)	1.33	1.36	1.525	1.45	1.53	1.65	1.525	1.68

FOR η_B:- REGRESSION EQUATION: $Y = 0.2185\ X + 6.015$
CORRELATION COEFFICIENT = 0.97

FOR η_p:- REGRESSION EQUATION: $Y = 0.0971\ X + 1.34$
CORRELATION COEFFICIENT = 0.895

ERRORS IN MEASUREMENT OF VISCOSITY = $\pm$ 0.2 cP

ERRORS IN MEASUREMENT OF Φ = $\pm$ 0.1 $mg.ml.^{-1}$

TABLE 58 FOR FIGURE 66

VARIATION OF CAMEL WHOLE BLOOD VISCOSITY, η_B, AND PLASMA VISCOSITY, η_p, WITH PLASMA FIBRINOGEN CONCENTRATION AT T = 30°C, γ = $230s^{-1}$ AND 45% HAEMATOCRIT

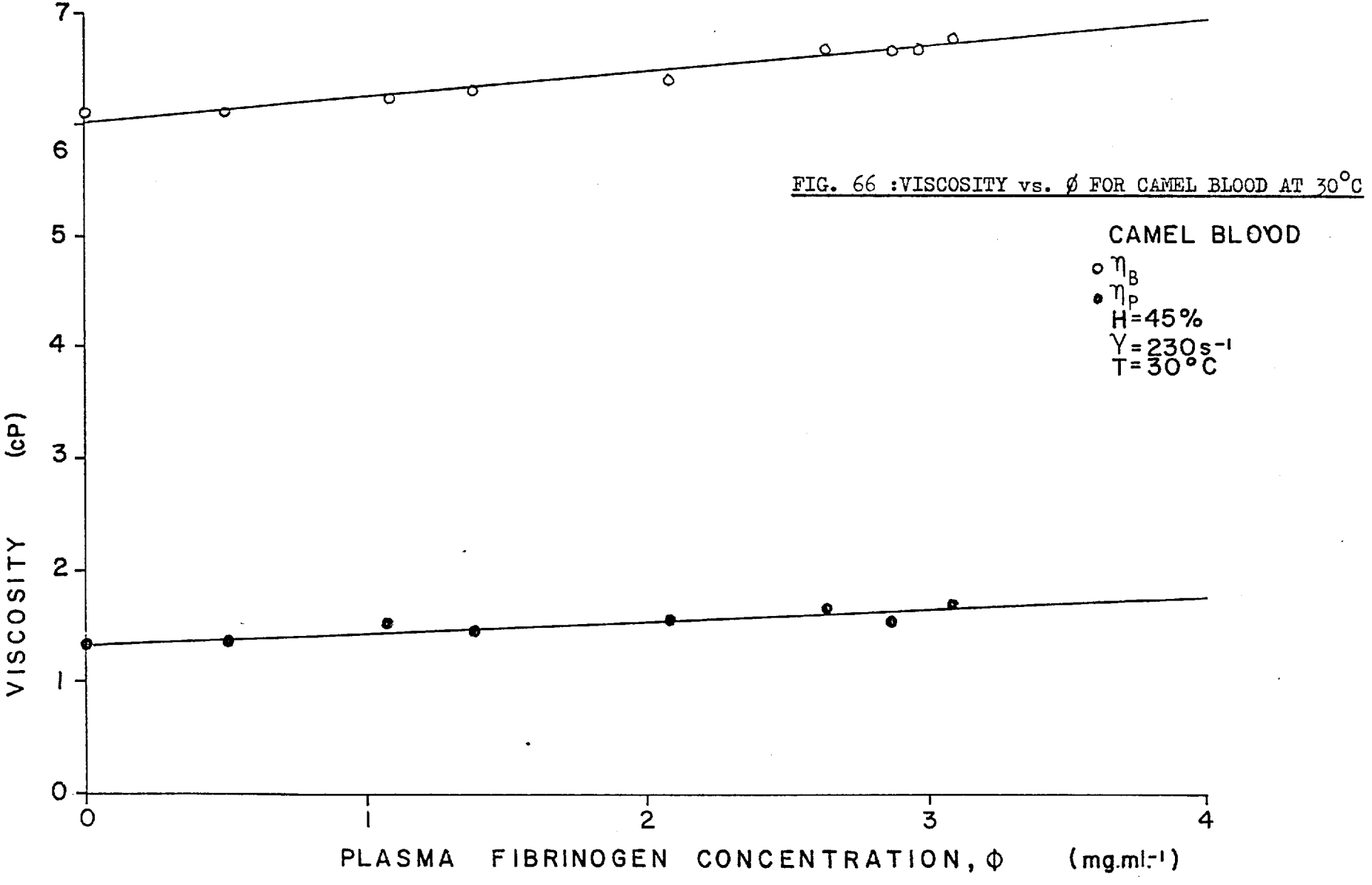


FIG. 66 :VISCOSITY vs. Ø FOR CAMEL BLOOD AT 30°C

	SHEAR RATE (s^{-1})				
	230	115	46	23	11.5
MAN	3.66 (cP)	4.0	4.8	5.6	7.0
HORSE	4.3	4.8	5.45	6.75	8.825
SHEEP	3.8	3.85	4.25	4.5	4.725
CATTLE	3.8	3.9	4.25	4.5	4.65
GOAT	3.9	4.0	4.3	4.55	4.82
CAMEL	4.1	4.15	4.35	4.65	5.05

TABLE 59 FOR FIGURE 67

WHOLE BLOOD VISCOSITY, η_B, AT DIFFERENT SHEAR RATES, 30% HAEMATOCRIT AND 37.5°C FOR MAN, HORSE, SHEEP, CATTLE, GOAT AND CAMEL

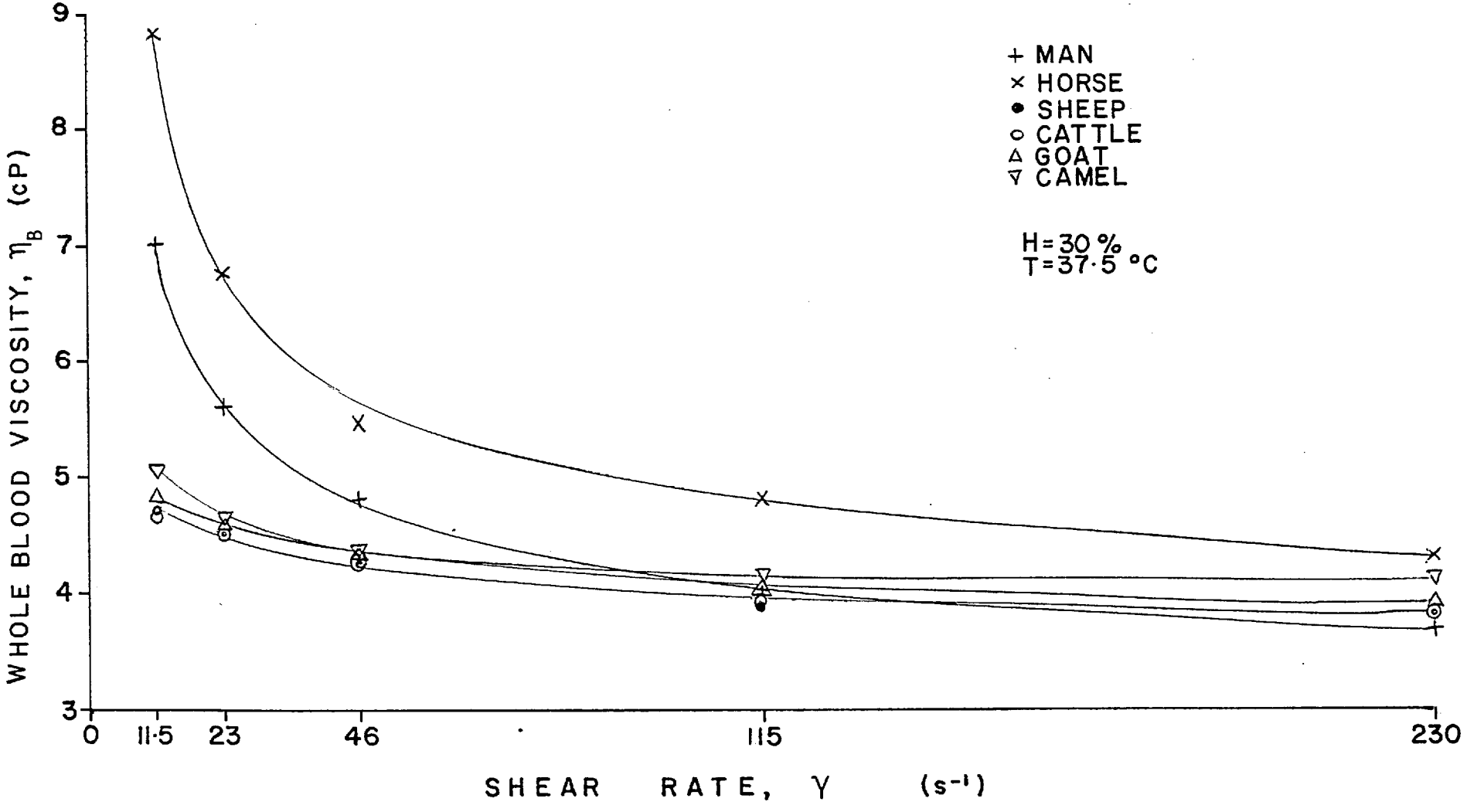


FIG. 67 : WHOLE BLOOD VISCOSITY FOR DIFFERENT SPECIES AT VARYING SHEAR RATES

$37.5^{o}C$ and high γ ($230s^{-1}$), human blood possesses the lowest η_B of the six species with sheep, cattle and goat having similar and higher η_B than man. Camel η_B is slightly higher again and the highest value of η_B being that of horse. As γ is decreased, sheep, cattle, goat and camel blood increase slowly with the former three species keeping their value of η_B similar. As γ is decreased η_B for human blood increases from being lowest of the six species at $\gamma = 230s^{-1}$ to being greater than η_B for sheep, cattle, goat and camel as $\gamma < 90s^{-1}$. The value of η_B for horse blood is highest at high γ ($230s^{-1}$), although horse red cells are much more flexible than the other species, and increases with decreasing γ, maintaining its highest value of η_B over the range of γ used.

The relative viscosities, η_r, of horse red cells suspension in plasma (whole blood), serum (defibrinated blood) and in saline at varying shear rates, γ, are shown in Fig. (68). Red cells are most flexible when suspended in plasma, and possess the lowest η_r at high γ ($230s^{-1}$) and highest η_r at low γ (below $15s^{-1}$). For red cells in serum, at high γ, its η_r is higher than that for whole blood but is lower for γ less than $75s^{-1}$. When red cells are suspended in saline, the red cell flexibility index is decreased to 2.2% min^{-1} at $37.5^{o}C$, compared to 42.5% min^{-1} for whole blood and 21.75% min^{-1} for defibrinated blood, at the same temperature. This decrease in flexibility index makes the red cells relatively inflexible in saline and hence

	SHEAR RATE, $\dot{\gamma}$ s^{-1}				
	230	115	46	23	11.5
RED CELLS IN PLASMA	3.2 (cP)	3.575	4.575	5.425	6.675
RED CELLS IN SERUM	3.45	3.775	4.45	5.25	6.45
RED CELLS IN SALINE	4.22	4.45	5.175	5.7	6.175

ERROR IN VISCOSITY MEASUREMENTS = ± 0.2 cP

TABLE 60 FOR FIGURE 68

VALUE OF RELATIVE VISCOSITY η_r AT DIFFERENT SHEAR RATES, $\dot{\gamma}$,
FOR HORSE RBC SUSPENDED IN PLASMA, SERUM AND SALINE AT 30°C AND HAEMATOCRIT OF 37%

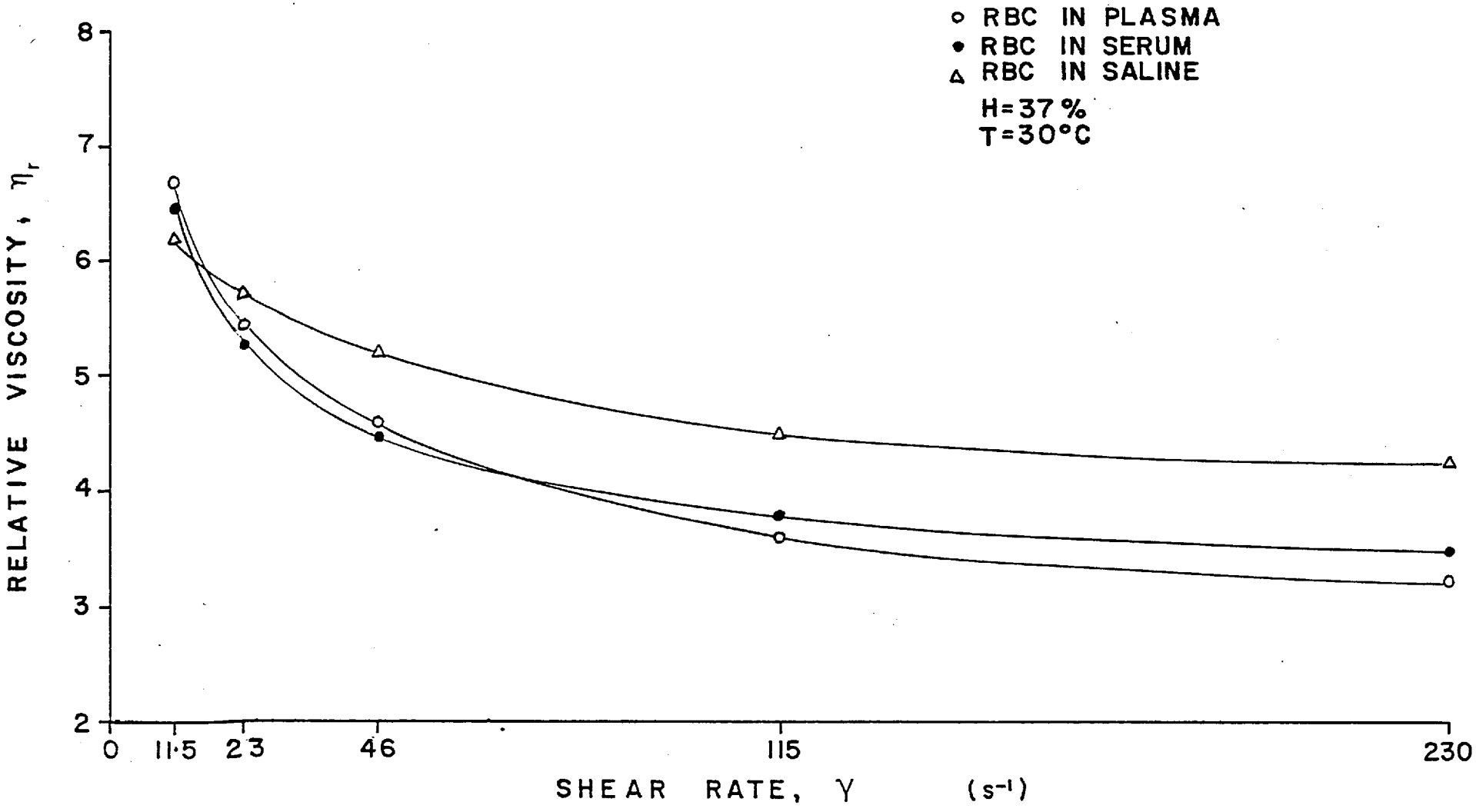


FIG. 68 : RELATIVE VISCOSITY OF DIFFERENT HORSE RBC SUSPENTIONS AT VARYING SHEAR RATES

its η_r is increased at high γ. As γ is lowered, η_r does not increase as rapidly as in the case of whole or defibrinated blood. For γ less than $15s^{-1}$, η_r for saline cells is lower in value than the other two suspensions.

(iii) Intrinsic Viscosity, KT'_c, and Red Cell Shape Factor, K

Fig. (69) shows the relationship between haematocrit, H, and relative viscosity, η_r, for blood from donor A, at 30^oC and $\gamma = 230s^{-1}$ (no aggregation). Also shown is the linear relationship between H and log η_r as described by equation 6.3. This relationship seems to be more linear for H = 0 to 30% than for higher values of H. By obtaining the regression equation relating H and log η_r, using the origin as a fixed point (i.e. at H = log η_r = 0), the value of log η_r at H = 1%, log η_{r1}, was calculated. The value of η_{r1} is then used to calculate the intrinsic viscosity of KT'_c using the following relationship

$$\eta_{r1} = 1 + KT'_cH_1 = 1 + KT'_c0.01$$

where H_1 is the 1% haematocrit, i.e. 0.01.

For human blood, red cell flexibility increases with plasma fibrinogen concentration, ϕ, in a linear relationship. By varying ϕ and measuring KT'_c at each

HAEMATOCRIT, H (%)	0	10	17.25	25	30	35	38	42	43	46.5	51	58
RELATIVE VISCOSITY, η_r	1	1.339	1.643	2.036	2.324	2.786	2.929	3.179	3.304	3.529	4.089	5.179
Log η_r	0	0.1268	0.2156	0.3088	0.3662	0.445	0.4667	0.5023	0.519	0.5477	0.6116	0.7143

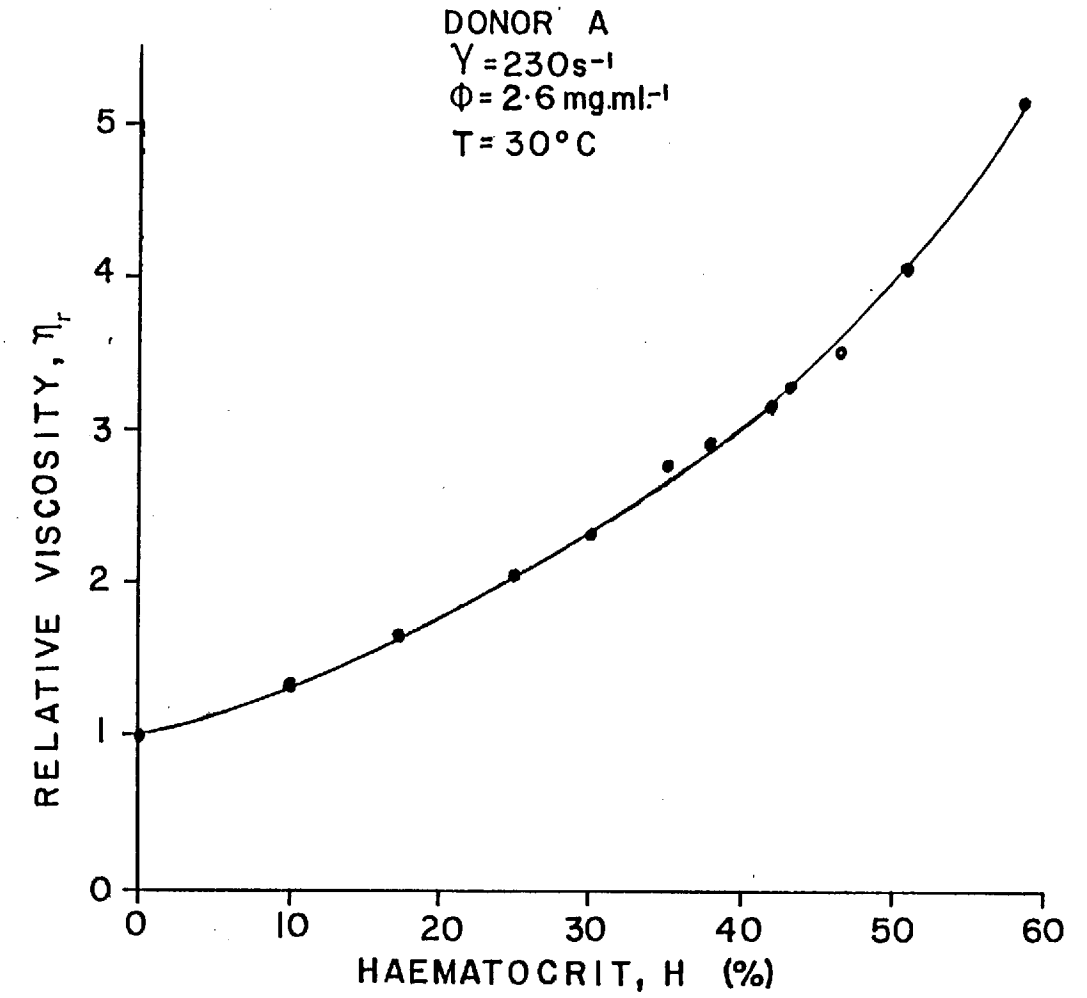
ERROR IN η_r = ± 0.15

CORRELATION COEFFICIENT = 0.99

ERROR IN H = ± 0.125%

TABLE 61 FOR FIGURE 69

VARIATION OF RELATIVE VISCOSITY η_r AND LOG η_r
WITH HAEMATOCRIT FOR HUMAN BLOOD (DONOR A) AT 30°C AND SHEAR RATE OF $230s^{-1}$



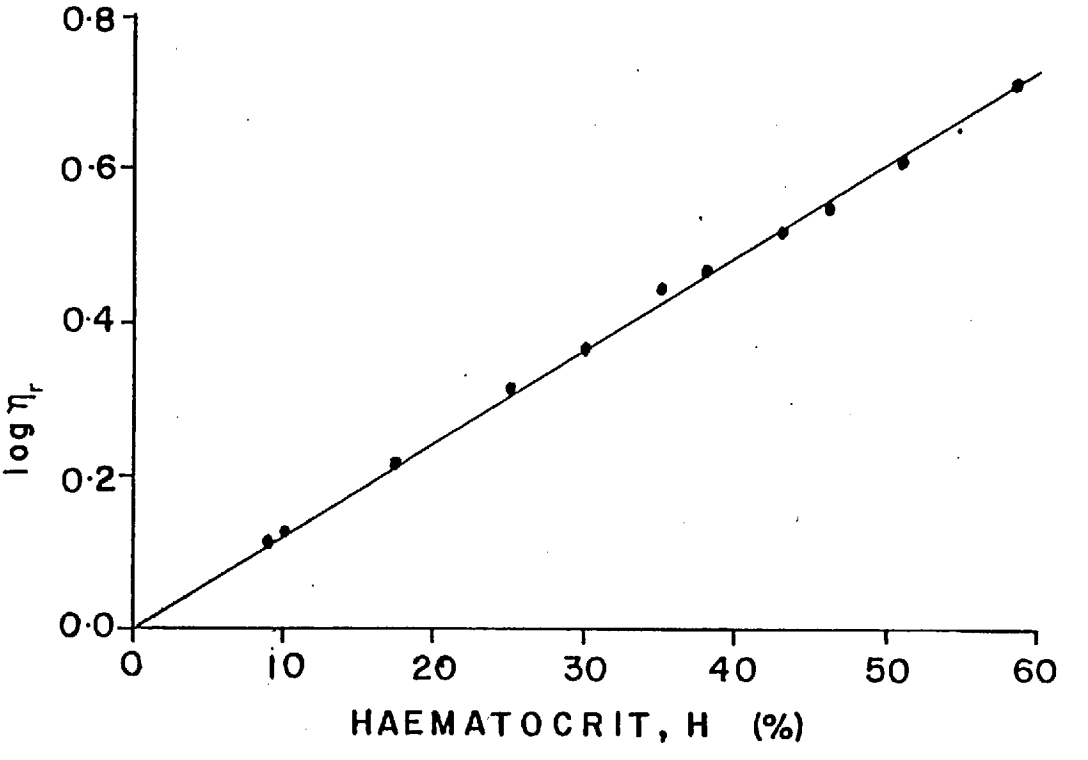


FIG. 69 : η_r AND LOG. η_r vs. H AT 30°C

value of Φ, the red cell flexibility is also being altered. Another way of altering the red cell flexibility is to heat harden the cells by heating blood samples for different intervals at 48.5°C, e.g. for 5, 10, 15 and 20 minutes. Alternatively formaldehyde fixing is used by suspending the red cells in an isotonic (pH = 7.4) Ringer-Locke/albumin solution of varying formaldehyde concentrations, i.e. 0.25, 0.5, 0.75 and 1% concentrations. The cells are then washed and resuspended in normal plasma. Heat hardening and formaldehyde fixing reduce red cell flexibility. To increase red cell flexibility, varying volumes of a concentrated isotonic (pH = 7.4) solution of human fibrinogen (Kabi Grade L) dissolved in Ringer-Locke solution are added to blood samples. The increase in Φ increases the red cell flexibility. Fig. (70) shows the values of the intrinsic viscosity KT'_c for blood of donor A at varying red cell flexibility index and 30°C where the red cell flexibility has been varied as explained above. As the red cell flexibility is increased from zero, the value of KT'_c decreases. At zero flexibility, $T'_c = 1$ and therefore KT'_c is equal to K, the red cell shape factor. From the regression equation used to fit the points to a line, the intercept of the line at zero flexibility gives a shape factor, K, equal to 3.34. It is also observed that within the measuring errors, the points for heat hardened and formaldehyde fixed red cells fit on the same line as normal red cells.

For all the species, 90 values of KT'_c was obtained from the H vs. $\log\eta_r$ plot.The correlation coefficient was < 0.75 in only three cases,which were were discarded as doubtful data.

INTRINSIC VISCOSITY KT_c' AT DIFFERENT RED CELL FLEXIBILITIES OF HUMAN (DONOR A) NORMAL, HEAT TREATED AND FORMALDEHYDE FIXED RED CELLS

NORMAL

RED CELL FLEXIBILITY INDEX (% min^{-1})	INTRINSIC VISCOSITY KT_c'
1.34	3.32
3.42	2.98
3.45	3.11
6.3	3.09
6.6	3.06
6.63	3.11
7.15	3.00
7.95	2.72
7.975	2.90
8.0	3.00
10.75	2.77
10.75	2.9

HEAT HARDENED

RED CELL FLEXIBILITY INDEX (% min^{-1})	INTRINSIC VISCOSITY KT_c'
4.05	3.11
4.55	3.06
5.75	3.11
6.1	3.16

FORMALDEHYDE FIXED

RED CELL FLEXIBILITY INDEX (% min^{-1})	INTRINSIC VISCOSITY KT_c'
0	3.33
1.325	3.34
4.0	3.32
4.68	3.03

REGRESSION EQUATION:- $Y = 0.049X + 3.343$

CORRELATION COEFFICIENT = -0.819

ERROR IN FLEX. INDEX = $\pm$ 0.5% min^{-1}

ERROR IN KT_c' = $\pm$ 0.2

TABLE 62 FOR FIGURE 70

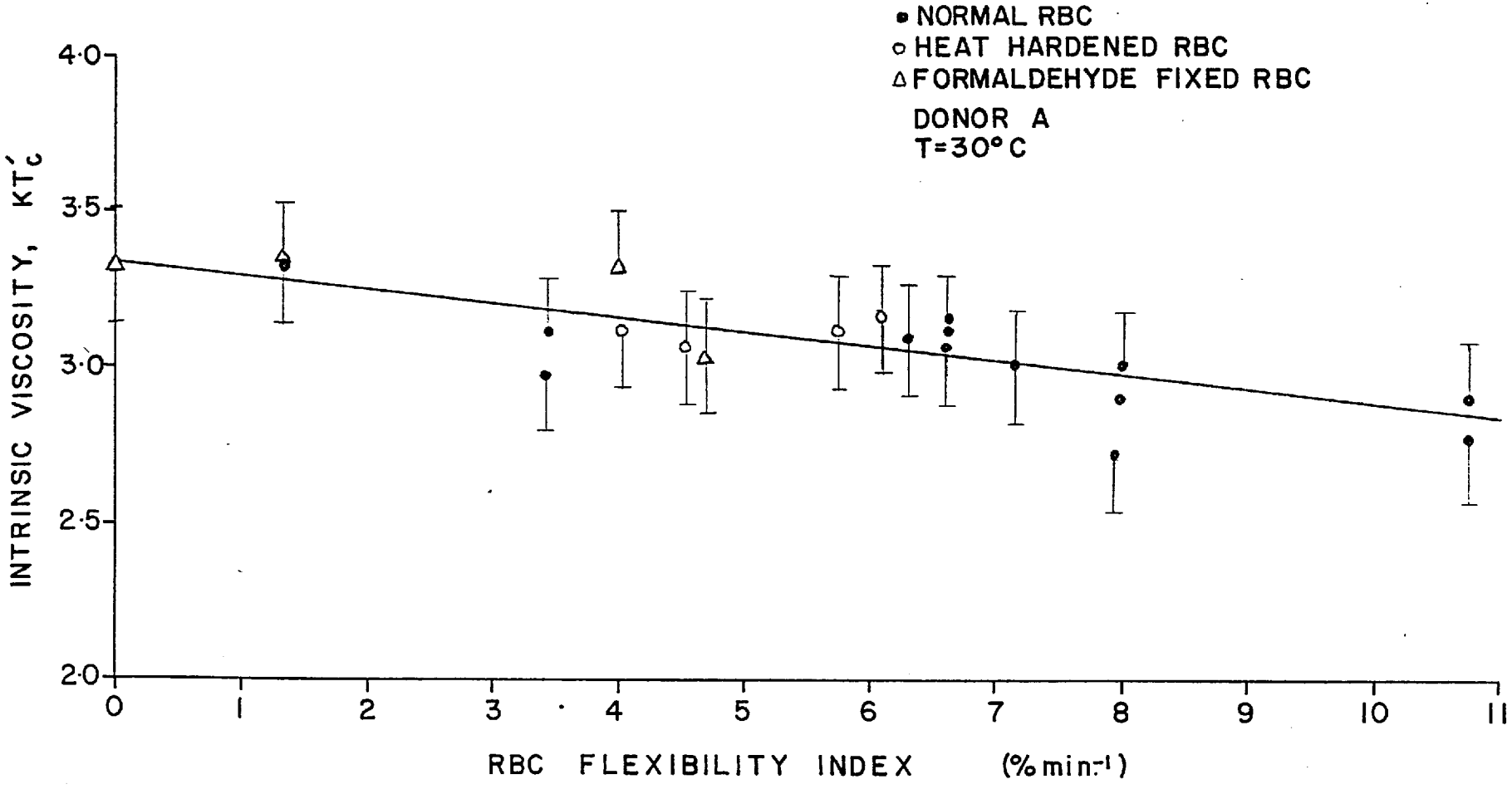


FIG. 70 : INTRINSIC VISCOSITY AT DIFFERENT RBC FLEXIBILITIES FOR DONOR A AT SHEAR RATE OF 230s^{-1}

By means of plasma dilution of blood samples with autologus serum to reduce the red cell flexibility, and addition of the human fibrinogen/Ringer-Locke solution to increase Φ , and hence flexibility, the values of KT'_c for varying red cell flexibility index are obtained for donors R, S, B and C. The results are shown on Figs. (71) to (74) respectively. All the donors used are healthy male adults. The shape factor, K, obtained from the value of KT'_c at zero flexibility by intercept of regression line, for donors R, S, B and C are 3.31, 3.22, 3.32 and 3.2 respectively.

Horse red cell flexibility can only be varied by suspending the red cells in plasma, serum or saline. Attempts were made to reduce the horse red cell flexibility by fixing the cells in varying concentrations of formaldehyde, as for human cells, and then resuspending in serum or plasma. As soon as the cells were resuspended in serum or plasma they regained their original flexibility in that suspending medium. Only the 2% formaldehyde concentration enabled the fixing of the red cells, making them inflexible. Using the four different values of red cell flexibilities for horse red cells, the values of KT'_c at the different flexibilities were obtained, Fig. (75). Considering red cell suspensions in plasma, serum and saline, the value of K, obtained as the intercept of the line at zero flexibility, would be 3.1. However, when the red cells were fixed in isotonic (pH = 7.4) 2% formaldehyde/Ringer-Locke solution, to which human

INTRINSIC VISCOSITY KT_c' AT DIFFERENT RED CELL FLEXIBILITY INDEX VALUES FOR NORMAL HUMAN RBC

TABLE 63
FOR FIGURE 71

DONOR R

RED CELL FLEX. INDEX (% min^{-1})	1	1.7	2.92	3.5	4.45	4.775	6.32	6.525	6.625
INTRINSIC VISCOSITY KT_c'	3.3	3.23	3.14	3.25	3.07	3.14	3.08	3.0	3.09

REGRESSION EQUATION:-
$Y = -0.0404X + 3.3142$

CORRELATION COEFFICIENT:-
-0.863

TABLE 64
FOR FIGURE 72

DONOR S

RED CELL FLEX. INDEX (% min^{-1})	2.03	4.57	6.1	7.374	7.7	11	14.94
INTRINSIC VISCOSITY KT_c'	3.28	3.0	3.13	3.01	3.04	2.98	2.93

REGRESSION EQUATION:-
$Y = -0.0219X + 3.221$

CORRELATION COEFFICIENT:-
-0.79

TABLE 65
FOR FIGURE 73

DONOR B

RED CELL FLEX. INDEX (% min^{-1})	1.5	2.525	3.25	3.95	3.95	4.4	6.85	6.85	9.75
INTRINSIC VISCOSITY KT_c'	3.265	3.23	3.175	3.16	3.2	3.0	3.01	3.16	2.88

REGRESSION EQUATION:-
$Y = -0.0418X + 3.322$

CORRELATION COEFFICIENT:-
- 0.841

TABLE 66
FOR FIGURE 74

DONOR C

RED CELL FLEX. INDEX (% min^{-1})	0.4	1.224	2.075	3.38	4.84	5.55	7.775
INTRINSIC VISCOSITY KT_c'	3.27	3.02	3.2	3.27	3.06	2.8	3.14

REGRESSION EQUATION:-
$Y = -0.0247X + 3.198$

CORRELATION COEFFICIENT:-
-0.387

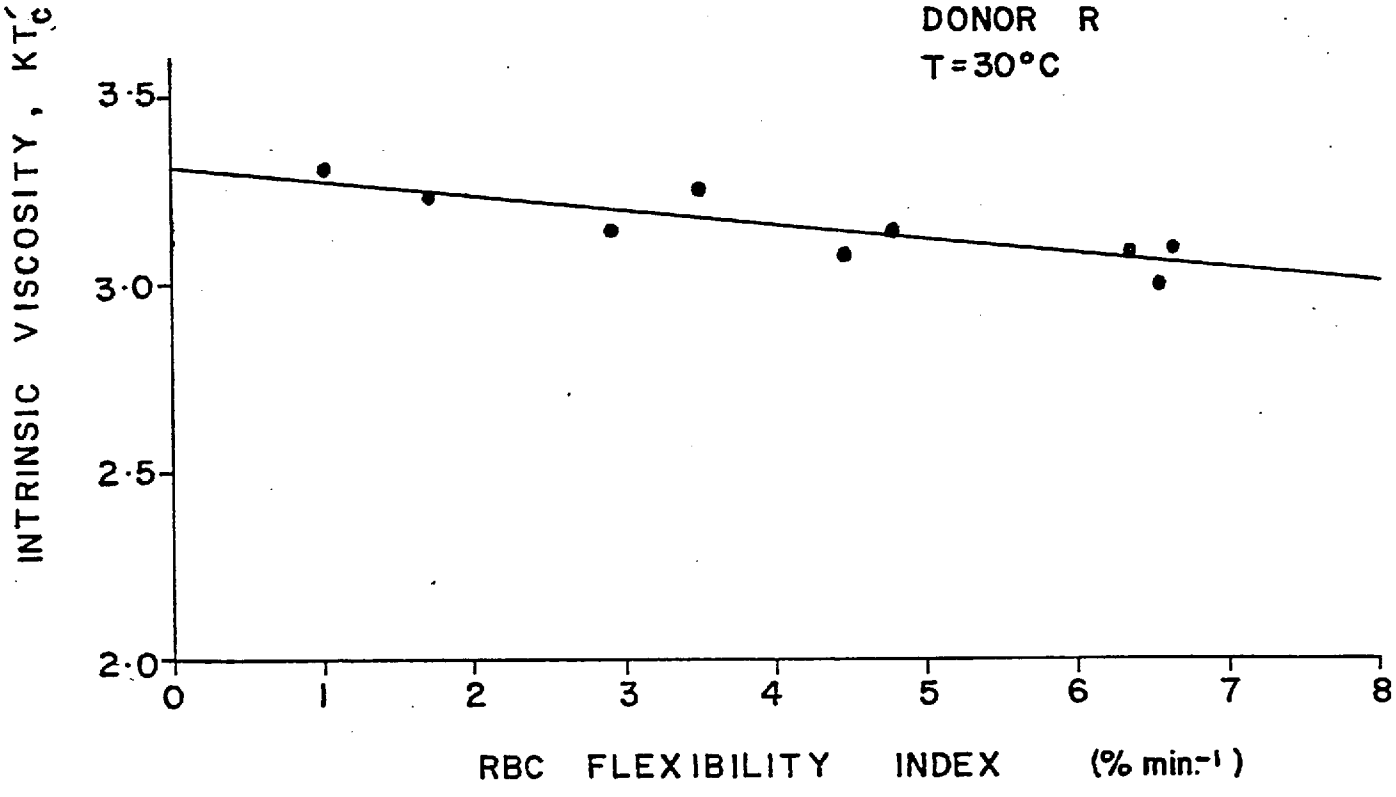


FIG. 71 : INTRINSIC VISCOSITY AT DIFFERENT RBC FLEXIBILITIES FOR DONOR R AT SHEAR RATE OF 230s^{-1}

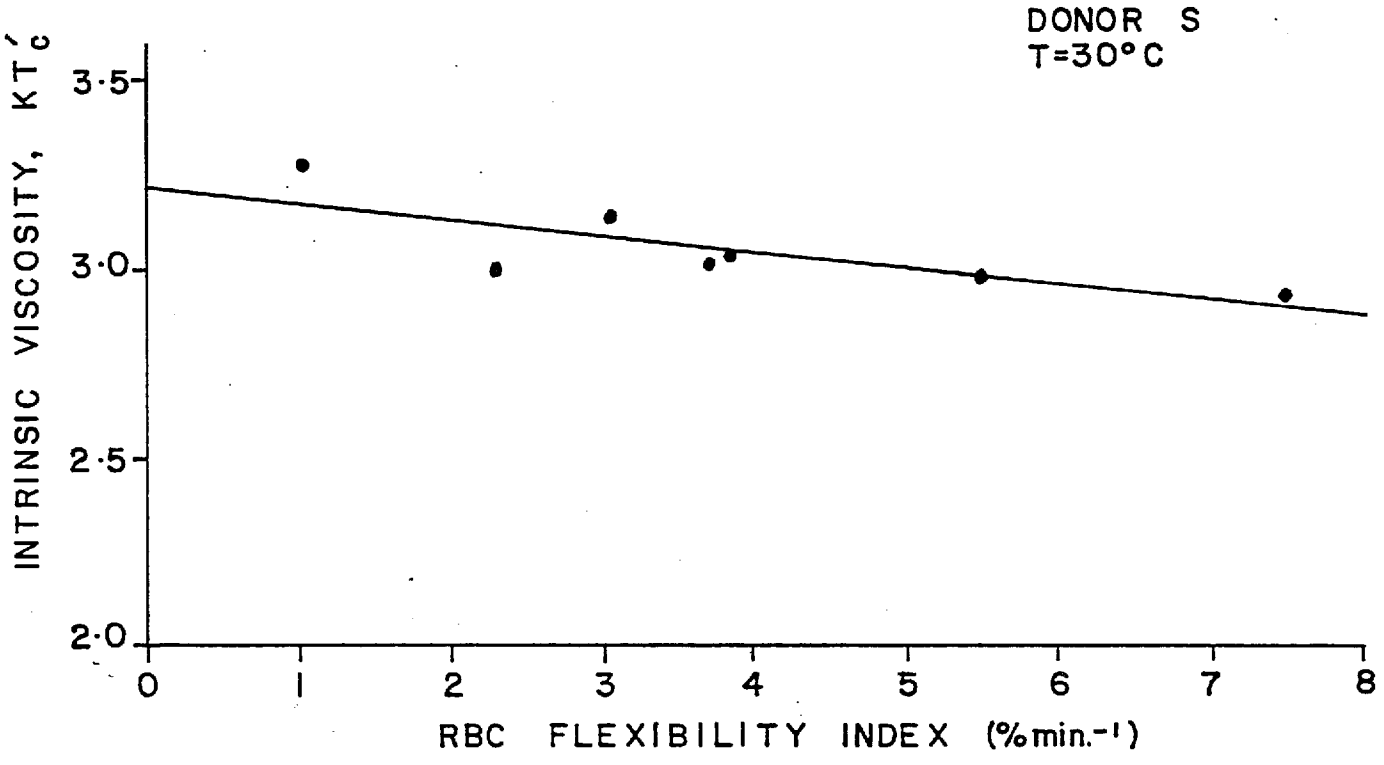


FIG. 72 : INTRINSIC VISCOSITY AT DIFFERENT RBC FLEXIBILITIES FOR DONOR S AT SHEAR RATE OF 230s^{-1}

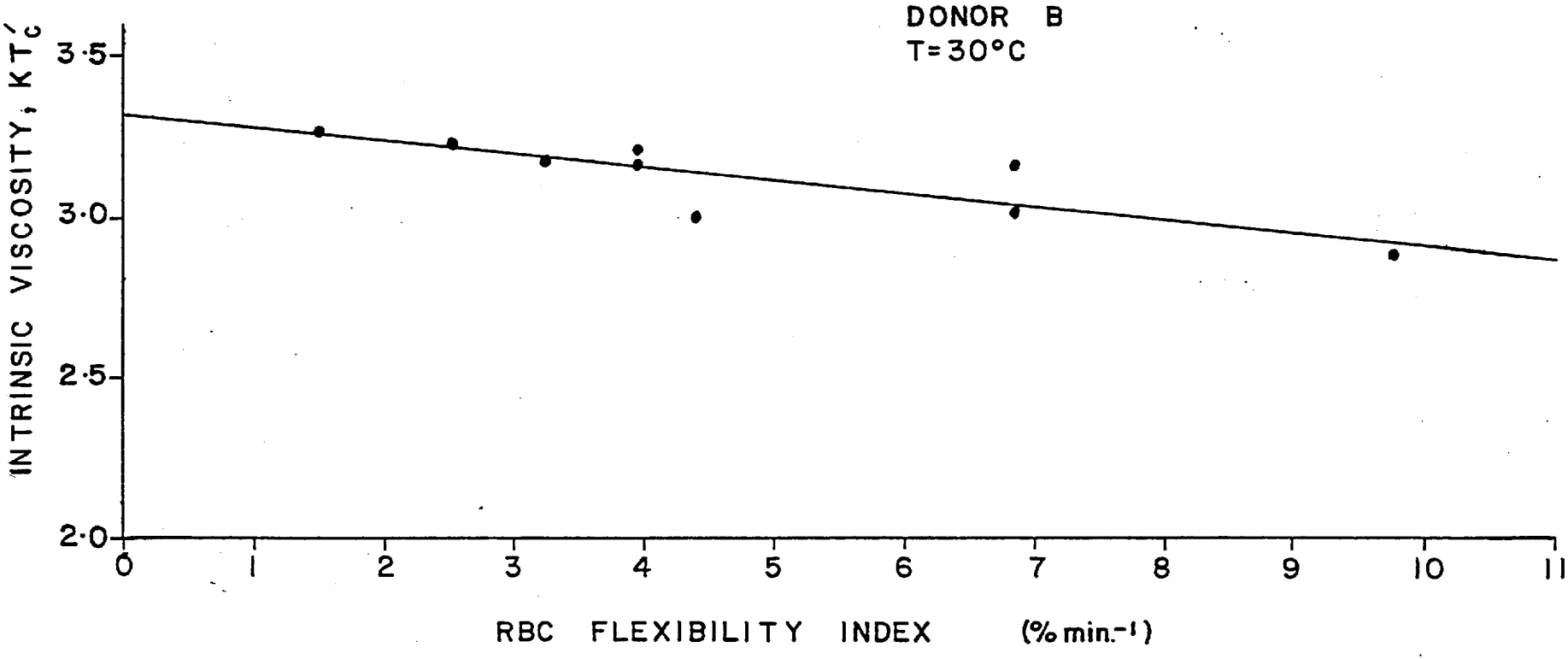


FIG. 73 : INTRINSIC VISCOSITY AT DIFFERENT RBC FLEXIBILITIES FOR DONOR B AT SHEAR RATE OF $230s^{-1}$

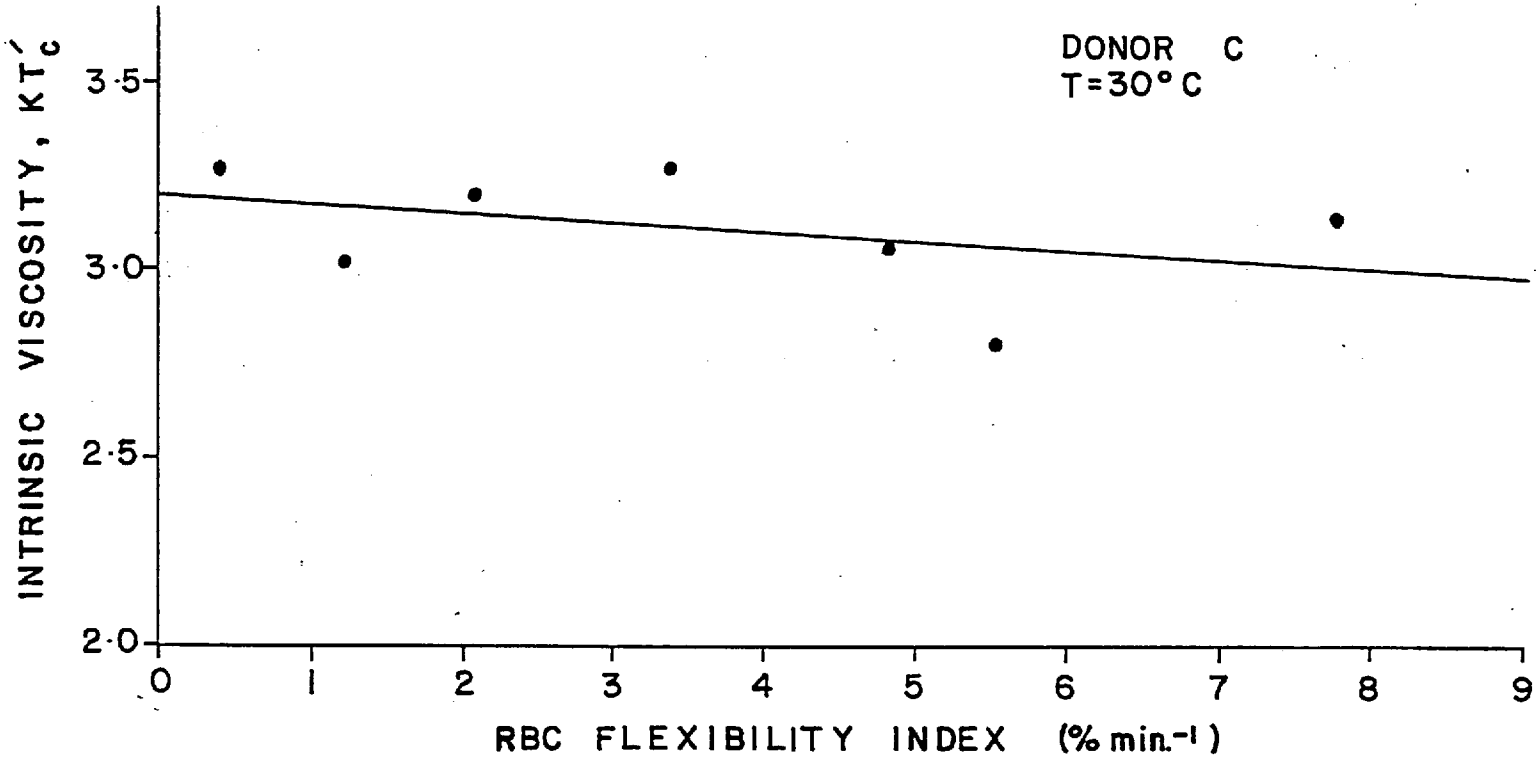


FIG. 74 : INTRINSIC VISCOSITY AT DIFFERENT RBC FLEXIBILITIES FOR DONOR C AT SHEAR RATE OF $230s^{-1}$

	RBC FLEX. INDEX (% $min.^{-1}$)	INTRINSIC VISCOSITY KT'_c
RED CELLS IN PLASMA	42.5	3.1, 3.12
RED CELLS IN SERUM	21.75	3.078, 3.115
RED CELLS IN SALINE	2.2	3.18, 3.21
FORMALDEHYDE FIXED RED CELLS	0.0	3.80, 3.81

ERROR IN FLEXIBILITY INDEX = 0.5% min^{-1}

ERROR IN KT'_c = ± 0.2

TABLE 67 FOR FIGURE 75

INTRINSIC VISCOSITY KT'_c AT DIFFERENT RED CELL FLEXIBILITY INDEX OF HORSE RED CELLS IN PLASMA, SERUM, SALINE AND FIXED IN 2% FORMALDEHYDE, AT 30°C AND 230s^{-1}

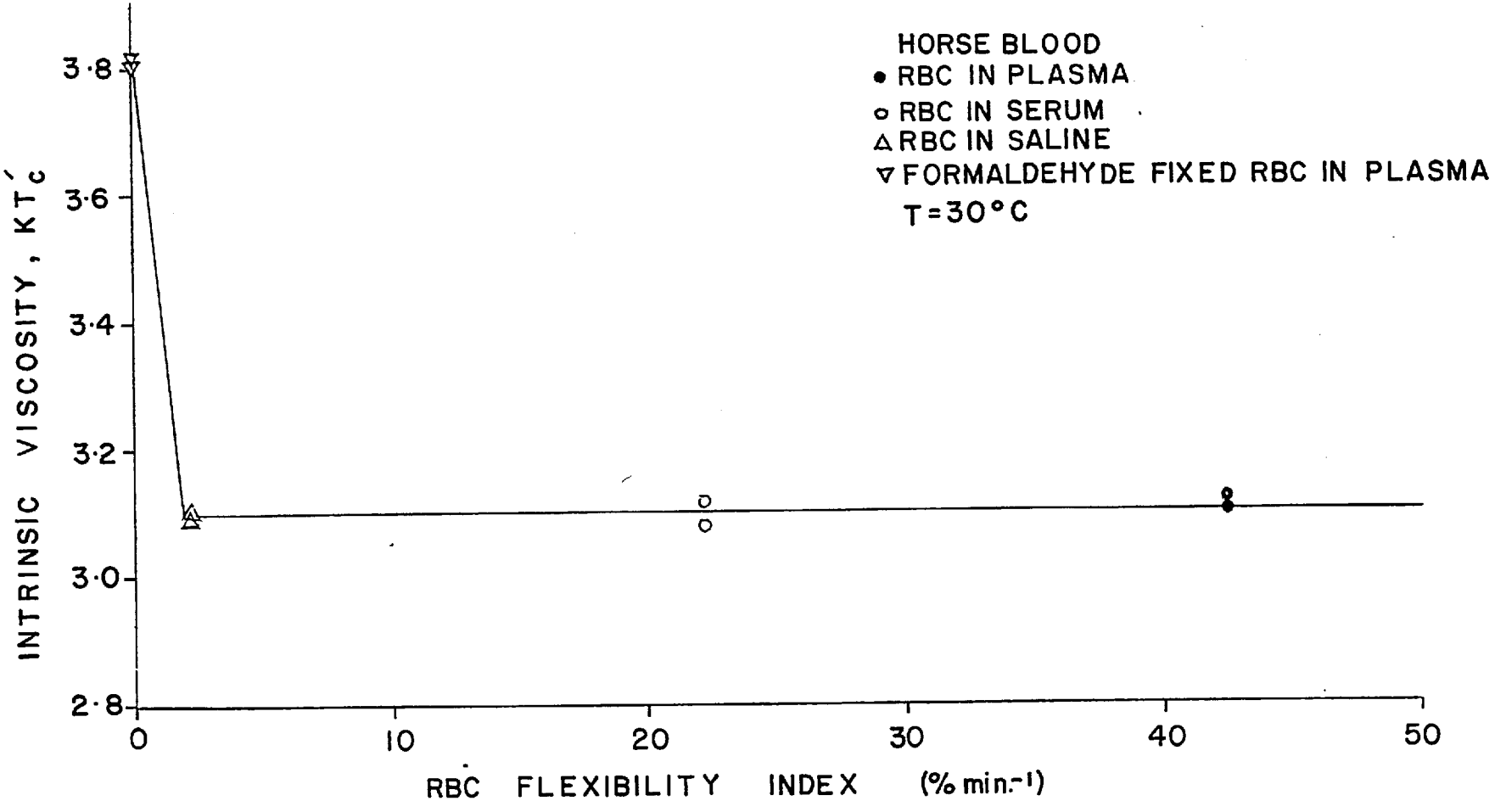


FIG. 75 : HORSE INTRINSIC VISCOSITY FOR RBC IN PLASMA, SERUM, SALINE AND FIXED CELLS AT SHEAR RATE OF $230s^{-1}$ AND 30°C

Albumin (Kabi) was added to a concentration of 2 $mg.ml.^{-1}$, and the red cells were washed and resuspended in autologus plasma, the value of K obtained was 3.8.

For sheep, cattle, goat and camel, the red cell flexibilities are low in value and are unaffected by changes in Φ , not even for $\Phi = 0$ (serum). If KT'_c is plotted against red cell flexibility, only one point per specie is obtained. Although the red cell flexibility is related to Φ by a zero gradient line in the case of the four species, plotting values of KT'_c against Φ would give several points to which a line could be plotted. A zero flexibility value of KT'_c was obtained for each specie by fixing the red cells in isotonic (pH = 7.4) 1% formaldehyde/Ringer-Locke/Albumin solution and then washing the cells and resuspending in autologus serum. Figs. (76) to (79) show the values of KT'_c obtained for normal and formaldehyde fixed red cells at varying Φ , for sheep, cattle, goat and camel respectively. The respective values of K are 2.86, 2.94, 3.1 and 2.9.

Microscopic observation revealed that the red cells of all the species investigated, except for the camel red cells, are round biconcave in shape, but differe in size and volume. The camel red cells are flat, oval and slightly biconcave in shape.

PLASMA FIB. CONC., Φ ($mg.ml.^{-1}$)	0	0.536	0.765	1.36	1.6	2.89	3.04	3.19
INTRINSIC VISCOSITY KT'_c	2.86	2.85	2.68	2.89	3.0	2.82	2.87	2.77

KT'_c FOR RED CELLS FIXED IN 1% FORMALDEHYDE = 2.9

ERROR IN MEASUREMENT OF Φ = $\pm$ 0.1 $mg.ml.^{-1}$

ERROR IN MEASUREMENT OF KT'_c = $\pm$ 0.2

TABLE 68 FOR FIGURE 76

SHEEP RED CELLS INTRINSIC VISCOSITY, KT'_c, AT DIFFERENT PLASMA FIBRINOGEN CONCENTRATIONS, Φ, AND 1% FORMALDEHYDE FIXED RED CELLS IN SERUM AT 30°C AND $\gamma = 230s^{-1}$

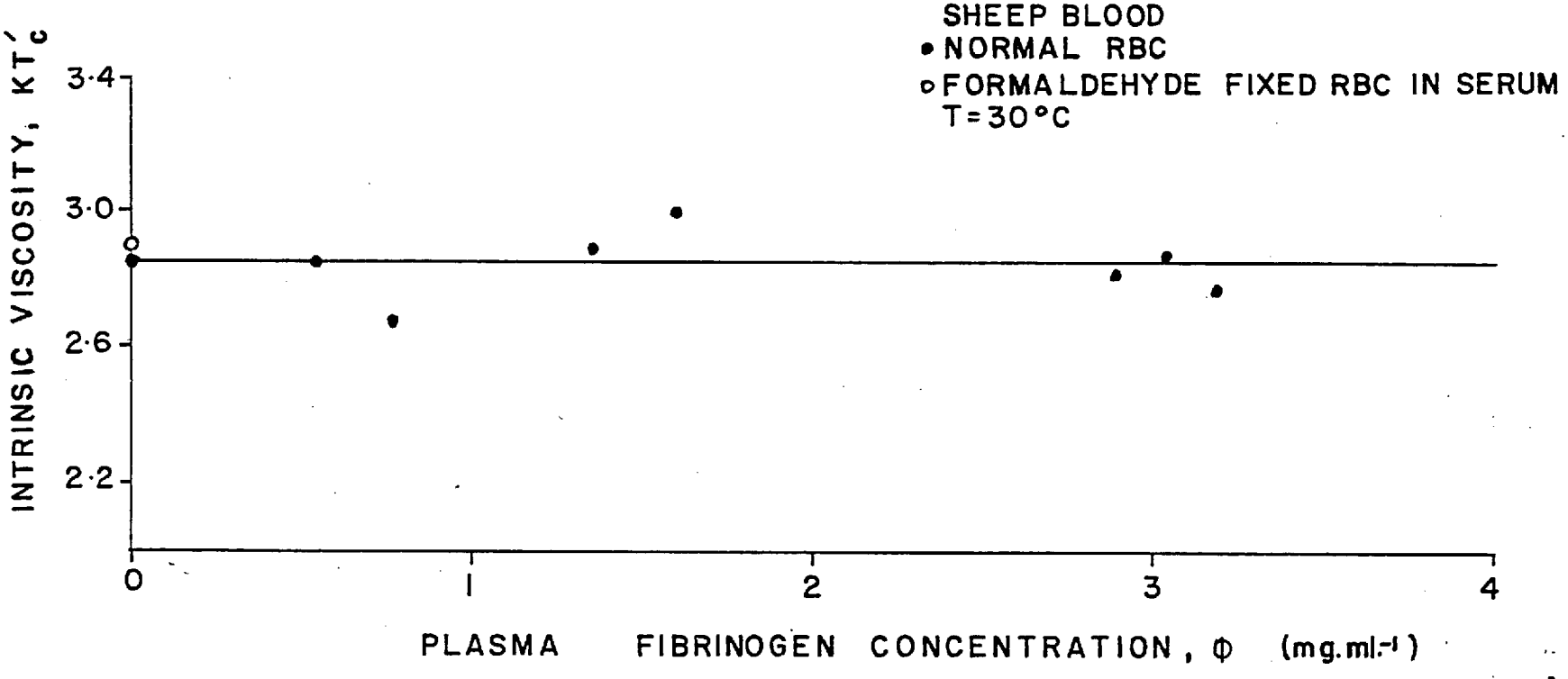


FIG. 76 : SHEEP INTRINSIC VISCOSITY AT DIFFERENT PLASMA FIBRINOGEN CONCENTRATIONS AND SHEAR RATE OF $230s^{-1}$ AND 30 C

PLASMA FIB. CONC., ($mg.ml.^{-1}$)	0	1.354	2.81	2.86	3.31	3.63	4.57	4.64
INTRINSIC VISCOSITY KT'_c	2.95	2.92	2.87	3.07	2.83	3.12	2.99	2.82

KT'_c FOR RED CELLS FIXED IN 1% FORMALDEHYDE = 2.97

ERROR IN MEASUREMENT OF Φ = $\pm$ 0.1 $mg.ml.^{-1}$

ERROR IN MEASUREMENT OF KT'_c 0 $\pm$ 0.2

TABLE 69 FOR FIGURE 77

CATTLE RED CELLS INTRINSIC VISCOSITY KT'_c AT DIFFERENT PLASMA FIBRINOGEN CONCENTRATIONS, Φ, AND 1% FORMALDEHYDE FIXED RED CELLS IN SERUM AT T 0 30°C AND $\gamma = 230s^{-1}$

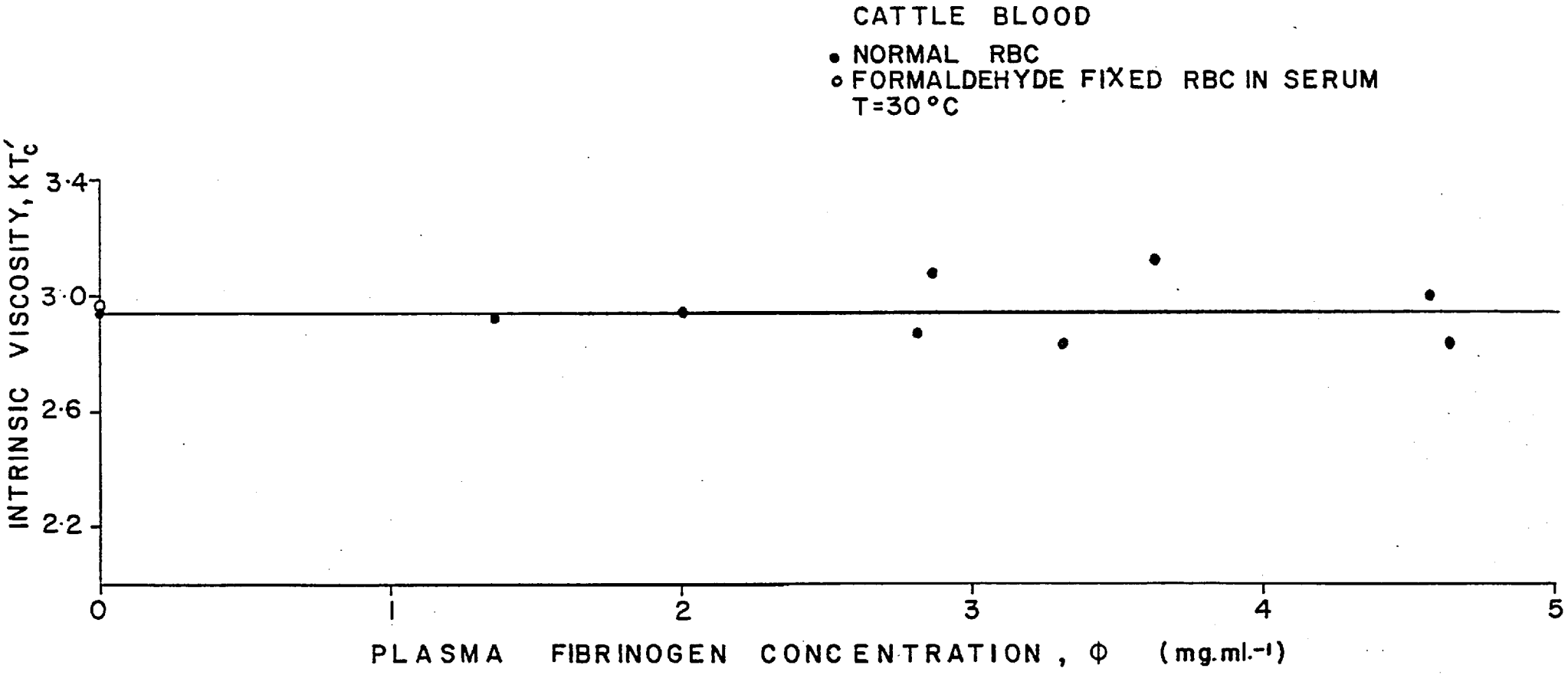


FIG. 77 : CATTLE INTRINSIC VISCOSITY AT DIFFERENT PLASMS FIBRINOGEN CONCENTRATIONS AND SHEAR RATE OF $230s^{-1}$ AND 30°C

PLASMA FIB. CONC., Φ ($mg.ml.^{-1}$)	0	1.375	2.6	3.25	3.4	3.9	4.23
INTRINSIC VISCOSITY KT'_c	3.1	3.05	3.2	2.9	3.05	2.85	3.12

KT'_c FOR RED CELLS FIXED IN 1% FORMALDEHYDE = 3.17

ERROR IN MEASUREMENT OF Φ = $\pm$ 0.1 $mg.ml.^{-1}$

ERROR IN MEASUREMENT OF KT'_c = $\pm$ 0.2

TABLE 70 FOR FIGURE 78

GOAT RED CELLS INTRINSIC VISCOSITY, KT'_c, AT DIFFERENT PLASMA FIBRINOGEN CONCENTRATIONS, Φ, AND 1% FORMALDEHYDE FIXED RED CELLS IN SERUM AT T = 30°C and γ = $230s^{-1}$

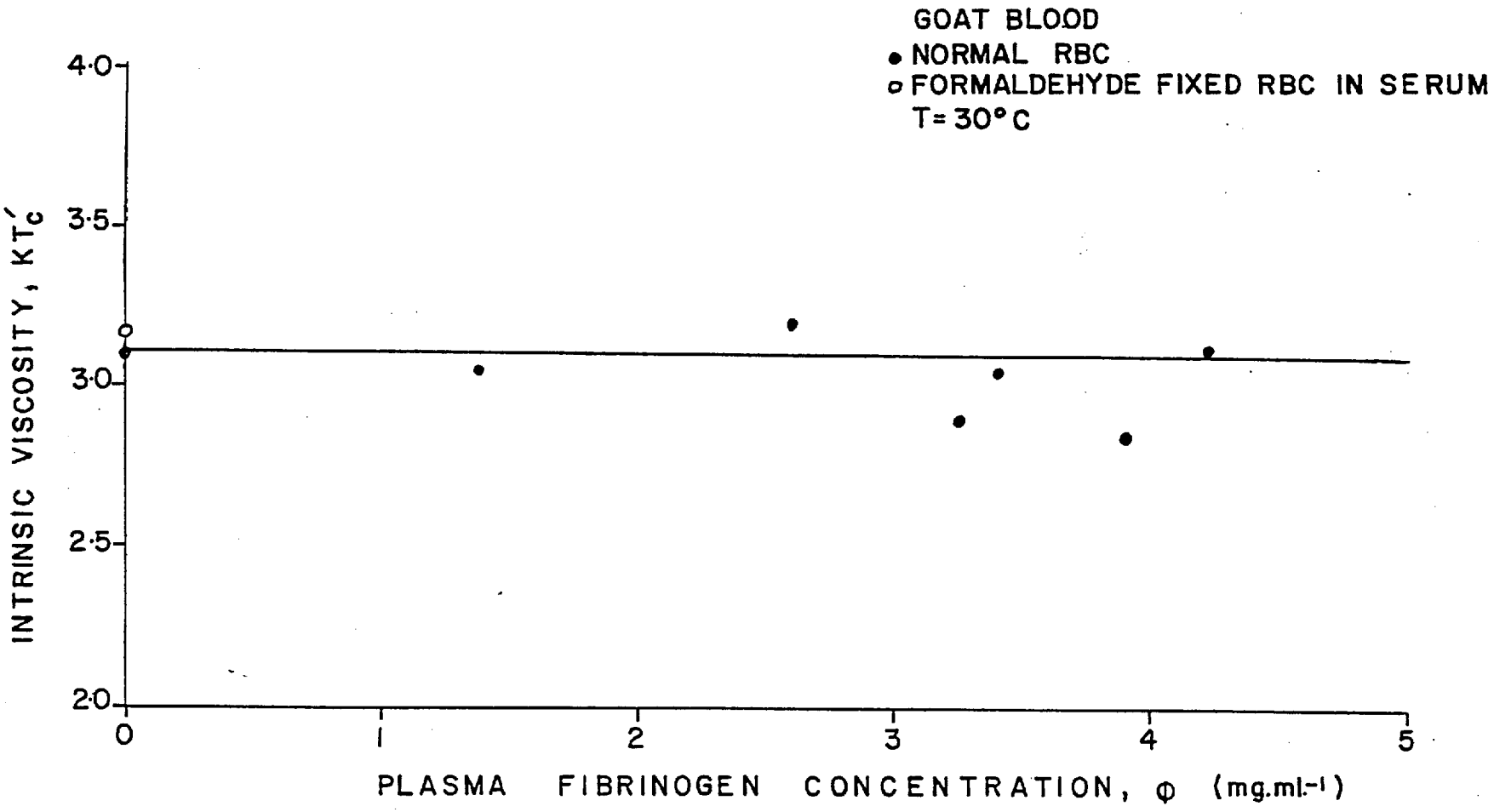


FIG. 78 : GOAT INTRINSIC VISCOSITY AT DIFFERENT PLASMA FIBRINOGEN CONCENTRATIONS AND SHEAR RATE OF $230s^{-1}$ AND 30°C

PLASMA FIB. CONC., Φ ($mg.ml.^{-1}$)	0	0.675	1.075	1.55
INTRINSIC VISCOSITY KT'_c	3.04 2.81	2.91 2.87	2.96 2.86	2.91 2.81

KT'_c FOR RED CELLS FIXED IN 1% FORMALDEHYDE = 2.9

ERROR IN MEASUREMENT OF Φ = $\pm$ 0.1 $mg.ml.^{-1}$

ERROR IN MEASUREMENT OF KT'_c = $\pm$ 0.2

TABLE 71 FOR FIGURE 79

CAMEL RED CELLS INTRINSIC VISCOSITY, KT'_c, AT DIFFERENT PLASMA FIBRINOGEN CONCENTRATIONS, Φ, AND 1% FORMALDEHYDE FIXED RED CELLS IN SERUM AT T = 30°C AND γ = $230s^{-1}$

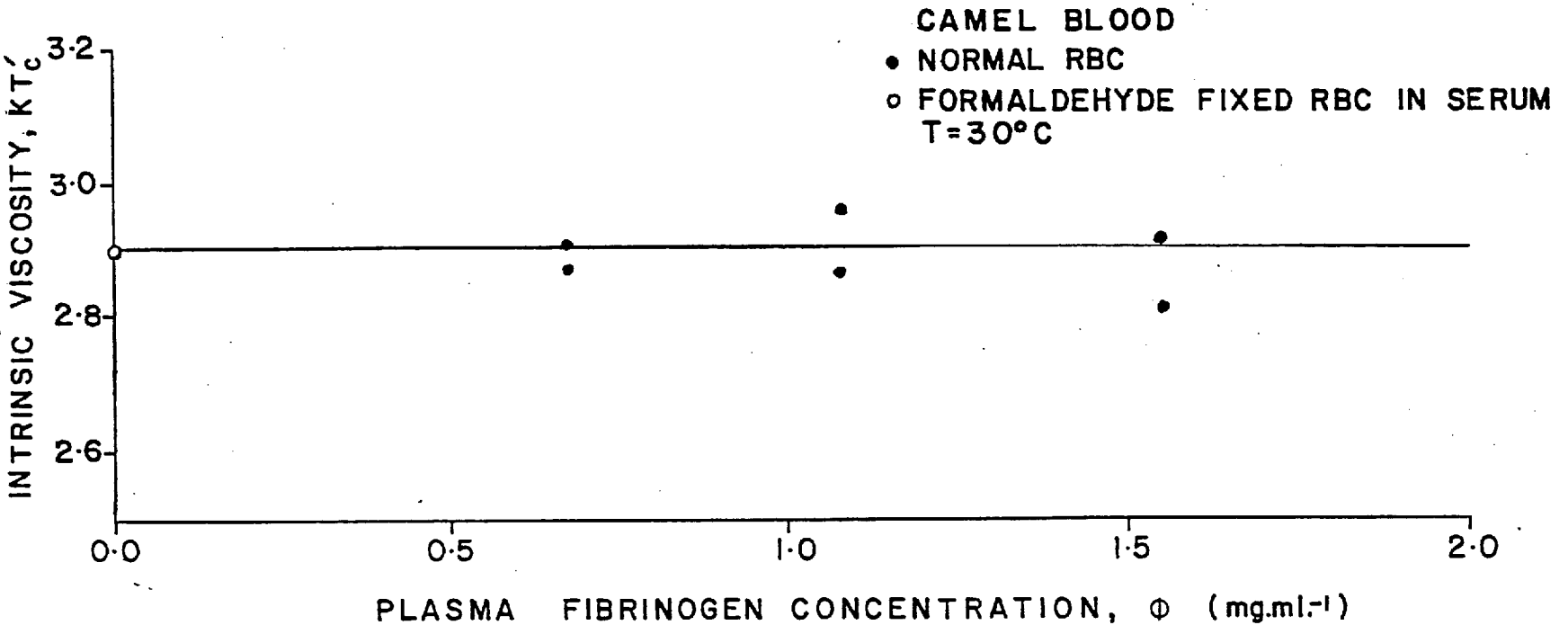


FIG. 79 : CAMEL INTRINSIC VISCOSITY AT DIFFERENT PLASMA FIBRINOGEN CONCENTRATIONS AND SHEAR RATE OF 230s^{-1} AND 30°C

Table (72) shows some of the data obtained for the six species, for comparative purposes.

IV DISCUSSION

For any plasma fibrinogen concentration, Φ , greater than 0.5 mg.ml.$^{-1}$, horse red cell flexibility index is very high, 42.5% min^{-1} at 30^{o}C. In serum, the red cell flexibility index is halved to 21.75% min^{-1}. For all values of Φ at 30^{o}C, the red cell flexibility index of sheep, cattle, goat and camel remain unchanged at 0.7, 0.88, 0.75 and 3.75% min^{-1} respectively. Human cells however, are inflexible in serum and increase their flexibility index linearly with increasing Φ . In this respect, the flexibility of the human red blood cell represents the intermediate range between the high extreme of horse erythrocytes, and the low extreme of sheep, cattle, goat and camel erythrocytes.

In human blood, we have already seen, Chapter 4, that as Φ increases, the effects of the corresponding increase in η_p and red cell flexibility on η_B, resulted in a compensatory process between η_p and the red cell flexibility taking place to keep η_B unaltered. Although η_p, in all the species investigated,

RHEOLOGICAL PARAMETERS FOR BLOOD OF MAN, HORSE, SHEEP, CATTLE, GOAT AND CAMEL

	MAN	HORSE	SHEEP	CATTLE	GOAT	CAMEL
RED CELL FLEXIBILITY INDEX (% min^{-1}) T = 30°C	6.75	42.5	0.7	0.88	0.75	3.75
NATURAL HAEMATOCRIT RANGE (%)	42-47	28-35	28-35	28-35	35-40	33-38
WHOLE BLOOD VISCOSITY η_B (cP); $\dot{\gamma} \cong 2305^{-1}$; T = 30°C	5.5 (H=45%)	4.3 (H=30%)	3.8 (H=30%)	3.82 (H=30%)	4.5 (H=36.5%)	4.5 (H=35%)
WHOLE BLOOD VISCOSITY η_B (cP); $\dot{\gamma} \cong 2305^{-1}$, T = 37.5°C	4.7	4.05	3.56	3.83	4.25	4.1
PLASMA VISCOSITY η_p (cP); $\dot{\gamma} = 2305^{-1}$, T = 30°C	1.475	2.1	1.85	1.9	1.4	1.5
PLASMA VISCOSITY η_p (cP); $\dot{\gamma} = 2305^{-1}$, T = 37.5°C	1.4	1.48	1.4	1.62	1.3	1.35
PLASMA FIBRINOGEN CONCENTRATION, ϕ (mg ml^{-1})	3.0	3.8	3.6	3.9	3.8	1.55
MCV (fl)	85	43	54	54	18	54
RED CELL SHAPE FACTOR, K	3.3	3.8	2.86	2.94	3.1	2.9

TABLE 72

increased with ϕ , the absence of an effect of ϕ on the red cell flexibility resulted in η_B increasing with ϕ .

In normal human whole blood, the presence of fibrinogen makes the red cells flexible. This flexibility decreases the whole blood viscosity, η_B, at high shear rates, γ ($230s^{-1}$), and increases η_B at low γ ($<46s^{-1}$) due to red cell aggregation and rouleaux formation. When the red cells are placed in serum, η_B at high γ is unaltered because the decrease in red cell flexibility is compensated for by fall in the value of η_p. At low γ , however, η_B does not increase significantly due to a decrease in red cell aggregation as a result of decreased flexibility and absence of fibrinogen.

Sheep, cattle, goat and camel red cells are relatively inflexible and their flexibility is unaffected by ϕ . At high γ , their red cell inflexibility increases their value of η_B, compared to η_B for human blood. Microscopic observation of sheep, cattle, goat and camel erythrocytes in whole blood revealed no aggregation present, Richter (1966) (for sheep and cattle). This can be seen from measurements of η_B at low γ , Fig. (67). For these four species, η_B does not increase greatly as γ is lowered, compared to human blood, indicating the absence of cell-protein interaction, i.e. aggregation. The slight increase in η_B at low γ is due to cell-cell interaction at low shear stress.

The very flexible horse red cells are expected to possess a lower value of η_B, at high γ, than for human blood. However, what in fact is observed is the reverse. Horse red cells have a very high degree of aggregation in whole and defibrinated blood, Fahraeus (1921 and 1929). This high degree of aggregation is able to resist shear rates of $230s^{-1}$ and needs a higher value of γ to break the aggregates to single cells. Fig. (68) shows that when horse whole blood is defibrinated, the decrease in red cell flexibility increases the value of η_B at high γ and slightly decreases it at low γ. When the cells are washed and resuspended in saline, they become relatively inflexible, increasing η_B at high γ, Fig. (68). At low γ, η_B decreases below that for whole and defibrinated blood, but remains significantly higher than its value at high γ. This means that in the absence of plasma proteins, there are strong interactions between horse red cells at low shear stress. These interactions are either cell-cell interactions or cell aggregation, or both. This, therefore, indicates that at low γ or shear stress, τ, cell-protein interactions are not the only or dominant process in red cell aggregation. The red cell flexibilities for red cells in saline suspensions, at $30^{o}C$ are 1.2, 4.0, 0.6, 0.85, 0.69 and 1.95% min^{-1} for man, horse, sheep, cattle, goat and camel respectively. Horse red cells are therefore relatively flexible in saline suspension, compared to the other species.

In a stable dispersion of one immiscible liquid in another, i.e. an emulsion, there is an interface between the two liquids. Since this interface has surface tension, the free energy of the system would be decreased if the emulsion droplets were to 'come together' to form droplets, or groups of droplets, of a smaller total surface area (Gibbs free energy theory). In a similar way to an emulsion, for horse red cells suspension in saline, the flexible red cells would decrease the total thermodynamic (system) energy, at low γ, by the cells forming aggregates or coming close together. The cell membrane surfaces would touch and interact through weak forces (e.g. Van der Waals forces) known as cell-cell interaction. The more flexible the membrane is, the greater the membrane surface area involved in the interaction, and hence the lower is the system energy state. The formation of clumps or aggregates, to reduce the system energy state, would increase the value of the suspension viscosity, η_B. Therefore, in the energy equation of a red cell suspension, given by Chien et al (1981), an extra term is needed to represent the attractive cell-cell interaction which act to reduce the energy state of the suspension, especially at low shear rates.

Heating, without sphering, and formaldehyde fixing of the red cells alters the red cell flexibility but does not change its shape. This is shown by the values of KT'_c, for heated and formaldehyde fixed red cells,

fitting on the same curve as the value of KT'_c for normal cells, Fig. (70), where the flexibility was altered by varying Φ, also Sirs (1981). For the five healthy adult male donors, the shape factor, K, varied in value between 3.2 and 3.34. The sheep, cattle and camel red cells were found to have equal MCV, 54 fl. (Table 72), and approximately equal value of K of 2.9. This is in spite of the fact that sheep and cattle red cells are biconcave discoidal in shape whereas camel cells are biconcave prolate ellipsoids in shape. The slightly higher red cell flexibility index of camel red cells, compared to that of sheep and cattle, is suspected to be due to the camel red cell being able to bend under force more easily because of its flat elliptical shape, rather than due to the cell membrane flexibility. This is supported by the findings that, at the same haematocrit and temperature, η_B for camel blood is greater than that for sheep or cattle at all shear rates, Fig. (67), although η_p for camel is lowest for the three species, Table (72). Hence the expected lower red cell flexibility for camel cells would increase the value of T'_c beyond that for sheep and camel, making the product KT'_c, in equation 6.1, greater for camel than for sheep or cattle bloods, under the same conditions. The greater value of KT'_c would give the observed higher value of η_B, Fig. (67).

Due to cattle red cells being slightly more flexible than sheep red cells, the value of T'_c for cattle would be slightly lower than for sheep. However, the K value

for cattle is slightly higher than for sheep. Therefore the product KT'_c would result in similar values of η_B as observed in Fig. (67).

The smaller goat red cells, with an MCV of 18 fl. and similar red cell flexibility index to sheep and cattle, has a higher K value of 3.1. The product KT'_c for goat blood would therefore be greater in value than that for sheep or cattle and therefore, would also have a greater value of η_B. This agrees with measured values of η_B for the three species, under similar conditions, Fig. (67).

Human red cells are more flexible than sheep, cattle, goat or camel red cells, and therefore have a lower value of T'_c. However, the K value of 3.4 is greater than that for the above-mentioned species. Here again we find that the increase in K is compensated for by the decrease in T'_c in the product KT'_c. At high shear rates, where no aggregation is present, the value of η_B is lower than for the above-mentioned species, Fig. (67). Possibly, this is because the decrease in T'_c is greater than for the other species, while at the same time the increase in K is not sufficient to increase KT'_c beyond that of the other species. This results in the lower value of KT'_c and hence η_B for human blood.

When the shear rate, γ, is decreased, human red cells tend to form aggregates and rouleaux. The degree of aggregation and rouleaux formation increases for

decreasing γ, below $\gamma = 46s^{-1}$, Schmidt-Schonbein (1968). In the absence of rouleaux, aggregates can result in a decrease in the value of K because aggregates of red cells can have a more spherical shape than individual red cells. However for large aggregates and rouleaux, where irregularities in shape exist, K is increased in value. When γ is decreased the increasing degree of aggregation increases K which in turn increases the value of η_B. When γ is decreased enough for rouleaux to form, K increases rapidly, increasing η_B rapidly as observed for $\gamma < 11.5s^{-1}$.

For horse blood, red cell aggregation is present even at high γ used ($230s^{-1}$). When horse red cells are fixed by formaldehyde, so as to become inflexible, the shape factor, K, was found to be 3.8. When normal cells are suspended in plasma, i.e. whole blood, the cells become very flexible, reducing the value of T'_c. When the cells are suspended in serum, the cell flexibility is reduced and T'_c is, therefore, increased. Similarly, by suspending the cells in saline, the cell flexibility is reduced further and T'_c is increased further, compared to cells in plasma and serum. Since the values of KT'_c for plasma, serum and saline red cells suspensions are equivalent, at 3.1, Fig. (75), this means that K has changed for each suspension. The value of K for zero cell flexibility is 3.8. If we assume that the decrease in KT'_c, from 3.8 at zero flexibility, to 3.1 for saline suspension is not due to a change in K but is due to the decrease in T'_c, since the cells are more flexible, then

the value of K must have increased for the serum, and further for the plasma, suspension. Since the change in K is not due to a change in γ, therefore, it must be due to increasing red cell aggregation in serum and further in plasma. The increasing aggregation is a result of increasing red cell flexibility, which results in the shape of the aggregates becoming more irregular in shape, hence increasing K.

V CONCLUSIONS

When human blood is compared to bloods from other mammalian species, there are several differences in the rheological parameters. As was shown, the red cell flexibility for sheep, cattle, goat and camel is low and is unaffected by changes in Φ. Horse red cells are very flexible in serum and the flexibility is doubled in the presence of fibrinogen. Changes in Φ, above $\Phi = 0$, does not alter the horse red cell flexibility. Human red cells, on the other hand, have low flexibility in serum, but increasing Φ increases the flexibility in a linear relationship.

Red cell aggregation is absent in sheep, cattle, goat and camel bloods. In horse whole and defibrinated bloods, red cell aggregation is present to a high degree. In human serum, red cell aggregation is absent,

but is present in the presence of fibrinogen and increases in degree with ϕ . Therefore, in terms of red cell flexibility and aggregation, human blood lies in the middle range between the high extremes of horse and low extremes of the other species bloods.

From the discussion, in section IV, it was shown that although there are differences in the red cell shape factor, K, and red cell flexibility factor, T'_c, between the different species, they interact and compensate for each others differences in determining η_B, derived by the Taylor equation. These interactions and compensatory processes explain the observed differences and similarities in η_B for the different species. From Table (72), it can be seen that as a result of the differences in rheological parameters, the natural haematocrits in the five species, other than man, are lower. This decrease results in similar values of η_B for all the species.

Sirs (1966) has shown that there is an inter-relation between red cell flexibility and the erythrocyte respiratory function, with the respiratory efficiency decreasing with inflexibility. The reason being that the more flexible the erythrocyte, the greater is the degree of haemoglobin mixing within the erythrocyte. This means that from the red cell flexibility index we would expect horse red cells to have the greatest respiratory function, followed, in order, by man, camel,

cattle, goat and lastly sheep. There is, however, a compensatory factor in the latter species, in that the cells are smaller than in humans.

If the human erythrocyte is represented by a semi-infinite layer of haemoglobin solution, of the same concentration as the erythrocyte content, and is enclosed between two membranes, of the same permaeability to gases as the erythrocyte membrane, the half thickness of this layer can be calculated from the gas diffusion equation developed by Roughton (1959). The half thickness of this layer was calculated to be 9.85×10^{-5} cm. for human erythrocytes, Sirs (1963b), and 7×10^{-5} cm. for sheep erythrocytes, Sirs (1963a). Hence the smaller size, MCV, of the sheep erythrocyte would increase the respiratory function compared to the larger human erythrocyte. This increase, due to the smaller MCV of sheep erythrocyte, compensates for the decrease in respiratory function due to the erythrocyte inflexibility in the species, other than man and horse, investigated in this study.

CHAPTER 7

STUDIES ON PROTEINS AFFECTING THE RED CELL FLEXIBILITY

I INTRODUCTION

Changes in serum globulins during pregnancy and disease in humans, have been investigated by many workers, as reviewed by Fahraeus (1929). The results of these investigations have shown that a pronounced parallelism exists between the amount of fibrinogen or serum globulins and blood ESR value. However, due to the complex processes involved in sedimentation of red cells, ESR values are difficult to analyse in terms of red cell flexibility.

The high degree of red cell aggregation and high ESR values of horse blood were observed by Fahraeus (1929), Richter (1966), Osbaldiston (1971) and many other workers. In Chapter 4, it has been shown that increased red cell aggregation in normal blood is dependent on increased red cell flexibility. This dependency has been shown to exist in human and other species bloods, in the previous chapter.

The causes of high red cell aggregation and flexibility of horse blood compared to human blood, and whether it is a property of the horse red cell membrane or a factor in the plasma, have not been investigated previously. It is this task which has been undertaken in the following study because of the pharmaceutical importance of a substance which can be used to increase human or other species red cell flexibility. Due to the problems of fractionation of plasma proteins and their identification, the investigation was a preliminary one, in which commercially available human and horse proteins were used. The range of

commercially available proteins were very limited and their purity is uncertain. For this reason a more systematic investigation would be needed in the future where purity and range of fractionates can be improved.

II METHODS

For each specie, the red cells were washed three times, in isotonic (pH = 7.4) Ringer-Locke solution, before being resuspended in the plasma of other species. The washing was carried out to remove any antibodies, which might react with the foreign plasma proteins. The absence of antibody reaction in the red cell suspension in a foreign plasma was checked by the following procedure. An equal volume of washed red cells from one specie was added into a volume of plasma of another specie. The suspension was mixed for 20 minutes and then centrifuged at 1000g for 10 minutes. The plasma was removed. This procedure of adding washed cells, mixing and then centrifuging, using the same plasma and fresh red cells was repeated a further two times. The reason for this procedure is to use the red cells to extract any antibodies in a given plasma. To the plasma, removed after the third centrifugation, fresh washed red cells were added and the suspension mixed for 20 minutes. As fast as possible, a small volume of the suspension was placed on a microscope glass slide, and the cells were observed microscopically for any red cell agglutination due

to antibody reaction. If agglutination was observed, the plasma and red cell combination of the two species is not used. If agglutination was absent, a further separation was carried out. This was performed by adding washed red cells to a fresh volume of the plasma, mixing the suspension, and as fast as possible after mixing, the suspension was observed microscopically. If agglutination was present, then the above previous procedure was used for the red cell-plasma combination suspension. If no agglutination was observed, then it is assumed that no antibodies exist in that particular specie plasma for the second specie's red cells.

Antibody reaction was also checked by measuring the packing rate, at the same haematocrit, of the two suspensions used above. If antibody reaction exist then the agglutinated red cells, which are very strongly bonded together, would alter the packing rate.

Beside antibody reaction, complement reaction in the plasma of one specie can react to the presence of red cells of a different specie. This reaction leads to haemolysis of the red cells. Haemolysis can be checked visually.

The serum of any specie was obtained by heating plasma at $56 \pm 1^{o}C$ for 10 minutes and then centrifuging the precipitated fibrin clot at 1000g for 30 minutes. This gives a clear serum solution.

The plasma fibrinogen concentration was varied by dilution with serum, to reduce Φ, or by addition of volumes of concentrated isotonic (pH = 7.4) Ringer-Locke/fibrinogen (Kabi) solution, to increase Φ. The preparation of this solution has been described in previous chapters.

The various human and horse proteins used in this study were obtained from Koch-Light Laboratories Ltd. (Colnbrook, Berks., England). The proteins and their fractions were as follows:

1) Human and horse α -globulins:- Cohn fraction IV
2) Human and horse β -globulins:- Cohn fraction III
3) Human and horse γ -globulins:- Cohn fraction II
4) Human α_1-globulins:- Cohn fraction IV_1

The proteins were dissolved in an isotonic (pH = 7.4) Ringer-Locke solution into which human albumin (Kabi) was dissolved to a concentration of $2g.l^{-1}$. The concentration of proteins were made to equal the concentration found in either the human or horse plasma.

Dialysis of plasma was carried out by placing the plasma in a size 18/32" Visking tubing (Scientific Instrument Centre Ltd., London), which allows substances of molecular weight less than 5000 to escape through the tubing wall. The plasma sample was dialized in either isotonic (pH = 7.4) saline or Ringer-Locke solution for at least four hours.

III RESULTS

When washed red cells from man, horse, sheep and cattle are suspended in each other's plasmas, no antibody reaction was observed. However, when human and horse red cells are suspended in sheep or cattle plasmas, complement reaction takes place and the red cells are lysed. The effects on the red cell flexibility index, at 30°C, of suspending each specie's washed red cells in plasmas of the other species, as well as autologus plasma, are shown in Table 73. When human red cells are suspended in horse plasma, its red cell flexibility index is increased from 6.75% min^{-1}, in autologus plasma, to 31.5% min^{-1} in horse plasma. This increase is very high even after the flexibility index is corrected for the increased Φ in horse plasma.

When horse red cells are suspended in human plasma, the flexibility index decreases from 42.5% min^{-1} in autologus plasma to 33% min^{-1} in human plasma. This decrease is greater than the decrease due to the lower Φ in human plasma. Suspending sheep or cattle red cells in other specie's plasmas does not significantly change the red cell flexibility index.

For horse red cells suspension in serum, if the serum is diluted with isotonic (pH = 7.4) Ringer-Locke solution, to dilute the serum proteins, the red cell flexibility index is also reduced, as shown in Fig. (80).

	HUMAN PLASMA $\emptyset$ = 2.68 mg.ml.$^{-1}$	HORSE PLASMA $\emptyset$ = 5.5 mg.ml.$^{-1}$	SHEEP PLASMA $\emptyset$ = 3.6 mg.ml.$^{-1}$	CATTLE PLASMA $\emptyset$ = 3.9 mg.ml.$^{-1}$
HUMAN RED CELL FLEXIBILITY INDEX (% min^{-1})	6.75	31.5	- (HAEMOLYSIS)	- (HAEMOLYSIS)
HORSE RED CELL FLEXIBILITY INDEX (% min^{-1})	33	42.5	- (HAEMOLYSIS)	- (HAEMOLYSIS)
SHEEP RED CELL FLEXIBILITY INDEX (% min^{-1})	0.63	0.9	0.7	0.67
CATTLE RED CELL FLEXIBILITY INDEX (% min^{-1})	0.65	0.89	0.83	0.88

TABLE 73

EFFECT OF INTERCHANGING PLASMAS OF DIFFERENT SPECIES ON EACH SPECIE RED CELL FLEXIBILITY INDEX

PERCENTAGE DILUTION OF SERUM WITH RINGER-LOCKE SOLUTION (%)	0	10	20	30	40	50	60	70	80	90	100
RED CELL FLEXIBILITY INDEX (% min^{-1})	22.4 21.45	22.3 21.5	20.0 19.45	17.05 15.9	12.03 11.35	7.7 6.62	4.25 4.0	3.5 2.92	2.45 2.01	1.25 1.0	0.85 0.65

TABLE 74 FOR FIGURE 80

EFFECT OF DILUTING HORSE SERUM PROTEINS WITH RINGER-LOCKE SOLUTION ON THE RED CELL FLEXIBILITY INDEX AT 30°C

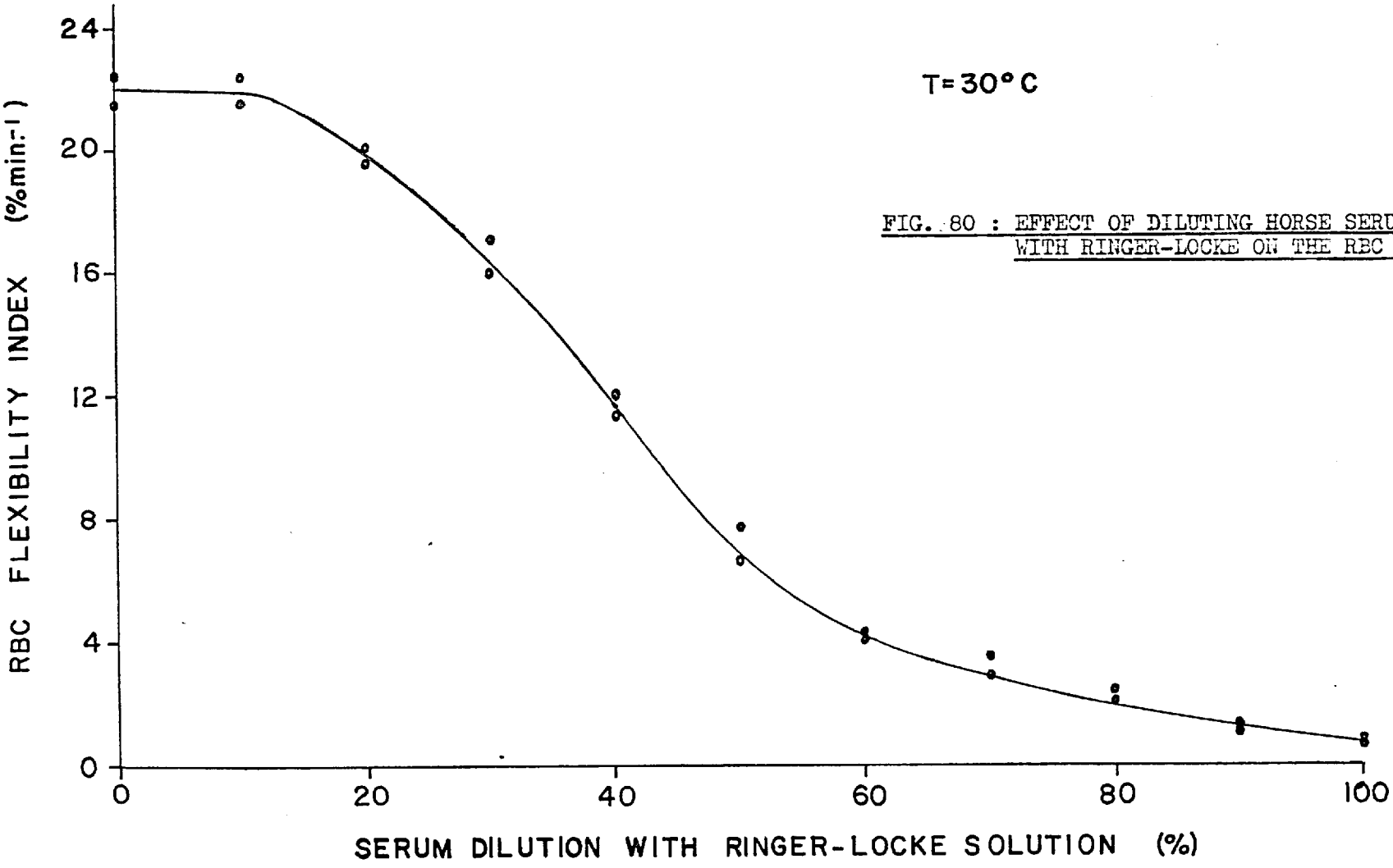


FIG. 80 : EFFECT OF DILUTING HORSE SERUM PROTEINS WITH RINGER-LOCKE ON THE RBC FLEXIBILITY

When Φ is varied, by serum dilution of plasma, in a suspension of human red cells in horse plasma, the red cell packing rates, at a haematocrit of 53%, varies as shown in Fig. (81). There is a slow increase in packing rate between $\Phi = 0$ to 2 mg.ml.$^{-1}$, after which there is a rapid increase and then a region of slow increase for $\Phi > 2.5$ mg.ml.$^{-1}$. The minimum value of the packing rate, at H = 53%, for red cells in serum, was found to be 16.36% min^{-1}. The flexibility index of normal human blood, at Φ = 2.68 mg.ml.$^{-1}$, was found to be 6.57% min^{-1}. At the same Φ value, the packing rate, at H = 53%, of human red cells in horse plasma was found to be 29.96% min^{-1}. When human red cells suspended in horse plasma are washed once in Ringer-Locke solution and resuspended in autologus plasma, its flexibility index is reduced to 5.38% min^{-1} at Φ = 2.5 mg.ml.$^{-1}$.

When Φ is varied, by serum and Ringer-Locke/fibrinogen (Kabi) solution dilution of plasma, in a suspension of horse red cells in human plasma, the red cell flexibility index is unchanged at about 33.25% min^{-1}. The flexibility index of normal horse blood was found to be 39.6% min^{-1}. If the horse red cells, suspended in human plasma, are washed once in Ringer-Locke solution and resuspended in autologus plasma, the flexibility index is increased to 39.4% min^{-1}, back to its normal blood value, Fig. (82).

In order to investigate the effect of different human globulins on the red cell flexibility, human α, α_1, β and γ globulins were added to suspensions of human red

EFFECT OF VARYING HORSE PLASMA FIBRINOGEN CONCENTRATION ON HUMAN RED CELL PACKING RATE AT 30°C

	HORSE PLASMA FIBRINOGEN CONCENTRATION, ø (mg ml^{-1})							
	0	1.15	2.07	2.15	2.75	3.89	4.9	5.5
HUMAN RED CELL PACKING RATE (H=53%) (% min^{-1})	16 16.73	16.3 17.15	20 20.9	24.5 27	28.67 31.25	32.6 33	30.07 31.5	33.3 33.3

RED CELL FLEXIBILITY INDEX FOR HUMAN BLOOD = 6.43, 6.7 % min^{-1}; ø=2·7 mg ml^{-1}
RED CELL FLEXIBILITY INDEX FOR HUMAN RED CELLS PLACED IN HORSE PLASMA THEN WASHED ONCE IN RINGER-LOCKE THEN REPLACED IN HUMAN PLASMA = 5.25, 5.5% min^{-1}, ø = 2.5 mg ml^{-1}
ERROR IN ø = ± 0.1 mg ml^{-1}; ERROR IN PACKING RATE = ± 1.5% min^{-1}

TABLE 75 FOR FIGURE 81

EFFECT OF VARYING HUMAN PLASMA FIBRINOGEN CONCENTRATION ON HORSE RED CELL

	HUMAN PLASMA AND SUSPENSION FIBRINOGEN CONCENTRATION, ø (mg ml^{-1})										
	0.0	1.0	1.72	2.4	2.725	2.86	3.6	4.45	5.125	6.77	8.1
HORSE RED CELL FLEX. INDEX (% min^{-1})	33.2 33.54	32.6 33	33 33.35	32.8 33	32.4 32.6	33 33.5	34 34.26	32.5 33	33.27 33.72	32.85 33.65	33.0 33.46

RED CELL FLEXIBILITY INDEX FOR HORSE BLOOD = 39.5, 39.7 % min^{-1}; ø = 6.3 mg ml^{-1}
RED CELL FLEXIBILITY FOR HORSE RED CELLS PLACED IN HUMAN PLASMA THEN WASHED ONCE IN RINGER-LOCKE THEN REPLACED IN HORSE PLASMA = 39.2, 39.6% min^{-1}; ø = 5.45 mg ml^{-1}
ERROR IN ø = ± 0.1 mg ml^{-1}; ERROR IN FLEXIBILITY INDEX = ± 1.5% min^{-1}

TABLE 76 FOR FIGURE 82

FIG. 81 : HUMAN RBC PACKING RATE vs. Ø FOR INTERCHANGED PLASMAS

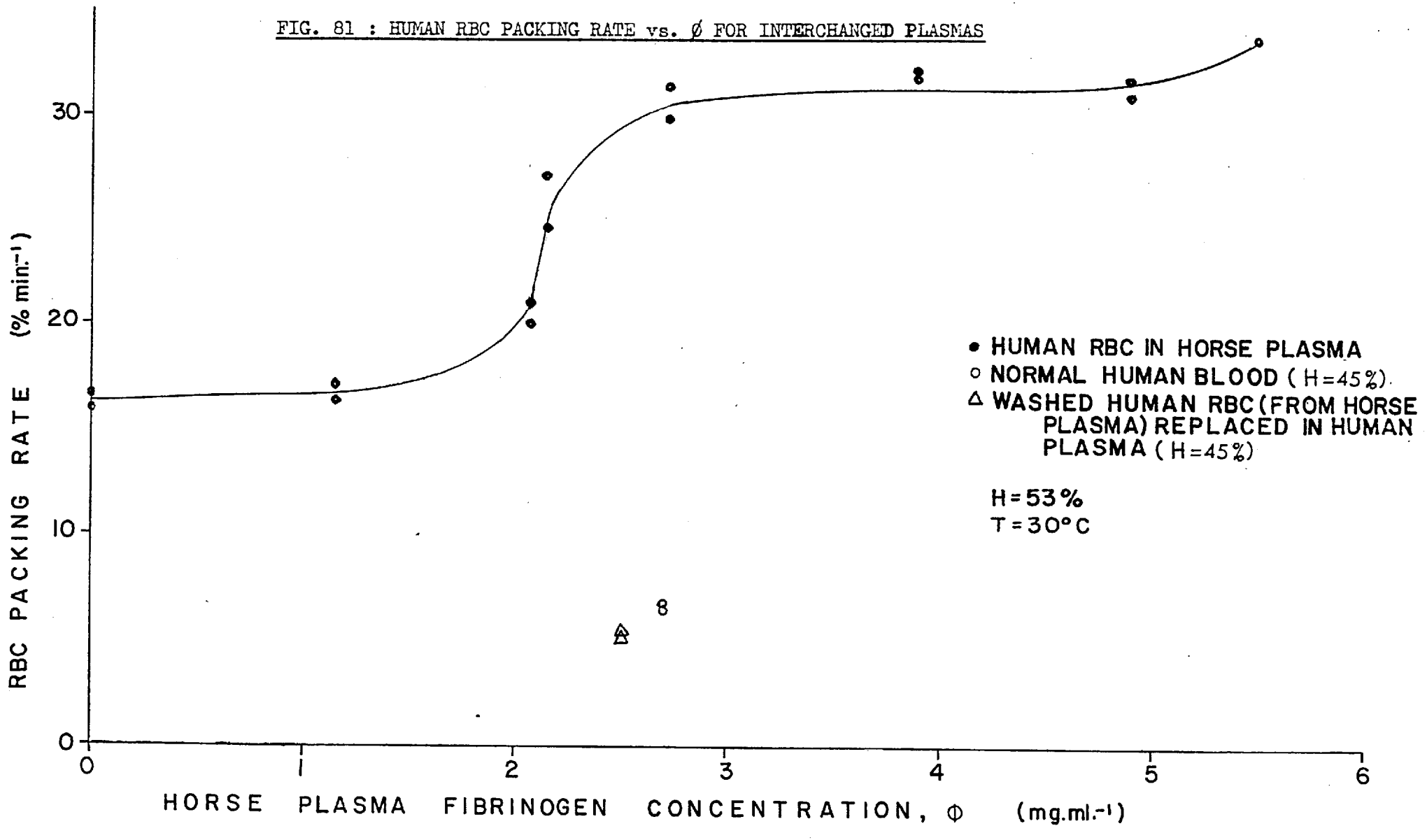
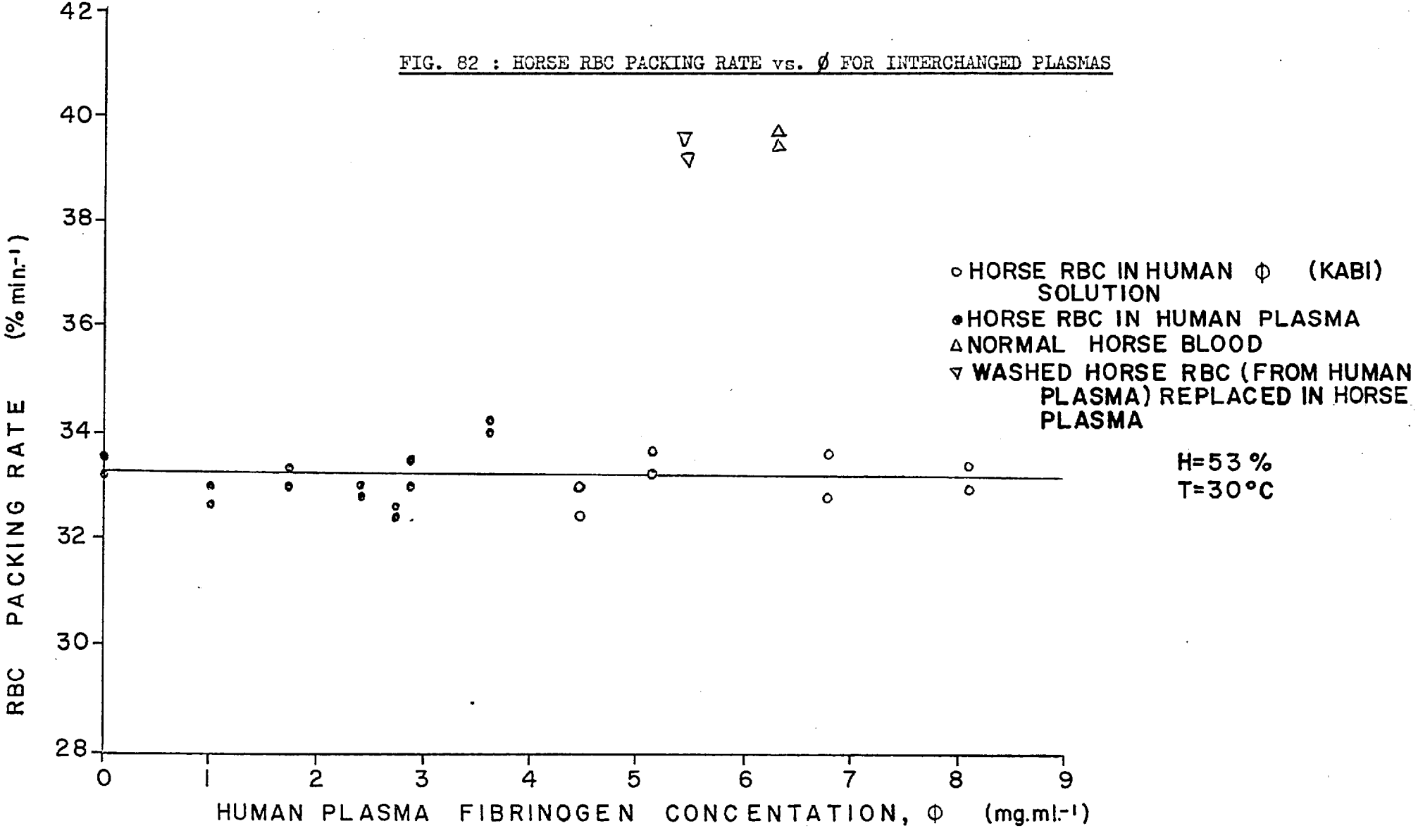


FIG. 82 : HORSE RBC PACKING RATE vs. Ø FOR INTERCHANGED PLASMAS



cells. These suspensions were in plasma, serum, Ringer-Locke/fibrinogen (Kabi)/albumin (Kabi) and Ringer-Locke/albumin solutions. Table 77 shows the effects of the different globulins on the red cell flexibility index for the different suspensions. The concentrations of the globulins were made to equal that found in normal plasma. Doubling the globulins concentrations in the plasma or serum suspensions had no effect. For the Ringer-Locke/fibrinogen/albumin suspension, the presence of α_1 globulin decreased the flexibility index but the other globulins had no effect. In the absence of fibrinogen, in the Ringer-Locke/ albumin suspension, the γ globulin slightly increased the flexibility index whereas the other globulins had no effect. For comparison purposes, the effect of the globulins were compared to the control sample, which is the red cell suspension without addition of the globulins. Due to the globulins being commercially produced, the possibility of antibody reaction between the plasma or serum suspension and the proteins were checked. No agglutination were observed microscopically in either suspension after the addition of the globulins. The absence of significant difference in the flexibility index values, compared to the control sample, confirms the absence of antibody reaction in both suspensions.

The effects of horse α, β and γ globulins on the flexibility of horse and human red cells are shown in Table 78. The control samples are suspensions without the addition of the globulins. The addition of α, β and γ globulins to normal horse blood and serum suspension,

	CONTROL	HUMAN PROTEINS			
		α 0.29 $g.l^{-1}$	α_1 0.29 $g.l^{-1}$	β 0.84 $g.l^{-1}$	γ 0.9 $g.l^{-1}$
RED CELLS IN PLASMA FLEXIBILITY INDEX (% min^{-1})	8.56 8.48	9.21 8.81	7.81 7.99	8.33 8.07	8.03 8.08
RED CELLS IN SERUM FLEXIBILITY INDEX (% min^{-1})	3.82 3.7	4.91 4.25	4.01 3.83	4.15 3.9	3.93 4.04
RED CELLS IN RINGER-LOCKE/ FIBRINOGEN (KABI)/ALBUMIN FLEXIBILITY INDEX (% min^{-1})	8.73 8.31	9.27 9.30	2.83 3.0	8.75 8.42	8.58 7.88
RED CELLS IN RINGER-LOCKE/ ALBUMIN FLEXIBILITY INDEX (% min^{-1})	2.78 2.85	2.33 2.38	3.49 3.72	2.79 2.57	4.16 3.84

TABLE 77

. EFFECT OF HUMAN α, α_1, β AND γ GLOBULINS ON HUMAN RED CELL FLEXIBILITY INDEX IN PLASMA, SERUM AND RINGER-LOCKE SUSPENSIONS AT 37.5°C

EFFECTS OF HORSE α, β AND γ - GLOBULINS ON HORSE AND HUMAN RED CELL FLEXIBILITY IN PLASMA, SERUM AND RINGER-LOCKE SUSPENSIONS AT 30°C

	CONTROL	HORSE PROTEINS					
		α 1.4 g.l.$^{-1}$	β 2.1 g.l.$^{-1}$	γ 1.4 g.l.$^{-1}$	$\alpha+\beta$	$\alpha+\gamma$	$\beta+\gamma$
HORSE RED CELLS IN PLASMA - PACKING RATE (H = 53%)	25.33 26.41	29.11 29.93	30.03 30.03	35.83 37.56	-	-	-
HORSE RED CELLS IN SERUM - PACKING RATE (H = 53%)	17.5 16.9	19.2 19.8	19.5 20.03	23.4 23.7	-	-	-
HORSE RED CELLS IN RINGER-LOCKE/ALBUMIN FLEXIBILITY INDEX (% min^{-1})	1.76 1.63	1.33 1.47	1.24 4.29	2.46 2.03	0.93 1.14	1.01 1.23	1.39 1.43
HUMAN RED CELLS IN R-L/FIBRINOGEN/ALBUMIN FLEXIBILITY INDEX (% min^{-1})	4.69 4.49	0.86 1.01	4.54 4.29	6.89 6.95	2.96 3.34	3.86 4.0	9.66 10.02
HUMAN RED CELLS IN RINGER-LOCKE/ALBUMIN FLEXIBILITY INDEX (% min^{-1})	2.0 1.63	1.33 1.67	1.24 1.67	2.46 1.93	0.94 1.23	1.01 0.96	1.39 1.43

TABLE 78

increases the red cell flexibility index, with the largest increase caused by the γ globulins. There were no antibody reaction observed for any of the additions of globulins. In the absence of normal horse plasma proteins, i.e. in a suspension of horse red cells in isotonic (pH = 7.4) Ringer-Locke/human (Kabi) albumin solutions, the addition of α, β or γ globulins as well as the combination ($\alpha+\beta$), ($\alpha+\gamma$) and ($\beta+\gamma$), had no significant effect on the red cell flexibility index, within experimental error.

For human red cells suspended in a Ringer-Locke/fibrinogen/albumin solution, the addition of horse α globulins reduced the cell flexibility, β globulins had no effect and γ globulins increased the flexibility. For globulin combinations, ($\alpha+\beta$) reduced the flexibility, ($\alpha+\gamma$) reduced it very slightly but ($\beta+\gamma$) increased the red cell flexibility to 9.84% min^{-1} (average of two values) compared to 4.59% min^{-1} for the control sample. In the absence of fibrinogen, i.e. red cells suspended in Ringer-Locke/albumin solution, the addition of globulins or their combinations, had no significant effect on the red cell flexibility index.

When horse or human red cells were suspended in dialized samples of horse plasma or serum, the red cell flexibility was unaltered compared to suspensions of undialized plasma or serum. This means that the factor in horse blood responsible for the high red cell flexibility has a molecular weight of more than 5000.

IV DISCUSSION

The flexibility of sheep and cattle's red cells is not affected by plasma proteins from each other's plasma or plasma from man and horse. When the more flexible human or horse red cells are suspended in sheep or cattle's plasma, red cell lysis occurs due to complement reaction. When horse red cells are suspended in human plasma, their flexibility is reduced. However, if the human plasma is washed off the horse red cells and the cells are then resuspended in their own plasma, they regain their original flexibility. This shows that there is some factor in horse plasma which is responsible for the high red cell flexibility. This factor can be washed off the red cell, and in its absence the red cell flexibility is reduced. The effect of this factor on red cell flexibility is dependent on its concentration. When serum is diluted with Ringer-Locke solution, the red cell flexibility is reduced, Fig. (80).

The factor in horse blood is responsible for the high red cell flexibility in plasma and serum. However in plasma, the presence of fibrinogen increases the cell flexibility further. When horse red cells are suspended in human serum, their flexibility is reduced. When human fibrinogen is added to the suspension, the red cell flexibility is not altered. Hence, human fibrinogen does not affect horse red cell flexibility in the absence of the factor present in horse blood.

When horse α , β and γ globulin concentrations in horse plasma or serum are increased, the red cell flexibility is increased, especially for γ globulins. In the absence of plasma proteins (other than albumin), addition of globulins to the red cell suspension has no affect on the red cell flexibility. Therefore, it seems that the effect of the unknown factor, in horse blood, on the red cell flexibility is enhanced by increased concentration of globulins, especially the γ globulin, only in the presence of normal plasma or serum proteins.

Suspending human red cells in horse serum or plasma increases their flexibility dramatically. When the horse fibrinogen concentration is increased the human red cell flexibility is increased in a non-linear fashion, see Fig (81). When the red cells, suspended in horse plasma, are washed once with Ringer-Locke solution and resuspended in autologus plasma, their flexibility is reduced to that of normal human blood value. This indicates again that the factor in horse blood, responsible for increasing the red cell flexibility, does not attach strongly to human red cells, and can be easily washed off.

Addition of human α , α_1, β and γ globulins to human red cells suspensions in plasma, serum and Ringer-Locke/albumin solution, had no effect on the red cell flexibility index. However, in the absence of plasma proteins, other than fibrinogen and albumin, the addition of α , β and γ globulins does not affect the red cell flexibility, but the addition of α_1 globulin reduces the

flexibility. Hence, human α_1-globulin is the only protein, of those investigated, which affect the human red cell flexibility but only in the presence of fibrinogen and albumin.

For human red cells suspension in Ringer-Locke/albumin solution, addition of horse α, β, γ and combination of all three, had no affect on the red cell flexibility. However, when fibrinogen is present, the horse α-globulin decreased flexibility, β-globulin had no effect and γ-globulin increased the flexibility. If α and β-globulins are present the decrease in flexibility is less than when α-globulin alone is present. If α and γ are present, the decrease is less than for the case of $(\alpha+\beta)$. However, when β and γ-globulins are present, the red cell flexibility is doubled, compared to the control sample.

Therefore, from these preliminary investigations to identify the factor in horse plasma and serum, responsible for increasing the red cell flexibility of horse and human red cells, the following points can be noted from the experimental results:-

1. The factor's molecular weight is greater than 5000.
2. The effect of this factor on the red cell flexibility is affected by dilution.
3. The factor is not strongly bound onto the red cell membrane, and can be easily washed off.

4. For horse red cells, the effect of this factor is enhanced by increases in concentration of α, β and γ globulins, only in the presence of other plasma or serum proteins. The increase is greater for γ than for α or β globulins.
5. For human red cells, horse globulins effect the red cell flexibility in the presence of fibrinogen, not in its absence.
6. In the presence of fibrinogen, human red cell flexibility is reduced by horse α-globulin, unaltered by β globulin and increased by γ-globulin. The increase is greater when both, the β and γ -globulins, are present in the suspension.

These points lead to the conclusion that the factor sought is either a single substance (or protein), or a combination of substances (or proteins) which interact together and with the red cell membrane. Such a combination is the horse β and γ globulins in the presence of fibrinogen, as was observed in my results.

CHAPTER 8

THE EFFECT OF SOME DISEASES AND DRUGS ON BLOOD RHEOLOGY

I INTRODUCTION

A) Preliminary Survey of Human Diseases

Fahraeus (1921 and 1929) observed that the red cell sedimentation rate, rouleaux formation and aggregation as well as whole blood viscosity varied in health and disease. The first systematic investigation of the changes in blood viscosity in disease, were made later, in the 1960s, following the advent of rotational viscometers, by Dintenfass (1962a and b). Since then, many investigators have measured the rheological parameters of blood from patients suffering from a variety of diseases and abnormal clinical situations. However, only limited data exists for the variation of the red cell flexibility, measured by the centrifuge method, for patients suffering from diseases. I have carried out a limited preliminary survey of the variation of the red cell flexibility index following treatment with drugs and in a variety of diseases. The red cell flexibility index values were then compared to normal blood values, at the varying plasma fibrinogen concentrations.

B) Prostacyclin (PGI_2)

Prostacyclin PGI_2 was the name given to PGX by Johnson et al (1976). It is the most potent endogenous inhibitor of platelet aggregation known, as well as a vasodilator. It is unstable and its antiaggregatory activity disappears within

15 seconds on boiling or within 10 minutes at 22°C, at neutral pH. In blood at 37°C prostacyclin has a half life of two to three minutes, Dusting et al (1978). Alkaline pH enhances its stability, Johnson et al (1976).

Prostacyclin is a principle product of arachidonic acid metabolism in blood vessel endothelium. It inhibits platelet aggregation by stimulating adenyl cyclase, resulting in an increase in platelet cyclic AMP, Gorman et al (1977) and Tateson et al (1977). The stable breakdown product of prostacyclin is prostacyclin 6-OXO-$PGF_{1\alpha}$ which has weak anti-aggregatory activity and is approximately 1000 times less potent in increasing cyclic AMP levels in platelets, Tateson et al (1977).

The stability of prostacyclin in solution depends on temperature, increasing its half life from 15 seconds at 100°C to 3.4 minutes at 25°C to 14.5 minutes at 4°C, Cho and Allen (1978). Alkalinity and concentration of albumin also increase the half life of prostacyclin, Wynalda and Fitzpatrick (1980). No difference in the stability of prostacyclin in plasma or whole blood was observed, Orchard and Robinson (1981), with its half life at 37°C and pH = 7.4 ranging between 14.2 to 23.3 minutes, compared to 2.8 ± 0.2 minutes in buffer alone. The increased stability of prostacyclin in the presence of albumin is attributed to the binding of the prostocyclin to albumin, decreasing the rate of decay of its antiaggregatory potency.

Bito and Baroody (1975) have observed that the rabbit red blood cell membrane is impermeable to prostaglandins. Similarly, Baroody and Bito (1981) discovered that the rabit red cell membrane were also impermeable to prostacyclin (PGI_2) and its breakdown product 6-Keto-$PGF_{1\alpha}$. Willems et al (1980) observed that prostacyclin (PGI_2) binds not only to albumin but also to human erythrocytes.

Belch et al (1981) measured the red cell deformability, using whole blood taken from patients at different intervals, after intravenous infusions of PGI_2, of different dosage. Their results show that the red cell deformability decreased for both in-vivo samples, and for in-vitro samples (incubated blood with varying concentrations of PGI_2), with the decrease being greater for greater concentrations of PGI_2.

Dowd et al (1981) measured the red cell deformability of a suspension of red cells in phosphate-buffered saline (pH = 7.4) containing 0.25% human albumin, into which varying amounts of PGI_2 were added. Their results show that the red cell deformability increased in the presence of PGI_2.

The controversy was further inflamed by Slott et al (1982), who measured the red cell deformability for a red cell suspension in Kreb's Buffer with 1% albumin at pH = 7.4 and 8.4 at $37^{o}C$. They found that for PGI_2 concentration of 500 $ng.ml.^{-1}$ there was no change in the red cell deformability compared to the control (with no PGI_2) present.

The differences in results regarding the effect of prostacyclin on the red blood cell deformability prompted the following investigation into whether there is an effect of PGI_2 on the mechanical property of the red cell, e.g. the red cell flexibility.

C) Dapsone

Dapsone (4, 4'-diaminodipenylsulphone) is widely accepted as one of the most effective treatments for dermititis herpetiformis (DH), a skin disease. The drug is taken orally by patients, in tablet form, and the dosage varies between 10 and 600 mg. daily. It is well absorbed and 50-80% of the drug is protein-bound in plasma.

Dapsone is known to cause haemolysis of the red cells, Lowe (1950), Ramanujam and Smith (1951) and Pengelly (1963), as well as haemolytic anemia, Hutchinson et al (1963), Cream and Scott (1970) and Manfredi et al (1979). The degrée of anaemia will depend upon the ability of the bone marrow to compensate by increased erythropoiesis, which might be impaired in patients with DH by malabsorption of iron, folate and vitamin B12. The folate deficiency is suspected to be caused by the dapsone interferring with the folate-acid metabolism in the gut, Fry et al (1967).

Cream and Scott (1970) observed that the red cell life span, in patients taking dapsone, was reduced, and that their average MCV was 144 fl compared to 95 fl for normals,

although none of the patients were folate or vitamin B12 deficient. They also observed that the red cell morphology in patients taking 100 mg. or more of dapsone daily was altered, with abnormalities such as red cell fragmentation and macrocytosis being most frequent.

In dapsone treated patients, Ramanujan and Smith (1951) observed cyanosis and methaemoglobinaemia. Methaemoglobin and sulphaemoglobin were found to be present in the blood of most patients receiving 100 mg. or more of dapsone daily. The presence of this non-functioning form of haemoglobin reduced the oxygen carrying capacity of the blood, and from their preliminary investigations, they suggest that the function of the remaining haemoglobin might be impaired.

In addition to dapsone-induced haemolysis, anemia and methaemoglobinaemia, dapsone has been reported to be the cause of hypoalbuminaemia, Kingham et al (1979) and Cowan and Wright (1981). Each dapsone molecule is suggested to bind to two albumin molecules, Riley and Levy (1973), and might act as a hapten and cause immunological destruction of albumin, causing the dapsone-induced hypoalbuminaemia, Cowan and Wright (1981).

A study was carried out, as described below, to investigate the haemorheological effects of dapsone, using blood samples from dapsone treated patients and comparing the results with those of normal, healthy subjects.

D) Warfarin and Navicular Disease in Horses

Jeremiah Bridges (1752) was probably the first to recognise a lameness in horses as a result of a disease of the navicular bone. The disease was named the "Navicular Joint Disease" by Turner, Youatt (1836). The navicular joint disease is associated with the small navicular bone just above the front hoofs, and to the rear of the limb, of the horse. The disease is clinically recognised by pain in the feet, leading eventually to a shortening of the forelimb stride, and the feet usually placed onto the ground toe first. This condition is nearly always present in both limbs. From a survey in 1962, navicular disease was diagnosed as the cause of about one-third of all cases of chronic forelimb lameness in horses, Colles (1982).

From the disease description of J. Bridges (1752), Moorcroft (1819) and Turner, it was generally accepted that the lameness involved ulcerations and erosions of the fibrocartilage of the navicular bone and lesions of the deep digital flexor tendon. However, Walley (1885) and more recently Hickman (1964) and Nemeth (1972) have suggested that changes in blood supply to the navicular bone might be an important factor in the aetiology of navicular disease. Colles and Hickman (1977) have shown that ischaemia of the navicular bone is due to progressive arterial occlusion (thrombosis). Recent investigations (Colles unpublished data) have established that the clearance of a contrast agent in blood flowing through the feet is extended from about 12 seconds in normals, to 1 or 2 minutes in navicular

disease. Besides correct shoeing and regular exercise, Colles (1979) found that anticoagulant therapy provided an improved form of treatment. The anticoagulant used was warfarin (a derivative of dicoumarin). From the horses treated with warfarin over a four year period, 77% have become sound while being treated, and remained sound for at least 12 months, Colles (1982).

Although the pharmacokinetics of warfarin in the horse is little known, it is used to prevent post-surgical thrombosis in man. The possibility of modification to the rheological properties of horse blood has prompted an investigation into the effect of warfarin in the treatment of the navicular disease, as described below.

II METHODS

A) Preliminary Survey of Human Diseases

Blood samples from patients suffering from different diseases were obtained from the hospital. The blood was obtained by vene-puncture with lithium heparin used as anticoagulant. The red cell flexibility index for each patient was measured by the automatc recording centrifuge method. The plasma fibrinogen concentration was measured by the thrombin clotting technique, Rampling and Gaffney

(1976). The MCV was calculated as the ratio of the haematocrit and red cell count per litre of blood, obtained using the Coulter Counter as described in Chapter 2.

B) Prostacyclin (PGI_2)

Sodium salt of the prostacyclin PGI_2 (Sigma Chemical Company Ltd., Dorset, UK) was dissolved in 0.01 M NaOH at -20°C. Solutions of different concentrations of PGI_2 were made up by adding varying volumes of PGI_2/NaOH solution to 5 ml volumes of Tris Buffer (pH = 7.4) into which human albumin (Kabi) was added to a concentration of $4gl^{-1}$. 10 µl. volumes of the PGI_2/buffer solution were added to 1 ml. samples of blood, very quickly, and then mixed gently, before measuring the red cell flexibility index at 37.5°C. The concentrations of PGI_2 in blood, used, were 10, 50, 100, 250, 400 and 600 $ng.ml^{-1}$.

C) Dapsone

Blood, obtained by vene-puncture with lithium heparin as anticoagulant, from patients suffering from dermitisis herpetiformis, was supplied by the hospital dermatology outpatient clinic. The red cell flexibility measurements for each blood sample was carried out using the centrifuge technique. The plasma fibrinogen concentration was measured by the thrombin clotting method. The MCV was obtained using

the red cell count, measured on the Coulter Counter. Viscometer measurements were performed on the Wells-Brookfield cone-plate viscometer.

D) Warfarin and Navicular Disease in Horses

Blood samples from ponies, cross-bred and thorough-bred horses were obtained from the Equine Research Station, Newmarket, Suffolk. Lithium heparin was used as anticoagulant. The blood was obtained by vene-puncture of the jugular vein. Work on the blood was carried out in the research station to enable analysis of bloods to be carried out within six hours of bleeding.

Unlike horse blood samples, obtained from the Wellcome Laboratories, Kent, for our previous investigations into horse blood rheology, the horse blood from the Newmarket horses presented us with a problem in viscosity measurements. The whole blood and plasma viscosity, measured on the Wells-Brookfield viscomter, for the Newmarket horses, were elevated in values but their relative viscosity was similar to values obtained using the Wellcome samples. The elevated values were suspected to be due to a fluid-air film of cryoproteins. This problem was overcome by using a capillary Coulter Viscometer (Couter Electronics Ltd., Bedfordshire). The high shear rate of the capillary viscometer, approximately $400s^{-1}$, was reduced to about $200s^{-1}$ by applying a constant back presence to the flow through the capillary, to slow down the flow speed. The

values obtained from the modified capillary viscometer for normal human and horse bloods were similar to those obtained previously on the Wells-Brookfield viscometer at a shear rate of $230s^{-1}$.

Red cell flexibility, Φ and MCV measurements were obtained using the measurement techniques described previously in Chapter 2.

III RESULTS

Table 79 shows the age, sex, disease diagnosed, Φ , red cell flexibility index and MCV for 26 patients suffering from a variety of diseases as indicated. The values of the MCV measured are within the normal range of 80-105 fl. with patient 17 having a larger value of 123 fl. and patient 20 having a lower value of 68.1 fl. The survey was carried out to obtain an indication of the variation of the red cell flexibility index for the different diseases compared to normal healthy subjects, used in previous chapters. In Fig. (83), it is shown that for most of the diseases investigated, the red cell flexibility index, at a given Φ , was greater than the range of values for normal healthy subjects' blood, except for cirrhosis of the liver, where the value of the flexibility index was decreased.

PATIENT	AGE	SEX	DISEASE DIAGNOSED	ϕ (mg ml^{-1})	RED CELL FLEXIBILITY INDEX (% min^{-1})	MCV (fl.)
1	59	M	MYOCARDIAL INFARCTION	6.75	15.71	-
2	49	M		4.07	9.57	-
3	59	M	ANGINA	4.52	9.36	-
4	62	M		4.6	9.26	86.6
5	62	M	HYPERTENSION	6.9	12.51	-
6	67	M		2.58	8.61	102.3
7	70	F		5.81	9.0	83.5
8	46	F		5.99	14.6	96.9
9	86	M	CHRONIC BRONCHITIS	5.97	10.55	-
10	80	M		3.98	11.4	-
11	77	M		5.01	9.35	87.8
12	78	M		5.24	10.76	90.6
13	67	M		9.14	14.89	83.8
14	68	F		7.42	9.79	-
15	43	F		4.1	9.11	-
16	61	F	ACUTE BRONCHITIS	3.65	20.9	95.7
17	71	M	PNEUMONIA	9.79	16.66	123.0
18	61	M		4.85	11.59	87.0
19	43	F		8.25	23.93	-
20	43	M	DIABETES	6.73	14.5	68.1
21	25	F	ACUTE ASTHMA	5.97	9.33	-
22	67	F	CIRRHOSIS OF LIVER	2.38	6.21	-
23	75	F	ANAEMIA (MACROCYTIC)	3.25	10.68	103.9
24	79	F	POLYCYTHEMIA	2.69	5.56	-
25	75	M	CHRONIC GASTRIC ULCER	4.38	9.35	84.5
26	37	F	TUBERCULOSIS	3.56	7.78	83.6

TEMPERATURE OF FLEXIBILITY INDEX MEASUREMENTS = 22°C

TABLE 79

RHEOLOGICAL PARAMETERS FOR BLOODS OF PATIENTS SUFFERING FROM VARIOUS DISEASES

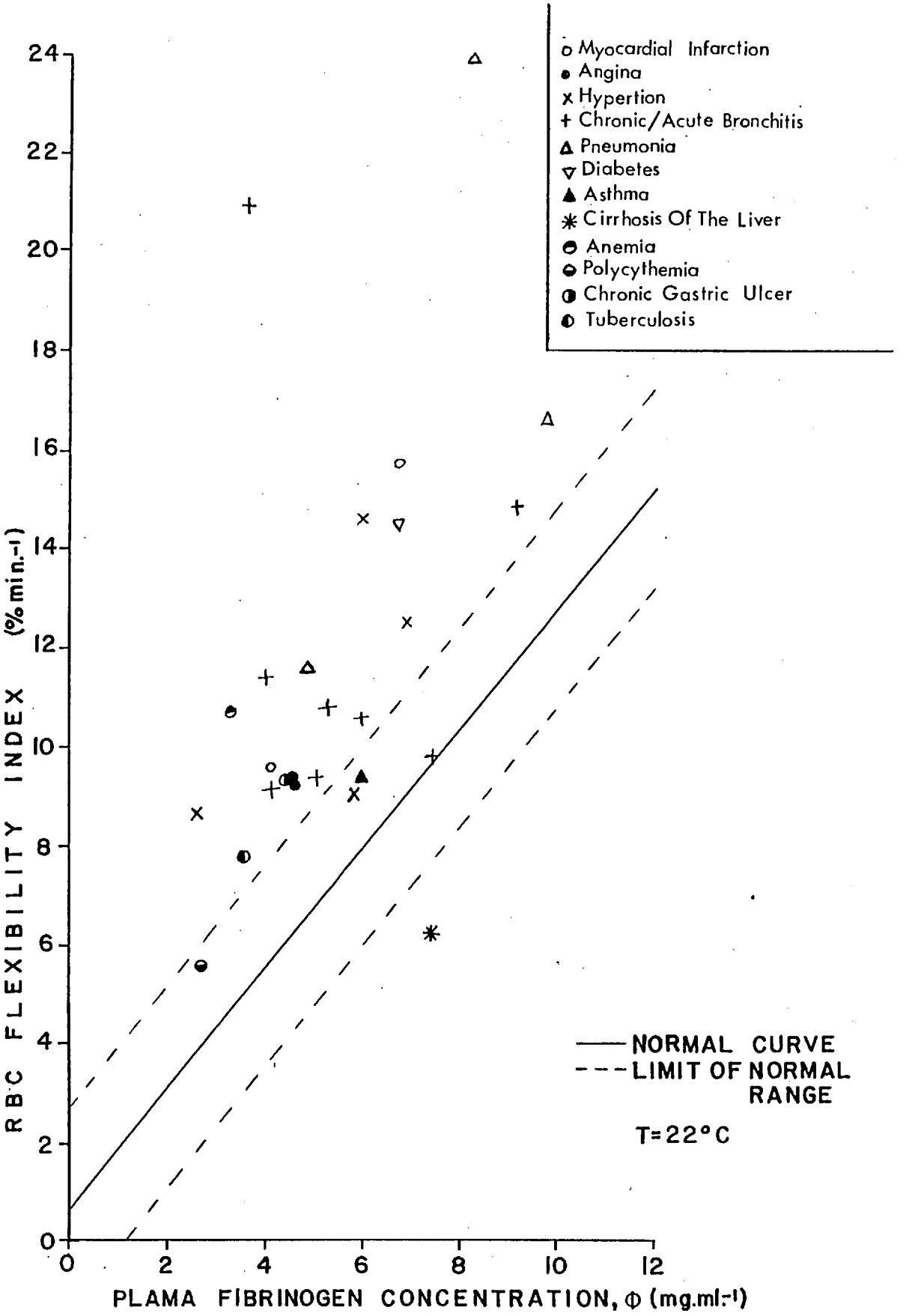


FIG. 83 : RBC FLEXIBILITY INDEX vs. ϕ FOR VARIOUS DISEASES

To investigate the reason for the increased red cell flexibility index in patients suffering from pneumonia (patients nos. 17 and 19) and from acute (patient 16) or chronic (patient 13) bronchitis, the red cells were washed and resuspended in their autologus serum and also washed and resuspended in Ringer-Locke/albumin solution. Table 80 shows that for both suspensions, the red cell flexibility index for the four patients' red cells suspensions was found to be significantly greater than values for a control suspension of normal healthy donor's red cells.

From Table 81 it is seen that varying the concentration of PGI_2 in blood from 10 to 600 ng.ml.$^{-1}$ had no effect on the values of the red cell flexibility index compared to the control sample.

Table 82 shows that the variation of η_B and η_r with haematocrit H for normal blood at 37.5°C. The linear relationship between H and log η_r is shown in Fig. (84). The linear relationship, using the natural logarithms, can be expressed as

$$\ln \eta_r = aH$$

i.e. $$\eta_r = e^{aH} = 1 + aH + \frac{a^2H^2}{2!} + \ldots$$

when H approaches zero

$$\eta_r = 1 + aH$$

	DISEASE DIAGNOSED	IN WHOLE BLOOD	IN SERUM	IN R-L/ALB.
CONTROL	HEALTHY NORMAL	4.35	1.03	0.81
PATIENT 17	PNEUMONIA	16.66	9.21	3.75
PATIENT 19	PNEUMONIA	23.93	12.6	5.23
PATIENT 13	CHRONIC BRONCHITIS	14.89	7.57	2.92
PATIENT 16	ACUTE BRONCHITIS	20.9	12.85	4.04

FLEXIBILITY INDEX VALUES ARE AVERAGE OF 3 VALUES MEASURED AT 22°C ERROR IN FLEXIBILITY INDEX VALUES = ± 0.5% min^{-1}

TABLE 80

FLEXIBILITY INDEX VALUES FOR PATIENTS' RED CELLS IN WHOLE BLOOD, SERUM AND RINGER-LOCKE/ALBUMIN SUSPENSIONS

	BLOOD PROSTACYCLIN CONCENTRATION ($ng.ml^{-1}$)						
	0 (CONTROL)	10	50	100	250	400	600
RED CELL FLEXIBILITY INDEX AT 25°C ($\% \ min^{-1}$)	5.4	5.37	5.6	5.41	5.5	5.37	5.32
	5.38	5.42	5.62	5.39	5.5	5.45	5.6
	5.5	5.6	5.35	5.4	5.43	5.42	5.55

TABLE 81

EFFECT OF DIFFERENT CONCENTRATION OF PROSTACYCLIN (PGI_2) ON THE RED CELL FLEXIBILITY INDEX OF NORMAL BLOOD AT 25°C

HAEMATOCRIT (%)	0	9	16	19.5	25	30	37
η_B (cP)	1.3	1.6	2.1	2.15	2.55	2.98	3.48
log η_r	0	0.0902	0.2083	0.2185	0.2926	0.3603	0.4276

η_p = 1.3 cP

TABLE 82 FOR FIGURE 84

VARIATION OF log η_r WITH HAEMATOCRIT FOR NORMAL BLOOD (DONOR A) AT 37.5°C AND SHEAR RATE OF 230s^{-1}

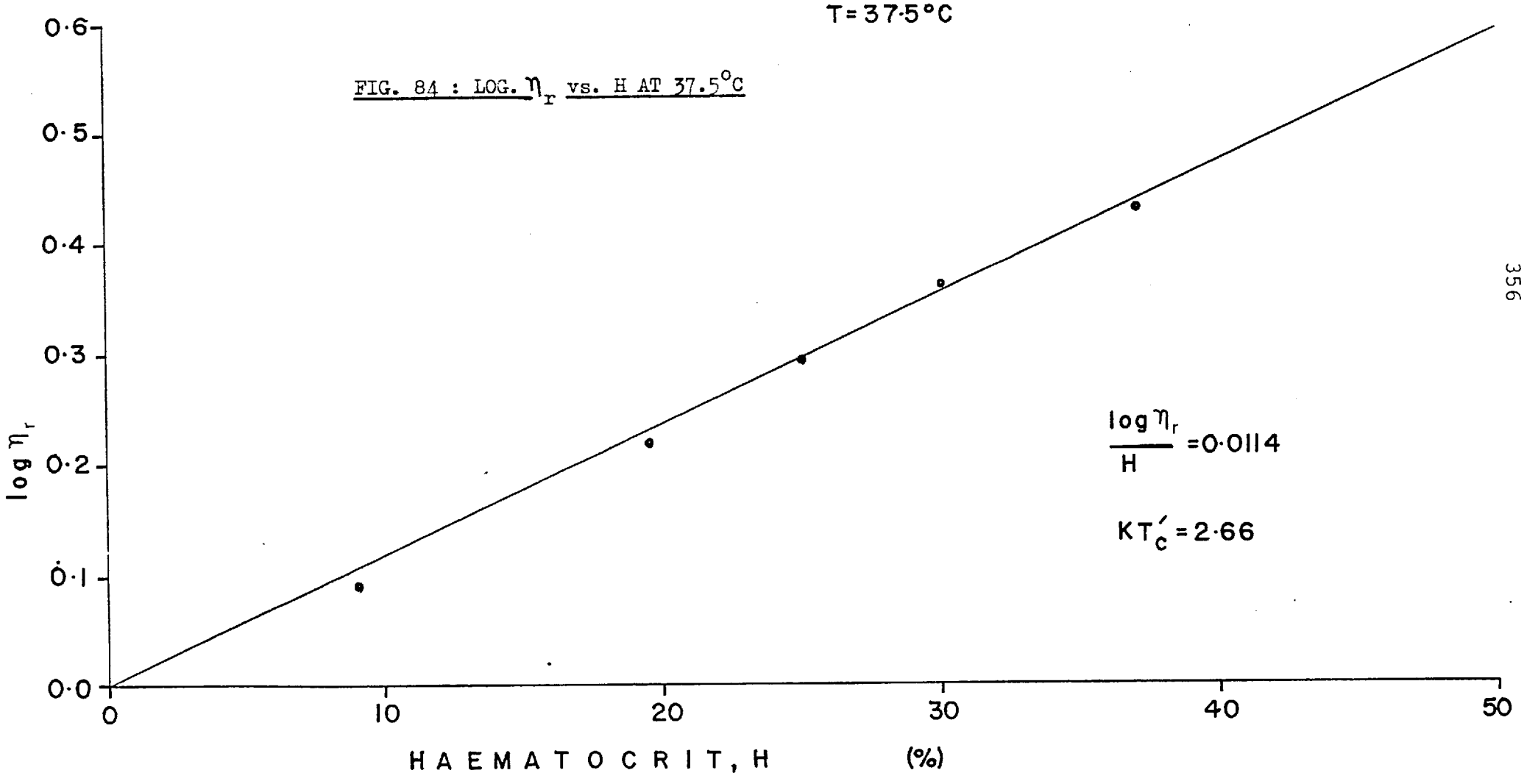


FIG. 84 : LOG. η_r vs. H AT 37.5°C

and from the modified Einstein equation

$$\eta_r = 1 + KT'_cH$$

therefore a is equivalent to KT'_c, hence

$$(\log \eta_r/H) \equiv KT'_c$$

For normal blood shown in Fig. (84) the value of KT'_c at 37.5^oC is 2.66.

For patients J.S. and L.G. treated with dapsone for DH, the variations of rheological parametors over the period of the survey of treatment, are shown in Table 83. J.S. was sampled before treatment, whereas L.G. had already started treatment. The variations of MCV, red cell flexibility index, Φ, η_B and η_p with time are shown in Fig. (85). In both cases, the red cell flexibility index and η_B decreased initially. The red cell flexibility for L.G. increased for the last sample after the initial decrease. Φ changed in opposite manners for the two patients, but η_p did not change appreciably.

The variations of the intrinsic viscosity, KT'_c, for J.S. and L.G. over the treatment period are shown in Fig. (86). For J.S. the value of KT'_c begins much greater than the normal blood value of 2.66 and decreases towards it by the third sample. There is a large increase for the fourth sample but the value returns to the normal level for the last (fifth) sample. Hence the treatment tends to return

THE VARIATION OF RHEOLOGICAL PARAMETERS WITH TIME FOR PATIENTS J.S. AND L.G. TREATED WITH DAPSONE WITH DERMITITIS HERPETIFORMIS

PATIENT AND DATE	DAPSONE DOSAGE (mg per day)	HAEMATOCRIT (%)	ϕ (mg ml^{-1})	RED CELL FLEX. INDEX (% min^{-1})	η_p (cP) ($\dot{\gamma}$= 2305^{-1})	η_B (cP) ($\dot{\gamma}$= 2305^{-1})	MCV (fl)	$\frac{\log \eta_r}{H}$	KT_c'
J.S. 29/1/82	Pre Treatment	37.25	2.17	9.58	1.3	4.1	94.3	0.0134	3.13
J.S. 12/2/82	100	37.0	2.84	8.86	1.39	4.0	117.0	0.0124	2.9
J.S. 25/2/82	100	37.0	2.92	7.85	1.3	3.47	132.4	0.0115	2.68
J.S. 26/3/82	100	32.5	2.89	7.46	1.22	3.4	125.1	0.0137	3.21
J.S. 23/4/82	100	34	1.64	6.02	1.425	3.45	116.8	0.0113	2.64
L.G. 5/2/82	100	41.5	4.82	13.5	1.6	4.65	108.3	0.0112	2.61
L.G. 26/2/82	100	39.5	3.56	10.56	1.55	4.0	101.6	0.0104	2.42
L.G. 23/4/82	150	34	2.62	9.76	1.47	3.88	101.6	0.0124	2.9
L.G. 11/6/82	150	36.5	2.04	0.09	1.455	3.53	92.9	0.0106	2.47
L.G. 15/10/82	100	38	2.61	10.1	1.45	3.5	88.6	0.0101	2.35

TEMPERATURE FOR VISCOMETRIC AND FLEXIBILITY INDEX MEASUREMENTS = 37.5°C

TABLE 83

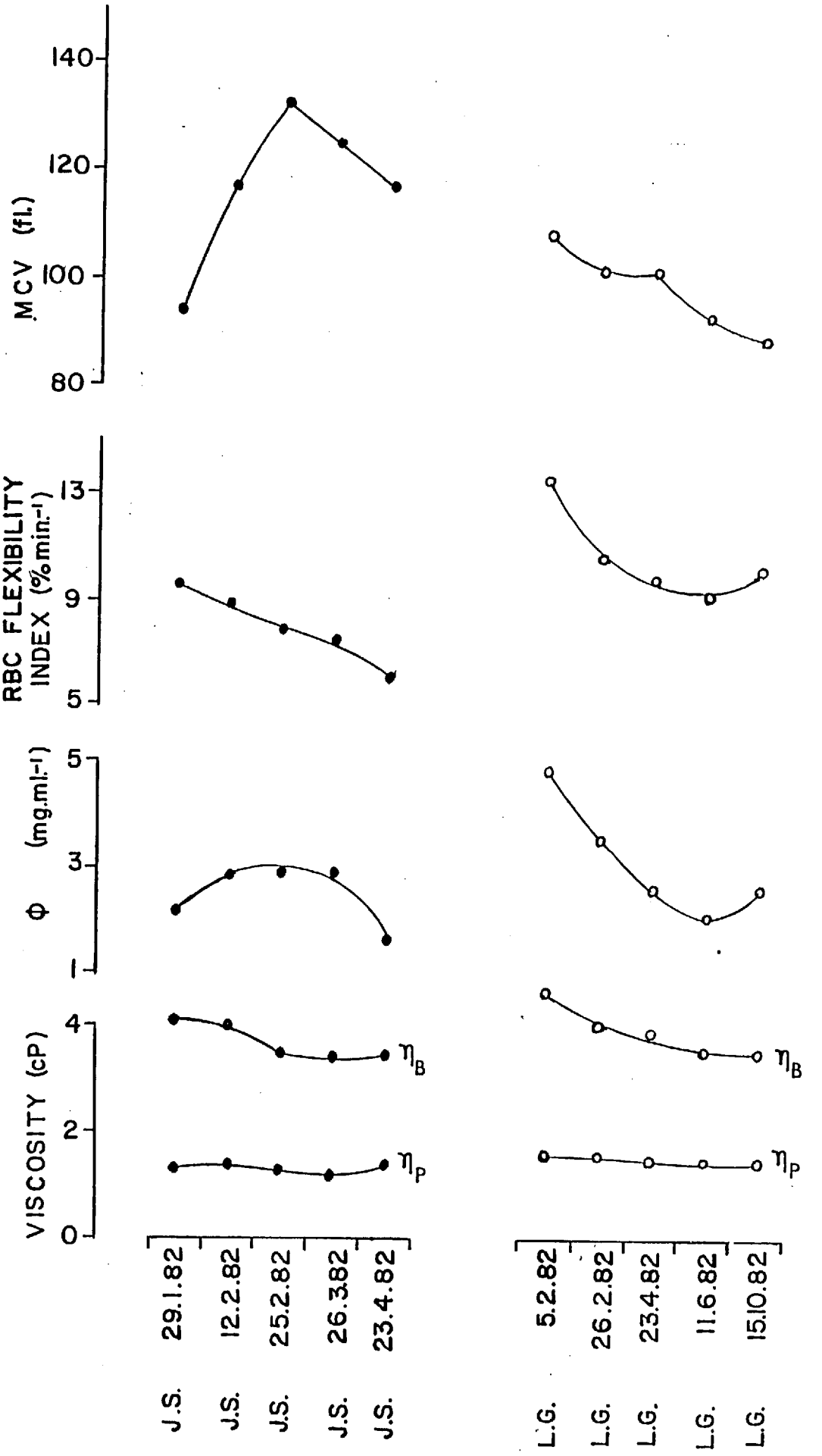


FIG. 85 : VARIATION OF RHEOLOGICAL PARAMETERS FOR J.S. AND L.G.

FIG. 86 : VARIATION OF KT'_c FOR PATIENTS J.S. AND L.G. FOR TREATMENT PERIOD

the value of KT'_c, for J.S., towards the normal value, with variations occuring through the period. For L.G. the value of KT'_c begins at approximately the normal value but is decreased for the second sample. KT'_c then increase above normal for the third sample and decreases for the fourth sample below normal value and lower still for the fifth sample. Hence the change in KT'_c, for L.G., over the treatment period, is to decrease below normal value.

For other patients of DH on dapsone therapy, Table 84 shows the values of the rheological parameters for a group of patients for which two blood samples were examined, at different times, and a group of patients for which only one blood sample was examined. The dapsone daily dosage varied between 25 mg. and 600 mg. Fig. (87) shows that when the variations of red cell flexibility index with Φ for patients on dapsone are compared to the values for normal blood, although there is a lower flexibility trend for scatter of values about the normal blood curve, there is no significant difference, with $p > 0.1$.

The scatter of values of KT'_c for dapsone patients about the normal blood value of 2.66, in Fig. (88), has no significant difference ($p > 0.1$). Again the trend of the scatter is towards a lower value of KT'_c than normal blood value.

In investigating the effect of warfarin on the blood rheology of horses in the treatment of Navicular Joint

PATIENT AND DATE	DAPSONE DOSAGE (mg per day)	HAEMATOCRIT (%)	ϕ (mg ml^{-1})	RED CELL FLEX. INDEX (% min^{-1})	η_p (cP)	η_B (cP)	MCV (fl)	$\frac{\log \eta_r}{H}$	KT'_c
T.C. 22.2.82	200	38	3.58	6.7	1.4	4.0	-	0.012	2.8
T.C. 29.10.82	200	40	1.79	4.99	1.35	3.6	95	0.0106	2.47
A.M. 26.3.82	25	42.5	1.77	7.74	1.4	4.08	94.5	0.0109	2.54
A.M. 19.11.82	100	44.5	2.3	5.17	1.3	4.15	86.9	0.0113	2.64
C.F. 30.4.82	100	30	4.21	7.36	1.325	3.2	64.1	0.0086	2.0
C.F. 1.10.82	100	30	6.33	5.43	1.325	2.9	91.7	0.0113	2.64
L.M. 7.5.82	100	42.5	2.99	9.15	1.4	4.0	80	0.0152	3.56
L.M. 3.12.82	100	41.5	3.07	7.29	1.34	3.78	87.19	0.0106	2.47
R.P. 14.5.82	100	43	3.63	5.23	1.5	3.85	92.9	0.011	2.57
R.P. 19.11.82	100	43	2.32	4.76	1.26	3.57	95.0	0.0105	2.45
M.L. 1.10.82	400	45	3.11	5.79	1.34	4.28	91.4	0.0117	2.73
M.L. 7.1.83	600	43	3.53	7.43	1.4	4.3	93.7	0.0108	2.52
F.B.	100	45	3.52	9.18	1.35	3.84	-	0.0101	2.35
S.P.	100	36	3.54	7.36	1.55	3.55	-	0.01	2.33
T.E.	50	36.5	-	10.11	1.625	3.95	100.6	0.0106	2.47
L.D.	100	38	1.57	5.51	1.375	4.1	92.4	0.0125	2.92
A.D.	100	41	2.91	6.74	1.3	3.88	-	0.0116	2.71
H.H.	200	48	4.25	8.32	1.46	5.28	81.7	0.0116	2.71
T.B.	100	45	4.44	8.16	1.5	4.45	84.3	0.0105	2.45
S.B.	100	35	2.83	7.28	1.49	3.65	78.7	0.0111	2.59
G.C.	100	42	3.14	7.4	1.45	3.75	89.1	0.0098	2.28
M.N.	100	35	2.03	5.07	1.25	3.24	95.3	0.0118	2.75
M.G.	150	44.5	-	6.34	1.35	4.15	97.1	0.011	2.57
W.Z.	500	31	2.45	4.44	1.2	2.7	129.1	0.0114	2.66

TABLE 84

(continued)

PATIENT	DAPSONE DOSAGE (mg per day)	HAEMATOCRIT (%)	ϕ (mg ml^{-1})	RED CELL FLEX. INDEX (% min^{-1})	η_p (cP)	η_B (cP)	MCV (fl)	$\frac{\log \eta_r}{H}$	KT_c'
T.W.	200	37	2.97	5.12	1.3	3.4	94.7	0.0113	2.64
I.C.	50	34	2.33	8.67	1.35	3.2	91.4	0.011	2.57
D.L.	50	40	1.9	5.17	1.415	4.09	87.6	0.0115	2.68
S.G.	100	40	3.04	5.38	1.375	3.67	79.3	0.0107	2.49
A.B.	50	39	2.39	7.05	1.3	4.1	73.2	0.0128	2.99
N.S.	200	40	2.86	6.82	1.355	3.95	78.8	0.0116	2.71
G.R.	100	42.5	2.59	5.41	1.375	3.885	111.2	0.0106	2.47
E.EA.	100	45	3.68	5.81	1.425	4.425	86.4	0.0109	2.54
E.EV.	100	39.5	2.34	10.44	1.35	3.45	81.9	0.0103	2.4

TEMPERATURE OF VISCOMETRIC AND FLEXIBILITY INDEX MEASUREMENTS = 37.5°C

TABLE 84 (continued)

RHEOLOGICAL PARAMETERS FOR DAPSONE TREATED PATIENTS

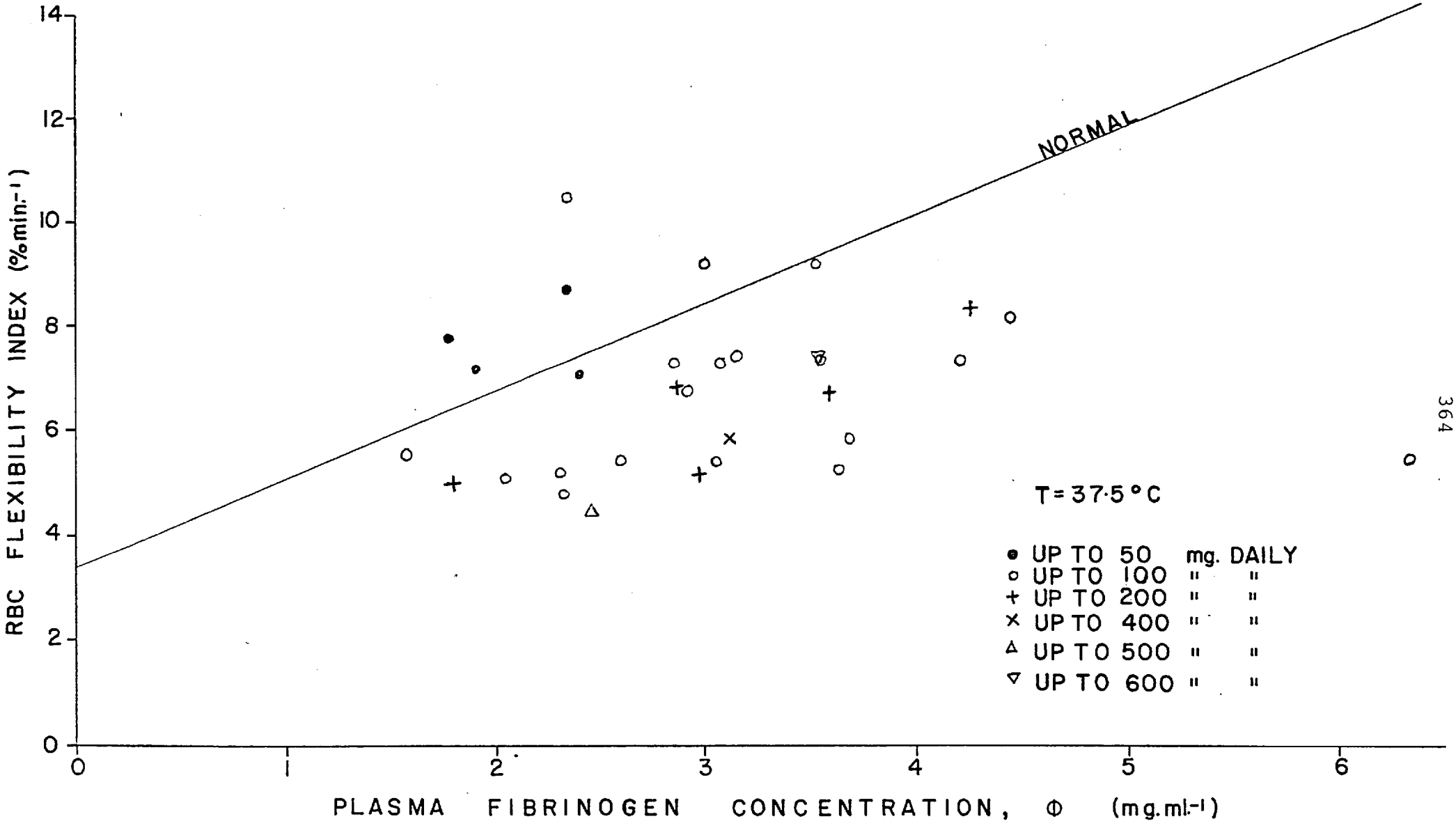


FIG. 87 : VARIATION OF RBC FLEXIBILITY INDEX WITH FIBRINOGEN CONCENTRATION FOR PATIENTS ON DIFFERENT DAPSONE DOSAGE

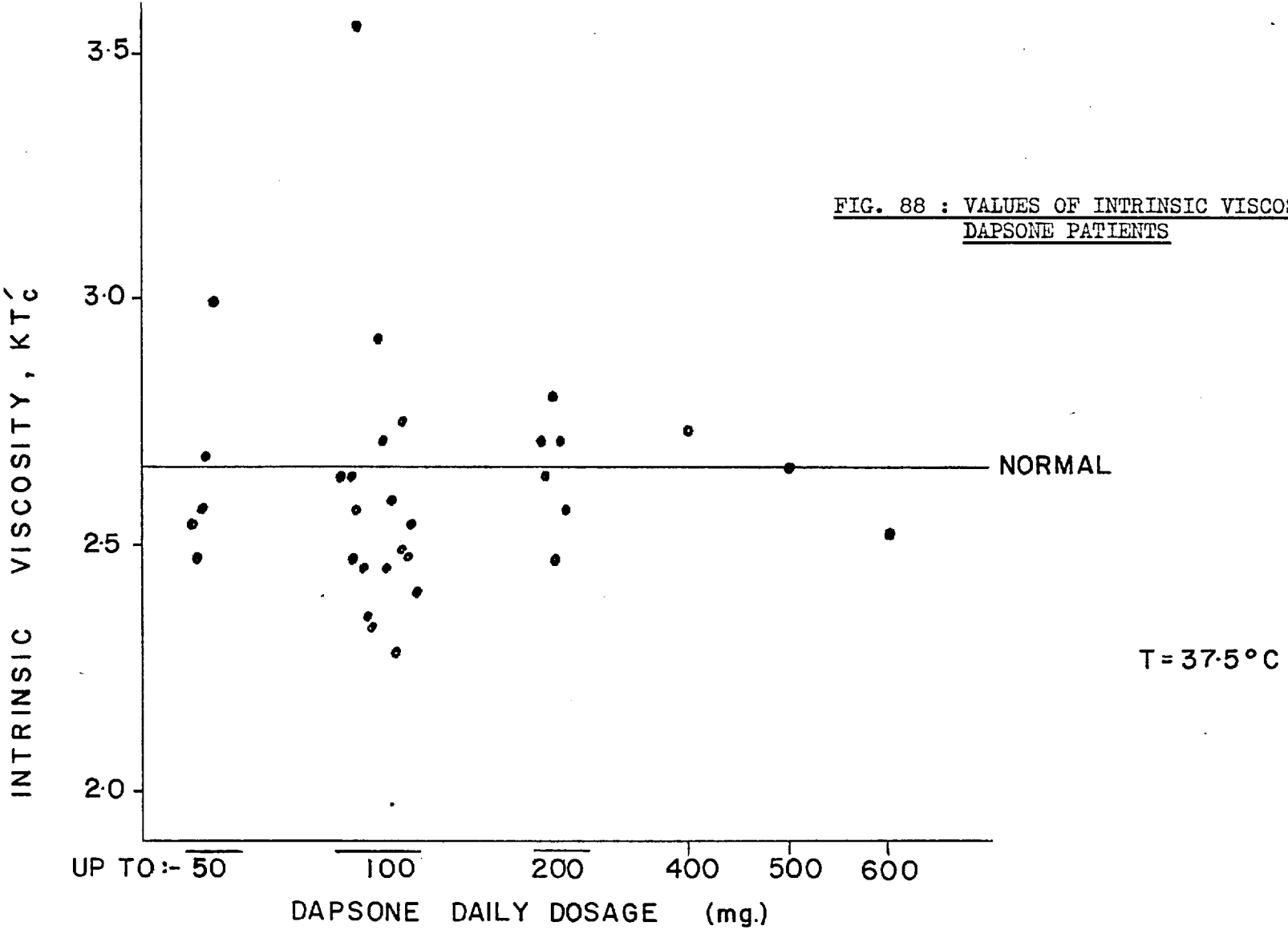


FIG. 88 : VALUES OF INTRINSIC VISCOSITY FOR DAPSONE PATIENTS

Disease, surveys and a trial were carried out on diseased, treated and normal healthy ponies, cross-bred and thorough-bred horses.

Table 85 shows the values of rheological parameters obtained for normal healthy (control) ponies. Tables 86 to 89 show the rheological parameters for four ponies; Windy, Bunting, Lisa and Galium respectively. These ponies were suffering from navicular disease, and a trail was carried out to find the effect of warfarin on their rheological parameters. The first blood sample was examined before the 5-day treatment period was started, and a blood sample was examined for each pony on each day of post-treatment, for the 5 days. In all four ponies, the intrinsic viscosity KT'_c varies greatly without a trend common to all four. However, there is a noticeable trend in the change occuring in the red cell flexibility index. For all four ponies the pre-treatment value of the flexibility index were higher than the values for the normal ponies and the final value at the end of the 5-day period, with variations in-between. Bunting (Table 87) shows the steadiest decline in red cell flexibility index with treatment period.

Table 90 shows the values of the blood rheological parameters in normal (control) thorough-bred horses. Their red cell flexibility index is in the range 27 to 33% min^{-1}. In untreated thorough-bred horses suffering from navicular disease, Table 91 shows that their flexibility index is raised to 38.5% min^{-1}, whereas in a long-term warfarin

PONY	H (%)	ϕ ($mg.ml^{-1}$)	MCV (fl)	η_B (cP)	η_p (cP)	η_r	RBC FLEXIBILITY INDEX (% min^{-1})	$\frac{\log \eta_r}{H}$	KT'_c
DANAKILL LAD	41	2.35	43.9	3.41	1.20	2.84	25.05	0.01106	2.58
PLOVER	33	3.22	44.2	2.71	1.32	2.05	23.35	0.0095	2.21
IVY	35	2.7	45.2	2.97	1.28	2.32	21.95	0.0104	2.42
GUS	33.5	2.2	47.1	3.43	1.24	2.77	21.13	0.0132	3.09
HOLLY	40	4.03	46.6	3.92	1.41	2.78	26.1	0.0111	2.59
MERLIN	34.4	3.06	46.3	2.63	1.16	2.27	24.9	0.0103	2.40
GROCK	31.3	2.59	43.2	2.38	1.145	2.08	25.95	0.0102	2.38

TEMPERATURE OF VISCOSITY AND FLEXIBILITY INDEX MEASUREMENTS = 37.5°C.

TABLE 85

RHEOLOGICAL PARAMETERS FOR NORMAL (CONTROL) PONIES

	DAYS OF WARFARIN TREATMENT					
	PRE TREATMENT	POST 1	2	3	4	5
HAEMATOCRIT %	35.0	37.3	37.5	38.0	34.5	34
η_B (cP)	3.42	2.98	3.09	3.04	3.11	2.89
η_p (cP)	1.42	1.38	1.40	1.36	1.27	1.30
η_r (cP)	2.42	2.15	2.21	2.24	2.45	2.22
KT'_c	2.57	2.07	2.14	2.14	2.64	2.38
ϕ ($mg.ml.^{-1}$)	3.83	2.57	3.83	2.51	4.0	3.39
RBC FLEXIBILITY INDEX (% min^{-1})	26.8	26.0	27.5	34.2	25.7	23.4
MCV (fl.)	44.2	44.8	45.6	45.3	45.2	45.6

TEMPERATURE OF VISCOSITY AND FLEXIBILITY INDEX MEASUREMENTS = 37.5°C

TABLE 86

RHEOLOGICAL PARAMETERS FOR 5-DAY WARFARIN TREATMENT OF WINDY (PONY)

	DAYS OF WARFARIN TREATMENT					
	PRE TREATMENT	POST 1	2	3	4	5
HAEMATOCRIT %	38.5	37.0	42.5	36.5	38.5	37.8
η_B (cP)	3.26	3.02	3.94	3.11	3.17	2.99
η_p (cP)	1.46	1.38	1.42	1.29	1.36	1.29
η_r (cP)	2.08	2.12	2.40	2.42	2.34	2.32
KT'_c	2.12	2.14	2.45	2.45	2.24	2.26
$\emptyset$ (mg.ml.$^{-1}$)	3.16	2.37	4.23	4.34	4.18	3.29
RBC FLEXIBILITY INDEX (% min^{-1})	30.0	26.0	24.7	28.5	27.5	24.1
MCV (fl.)	47.5	48.4	48.0	48.8	49.2	48.0

TEMPERATURE OF VISCOSITY AND FLEXIBILITY INDEX MEASUREMENTS = 37.5°C

TABLE 87

RHEOLOGICAL PARAMETERS FOR 5-DAY WARFARIN TREATMENT OF BUNTING (PONY)

	DAYS OF WARFARIN TREATMENT					
	PRE TREATMENT	POST 1	2	3	4	5
HAEMATOCRIT %	36.5	34.5	35.5	35.5	33.5	34
η_B (cP)	3.03	2.67	2.9	2.72	2.82	3.09
η_p (cP)	1.35	1.29	1.34	1.27	1.24	1.27
η_r (cP)	2.24	2.07	2.17	2.13	2.26	2.79
KT'_c	2.23	2.13	2.21	2.15	2.46	3.06
ϕ ($mg.ml.^{-1}$)	2.81	4.02	4.74	5.5	3.92	3.39
RBC FLEXIBILITY INDEX (% min^{-1})	28.7	28.5	33.7	30.2	29.8	24.1
MCV (fl.)	48	47.4	47.9	47.3	48	46.7

TEMPERATURE OF VISCOSITY AND FLEXIBILITY INDEX MEASUREMENTS = 37.5°C

TABLE 88

RHEOLOGICAL PARAMETERS FOR 5-DAY WARFARIN TREATMENT OF LISA (PONY)

	DAYS OF WARFARIN TREATMENT					
	PRE TREATMENT	POST 1	2	3	4	5
HAEMATOCRIT %	42.5	46	47	44.5	45	42.5
η_B (cP)	4.27	4.3	4.8	4.45	4.25	3.79
η_p (cP)	1.59	1.54	1.53	1.36	1.44	1.36
η_r (cP)	2.70	2.79	3.14	3.28	2.94	2.79
KT'_c	2.38	2.26	2.47	2.71	2.42	2.45
ϕ ($mg.ml.^{-1}$)	3.4	3.86	5.3	4.24	3.86	3.24
RBC FLEXIBILITY INDEX (% min^{-1})	26.8	25.5	30.2	31.9	30.3	24.1
MCV (fl.)	46.2	48.4	48.5	48.2	48.4	47.5

TEMPERATURE OF VISCOSITY AND FLEXIBILITY INDEX MEASUREMENTS = 37.5°C

TABLE 89

RHEOLOGICAL PARAMETERS FOR 5-DAY WARFARIN TREATMENT OF GALIUM (PONY)

THOROUGH-BRED HORSE (CONTROL)	H (%)	$\emptyset$ ($mg.mL^{-1}$)	MCV (fl.)	η_B (cP)	η_P (cP)	η_r	RBC FLEXIBILITY INDEX (% min^{-1})	$\frac{\log \eta_r}{H}$	KT'_c
JENNY	38	4.33	45.1	3.01	1.26	2.39	32.4	0.001	2.32
LISKA	49	3.77	48	4.04	1.20	3.37	27.4	0.0108	2.51
JUNIOR	38	3.71	41.8	3.01	1.255	2.46	30.0	0.0103	2.39

TEMPERATURE OF VISCOSITY AND FLEXIBILITY INDEX MEASUREMENTS = 37.5°C

TABLE 90

RHEOLOGICAL PARAMETERS FOR NORMAL (CONTROL) THOROUGH-BRED HORSES

THOROUGH-BRED HORSE	H (%)	$\emptyset$ ($mg.ml.^{-1}$)	MCV (fl.)	RBC FLEXIBILITY INDEX ($\% min.^{-1}$)
HALF ROYAL	33.2	3.18	42.6	38.5
DA CAFIO	47.7	3.29	46.7	38.5

TEMPERATURE FOR FLEXIBILITY INDEX MEASUREMENT = 37.5°C

TABLE 91

RHEOLOGICAL PARAMETERS FOR TWO UNTREATED
THOROUGH-BRED HORSES SUFFERING FROM NAVICULAR DISEASE

treated thorough-bred horse, Table 92, the flexibility index is reduced to 24.1% min^{-1}. Again, there is no noticeable trend in the values of KT'_c.

The red cell flexibility index values in normal (control) cross-bred horses, Table 93, are similar to those of thorough-bred horses, in the range 27 to 33% min^{-1}. Long-term warfarin treated cross-bred horses, Table 94, have a reduced flexibility index in the range 23 to 26% min^{-1}, similar to the long-term treated thorough-bred horse, Farimar, in Table 92. Here, again, there is no noticeable trend in the change in the values of KT'_c for normal and treated horses.

IV DISCUSSION

As was shown in Fig. (83), disease in man affects the blood rheology. The red cell flexibility index in the diseases I have surveyed was either decreased (cirrhosis of the liver), unchanged or increased. The red cell flexibility index in the case of cirrhosis of the liver is expected to decrease because in this condition, the blood protein synthesis of the liver is affected, leading to lower concentrations of plasma proteins which affect the flexibility of the red cell. Of the other diseases, the most significant increase in red cell flexibility are in pneumonia and bronchitis. In both of these conditions, the respiratory function is

THOROUGH-BRED HORSE	H (%)	ϕ ($mg.ml.^{-1}$)	MCV (fl.)	η_B (cP)	η_p (cP)	η_r	RBC FLEXIBILITY INDEX ($\% \ min^{-1}$)	$\frac{\log \eta_r}{H}$	KT'_c
FARIMAR	41.5	3.14	43.6	3.47	1.19	2.92	24.1	0.0112	2.61

TEMPERATURE = 37.5°C

TABLE 92

RHEOLOGICAL PARAMETERS FOR A THOROUGH-BRED HORSE ON LONG-TERM WARFARIN TREATMENT FOR NAVICULAR DISEASE

CROSS-BRED HORSE (CONTROL)	H (%)	ϕ ($mg.ml.^{-1}$)	MCV (fl.)	η_B (cP)	η_p (cP)	η_r	RBC FLEXIBILITY INDEX ($\%\ min.^{-1}$)	$\frac{\log \eta_r}{H}$	KT'_c
MIDNIGHT SAFARI	38.5	3.94	47.5	3.55	1.29	2.75	32.45	0.0114	2.66
DUKE	33	4.03	43	2.58	1.21	2.13	30.1	0.001	2.32
FAIRCLOUGH	34	4.1	42.1	2.66	1.15	2.31	27.5	0.0107	2.5

TEMPERATURE FOR VISCOSITY AND FLEXIBILITY INDEX MEASUREMENTS = 37.5°C

TABLE 93

RHEOLOGICAL PARAMETERS FOR NORMAL (CONTROL) CROSS-BRED HORSES

CROSS-BRED HORSE	H (%)	ϕ ($mg.ml.^{-1}$)	MCV (fl.)	η_B (cP)	η_p (cP)	η_r	RBC FLEXIBILITY INDEX ($\%\ min.^{-1}$)	$\frac{\log \eta_r}{H}$	KT'_c
RED LAW	41	2.39	48.4	3.41	1.31	2.6	25.2	0.0101	2.36
MAYFAIR TRAMP	38.5	2.06	41.1	3.17	1.23	2.58	26	0.0107	2.49
ANGRAM	34.4	2.29	46.2	-	-	-	23.1	-	-

TEMPERATURE = 37.5°C

TABLE 94

RHEOLOGICAL PARAMETERS FOR CROSS-BRED HORSES
ON LONG-TERM WARFARIN TREATMENT OF NAVICULAR DISEASE

affected and hence the oxygen exchange between the lungs and the blood is expected to be reduced. To compensate for this decrease in oxygen exchange, the body is stimulated to increase the red cell flexibility in two possible ways. The first is to make the red cell membrane more flexible during the erythrocyte production, possibly in the marrow, and secondly to stimulate the production of a plasma factor other than fibrinogen which interacts with the red cell to increase its flexibility. This factor is similar to the factor present in horse blood, and discussed in the previous chapter. These two methods of increasing the red cell flexibility are suggested because of the evidence of increased flexibility of red cells suspended in serum and saline (compared to normal cells) in the case of pneumonia and bronchitis as was shown in Table 80.

Alteration of the red cell flexibility in disease would be expected to alter other parameters such as viscosity and red cell shape factor, K. However, these parameters were not measured in my preliminary survey and should be the subject of a future detailed study.

The effect of prostacyclin (PGI_2) on the red cell was reported to increase its deformability by Belch et al (1981), decrease it by Dowed et al (1981) and to have no effect by Slott et al (1982). In the three investigations the red cell deformability was measured using the filtration technique. Besides the Fahraeus-Lindqvist effect, the presence of white blood cells, which have the effect of giving misleading data, could account for the three

different results using the same technique. By using the centrifuge technique, I have avoided these problems as well as having the advantage of using whole blood in its physiological state (whole blood was also used by Belch et al). From my data, I could not find any effect of PGI_2 on the red cell flexibility and therefore suspect that the vasodilatory action of PGI_2 and its inhibition of platelets aggregation are the dominant processes in its effect on the blood circulation.

In the dapsone therapy for the treatment of dermititis herpetiformis (DH), two patients, J.S. and L.G., were followed over a period of several months, in the investigation into the effect of dapsone on the blood rheological parameters. J.S. which was investigated before starting on dapsone and post treatment, has shown a great increase in the value of his red cell MCV from 94.3 to 132.4 fl. over a one month period before decreasing, over the following two months, to 116.8 fl., Table 83.

The dapsone treatment of patient L.G., over the 8 months period of investigation, resulted in his MCV decreasing gradually from 108.3 to 88.6 fl. As was shown in Chapter 5, the increase in red cell MCV, due to either hypotonicity or acidity, resulted in a decrease of the red cell flexibility index, and obviously the MCHC, due to dilution of the intracellular contents. However, the change in red cells density could be avoided when comparing samples by increasing the haematocrit to maintain a constant value of the product $(\rho_c - \rho_p)h'$, Table 35. Unfortunately no

measurements of the red cells or plasma densities were carried out during the investigations for both patients, and hence the data is insufficient to corret for this factor in the values of the red cell flexibility index.

Considering the available data, we can see that for patient J.S., there is an initial decrease in red cell flexibility, probably due to increasing MCV, but as the MCV starts to decrease, after the third sample, the red cell flexibility continues to decrease, probably as a result of decrease in Φ. The initial decrease in red cell flexibility would increase the value of T'_c but the increase in MCV would decrease the value of K. The net result on KT'_c is for its value to decrease, probably due to the decrease in K resulting from drastic increase in MCV. The overall effect on η_B is to decrease it in value. In the latter samples, the value of KT'_c changes appreciably about the normal value, but the value of η_B is maintained. This is probably due to the compensatory effect of decreasing Φ and red cell flexibility on η_B.

For patient L.G., the flexibility index decrease and increase is accompanied by the same change in Φ. Additionally there is the effect of decreasing MCV on the cells density and haemoglobin concentration, which was not corrected for. However, since the overall trend of KT'_c is to decrease, η_B would decrease, as observed. Hence, for the two patients, J.S. and L.G., the dapsone effects on

their blood rheology is to decrease their whole blood viscosity, η_B, by altering the red cell flexibility and shape, that is by decreasing the value of KT'_c.

From the survey of other DH patients, it was seen that although there was no significant difference, the dapsone treatment tended to decrease both the red cell flexibility index, Fig. (87), as well as the intrinsic viscosity, KT'_c, Fig. (88). This trend would agree with that observed for J.S. and L.G. but obviously a more detailed and comprehensive study is needed before such behaviour could be established.

From values of blood rheological parameters for ponies and horses which are healthy, suffering from navicular disease or being treated, there are two observations. The first, is that disease or warfarin treatment does not seem to affect the intrinsic viscosity as for humans treated with dapsone. The second, that navicular disease seems to increase the horse's or pony's red cell flexibility index, resulting in an increase in red cell aggregation, which is present in horse blood even at high shear rates, as was shown in Chapter 6. The increased red cell aggregation causes the blood flow to decrease and become 'sluggish', leading to necrosis of tissues in the navicular joint, as is characteristic of this disease. By decreasing the red cell flexibility, warfarin treatment decreases the degree of red cell aggregation, and improves the blood flow in the navicular joint. As a result of decreasing the red cell

flexibility, the whole blood viscosity, η_B, is not increased because of the compensatory effect of decrease in the degree of aggregation.

Being an anticoagulant, warfarin also avoids thrombus formation in the blood vessels of the navicular joint, thus improving the blood flow.

CHAPTER 9

THE EFFECT OF ANAESTHETICS AND HIGH PRESSURE ON THE RED BLOOD CELL FLEXIBILITY

I INTRODUCTION

Since the 1890s, following the investigations of Meyer (1899) and Overton (1901), it has been known that the potency of an anaesthetic increased proportional to its partition coefficient (concentration ratio) between olive oil and water. The correlation of anaesthetic potency with its partition coefficient has been found to be more successful than its correlation with physical properties of the anaesthetic molecule, Richards (1980).

The good correlation between solvent/water partition coefficient and anaesthetic potency (expressed as reciprocal of that aqueous concentration needed to produce general anaesthesia) for polar and apolar solvents, Franks and Lieb (1978), suggest that the anaesthetic site composed, at least partly, of polar and apolar portions of a protein, i.e. the site on the protein is amphiphilic.

The Ostwald solubility coefficients for various anaesthetic agents in water, oil, various fluids and biological tissues have been investigated and listed for normal conditions of pressure and temperatures, Larson et al (1962) and Steward et al (1973).

Johnson and Flagler (1950) were the first to report an observation that at hydrostatic pressures of about 150 atmospheres or higher, the narcotic effects of anaesthetics, in tadpoles, are reversed. Since then other workers, Lever et al (1971) and Halsey and Wardley-Smith

(1975), have reported the same effects. Since pressure acts to reduce volume, the total volume of anaesthetic site plus anaesthetic molecule is increased when they interact. Although such increase may only be a secondary effect to the mechanism of anaesthetics, some investigators suggest that it is the primary effect. Such investigators suggest that "anaesthesia occurs when the volume of a hydrophobic region is caused to expand beyond a certain critical amount by the absorption of molecules of an inert substance. If the volume of this hydrophobic region can be restored by changes of temperature or pressure then the anaesthesia will be removed", Miller and Smith (1973). According to this critical volume hypothesis, as the total pressure is raised, more anaesthetic is needed, to counteract the reduction in volume caused by pressure. This hypothesis is supported by the findings that there are good agreements between the predictions of the critical volume hypothetsis and the pressure reversal data using a simple binding model of the helium gas molecule as the anaesthetic agent, Miller et al (1973 and 1978). However, the possibility that pressure and anaesthetic act on different sites, with the pressure acting to increase excitability of the membrane to counteract the general decrease of excitability caused by anaesthetic, has been partly investigated by Miller et al (1978), but is in need of further substantiation.

Meyer (1901) found that aqueous potencies for tadpoles varied with temperature. Allot et al (1973) and several other workers, found that oil/gas partition coefficients of several anaesthetic agents in solvents and biological

media, decreased with increasing temperature. This observation can be explained in the simple binding model by the increasing temperature decreasing the gas phase potency, because of the tendency of heat to drive the anaesthetic off the site into the gas phase.

Using the magnetic resonance technique, Metcalfe et al (1968), showed that anaesthetics could increase the fluidity of membranes and lipid bilayers. The results led to the theory that by increasing the fluidity of the membrane lipids, anaesthetics perturbed the function of some crucial membrane protein such as nerve channels. However, Boggs et al (1976) and Richards et al (1978) could not find correlation between anaesthia and lipid bilayer structural changes. Franks and Lieb (1979), using X-ray diffraction, have demonstrated that although at very high anaesthetic concentrations the lipid bilayer showed increased disorder, while maintaining constant membrane thickness, at up to ten times the surgical concentrations of anaesthetics, no significant change is observed in the lipid bilayer and hence concluded that the lipid bilayer per se is not the primary site of action of general anaesthetics. Increasing temperature increases the membrane lipid bilayer fluidity but as was mentioned above the anesthetic solubility is decreased at the same time.

The permeability of lipid vesicles to cations has been shown to be increased in the presence of clinical concentrations of anaesthetics by up to 20% to 50% of normal value, Bangham et al (1965), Johnson and Bangham

(1969) and Pang et al (1979). This increase was explained to be due to the anaesthetic increasing the cation permeability at the cation permeability barrier, located at the aqueous-lipid interface. This was caused as a result of the anaesthetic increasing the freedom of movement of groups in the lipid molecule near the interface. The increased cation permeability in the presence of polar anaesthetics was found to be reversed at high pressures, Johnson et al (1973). The process of cation transfer across lipid bilayers is suggested to be a result of the anaesthetics causing a collapse of pH gradients across catecholamine - containing synaptic vesicles, Bangham and Mason (1980). However, experimental results by Gutknecht and Walter (1981) and Nozaki and Tanford (1981) suggest that the lipid bilayer permeability to protons, H^+ in this case, is too small or slow to support the explanation given by the above-mentioned workers for the effect of anaesthetics.

From these investigations into the mechanism of anaesthesia and the reversal of its effects by high pressure, one can see that although each theory can provide some evidence to support its conclusions, none of them gives a complete satisfactory explanation. However, one thing on which all workers in this field agree on is that the site of anaesthetic action on the membrane is a protein. The action of the anaesthetic molecule on its target site protein is to bind to it and inhibit its function somehow. The effect of high pressure, i.e. reversal of the anaesthetic effect, could very well be due to the pressure

'squeezing' the anaesthetic molecule away from its target site, Franks and Lieb (1982).

The phenomena of pressure reversal of the anaesthetic effect is of great importance for man in conditions of deep-sea diving. The high-pressure nervous syndrome (HPNS), which is a form of convulsions and tremors experienced by man and mammels exposed to pressures between 30 and 150 atmospheres, was first reported by Bennett (1967). The same phenomenon was reported to occur as repetitive impulse generation in isolated crayfish axons, at pressures from 1 to 200 atmospheres, Kendig et al (1978a), with low temperature acting in the same way as high pressure in increasing the impulse generation frequency. Hunter and Bennett (1974) observed that HPNS in man is augmented by rapid compression and is unaffected by temperature changes. They also suggest that HPNS is a manifestation of some aspect of hydrostatic pressure per se, and that prevention would be achieved by use of a mixture of inert and anaesthetic gases for breathing. The breathing mixture of gases found to avoid HPNS in human divers was $He-O_2-N_2$ mix, or Trimix, Bennett et al (1974). Kendig et al (1978b) observed that anaesthetics other than the inert gases inhibit the pressure-induced repetitive impulse generation in the isolated Crayfish nerves.

Because of the importance of the phenomenon of pressure reversal of anaesthetics effects in deep sea diving and the problems of HPNS, the following investigation, described below, was carried out. To find out whether the mechanism

of anaesthetics and pressure reversal is a physico-mechanical effect on the lipid bilayer, human erythrocytes were equilibrated with anaesthetics at normal and high pressures, and the red cell flexibility index was measured. If changes do occur in the structure of the lipid bilayer, its manifestation would be expected to result in a change in its mechanical properties, i.e. membrane flexibility.

II METHODS

(A) Anaesthetics

Five types of anaesthetics were used in this study. Three were fluids (at 20^{o}C); diethyl ether, chloroform and halothane. Two were gases; nitrous oxide and cyclopropane. The diethyl ether and chloroform were Analar grade (British Drug Houses Ltd., Poole, UK), the halothane was inhalation grade (May and Baker Ltd., Dagenham, UK), and the nitrous oxide and cyclopropane were obtained in pure gase form (British Oxygen Company Ltd., London, UK).

The concentration of anaesthetics needed to produce anaesthesia in man, Pryor and Bush (1973), are as follows. For ether, used in a mixture with oxygen and nitrous oxide, 20% ether vapour is needed. For halothane, mixed with oxygen and nitrous oxide, 1% to 4% vapour is needed. For

nitrous oxide, mixed with oxygen, 75% is needed. For cyclopropane, mixed with oxygen, 15% to 25% is needed. Chloroform is a very powerful anaesthetic, but due to its undesirable side-effects, it is not generally used on man. However due to its high Ostwald solubility coefficient in human blood, Table 95, it is expected to produce anaesthsia for concentrations up to 25% chloroform vapour, mixed with oxygen.

(B) Equilibration of Blood with Anaesthetics

A 10-litre glass flask was evacuated, and anaesthetics were introduced into it, by direct injection, to vapourise the anaesthetics (in the case of the fluids). The gas anaesthetics were introduced into the evacuated flask by connecting the flask directly to the gas cylinders. The concentration of anaesthetic in the flask was calculated in terms of its partial pressure as the flask is filled.

The vapour partial pressure is calculated using the gas equations

$$PV = nRT \quad \text{and} \quad \frac{P_o V_o}{T_o} = \frac{P_1 V_1}{T_1}$$

where n, the number of gram moles is calculated from the expression

$$n = \frac{m\rho}{n_o}$$

ANAESTHETIC	PHYSICAL DESCRIPTION AT 20°C	CHEMICAL STRUCTURE	MOLECULAR WEIGHT (gm.)	DENSITY AT 20°C (gm./ml.)	OSTWALD SOLUBILITY COEFFICIENTS* AT 37°C IN			
					WATER	OLIVE OIL	BLOOD	PLASMA
DIETHYL ETHER	LIQUID	$(C_2H_5)_2O$	74.12	0.714 to 0.718	13.0	65.0	12.0	12.27
CHLOROFORM	LIQUID	$CHCl_3$	119.4	1.474 to 1.479	4.0	400	8.0	10.3
HALOTHANE	LIQUID	$C_2HBrClF_3$	197.4	1.872 to 1.877	0.80	2.79	2.4	220
NITROUS OXIDE	GAS	N_2O	44.01	-	0.47	0.454	0.47	1.4
CYCLOPROPANE	GAS	C_3H_6	42.08	-	0.21	11.5	0.55	0.29

* VALUES OBTAINED FROM STEWARD ET AL (1973)

TABLE 95

PHYSICAL AND CHEMICAL PROPERTIES OF FIVE ANAESTHETICS USED

where m is the volume of fluid injected into the flask, ρ is the anaesthetic density and n_o is its molecular weights in grams, Table 95. P_o and T_o are the standard pressure (760 mm.Hg) and temperature (273°K) respectively, and V_o is the volume occupied by a gram mole at P_o and T_o (22.4 litres). T in this case is the room temperature (295°K) and V is the flask volume (10 litres).

Different concentrations of each anaesthetic was used to equilibrate blood. After filling the flask with the desired anaesthetic concentration, the pressure inside the flask is brought up to atmospheric pressure by adding 5% CO_2 and the rest is made up with air. A positive pressure of 10 mm.Hg is created inside the flask by adding N_2 from a pressurized N_2 cylinder.

The anaesthetic/Co_2/O_2 mixtures were used to equilibrate normal blood at room temperature (22°C) and at 37.5°C. The blood was divided into five 1 ml. samples, with each sample equilibrated for different periods. The equilibration periods were 5, 15, 30, 60 and 90 minutes. The samples were continually agitated while placed in a water bath at the chosen temperature.

Control blood samples were equilibrated with 5% CO_2, 20% O_2 and 75% N_2, for the same maximum period as the anaesthetics samples, i.e. 90 minutes.

(C) Effect of Anaesthetics on Red Cell Flexibility

To investigate the effect of anaesthetics on the red cell flexibility index, the red cell packing rate was measured for samples equilibrated for different periods with each of the four different anaesthetics at different concentrations and temperature. For comparison, the red cell packing rate was also measured for the control samples at the two temperatures used. The red cell packing rate was measured on the automatic recording centrifuge described in Chapter 2.

(D) Effects of Anaesthetics at High Pressure on Red Cell Flexibility

To pressurise blood-filled capillary tubes up to 100 atmospheres, while measuring the red cell packing rate, a hydraulically operated aluminium pressure chamber was designed and built, see Plate 3 and schematic Fig. (89). In this pressure chamber the blood-filled capillary tube is placed in an environment of siliconeoil (3 in 1 oil, E.R. Howard Ltd., Ipswich, UK). The chamber is then sealed and filled with oil to remove air bubbles. To increase the pressure, the piston is screwed in and the pressure is read on the pressure guage. The chamber is designed to fit onto the centrifuge head, so as to enable centrifugation of blood samples on the duraluminium centrifuge head as well as the pressure chamber. The radius of the bottom of the capillary tube inside the chamber is equal to the radius to



PLATE 3 : HIGH-PRESSURE CHAMBER

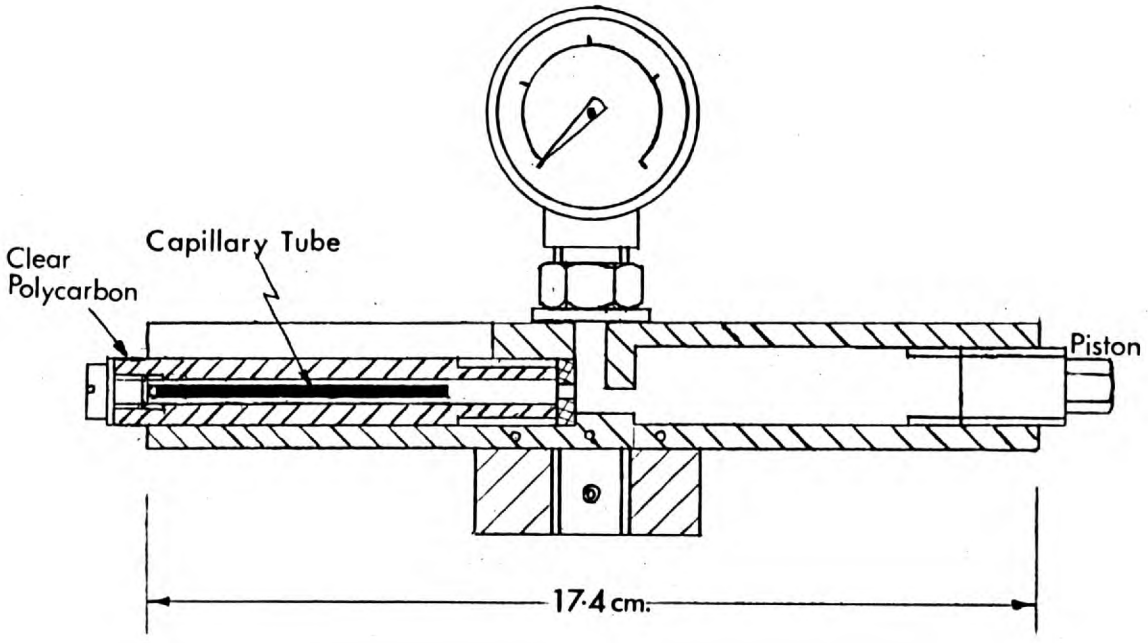


FIG. 89 : SCHEMATIC DIAGRAM OF HIGH-PRESSURE CHAMBER

the bottom of a capillary tube on the centrifuge head. Therefore, the centrifugal forces are equal. The density of oil is less than that of plasma and therefore there are no mixing of the oil with blood during centrifugation.

To avoid air being dissolved into the blood at high pressures, the following procedure was adopoted with all blood-filled capillary tubes placed into the chamber. The capillary tube was filled with blood and the clean end of the tube was sealed in a bunsen flame. The blood was then pushed to the bottom of the tube (to the sealed end) by gently tapping the tube. The empty length of the tube was then broken off to leave a sealed capillary tube completely filled only with blood.

To find whether the oil environment affected the red cell flexibility measurement at atmospheric pressure, blood samples were placed into the unpressurized chamber in an oil environment, then the packing rate was measured. This was compared to a blood sample placed in the unpressurized chamber with all the oil removed. It was not possible to carry out the same test for high pressures as the only pressure medium available, which is safe and inert, was siliconeoil.

The effect of rapid compression on the red cell flexibility was measured by compressing the blood rapidly then measuring the packing rate. Effect of rapid decompression was measured by pressurizing the blood gently, equilibrating the blood for half-an-hour, while mixing on

the rotary mixer (described in Chapter 2), to which the pressure chamber is attached, then rapidly depressurizing the blood from 100 atms (1500 p.s.i.) to 1 atms and measuring the packing rate.

The effect of high pressure on the packing rate of blood equilibrated with anaesthetics for 1½ hours was investigated by pressurizing the blood samples up to 1500 p.s.i., mixing the blood for half-an-hour on the rotary mixer, then measuring the packing rate.

The packing rate was measured in the same way as for samples placed on the duraluminium head, i.e. by plotting from negatives of the camera shots (described in Chapter 2). The difference in colour and defractive index (due to difference in density) between plasma and silicon oil, enables the interface to be seen photographically.

The haematocrit for blood samples was measured by centrifugation for 4 minutes at 13,000 g on the Hawksley Microhaematocrit centrifuge.

III RESULTS

(A) Effect of Anaesthetics on the Red Cell Flexibility

(i) Diethyl ether

Table 96 shows the values of the red cell flexibility index, measured in duplicate, for blood samples equilibrated for 0 (control sample), 5, 15, 30, 60 and 90 minutes at 22° and 37.5°C with concentrations of 4%, 10%, 20% and 50% ether. In each case the ether vapour was mixed with 5% CO_2 and air. The results show that within the measuring error of $\pm$ 0.5% min^{-1} for the red cell flexibility index, equilibration of blood with the anaesthetic has no effect on the red cell flexibility.

(ii) Chloroform

For blood samples equilibrated for periods between 0 (control) and 90 minutes with concentrations of 4%, 20% and 30% chloroform vapour (mixed with 5% CO_2 and air), Table 97 shows that chloroform has no effect on the red cell flexibility at 22° or 37.5°C.

(iii) Halothane

From Table 98, again it is shown that for bloods equilibrated with 4%, 10% and 20% halothane, for different periods and temperature, the red cell flexibility index is unaltered by the anaesthetic.

ANAESTHETIC CONCENTRATION	22°C						37.5°C					
	ANAESTHETIC EQUILIBRATION TIME (mins.)						ANAESTHETIC EQUILIBRATION TIME (mins.)					
	0	5	15	30	60	90	0	5	15	30	60	90
4%	5.63 5.2	5.22 5.4	5.3 5.4	5.4 5.51	5.24 5.4	5.51 5.74	7.72 7.86	7.72 7.6	7.04 7.18	7.22 6.96	7.86 7.52	8.12 7.58
10%	5.64 6.08	5.54 5.54	5.8 5.97	5.73 5.94	5.43 5.63	5.37 5.76	8.28 8.17	8.13 8.9	7.94 7.85	8.14 7.94	7.33 7.86	8.13 7.91
20%	3.98 4.56	4.72 4.8	4.45 4.53	4.38 4.68	4.31 4.88	4.57 4.33	8.23 8.68	9.15 9.15	8.98 8.87	7.7 7.89	8.2 9.06	8.19 8.68
50%	4.89 4.95	5.01 5.21	5.21 4.85	5.13 5.11	5.21 4.98	4.88 5.03	7.95 8.02	8.11 8.21	7.95 7.87	8.14 8.12	7.89 8.17	8.14 7.98

TABLE 96

RED CELL FLEXIBILITY INDEX VALUES (% min^{-1}) FOR BLOOD EQUILIBRATED FOR DIFFERENT PERIODS WITH DIFFERENT CONCENTRATIONS OF DIETHYL ETHER AT 22°C AND 37.5°C

ANAESTHETIC CONCENTRATION	22°C						37.5°C					
	ANAESTHETIC EQUILIBRATION TIME (mins.)						ANAESTHETIC EQUILIBRATION TIME (mins.)					
	0	5	15	30	60	90	0	5	15	30	60	90
4%	4.73 5.13	5.56 5.13	5.15 5.14	4.89 4.93	5.53 4.98	4.82 5.04	7.73 7.91	8.29 8.08	8.2 7.98	7.62 8.11	7.9 8.05	7.62 8.12
20%	4.79 5.2	4.87 4.59	4.87 4.52	4.83 4.8	4.62 4.61	5.32 5.01	7.69 7.86	7.77 8.35	8.06 7.75	8.45 7.79	7.69 7.61	7.68 7.25
30%	5.49 4.89	4.82 4.87	4.82 5.2	4.62 5.04	4.78 5.17	5.16 4.67	8.2 7.98	7.75 7.84	7.93 7.66	8.22 8.17	8.03 7.96	7.78 8.01

TABLE 97

RED CELL FLEXIBILITY INDEX VALUES (% min^{-1}) FOR BLOOD EQUILIBRATED FOR DIFFERENT PERIODS AT DIFFERENT CONCENTRATIONS OF CHLOROFORM AT 22° AND 37.5°C

ANAESTHETIC CONCENTRATION	22°C						37.5°C					
	ANAESTHETIC EQUILIBRATION TIME (mins.)						ANAESTHETIC EQUILIBRATION TIME (mins.)					
	0	5	15	30	60	90	0	5	15	30	60	90
4%	4.61 4.64	4.82 4.79	4.23 4.5	4.61 4.99	4.65 4.36	4.58 4.89	8.58 8.78	8.9 8.79	8.57 8.14	8.92 8.59	8.29 9.01	8.65 8.43
10%	4.85 4.97	4.79 4.99	5.2 4.61	4.94 4.64	4.91 5.28	5.22 4.8	7.9 7.84	8.14 7.84	7.61 7.88	8.15 7.69	7.56 7.84	7.99 8.0
20%	5.23 5.51	5.68 5.61	5.33 5.01	5.5 5.59	5.0 4.87	5.69 5.47	8.11 8.19	8.32 8.67	7.85 7.89	8.15 8.27	8.13 8.13	8.29 8.58

TABLE 98

RED CELL FLEXIBILITY INDEX VALUES (% min^{-1}) FOR BLOOD EQUILIBRATED FOR DIFFERENT PERIODS WITH DIFFERENT CONCENTRATIONS OF HALOTHANE AT 22°C AND 37.5°C

(iv) Nitrous oxide

Again for blood equilibrated with 75% nitrous oxide, for different periods and temperatures, Table 99, shows that the red cell flexibility is unaltered.

(v) Cyclopropane

For blood equilibrated with 30% cyclopropane, for different periods and temperatures, the red cell flexibility index is unaffected by the anaesthetic, Table 100.

(B) Effect of Rapid Compression and Decompression on the Red Cell Flexibility

At atmospheric pressure, the red cell flexibility index of blood samples placed in an environment of silicon oil was not altered compared to samples in ordinary air environment, Table 101. Due to the inertness of silicone oil (in terms of its chemical reaction with blood) at atmospheric pressure, it is assumed that at high pressures of 100 atms, it would still have no effect on the red cell flexibility. This assumption is probably supported by the findings that rapid compression and decompression of blood, in siliconeoil environment, has no effect on the red cell flexibility index, Table 101.

ANAESTHETIC CONCENTRATION	22°C						37.5°C					
	ANAESTHETIC EQUILIBRATION TIME (mins.)						ANAESTHETIC EQUILIBRATION TIME (mins.)					
	0	5	15	30	60	90	0	5	15	30	60	90
75%	5.26 5.71	5.15 5.15	4.99 4.02	5.68 4.83	4.87 4.78	5.02 4.97	8.31 8.03	7.75 7.99	7.75 7.87	8.07 8.25	8.39 8.18	8.06 8.34

TABLE 99

RED CELL FLEXIBILITY INDEX VALUES (% $min.^{-1}$) FOR BLOOD EQUILIBRATED FOR DIFFERENT PERIODS WITH 75% NITROUS OXIDE AT 22°C AND 37.5°C

ANAESTHETIC CONCENTRATION	22°C						37.5°C					
	ANAESTHETIC EQUILIBRATION TIME (mins.)						ANAESTHETIC EQUILIBRATION TIME (mins.)					
	0	5	15	30	60	90	0	5	15	30	60	90
30%	5.67 5.75	4.94 5.62	5.12 5.40	5.38 4.92	5.47 5.61	5.08 5.81	7.35 7.64	7.2 7.48	7.88 7.14	8.02 8.33	7.9 8.23	7.75 8.21

TABLE 100

RED CELL FLEXIBILITY INDEX VALUES (% $min.^{-1}$) FOR BLOOD EQUILIBRATED FOR DIFFERENT PERIODS WITH 30% CYCLOPROPANE AT 22°C AND 37.5°C

(C) Effect of Anaesthetics at High Pressure (100 atms) on the Red Cell Flexibility

Blood, equilibrated with various concentrations of five anaesthetics and air, is pressurized to 100 atms, the red cell flexibility index at high pressure is not altered from the values of 1 atms., Table 102. Therefore high pressure or anaesthetics at high pressure have no effect on the red cell flexibility.

IV DISCUSSION

The absence of effects of anaesthetics, at atmospheric pressure, on the red cell flexibility suggests that anaesthetics do not affect the structure of the lipid bilayer. These findings agree with the X-ray diffraction studies of lipid bilayers, equilibrated with anaesthetics, of Franks and Lieb (1979) who observed that there was no significant change in the lipid bilayer due to anaesthetics. Hence my conclusion agrees with theirs, that 'the lipid bilayer per se is not the primary site of action of general anaesthetics'. This conclusion leaves the question of where do anaesthetics act unanswered but perhaps points to the theory that anaesthetics act to alter membrane permeability to cations, Bangham et al (1965), Johnson and Bangham (1969), Pang et al (1979) and Bangham and Mason (1980).

	RED CELL FLEXIBILITY INDEX AT 22°C ($\% \text{ min}^{-1}$)	AVERAGE VALUE ($\% \text{ min}^{-1}$)
CONTROL SAMPLES IN AIR AT 1 ATMS.	5.56; 5.43; 5.17; 5.21	5.34
SAMPLES IN SILICON OIL AT 1 ATMS.	5.23; 5.54; 5.33; 5.17	5.32
SAMPLES RAPIDLY COMPRESSED FROM 1 ATMS. TO 100 ATMS.	5.04; 5.63; 5.54; 4.99	5.3
SAMPLES RAPIDLY DECOMPRESSED FROM 100 ATMS. TO 1 ATMS.	5.28; 5.14; 5.67; 5.32	5.35

TABLE 101

EFFECT OF OIL ENVIRONMENT AND RAPID COMPRESSION AND DECOMPRESSION ON THE RED CELL FLEXIBILITY INDEX OF HUMAN BLOOD

ANAESTHETIC	CONCENTRATION %	RED CELL FLEXIBILITY INDEX AT 1 ATMS (% min^{-1})	RED CELL FLEXIBILITY INDEX AT 100 ATMS (% min^{-1})
AIR	100	5.56; 5.43; 5.17; 5.21	5.45; 4.97; 5.55; 5.32
DIETHYL ETHER	4 10 20 50	5.6; 5.42; 5.71; 5.02; 5.45 5.64; 6.08; 4.98; 5.93; 5.47 4.87; 5.5; 5.81; 5.61; 5.73 6.01; 6.23; 5.97; 5.74; 6.13	5.71; 4.96; 4.97; 5.43; 5.51 6.13; 6.27; 5.12; 5.04, 5.52 5.44; 5.78; 5.63; 5.47; 4.91 5.53; 5.41; 6.39; 6.37; 5.94
CHLOROFORM	4 20 30	4.71; 5.08; 5.18; 4.87; 5.02 4.73; 5.41; 4.87; 5.31; 5.27 5.21; 4.97; 5.23; 4.81; 5.47	5.51; 5.24; 5.44; 4.78; 5.08 5.14; 5.17; 5.05; 4.96; 5.07 4.88; 4.97; 4.79; 4.99; 5.5
HALOTHANE	4 10 20	4.62; 4.78; 4.92; 4.81; 4.7 6.28; 6.53; 6.04; 5.97; 6.1 5.78; 6.03; 5.88; 6.32; 5.91	5.03; 4.51; 4.88; 4.92; 4.5 5.89; 6.11; 6.43; 5.97; 6.01 6.05; 6.24; 5.99; 5.87; 6.13
NITROUS OXIDE	75	4.89; 4.76; 4.91; 4.65; 5.01	4.77; 4.82; 5.21; 4.62; 5.01
CYCLOPROPANE	30	5.52; 6.12; 5.04; 6.11; 5.78	5.81; 5.77; 6.13; 5.42; 5.83

TEMPERATURE FOR FLEXIBILITY INDEX MEASUREMENTS = 22°C

TABLE 102

RED CELL FLEXIBILITY INDEX VALUES, AT ATMOSPHERIC AND HIGH PRESSURE, FOR BLOOD EQUILIBRATED WITH DIFFERENT ANAESTHETICS AT DIFFERENT CONCENTRATIONS FOR 1½ HOURS AT 22°C

Rapid compression of human or animal subjects, increases the intensity of high-pressure nervous syndrome (HPNS), Hunter and Bennett (1974) and Brauer et al (1975). Rapid decompression causes, in man, what is known as the 'bends', which is a painful destruction of tissues, especially the soft tissues in bone joints, bubbles in the blood, and haemorrhage from the mouth, ears and nose. The cause of this condition is due to the dissolved gases, under pressure, destroying the tissues as they are released by sudden decompression. Although the effects of decompression can be explained in terms of the sudden release of dissolved gases, the effects of rapid compression on the nervous system is not understood. In both cases my findings suggest that the red cell membrane flexibility is not affected by the rapid change in pressure and hence the erythrocyte membrane lipid bilayer can either withstand rapid changes in pressure or adapts very quickly, leaving it in both cases structurally unaltered.

The ability of the erythrocyte membrane lipid bilayer to withstand or adapt to high pressure is seen from my results of the red cell flexibility index at high pressure. In the presence or absence of anaesthetics, the red cell flexibility index at high pressure is not changed from values at atmospheric pressure. This suggests that the phenomenon of pressure reversal of anaesthetics effects is not due to changes in the mechanical properties of the red

cell membrane lipid bilayer. This again supports the suggestion that the site of action of anaesthetics is not on the lipid bilayer.

When mammalian red cells, suspended in saline, are subjected to pressures of the order of 1500 atms. for 15 minutes, Haubrich (1937) observed that all the cells are converted to spheres. Although this phenomena is not fully understood, the order of pressure needed to sphere the cells explains why no changes were observed in the red cells' mechanical properties at the 100 atms. pressure used in the above investigation.

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Measurement of erythrocyte deformability using a stroboscopic recording centrifuge

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Abstract. A stroboscopic recording centrifuge technique is described which can measure deformability of red blood cells in terms of the rate of packing at low g. It can be used to measure several whole blood samples simultaneously, with accurate control of the speed of rotation and recording time. Only 0.05 ml of blood is required per measurement, and no prior treatment or preparation of the sample is necessary. The calibration procedures to allow for variations of the haematocrit and plasma fibrinogen level are explained.

1. Introduction

Erythrocyte deformability is an important component of blood viscosity and one of the main factors determining blood flow through the microcirculation. The rate at which oxygen can be exchanged with haemoglobin in intact erythrocytes is also dependent on the flexing and internal mixing within the cells (Sirs 1968a). The centrifuge method for measuring erythrocyte deformability, in terms of the initial packing rate at 200 to 600g acceleration, gives an index which can be directly related to whole blood viscosity (Sirs 1981) and the rate of oxygen exchange. Two methods for measuring the packing rate have so far been used, a stop–start procedure (Sirs 1968b) and an automatic recording technique (Sirs 1970). The stop-start procedure suffers from the disadvantages that neither the speed nor the time of rotation can be accurately controlled, though several samples can be studied simultaneously. This technique is comparative, and a control sample is included so that experiments can be inter-related from day to day. The automatic centrifuge method records the rate of packing photographically, and provides accurate control of the time and speed of rotation, but, by utilising the light transmitted through the tube, only one blood sample can be studied at a time. This limits the use of the technique, and surveys of patients are laborious. The stroboscopic recording technique described in this paper combines the advantages of precise timing and speed control, with the measurement of numerous blood samples simultaneously. It is relatively inexpensive and provides a simple procedure which can be used for routine clinical investigations.

2. Materials and methods

A schematic representation of the stroboscopically illuminated centrifuge system is shown in figure 1. It consists of a centrifuge, a stroboscope that is electrically triggered as the centrifuge rotates, and a standard 35 mm reflex camera (Olympus OM 10),

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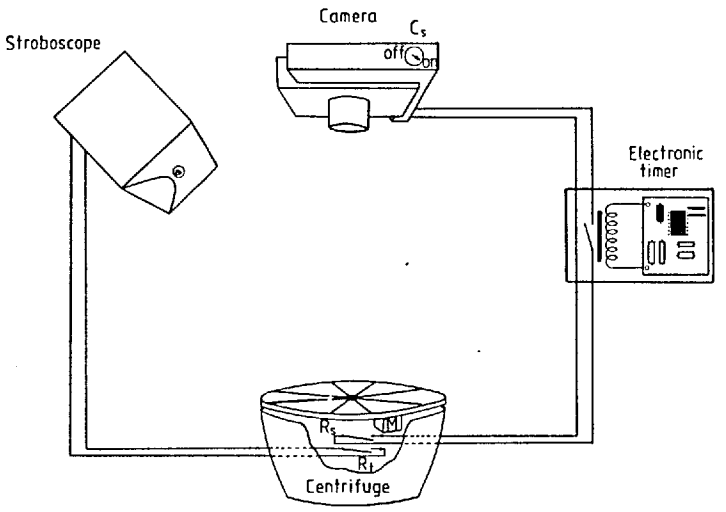


Figure 1. Schematic diagram of the stroboscopic recording centrifuge. Reed switches R_s and R_t synchronise camera and stroboscope respectively to the centrifuge rotation.

which is controlled by a timer to take pictures every 30 s and then automatically wind on. A photographic record is obtained of the length of the red-cell column at given time intervals, by synchronously illuminating the centrifuge head with the high intensity strobe lamp. The centrifuge was assembled by attaching a duraluminium head, from a Hawksley microhaematocrit centrifuge, to a squirrel-cage GEC 1/4 horse-power motor. The power of the motor is sufficient to ensure it runs constantly at 1480 RPM, and reaches this speed within 3 s of switching on. A small magnet attached to the underside of the duraluminium head is used to close transiently two magnetic dry-reed switches (RS Components type 338-153) as it passes over them each cycle. The first magnetic relay is connected in series with a timer relay, controlled by an electronic delay circuit prior to being connected to the camera. The timer relay is closed for 0.1 s every 30 s. Assuming the timer relay is closed, the operation of the first magnetic reed-switch triggers the camera to open the shutter for 1/30 s. Following a further rotation of the centrifuge head by one third of a revolution, in about 0.015 s, the magnet then passes over and closes the second reed-switch, triggering the strobe lamp (Strobe Automation Ltd). This produces a high-intensity flash of about 10^{-5} s duration every cycle. The camera shutter closes when the head has rotated about two thirds of a revolution, relative to the initial closing of the first magnetic relay. The optimum exposure on Ilford FP4 film is obtained by varying the lens aperture, from about F2.8 to 4.0. After each exposure the winder motor, attached to the camera, automatically advances the film for the next operation. During the 0.3 s required to advance the film, the camera does not respond to any further pulses. The stroboscopic recording centrifuge is operated in a dark room, using safety lights.

After developing the exposed film the percentage packing at known times in terms of the length of the red-cell column to the total length of the cells plus plasma, is obtained by projecting the photographic record onto graph paper, using an enlarger. This is the most tedious part of the procedure, and it is intended to eventually avoid this stage by using photocells to record the light transmitted through the plasma layer and connecting directly to a pen recorder. An index of erythrocyte deformability is

obtained by measuring the initial slope, in % min^{-1}, from the curve of percentage haematocrit against time.

To provide temperature control during centrifugation the centrifuge head and sample tubes are enclosed in a box, with a glass top to permit observation. The air space can be heated or cooled with a heater and air blower over the temperature range 15–50 °C (±1 °C). A partition separately enclosed the squirrel-cage motor, which is air cooled by a fan.

Blood samples are obtained by vene-puncture, using heparin to prevent coagulation, and measurements made within four hours of collection. It has been shown by collecting blood samples with no anticoagulant and measuring the packing rate immediately, by using blood from haemophilic patients, and by varying the amount of heparin used, that this anticoagulant has no discernable effect on the packing rate at a concentration of 12.5 units of heparin per ml of blood. Adding high concentrations of heparin *in vitro* can give rise to abnormally high packing rates. Only 0.05 ml of blood is required for each determination of the erythrocyte deformability. Measurements are also made of the PCV of the blood sample, by centrifuging at 13 000*g* for 3 min, and of the plasma fibrinogen concentration. The latter was determined by adding 20 units of thrombin to 0.2 ml of plasma. The resulting clot was removed on a stick and dried, then dissolved in 5 ml of 4M urea in 0.1M NaOH. The concentration of dissolved protein was ascertained with a UV spectrophotometer.

Relative to the force required to disrupt Rouleaux formation, the pressure on the cells during centrifugation is high, and comparable to the pressures occurring in the microcirculation. The pressures arises from the weight of cells in contact with each other, being supported by the base of the centrifuge tube. Allowing, by Archimedes principle, for the buoyancy of the cells in plasma, the pressure at the bottom of the cell column is given by $h'(\rho_c-\rho_p)g' \times 10^{-1}$ Pa (Sirs 1968a). With a speed of rotation of 1480 RPM and a mean radius of the cell column about the point of rotation of 7 cm, the acceleration g' is equal to 172*g*. Taking the cell column length, h', as half the total length of cells plus plasma, equal to 2.5 cm, the cell, ρ_c, and plasma, ρ_p, specific gravities as 1.10 and 1.03 respectively, the pressure acting on the cells is 22.0 mmHg.

3. Results

Typical photographic records of the centrifuge head and sample tubes, at given time intervals are shown in figure 2. Using a series of such records a curve of the haematocrit with time can be constructed as indicated (figure 3). The initial slope of these curves, in terms of packing rate (in % min^{-1}) provides an index of erythrocyte deformability. The packing rate depends on the PCV, and to allow for this a calibration curve is obtained of the packing rate at various haematocrits. This is simply produced by diluting the blood sample with its own plasma or removing plasma. A series of results, on blood samples from several subjects, can be used to give the general calibration curve shown in figure 4, by taking the packing rates at different haematocrits relative to that at 45% haematocrit. The deformability of erythrocyte in blood of less than 35% haematocrit cannot be directly measured by this technique, as the implicit requirement that the cells are in contact with each other throughout the centrifugation is then no longer valid. A measure of erythrocyte deformability in patients with low haematocrits can be obtained by first gently centrifuging and removing some plasma to bring the haematocrit above 35%. Using the calibration curve a direct comparison

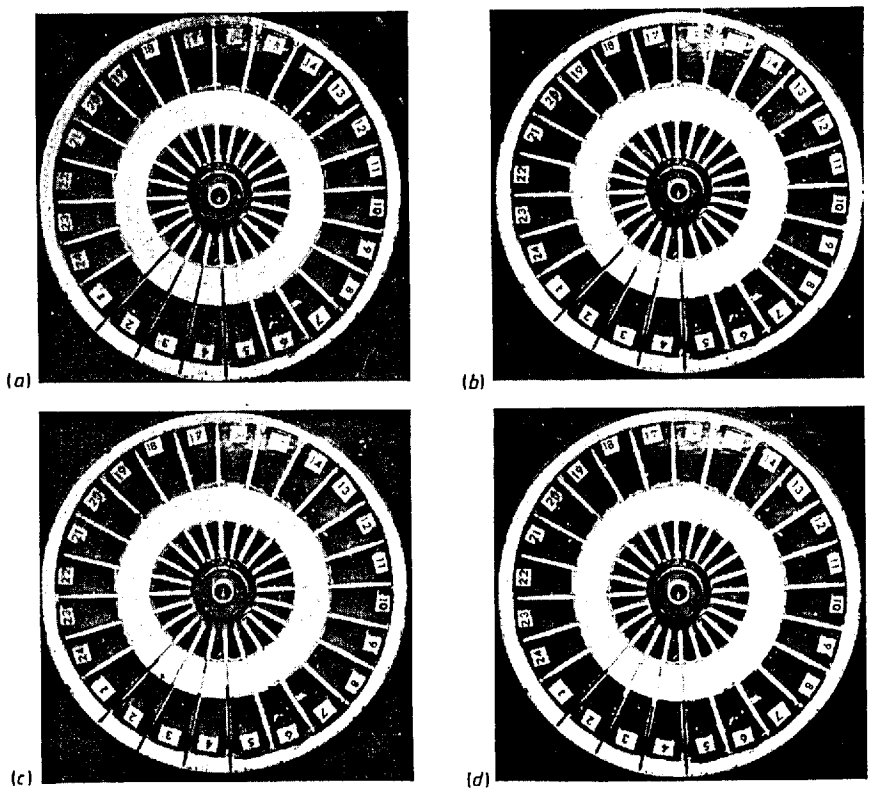


Figure 2. Four blood samples from the same individual at different lengths of RBC and plasma columns. Photographs are at different times during centrifugation, (*a*) 0.5 min; (*b*) 1.5 min; (*c*) 4 min; (*d*) 6 min.

of the erythrocyte deformability between individuals and in disease can be obtained by adjusting the measured packing rate to an haematocrit of 45%.

It became apparent in the earlier experiments, that erratic differences in the packing rate occurred when the microhaematocrit tubes were loaded onto the centrifuge head while other blood samples were prepared. The packing rates for identical blood

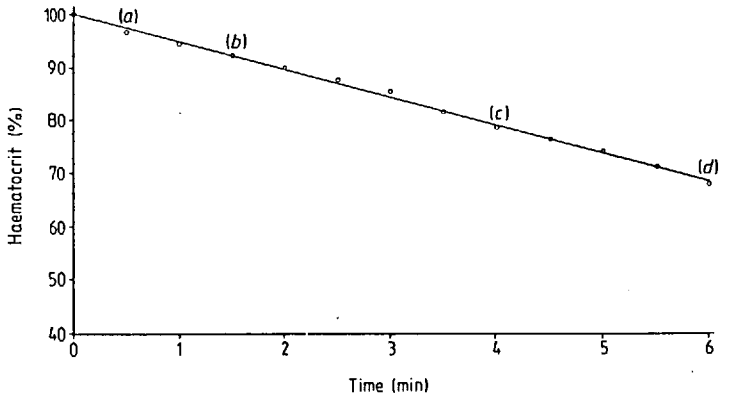


Figure 3. Typical curve of haematocrit change plotted against time, for blood samples of figure 2 during centrifugation. All four measurements, for blood sample in position 3, fit on the same curve within $\pm\frac{1}{2}\%$.

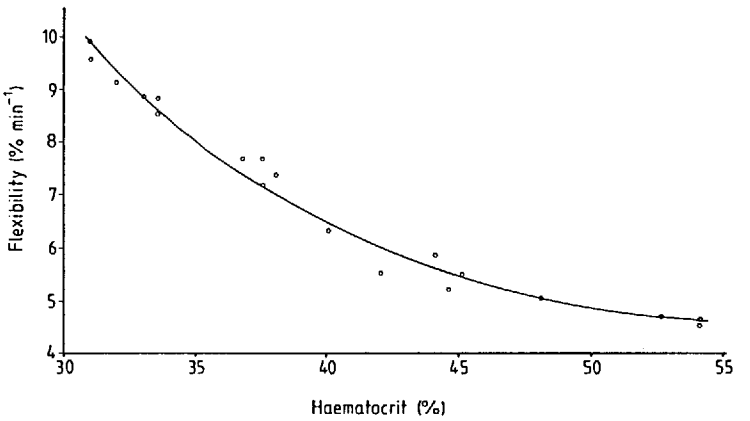


Figure 4. The relative change of flexibility with haematocrit, obtained by scaling curves from several normal individuals, after being made to correspond at 45% haematocrit.

samples, which were centrifuged immediately or left for 15 min and 25 min on the centrifuge head, were 5.4, 13 and 18% min^{-1} respectively. This was due to the settling of cells under gravity giving an unequal distribution over the tube cross-section. To overcome this problem a mixer was made, consisting of a wooden disc, rotated in the vertical plane by a 15 RMP motor, round the circumference of which the centrifuge tubes were attached horizontally with Plasticine. The efficiency of this device was checked by comparing the packing rate of blood samples which had been left on the mixer for up to 30 min with a fresh sample. No significant differences (±0.5% min^{-1}) in the packing rates were then observed. Routinely, blood samples are transferred into the microhaematocrit tubes which are then sealed and placed on the mixer. When all the tubes are ready, they are quickly transferred to the recording centrifuge and the erythrocyte deformability measured.

After a correction has been made for the haematocrit, further allowance must be made for the influence of the plasma fibrinogen concentration on deformability. Using a single blood sample, the effect of fibrinogen can be assessed by diluting the blood in its own serum. The fibrinogen was removed from the plasma, to produce serum, by heating at 56 °C for 10 min. Finally the clot was separated from the serum by centrifugation. Elevated plasma fibrinogen concentrations were studied by adding a concentrated solution of Kabi Grade L fibrinogen. This solution was prepared by dissolving 280 mg of the dried fibrinogen in 10 ml distilled water, and by incremental addition of NaCl followed by measurement of the freezing point depression, adjusted to a tonicity of 300 m-osmole. The pH was then standardised at 7.4, by adding 0.15 M HCl or 0.15 M NaOH as required. A maximum concentration of 10 mg ml^{-1} of fibrinogen solution could be obtained in this way. A minimum volume of the concentrated solution was added to the blood samples to obtain the required plasma level. Control experiments, in which the solution was added to blood after removing the fibrinogen, indicated that no significant adverse effects occurred using this procedure. The linear variation of packing rate with fibrinogen concentration, using blood from a subject representative of healthy adults, is shown in figure 5. The erythrocyte deformability is considered normal if the packing rate falls within ±1% of the value given on the line for the patient's fibrinogen level. If the value falls below this line the erythrocytes are less flexible, as occurs in many diabetic subjects. When the value

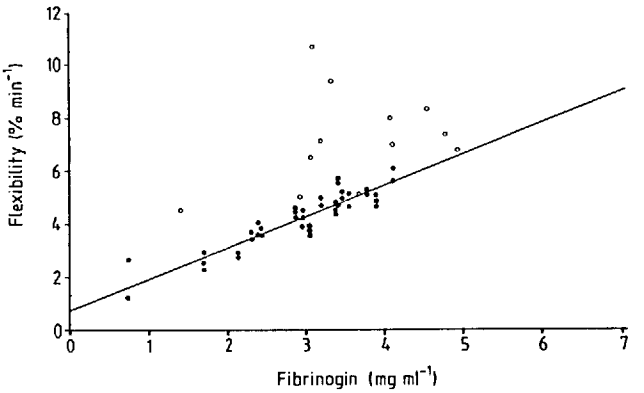


Figure 5. Flexibility at 45% haematocrit plotted against fibrinogen concentration for normal individuals (closed circles). Also shown is the flexibility of twelve hypertension patients (open circles).

is above the line the cells are more flexible, with an increased disposition to red cell aggregation. An example of its clinical application, using blood samples from twelve hypertensive patients, is also shown in figure 5.

4. Discusslon

The results obtained with the stroboscopic recording centrifuge are consistent with those obtained with much more expenditure of effort and time by the single sample recording method (Sirs 1970). The technique has been used routinely to study the deformability of erythrocytes in normal and clinical subjects over the past year, with no problems. It is proving a powerful method for the rapid survey of the changes of blood rheology in disease.

Résumé

Utilisation d'une centrifugeuse à mesure stroboscopique pour la mesure de la déformabilité des érythrocytes.

Nous décrivons une technique utilisant une centrifugeuse à mesure stroboscopique, qui permet de mesurer la déformabilité des globules rouges du sang en terme de taux d'aggrégation à faible g. Elle peut être utilisé pour mesurer simultanéiment plusieurs échantillons de sang complet avec une commande exacte de la vitesse de rotation et du temps de mesure. Elle ne nécessite que 0,05 ml de sang par mesure et ne demande aucun traitement ni préparation anterieurs. Nous expliquons les procédures de calibration permettant les variations de l'hématocrite et du taux de fibrinogènes dans le plasma.

Zusammenfassung

Messung der Erythrozytendeformierbarkeit mit einer stroboskopischen Aufnahmetechnik.

Ein stroboskopisches Aufnahmeverfahren wird beschrieben, eas die Deformierbarkeit von roten Blutzellen über die Verdichtungsrate bei niedrigem g mißt. Es kann zur simultanen Messung mehrerer Gesamtblutproben verwendet werden, wobei die Rotationsgeschwindigkeit und die Aufnahmezeit genau kontrolliert wird. Pro Messung sind nur 0.05 ml Blut erforderlich. Eine Vorbehandlung der Probe ist nicht nötig. Die Kalibrierverfahren, die Schwankungen des Hämatokrit und des Plasma-Fibrinogengehaltes erlauben, werden erklärt.

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ABSTRACT No. 10

MICROVASCULAR RESEARCH 24, 232–239 (1982)

Abstracts of Papers Presented at a Meeting of the British Microcirculation Society

April 7 and 8, 1982, St. Bartholomew's Hospital, London, England

1. *Vascular Patency after Reconstructive Surgery.* J. S. P. LUMLEY, Surgical Professorial Unit, St. Bartholomew's Hospital, London EC1A 7BE, England.

The introduction of reconstructive vascular techniques has forced vascular surgeons to direct their attention to postoperative blood vessel patency, rather than to the previous preoccupation with haemostasis. Threats to the maintenance of patency may be considered under the headings of Virchow's triad—vessel wall damage, hypercoagulability of the blood, and stasis. Current techniques are largely empirical, but advances in understanding of these basic mechanisms should lead to improvements in management. A clear understanding of the antithrombotic function of normal endothelium and the effect of subintimal proliferation on this property could influence the surgeon when deciding on the depth of endarterectomy. Histochemical changes may be related to vessel wall damage, platelet aggregation or local thrombus formation and an understanding of their nature could influence the use of anticoagulant or antiplatelet drugs applied locally or systemically during and after surgery. While endothelial proliferation and migration can provide neointimal cover to small denuded areas, this mechanism is inadequate for larger endarterectomised segments. The nature of the neointima in these situations deserves further attention as does the timing of its deposition and cellular content, since they may have marked influence on subsequent vessel patency and may also determine appropriate medication. The potential inflammatory response to excess pressure on the vessel wall, needle puncture sites, and suture and graft material, is still itself incompletely understood. Synthetic graft materials carry specific problems in relation to their optimal compliance and porosity, the latter determining tissue ingrowth and possible thrombogenicity of the new lining. While changes in the blood may be closely related to vessel wall damage, in respect to platelet aggregation a number of other haemorheological factors are at present receiving attention. These include studies on red cell deformability, serum fibrinogen levels, viscosity, and coagulability. Stasis, the third factor of the triad, is prevented by adequate flow through the vessel. This, in turn, being determined by the extent of proximal and distal disease and the resultant pressure gradient and vessel diameter. In controlling atherosclerotic disease one has to consider the risk factors and possible contribution of abnormal lipids, hypertension, smoking, and the value of regular exercise.

2. *Healing of Rabbit Aortic Endothelium following Injury.* L. N. WALKER, M. M. RAMSAY, AND D. E. BOWYER, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, England.

Studies of mechanical injury to aortic endothelium have produced conflicting results as to the role of platelets and smooth muscle cells in the repair process. We have studied the healing of rabbit aortic endothelium following narrow injuries which expose different components of the vessel wall. The effect of hypercholesterolaemia, a risk factor for atherosclerosis, on the repair process has also been investigated. Superficial and deep injuries were produced using a nylon catheter containing either a nylon or wire filament, respectively. The repair process was observed using scanning and transmission electron microscopy. When endothelial cells were removed without damage to the media (superficial injury), a sparse monolayer of platelets and isolated leukocytes adhered to the denuded area. Reendothelialization was achieved at 48 hr by endothelial cell migration and division;

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fluctuation frequencies are determined by the technique of photon correlation spectroscopy. Details of the experimental methods and the results of initial applications will be described. Changes in cutaneous blood flow in different body sites are directly observable as well as the dependence of the blood flow index on temperature. Comparison with the epicutaneous xenon clearance technique has shown a close correlation. The method is noninvasive and enables quick, continuous or repeated measurements to be made of blood flow at any part of the body surface.

9. *The Use of the Laser Doppler Flowmeter in Reconstructive Microsurgery.* B. M. JONES AND L. H. SMAJE.

Systems using the Doppler shift of laser light backscattered from moving red cells are useful for cutaneous capillary blood flow measurement, the results correlating well with xenon clearance methods (Holloway and Watkins, 1977, *J. Invest. Dermatol.* **69**, 306). We have used a compact laser Doppler flowmeter (Nilsson *et al.*, 1980, *I.E.E.E. Trans. Biomed. Eng.* **27**, 12) as a monitor in nine microvascular free flaps in patients. Flowmeter output was registered on a chart recorder and the tracings were analysed retrospectively and flow values expressed as a percentage of a standard 10-V calibration deflection. Six flaps had an uncomplicated course and had increasing flow readings in the early postoperative period, which then stabilised. One flap failed because of venous occlusion. This showed a dramatic and sustained reduction in flow between Hours 4 and 14, when it was reexplored. At this stage in the investigation nurses had been instructed to undertake and act on their usual clinical observations only. In a later flap, readings diminished between Hours 30 and 34. Skin sutures which were compressing the vascular pedicle were removed and there was an immediate sevenfold increase in flow. Despite some superficial necrosis, the flap was saved. The last case suffered necrosis of its distal one-third. The flow had diminished between Hours 14 and 19, but a decision not to explore was made on clinical grounds. This instrument shows great promise as a monitor for microvascular surgery. Impairment of flow to a flap appears to be detected earlier and more reliably than is the case using clinical criteria alone.

10. *Blood Rheology in Man, Horse, Sheep, and Cattle.* T. AMIN AND J. A. SIRS, Department of Biophysics, St. Mary's Hospital Medical School, Paddington, London W2 1PG, England.

The majority of research on blood rheology has concentrated on human subjects in health and disease. Though information regarding blood viscosity and erythrocyte aggregation is available for various animal species there is scant information as to the variation of erythrocyte deformability, which is of interest relative to changes observed in human disease. A comparative survey has been undertaken of blood and plasma viscosities, plasma fibrinogen concentration, and erythrocyte deformability in man, sheep, cattle, and horse. Compared with man, cattle and sheep have relatively inflexible red blood cells while horse has very flexible cells. The packing rates at 200 g and 30° are 6.75, 42.5, 0.7, and 0.88% min^{-1} for man, horse, sheep, and cattle, respectively. Unlike man, the erythrocyte deformability of sheep, cattle, and horses are not significantly altered by fibrinogen concentration. The high flexibility of horse erythrocytes can only be lowered by repeated washing, and is associated with an unknown factor that is not fibrinogen. The blood viscosities of man, horse, sheep, and cattle at 230 sec^{-1}, 37.5°, and haematocrit of 45% are 4.5 ± 0.3, 5.8 ± 0.2, 5 ± 0.2, and 6 ± 0.2 cP, respectively. Horse blood viscosity is high at all shear rates due to considerable aggregation, which is probably avoided in horses at rest by their lower haematocrit of 30–35%. The red cell shape factors, K, obtained from whole blood and plasma viscosities, are 2.84, 2.94, 3.8, and 3.3 for sheep, cattle, horses, and man, respectively. The viscosity of sheep's blood is not as high, relative to man, as one would expect with their comparatively inflexible cells. The reason for this appears to be that the shape factor, K, for sheep cells is lower, off-setting the detrimental effect of inflexibility.

11. *Morphological Aspects of the Synovial Microcirculation.* A. D. KNIGHT AND J. R. LEVICK, Department of Physiology, St. George's Hospital Medical School, London SW17 ORE, England.

The distribution and ultrastructure of capillaries (microvessels of diameter < 10 μm) were examined by light and electron microscopy in sections of synovium from the knees of 5 rabbits. Capillary distribution was uneven, with 72.10^3 vessels/cm^2 section in areolar synovium, $58.10^3/cm^2$ in adipose synovium, and $0.9.10^3/cm^2$ in fibrous synovium. Nineteen percent of the length of the synovial surface

ASSOCIATION FOR VETERINARY CLINICAL PHARMACOLOGY AND THERAPEUTICS (A.V.C.P.T.). NEWSLETTER AND PROCEEDINGS, VOLUME 8, MARCH 1984, 109-112.

The effect of warfarin on blood rheology in horses.

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The blood rheology of horses is unusual in several respects.The erythrocytes are very flexible(1),which in turn is associated with relatively high levels of aggregation and Rouleaux formation,and a rapid ESR(2).Though the increased flexibility contributes to a slightly lower whole blood viscosity at high shear rates ,relative to man,the viscosity at low shear rates is considerably higher.In man, high flexibility and increased viscosity at low shear rates,only occur in pathological situations, where aggregation and sludging may impair blood flow.The horse has the ability to lower its haematocrit at rest, to 30-35%, by sequestration of cells in the spleen, and this in turn reduces the problems of aggregation that are so detrimental in man.During exercise,and at an associated higher shear rate,the spleen contracts raising the haematocrit to about 45%.The advantages to the horse, and greyhounds which have red cells with similar properties,are the slightly lower viscosity at high shear rates, facilitated flow of erythrocytes through capillaries and the increased rate at which flexible erythrocytes can exchange oxygen.

The objectives of the present study were to measure rheological factors in different breeds of horse, and investigate if rheological changes could explain the efficacy of low dose Warfarin used in treating Navicular disease(3)(4). Measurements of blood and plasma viscosities have been made at 37.3C, using a modified Harkness capillary viscometer.A cone-on-plate viscometer gave erratic and high readings with horse blood.This is a variable factor from one horse to another, and appears to be associated with the formation of a thin gelatinous layer at the air-blood interface.Blood samples were obtained by venepuncture,using heparin to prevent coagulation. Erythrocyte flexibility was determined using the stroboscopic

centrifuge technique(5); the plasma fibrinogen level by a thrombin clot method(6);and the PCV by spinning at 13,000g for 3min.

The erythrocyte flexibility varies with breed,being significantly lower in ponies(mean 23.8%/min), than crossbreds(30%/min) and thoroughbreds(30%/min). The corresponding plasma viscosities were 1.34,1.22 and 1.23cp respectively.The mean blood viscosities, corrected to a PCV of 35%, were 3.08,2.89 and 2.89cp for ponies, crossbreds and thoroughbreds respectively.These compare with a flexibility of 7.3%/min, plasma viscosity of 1.22cp and blood viscosity of 2.95cp, under comparable conditions, for a healthy young man.The decrease in the blood viscosity of the horse is not as high as one would expect for such a large increase of erythrocyte flexibility, due to an increase of the cell shape factor, K(1). The highly flexible horse erythrocytes can be made relatively rigid by repeated washing with and resuspension in isotonic saline.The cell flexibility is restored to its initial high value ,if, after washing the cells are resuspended in horse plasma.Human cells suspended in horse plasma have an enhanced flexibility. This is not due to horse fibrinogen,horse serum has the same effect. It has been established that the factor in horse plasma is a protein, but which particular protein has still to be resolved.

Over the last seven years there has been an increasing use of warfarin in the horse, primarily for the treatment of Navicular disease.A number of cases have responded satisfactorily in which the one stage prothrombin time would not have been considered significantly lenthened in man.One possible explanation of the effect of warfarin in Navicular disease, is that it improves blood flow by modifying the rheological properties of blood.In the previous communication,Dr Colles reported a small but significant drop in plasma viscosity.We have now extended the investigations to include blood viscosity and erythrocyte flexibility.In two horses with long standing and untreated Navicular disease, both the erythrocyte flexibility (38.5%/min) and plasma viscosity are elevated relative to healthy controls.By comparison, six horses suffering from Navicular disease and treated with low dose warfarin,had almost normal values for these parameters (means 28%/min and 1.25cp). A

preliminary study has been undertaken of the effect of low dose warfarin treatment on four normal ponies. After one week of treatment, there was a fall of blood viscosity at high shear rates (eg 3.26 to 3.00cp), a decrease of plasma viscosity (eg 1.46 to 1.29cp), and a reduction of erythrocyte flexibility (eg 30 to 24.1%/min) in all ponies; with no significant change of PCV, MCV, or plasma fibrinogen levels. Incubating blood 'in vitro' with warfarin does not have any effect on the erythrocytes or blood viscosity. The explanation of these changes is not known, but they are consistent with the possibility that blood flow to the Navicular bone is improved by the reduction in aggregation, following the decease in flexibility, in the venous return. These findings have implications to our understanding of the pharmocological action of warfarin and could have application to the use of the drug in man.

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ABSTRACT SUBMITTED TO THE BRITISH MICROCIRCULATION SOCIETY MEETING - OXFORD 5 & 6 TH APRIL 1984

The influence of erythrocyte deformability on blood viscosity at high and low shear rates. T.A.AMIN and J.A.SIRS, Department of Physiology and Biophysics, St. Mary's Hospital Medical School, University of London, Paddington, London. W2 1PG

It is commonly stated that aggregation of erythrocytes, at low shear rates, is promoted by fibrinogen acting as a binding agent between cells. We would like to present some experimental evidence which suggests that fibrinogen only acts indirectly by modifying erythrocyte flexibility, and it is the latter factor which is decisive. The viscosity of blood samples with an haematocrit of 45%, were measured at 37C, over shear rates from 11.5 to 230 /s, using a Wells-Brookfield viscometer, and erythrocyte flexibility by the centrifuge technique. The erythrocyte flexibility and aggregation were changed by suspending the cells in plasma, serum and Ringer-Locke solution, and by incubating at 48.5C for varying lengths of time. At high shear rates, the relative viscosity increases with decrease of flexibility. At low shear rates, a decrease of flexibility initially decreases the relative viscosity. Further decrease of flexibility then leads to an increase of relative viscosity. These changes do not correlate with the presence or absence of fibrinogen, and are independent of the suspending medium. The phenomena can be explained on the basis that at high shear rates the relative viscosity depends on the erythrocyte flexibility and shape, and the haematocrit. At low shear rates there is an additional contribution due to aggregation. Whereas at high shear rates decreased flexibility directly increases the relative viscosity, at low shear rates this is initially offset by an associated reduction in aggregation. Only when the cells become relatively inflexible does the viscosity rise at low shear rates.

ABSTRACTS SUBMITTED TO THE 3RD WORLD CONGRESS FOR MICROCIRCULATION - OXFORD 9-14 TH SEPTEMBER 1984

Anaesthetics, High pressure and Erythrocyte flexibility.

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The action and potency of an anaesthetic is related to its oil-water solubility. Anaesthesia can be reversed in animals, by increasing the atmospheric pressure. Human subjects can only adapt slowly to high-pressures, during deep-sea diving, or they suffer nervous tremours on compression and the bends on decompression. It has been suggested that these effects are due to physical changes in the plasma membrane, possibly associated with changes of fluidity or mechanical structure. We have attempted to detect any change of membrane rheology, by measuring erythrocyte flexibility using the centrifuge technique, following the equilibration of blood with anaesthetic agents, the application of pressures up to 100 atmos., and the combination of both. Hydraulic pressure was applied by mounting micro-capillary tubes in a small chamber, that could be sealed under pressure, and then attached to the head of a centrifuge, operated at 200g, to measure the packing rate. We could detect no changes of packing rate using di-ethyl ether, chloroform, halothane, cyclopropane and nitrous oxide, at higher than surgical levels. Neither rapid compression or decompression, nor application of high pressures to blood equilibrated with an anaesthetic had any effect. We conclude that the action of these agents is not due to rheological or mechanical effects in the membrane.

The effects of tonicity and pH on blood rheology.

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Changes in the tonicity and pH of normal blood alter its rheological behaviour. After altering the plasma tonicity, over the range 180 to 600 mosm, and the pH, over the range 6.5 to 8.1, it was found that the erythrocyte packing rate, measured by the centrifuge technique, blood viscosity, cell shape factor, K, and MCV were altered. For hypotonic and hypertonic conditions, and acid pH, the erythrocyte flexibility is reduced, with a corresponding increase of the relative blood viscosity. However, when blood is alkaline, there is an increase of flexibility, and decrease of relative viscosity. The shape factor decreased in acidic and hypotonic bloods, with the cells becoming more spherical; and increased under alkaline and hypertonic conditions, consistent with the observed viscosity changes. It is believed that the increase of erythrocyte flexibiliy at high pH is due to alterations in the cell membrane.