THE HEMORHEOMETER: A NEW FILTRATION APPARATUS

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SUMMARY

An increasing interest in the rheological properties of Red Blood Cell (RBC) has arisen during the last decade and the Erythrocyte Deformability (E.D.) has been recognized as a crucial property at the microcirculation level. Many techniques have been proposed to measure this parameter. Whole blood techniques (such as Teitel'paper filter or Reid & Dormandy's) may have some interest in the sense that they can be considered as a global physio-pathological test. The «hemorheometer» is a new filtration apparatus which has been developped in order to perform reproductible significant E.D. measurements. It is based on the initial filtration rate of a diluted RBC suspensions through 5 μm Nucleopore filter pores. Only about 400 RBC pass through each pore and the measurements are made in a few seconds, this method is scarecely influenced by sedimentation effects and filter plugging. Already described [1] and tested in several leading Haemorheology Laboratories, the Hemorheometer is now available for research and routine work.

DEDICATION

This presentation in the 2nd Symposium on Clinical Haemorheology (Lisbon) is dedicated to the memory of Professor HANSS, a pioneer in the field of Biorheology and the inventor of the «Hemorheometer». Professor HANSS was one of the founders of the French Clinical Haemorheology Society and became its vice-president. He will be remembered as having a major contributor to biorheology research. His untimely death on August 3, 1984 was a cause of deep regret for his colleagues and great loss to science.

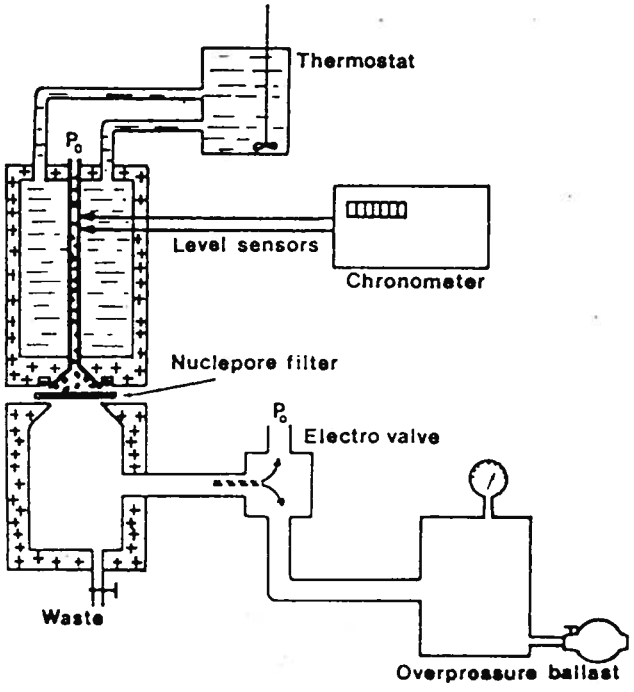

Figure 1: General outlay of the hemorheometer.

INTRODUCTION

The erythrocyte deformability (E.D.) has been recognized as a crucial property at the microcirculation level, as has been so nicely demonstrated by Branemark.[2] and from an hemorheological standpoint, its reduction, in other circumstances than in certain hematological disorders, is the common denominator between the various cardiovascular risk factors.[3] Accordingly, a number of different experimental techniques have been devised in recent years to use microsieving through 5 μm or 3 μm polycarbonate pores filter as a test of filterability of the RBC suspensions. And, in the same way, various indice of E.D. have been proposed to express the results of these tests.

Studies were carried out to evaluate:

1. The variations of the index of rigidity of the cell (I.R.) given by the hemorheometer in different cases; normal RBC, pathological RBC and RBC treated with pentoxifylline.
2. The correlation between I.R. and β (indice of E.D. measured by the initial pressure induced at constant flow).[4]

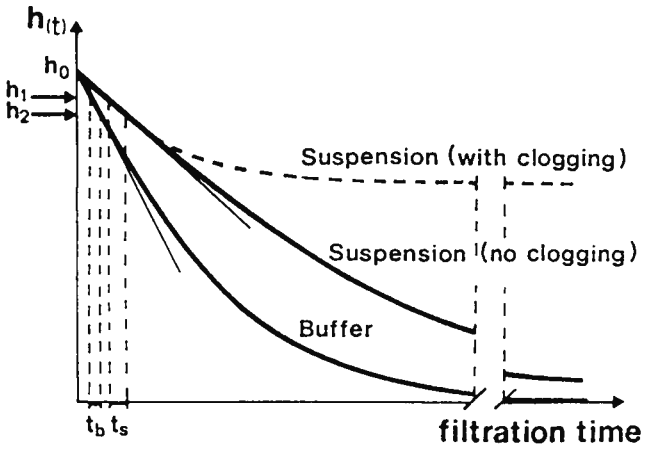

Figure 2: Filtration curves in different cases.

2. DESCRIPTION OF THE HEMORHEOMETER AND ITS PRINCIPLE OF MEASUREMENT

The hemorheometer is built around two plastic parts (Fig. 1). The upper plastic block is fitted with a central capillary surrounded by water circulating from a thermostat. The capillary is terminated by a conical section, under which, is situated the Nuclepore membrane.

The central capillary can be filled either with RBC suspension or a suspending medium (buffer). Two level-detectors (situated near the top of the capillary and separated from each other by 9 mm) can activate and stop an electronic chronometer as the meniscus of the liquid passes by.

The lower plastic block holds the membranes, under which a positive pressure of about 6, 7 cm water can be applied. A simple pumping and metering device allows for this positive pressure. By suddenly releasing this overpressure, the filtration begins at a precisely known-time.

As the filtered volume is small, about 50 μl, the time laps is short (typically in the range of 0.5 to 1 second) therefore excluding any supurious sedimentation effects. The filter can be reused many times after washing.

The principle of the hemorheometer is based on its initial flow rate measurement of a diluted suspension. So if we measure the initial portion of the curve, we can see (Fig. 2) that in the case of clogging suspension, the measurement of a lapse of time is still significant. In contraste, the time needed to measure the flow of a larger suspension volume may become infinitely large, thus without any meaning as regards the intrinsic RBC rheological properties.

The results are given as a rigidity index (IR):

$$IR = \frac{ts - tb}{tb \cdot H}$$

where ts and tb are time lapses for the suspension and the buffer, measured with the same filter at a given haematocrit.

This formula is similar to well-known concept of «reduced viscosity» in polymer chemistry. In the range of low concentrations this parameter becomes concentration independent and represents the «intrinsic viscosity» of the suspension.

3. RESULTS AND DISCUSSIONS

1. Variation of IR as a function of haematocrit (Table 1)

The instrument is largely insensible to well-known secondary phenomena (clogging, sedimentation...) which are commonly found in many filtration techniques. This is because only a small initial portion of the filtration curves used, as we can see on the figure 2.

However, in order to know if the rigidity index is insensitive to RBC interactions, it is necessary to verify that IR is independent of haematocrit for normal RBC. This is reflected in the table 1 that the rigidity index given by the hemorheometer is independent of the RBC interactions.

2. Variation of IR as a function of osmolarity (Table 2)

IR seems very sensitive to deformability changes as shown by rapid increases induced by osmolarity changes: with NaCl, IR increases rapidly when the osmolarity values are over 400 mOsm or under 200 mOsm, with saccharose, the increase under 200 is similar to NaCl results, but IR increases steadily when osmolarity is higher than 300 mOsm.

The discrepancy between results obtained with NaCl and saccharose could be related to the ionic movements induced by high NaCl concentration.

3. Variation of IR on diabetic patients (Table 3 and table 4)

The table 3 presents a study on diabetic patients. We have found that the IR is significantly increased in diabetic patients using heparin as anticoagulant.

On the EDTA, the IR is also higher for diabetic patients, but the statistical significance is lower. In the heparin samples, the RBC's are probably slightly more rigid when the diabetic patient is insulin-dependent as we can see in table 4.

4. Variation of IR on cirrhotic patients (Fig. 3)

The figure 3 shows the histograms of cirrhotic patients as compared to those of control patients. The mean values of the IR for cirrhotic patients is about 30% higher. This difference is statistically significant.

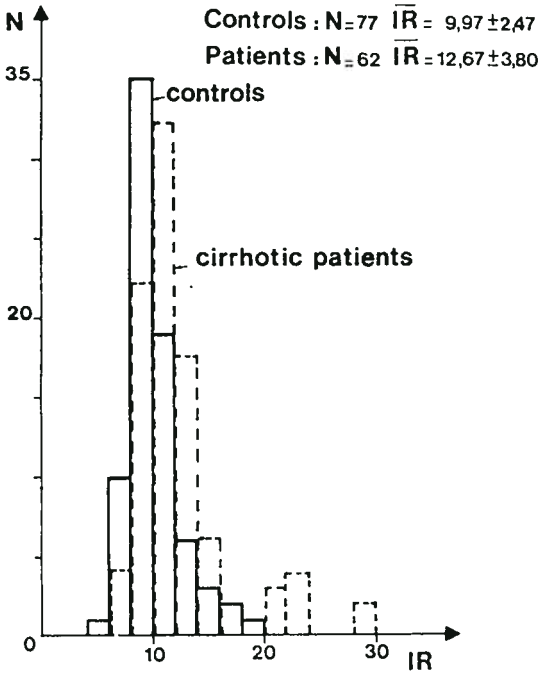

Figure 3: Variation of IR on cirrhotic patients.

Table 1 **Variation of IR as a function of hematocrit.**

H%	0.5	1	2	4	6	8
IR	10.9	11.1	10.1	11.0	11.1	10.9

Table 2 **Variation of IR as a function of osmolarity.**

mOsm	170	190	200	230	250	300	350	390	400
IRsaccharose	69.9	8.0	7.4	8.7	7.3	11.3	36.7	125.6	361.5
IRNaCl	360.0	22.0	11.8	6.1	8.1	8.6	11.8	14.7	20.2

Table 3 **Variation of IR on diabetic patients.**

		Diabetic patients	Controls	
Heparin	N	311	77	
	<IR>	11,44	9,97	p<0,001
	σ	2,82	2,47	p<0,001
	SEM	0,162	0,282	
EDTA	N	293	30	
	<IR>	15,01	30,06	NS
	σ	6,68	2,52	p<0,01
	SEM	0,388	0,461	

Table 4 **Variation of IR on diabetic patients.**

	Heparin				
	N	<IR>	σ	SEM	
IDD	100	11,86	3,07	0,307	p<0,05
NIDD	119	11,09	2,39	0,219	

Table 5 **N. RBC suspended in Tris-buffer.**

	N	MEAN	SD	MIN.	MAX.
IR	47	10.57	2.65	7.67	20.99
IRpent	47	9.84	2.10	7.18	18.06

Table 6 **Hb AS suspended in Tris-buffer with 3 Ca++ concentrations.**

Calcium	N	IR	IRpent	% improvement
0 mM	4	15.5 ±2.2	14.8 ±3.0	2.5 %
2 mM	4	17.9 ±2.4	16.8 ±1.6	5.3 %
4.3 mM	4	19.9 ±2.0	18.0 ±1.2	14.0 %

5. Variation of IR in different hemoglobin disorders (Fig. 4a and Fig. 4b)

The histogram (Fig. 4a) shows that for physiological oxygen levels and normal osmolality, there is a clear difference between control population and those with AS and SS hemoglobin disorders. We can observe that there is no over lap between the control, the AS and SS populations.

In the histogram of the beta-thalassemia syndromes (Fig. 4b), we can also observe a clear discrimination between the RBC of the control, the heterozygote and the homozygote popullations.

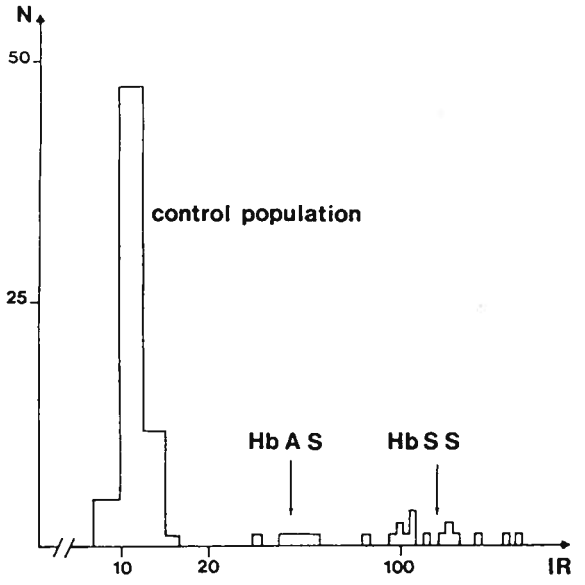

Figure 4a: Variation of IR in different hemoglobin disorders.

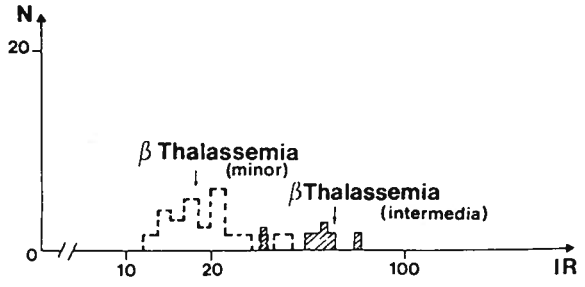

Figure 4b: Variation of IR in different hemoglobin disorders.

6. Variation of IR induced by pentoxifylline

a) Normal RBC suspended in Tris-buffer (Table 5)

Some hemorheological substances as pentoxifylline and xanthines [5] are also tested on this apparatus. The instrument seems to be sensitive to the IR variation due to the effects of these different substances. In the case of pentoxifylline, we can predict an improvement of 6,96 % on normal RBC suspended in Tris-buffer. This improvement is statistically significant ($p < 0.0001$).

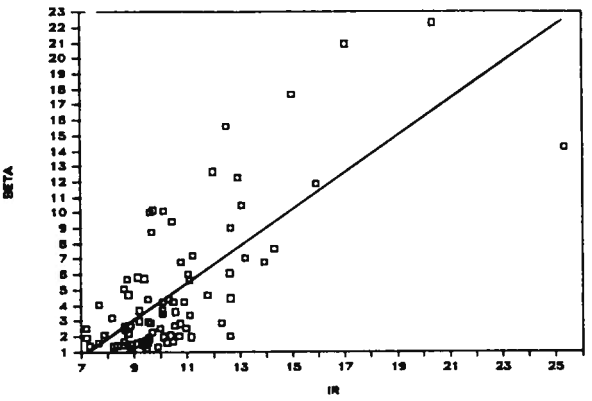

Figure 5: Correlation between IR and β.

b) Hemoglobin SS suspended in Tris-buffer with Ca++ concentrations (Table 6)

Experiments have been performed with the following values = haematocrit: 8 %, calcium concentration: 0; 2; 4.3 mM; pentoxifylline: 7.10^{-4} M.

Table 6 shows that although the response with pentoxifylline is variable in each case, the E.D. of the treated cell is improved independently of the calcium concentration. The improvement is more prononced when the initial IR is higher.

7. Correlation between IR and β

We have been able to demonstrate the existence of a close correlation between measurements made using a Hanss' apparatus and β: the E.D. indice obtained by measuring the initial pressure at constant flow.[4]

$\beta = 1.19$ IR + 7.65 $N = 86$ $r = 0.728$ $p < 0.001$

CONCLUSIONS

The independence of IR on the haematocrit ($H < 8$ %) is a good indication, reflecting that the measurement is independent of the RBC's interactions; indeed the assumption that IR determines some individual intrinsic rheological properties of the «average» RBC is justified. This condition is particularly important to evaluate the average transit time of a given RBC suspension through a 5μm pore filter. The overall precisions of IR is in order of 8 %. This precision could be increased slightly by making the filtration measurement at a constant temperature. The results on different cases in this study were obtained thanks to the sensitivity and the reproductibility of the initial flow-rate method used by the hemorheometer.

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