

# Cellular aggregation and destruction during blood circulation and oxygenation

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**Wright, G. and Sanderson, J. M. (1976).** *Thorax*, 31, 405–409. **Cellular aggregation and destruction during blood circulation and oxygenation.** Platelet and leucocyte aggregation and severe haemolysis may occur in blood during the preparation of an extracorporeal circuit for open-heart surgery. Experiments with dog blood showed that both processes result from bubble oxygenation but not from circulation of diluted blood and that they appear to be inhibited by the presence of acid citrate dextrose and heparin, or citrate phosphate dextrose and heparin, but not by heparin alone.

It has been suggested that platelet and leucocyte aggregates may be responsible for tissue damage during extracorporeal circulation for open-heart surgery (Swank and Porter, 1963; Sachdev *et al.*, 1967). Various factors have been blamed for the formation of these cellular aggregates. According to Allardyce, Yoshida, and Ashmore (1966), Ashmore, Sviteck, and Ambrose (1968), Ashmore *et al.* (1972) and Rittenhouse *et al.* (1972), cellular aggregates formed when blood was continuously circulated and oxygenated in an extracorporeal circuit. The development of aggregates was measured as an increase in the screen filtration pressure (SFP) of the blood using the technique of Swank *et al.* (1964). On the other hand, Swank and Porter (1963) measured a decrease in the SFP under similar conditions. Because of this difference, the factors that might be responsible for cellular aggregation were examined.

## MATERIAL AND METHODS

**CLINICAL PRE-PERFUSION STUDIES** For open-heart operations on human patients the extracorporeal circuit consisted of a barely occlusive single roller Mark IV Rygg pump, a Rygg stainless steel torpedo-type heat exchanger, a Standard Low Prime Rygg-Kyvsgaard bubble oxygenator, a 1 m length of 10 mm or 12 mm internal diameter silicone rubber pump insert, and a 4.572 m length of 9.4 mm internal diameter polyvinyl chloride tubing

forming a closed arteriovenous circuit. A Pall 40  $\mu$  woven polyester extracorporeal microfilter and bypass loop were placed in the arterial line. The circuit was primed with two packs, each containing 450 ml of whole blood anticoagulated with 67.5 ml acid citrate dextrose NIHA (ACD) or 63 ml citrate phosphate dextrose (CPD), 2500 IU mucous heparin, and 3 ml 20% calcium chloride per blood pack, 2 l. Ringer-lactate solution (Hartmann's), 50 ml 8.4% sodium bicarbonate, and one million units benzyl penicillin. In some cases, the blood was microfiltered as it entered the circuit. The prime was oxygenated for approximately 5 min with a mixture of 96% oxygen and 4% carbon dioxide at a flow rate of 4 l/min and then circulated at 800 ml/min for 25–115 min at 30°C.

Blood samples were collected from the whole blood packs and from the circuit prime after 5 min circulation to mix the components and after a further 20–110 min circulation. Measurements were made of the packed cell volume (PCV) and SFP according to Swank *et al.* (1964). Red cells were haemolysed with 1% cocaine in 0.2% sodium chloride for platelet and leucocyte counts by phase microscopy. Blood smears were stained for platelet and leucocyte aggregates with Leishman-Giemsa.

**DOG BLOOD CIRCULATION STUDIES** For experiments with dog blood, the extracorporeal circuit con-

sisted of a Travenol ILF bubble oxygenator, a barely occlusive Sarns double roller pump, a Bio-Med Engineering torpedo-type heat exchanger, and a Travenol 3LF pump set. The circuit was primed with 500 ml of fresh blood collected from the femoral artery or jugular vein of 37 adult mongrel dogs, 11·3–36·3 kg body weight, into either 75 ml ACD, 70 ml CPD or 30 ml heparin solution, all supplied in Fenwall blood packs. The blood passed through a standard 170  $\mu$  blood clot filter (Fenwall HB92D) to enter the circuit. The heparin concentration was equated in all groups by adding 2250 IU mucous heparin to the circuits containing ACD and CPD blood. The total volume of the prime was then made up to 730 ml with Ringer-lactate solution.

Seven preliminary experiments were performed, followed by 10 experiments in each anticoagulant group. In each group, five of the circuits were primed with arterial blood and five with venous blood. The prime was circulated for 5 min at 1 l/min to mix the components and for a further 4 h at 1 l/min with a pure oxygen flow of 3 l/min at 37°C. Blood samples were obtained from the circuit at 30-min intervals for the tests listed above, for measurements of whole blood haemoglobin and plasma haemoglobin concentrations, and for blood hydrogen ion concentration and gas analysis using Astrup microequipment.

RESULTS

**CLINICAL PRE-PERFUSION STUDIES** Fresh blood samples collected from 23 human donors into ACD or CPD had SFPs of 1·6–5·1 kPa. The blood used to prime the circuit for open-heart operations was 2–8 days old ACD or CPD blood (Table). Of 78 ACD blood samples 4–8 days old, 20 (25·6%) had SFPs within the range for fresh blood, whereas of 128 CPD blood samples 4–8 days old, only

one (0·8%) was below 5·1 kPa. This difference was statistically significant ( $p<0\cdot001$ ). There were also significant differences between the PCVs ( $\bar{x}=0\cdot46$  for ACD and 0·34 for CPD;  $p<0\cdot001$ ) and platelet counts ( $\bar{x}=138\times10^9/l$  for ACD and  $169\times10^9/l$  for CPD;  $p<0\cdot001$ ). Neither of these latter differences correlated with the difference between the SFPs ( $r=-0\cdot19$  for PCV and 0·12 for platelets). The differences between the PCVs may be due to a sampling error since it is difficult to achieve a uniform suspension of stored red cells. The differences between the platelet counts could be due partly to the use of a relatively higher volume of ACD than of CPD solution, or to the technical error introduced in platelet counting by the presence of platelet aggregates. The addition of 2500 IU mucous heparin and 3 ml calcium chloride to 40 packs of 5-day-old CPD human blood did not result in any significant change in the PCV, SFP, platelet or leucocyte counts.

The passage of 10 packs of the heparinized and recalcified human CPD blood through a standard 170  $\mu$  clot filter reduced the SFP slightly without affecting the other parameters (Fig. 1). Microfiltration had little effect upon the PCV but it reduced the SFP, platelet, and leucocyte counts; the effects being more pronounced in the ordered 40  $\mu$  woven polyester fibres (Pall) < 27  $\mu$  polyurethane foam (Bentley) < Dacron wool (Swank) for all three parameters. The standard clot filter allowed tight platelet and leucocyte aggregates up to 180  $\mu$  diameter to pass through. The maximum size of tight aggregate that passed through a Dacron wool filter was 55  $\mu$  diameter but aggre-

TABLE SCREEN FILTRATION PRESSURES OF ACD AND CPD HUMAN BANK BLOOD				
Age of Blood (days)	ACD		CPD	
	No. of Observations	SFP (mean $\pm$ SE) (kPa)	No. of Observations	SFP (mean $\pm$ SE) (kPa)
2	0	—	4	4·5
3	0	—	3	25·1
4	14	10·8 $\pm$ 2·9	4	40·7
5	40	20·0 $\pm$ 3·9	93	49·3 $\pm$ 2·8
6	8	18·6 $\pm$ 9·2	5	37·9
7	14	30·0 $\pm$ 8·6	22	53·7 $\pm$ 5·5
8	2	41·7	4	44·6

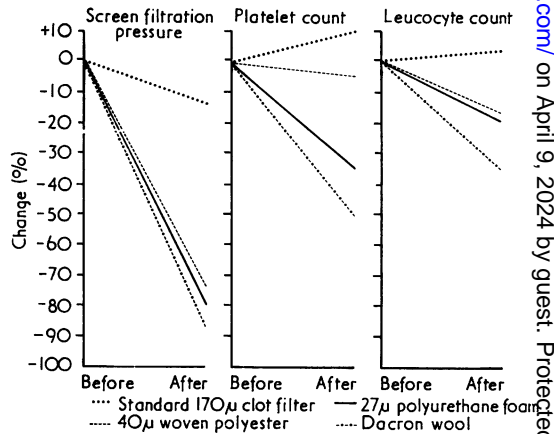


FIG. 1. Cellular effects of human blood filtration.

gates up to 120  $\mu$  diameter passed through the polyurethane foam and woven polyester microfilters. All of these dimensions exceed the nominal pore sizes of the filters. The aggregates seen in the blood samples obtained from the distal end of the filters appeared to be identical with those taken from the proximal end. However, it is remotely possible that some aggregates were formed within the filter.

Although there was a statistically significant difference between the SFPs of the ACD and CPD human blood used to prime the circuit there did not appear to be a corresponding difference in SFP between the ACD and CPD blood when mixed with the other solutions in the circuit.

Circulation of the diluted human blood prime reduced the SFP from  $2.3 \pm 0.2$  kPa to  $1.8 \pm 0.1$  kPa for ACD blood and from  $2.1 \pm 0.3$  kPa to  $1.6 \pm 0.1$  kPa for CPD blood. These differences were not significant ( $p > 0.05 < 0.10$ ). The SFP was reduced by approximately the same amount whether a 40  $\mu$  woven polyester extracorporeal microfilter was included in the circuit ( $2.2 \pm 0.3$  kPa to  $1.7 \pm 0.1$  kPa) or bypassed ( $2.8 \pm 0.6$  kPa to  $2.3 \pm 0.6$  kPa). However, a 10-min period of circulation through a 40  $\mu$  woven polyester extracorporeal microfilter following 10–60 min of circulation with no filter in line further reduced the SFP from  $2.3 \pm 0.6$  kPa to  $1.7 \pm 0.3$  kPa. None of these changes was statistically significant. The PCV, platelet, and leucocyte counts did not appear to be affected by circulation.

In 10 cases, samples of the blood and other solutions used to prime the circuit were mixed in polystyrene blood tubes in the same proportions as in the circuit. The mean SFP of these mixtures was  $22.9 \pm 7.0$  kPa compared with  $2.8 \pm 0.6$  kPa for the circuit primes ( $p < 0.01$ ). Many platelet and leucocyte aggregates were seen in the mixtures but there were few or none in the primes.

**DOG BLOOD CIRCULATION STUDIES** Fresh anticoagulated dog blood samples had SFPs of  $7.6 \pm 1.2$  kPa for ACD,  $4.7 \pm 0.3$  kPa for CPD, and  $17.9 \pm 7.4$  kPa for heparin. The SFP was not significantly altered by the circulation and oxygenation of the circuit primes containing ACD–heparin or CPD–heparin blood, but the SFP of circuit primes containing blood anticoagulated with heparin alone was significantly higher than for ACD–heparin blood after 30 min (Fig. 2). Thereafter the SFP changes in circuit primes containing heparinized blood were very variable but the peak values exceeded 13.3 kPa in five of the 10 experiments.

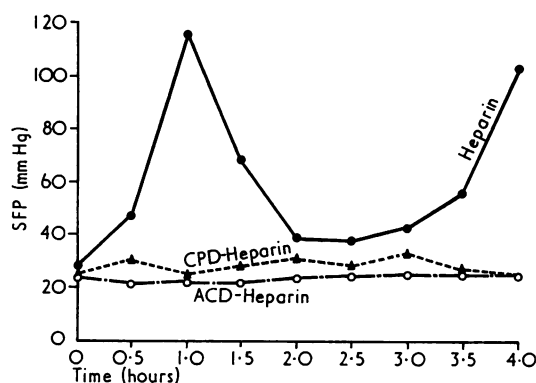


FIG. 2. Screen filtration pressure changes during dog blood circulation and oxygenation.

SFP values above the normal range were found in nine of the 10 experiments with heparinized blood but in none of the experiments in the other two groups. Small (2–35  $\mu$ ) and large (35–108  $\mu$ ) loose and tight platelet and leucocyte aggregates were seen in the smears prepared from blood samples with high SFPs. Tight aggregates were distinguished from loose ones by the apparent fusion of the platelet membrane and the presence of a central dense-staining mass.

The rates of haemolysis were markedly different in the three groups (Fig. 3). Measured mean values of the increase in plasma haemoglobin concentration per 100 passages of the prime through the

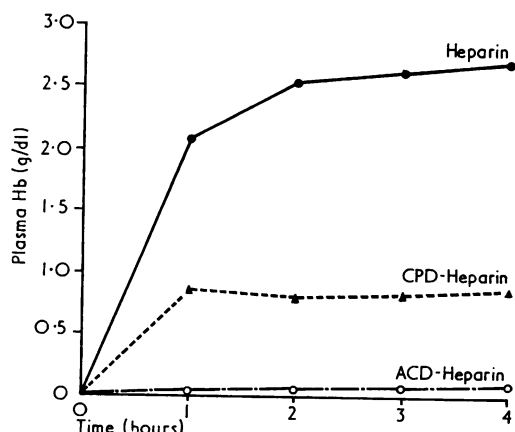


FIG. 3. Haemolysis during dog blood circulation and oxygenation.

circuit averaged over the four-hour period were  $24.2 \pm 3.5$  mg/dl (ACD-heparin),  $266.7 \pm 74.8$  mg/dl (CPD-heparin), and  $810.7 \pm 18.8$  mg/dl (heparin alone). These differences were all statistically significant and correlated with corresponding decreases in the PCV. Considering all three experimental groups together, 78.3% of the haemolysis occurred during the first hour of circulation and oxygenation. Platelet counts decreased by 17.4% (ACD-heparin), 26.1% (CPD-heparin), and 26.8% (heparin alone). Leucocyte counts decreased by 7.9% (ACD-heparin), 2.0% (CPD-heparin), and 6.2% (heparin alone). Similarly the differences between the hydrogen ion concentrations of the primes were maintained with little relative change. Regrouping the data to compare arterial and venous blood primes revealed no statistically significant differences between the SFP, PCV, plasma haemoglobin concentration, or platelet or leucocyte counts.

Similar changes in the SFPs and plasma haemoglobin concentrations were seen when identical blood and Ringer-lactate primes were circulated at the same blood and gas flow rates but using 96% oxygen and 4% carbon dioxide instead of 100% oxygen for oxygenation. These changes were not seen when the primes were circulated at the same blood flow rate but with no gas flow.

#### DISCUSSION

The results of the experiments with dog blood show that the continuous circulation and oxygenation of diluted blood in an extracorporeal circuit caused slight red cell, platelet, and leucocyte destruction when the blood was collected directly into ACD or CPD solutions. When the blood was collected directly into heparin, the red cell destruction was much more severe and platelet and leucocyte aggregates were formed. These changes were dependent upon the flow of gas through the prime. Platelet and leucocyte aggregation did not appear to be related to the arterial or venous origin of the blood as it is in stored blood (Wright and Sanderson, 1974; Wright, 1975).

Allardyce *et al.* (1966), Ashmore *et al.* (1968), Ashmore *et al.* (1972), and Rittenhouse *et al.* (1972) recorded increases in the SFP of dog blood circulated and oxygenated in a disc oxygenator. Allardyce *et al.* (1966) and Ashmore *et al.* (1972) used blood collected directly into heparin but Ashmore *et al.* (1968) added heparin to ACD blood, and Rittenhouse *et al.* (1972) used blood anticoagulated with ACD alone. Ashmore *et al.* (1968) noted that the increase in SFP did not occur in the

absence of a high concentration of oxygen in the oxygenator, and Swank and Porter (1963) reported a decrease in the SFP of blood collected into heparin and circulated without oxygenation in a disc oxygenator.

Thus it appears that oxygenation of heparinized dog blood in a bubble oxygenator can be responsible for platelet and leucocyte aggregation and severe haemolysis, and that the presence of ACD or CPD in the priming solution provides an adequate protection against these effects. The red cells in dog blood anticoagulated with ACD appear to be less fragile than those anticoagulated with CPD. The platelet and leucocyte aggregation found by Ashmore *et al.* (1968) and by Rittenhouse *et al.* (1972) in the presence of ACD may have been due to their use of the more traumatic disc oxygenator or to some other experimental difference.

The circulation of human or dog blood with a roller pump does not appear to cause platelet and leucocyte aggregation. On the contrary, there is a strong tendency for any aggregates present in the donor blood to be disintegrated by the pump. Consequently, during the combined circulation and oxygenation of blood in an extracorporeal circuit, a dynamic equilibrium may be achieved between the two opposing forces, the equilibrium position being shifted in favour of the disintegration of aggregates in blood anticoagulated with ACD or CPD. It appears that these anticoagulants are not so effective in preventing the cellular aggregation that takes place during blood storage, but that ACD is to be preferred to CPD in this respect.

Based upon these findings, our current practice is to prime the extracorporeal circuit with a Ringer-lactate solution, and when a part-blood prime is required, ACD-heparin or CPD-heparin but never heparin alone is used as the anticoagulant. The prime is oxygenated for less than 5 min before starting cardiopulmonary bypass. Under these conditions no platelet and leucocyte aggregates are formed and haemolysis is negligible.

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