Reduction of Hemolytic Blood Damage with Dextran

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Mechanical damage to red cells, relevant to all blood flow processes, is typically quantified by tracking hemoglobin release under specified conditions of shear stress or shear rate and device geometrical parameters (Nevaril et al., 1968; Leverett et al., 1972; Blackshear, 1972; Offeman and Williams, 1979). Shear stresses below 150 N/m^2 are considered to be in the low stress regime; it is in this range that cell interactions with device surfaces dominate the degree of hemolysis, nearly independent of the fluid stress magnitude but dependent upon exposure time to the stress (Shapiro and Williams, 1970; Leverett et al., 1972; Williams, 1973). This is the stress condition prevailing in the present study. Shear stresses above 150 N/m^2 can cause erythrocyte damage almost instantaneously, independent of surface interactions, and represent such severe cell damage (rupture and fragmentation) that this condition is not usually relevant to clinical practice.

Mitigation of flow-induced blood damage has obvious practical importance. Engineering studies have been directed primarily toward understanding factors related to hydrodynamics (Beissinger and Williams, 1984) and device materials (Monroe et al., 1980). The possibility that chemical additives to the blood might alleviate the problem has not been extensively explored.

Introduction of adenosine into blood preservatives led to reduction of hemolysis in bloods stored with those agents (Tadano et al., 1977), most likely because of the role adenosine plays in the cell metabolism. An extended investigation in our laboratory (Offeman and Williams, 1979; Lijana and Williams, 1986; Lijana et al., 1987) demonstrated that low molecular weight antibiotics and a common asthma drug (theophylline) reduced hemolytic damage up to about 20% (within physiological dose magnitudes). Here, the benefit is correlated with the extent of agent absorption by cell membranes and coincides with reductions in cell deformability.

Polymeric character alone seems insufficient as a predictor of an additive's influence. Large-molecule antibiotics are known to be hemolytic (Butler and Cottlove, 1971) and polyadenylate is also harmful, while polycytidylate is protective (Lijana et al., 1987); the latter two compounds are synthetic polynucleic acids. Blood proteins seem to be helpful by depositing on device surfaces and generally providing the cells with a physiological environment in cell suspensions (Bernstein, 1971; Nichols and Williams, 1976); albumin and gamma globulin have been the most successful.

One important polymeric blood additive has not been fully examined for its influence on shear-induced hemolysis: dextran. (The need for plasma substitutes that can be used as temporary replacement for lost blood is often fulfilled by dextran solutions.) Dextrans are polysaccharides of d-glucose units linked by $\alpha(1,6)$-glycoside bonds. The dextran used for this work was dextran 70 (D70), having an average molecular weight of 70,000 to approximate that of human plasma albumin. High molecular weight dextrans (e.g., D70) enhance erythrocyte aggregation, whereas low molecular weight dextrans (e.g., D40) exhibit a disaggregating effect (Sewchand and Canham, 1979; Sewchand and Bruckschwaiger, 1980; Jan et al., 1982). Regarding cell mechanical properties, the most recent studies are those of Nash and Meiselman (1983), who concluded that dextran by itself has little effect on red cell membrane elasticity or cell shape, and Tran-Son-Tay et al. (1985), who found that cell membrane viscosity is only minimally affected by dextran.

In what follows, we present the results of a preliminary study of D70 dextran added to whole blood. Remarkably, hemolysis is found to be almost totally suppressed in low-stress shear flow.

Experimental Procedure

Units of whole human blood from healthy donors were obtained when legally expired, having been stored at 4°C for 21 days with preservative citrate-phosphate-dextrose (CPD) solution. Three different bloods were tested in this dextran study; from our experience with blood from hundreds of donors used in our previous work, these three bloods were recognizable as

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entirely normal and representative, according to standard chemical evaluation.

The shearing device has been described in detail in our previous work. Polyethylene disks of 10.0 cm dia. are spaced 1.0 mm apart during the testing, the gap being filled with blood; excess blood is contained in a reservoir around the disk rim. The upper disk rotates at 350 rpm and the lower disk is stationary, flush with the reservoir floor. Flow between the disks is laminar, with a spiral pattern because of radial velocity superimposed by centrifugal force on the basically rotational flow. Blood is carried outward along the top disk and back in along the bottom disk; this vigorous action prevents cell settling and assures that released hemoglobin is quickly and uniformly distributed. The intradisk fluid mechanics are known (Beissinger and Williams, 1984); shear stress varies radially from zero at the centerline to 13 N/m^2 (as computed using a viscosity of 5 mPa · s) at the upper disk rim. This low range of shear stress approximates quite well the stresses of the human circulatory system; erythrocyte stresses are far from the high-stress threshold.

The day before testing, blood was filtered into polypropylene (PP) tubes in aliquots of 30 mL through a large-weave nylon filter (Pliapak Blood Administration Set, Abbott Labs, Chicago, IL) for removal of possible microclots. To achieve the desired plasma concentration of D70 (Pharmacia Fine Chemicals, Uppsala, Sweden), solutions in normal saline were added as appropriate to the PP tubes using sterile needles and syringes, and then the D70-containing and control tubes and the blood bag were replaced in 4°C storage.

On the day of shear testing, a PP tube was warmed for 30 min in a room-temperature water bath. During this period, the shearing device was aligned and a moist nitrogen flow through it was established. After gentle inversions of the PP tube to homogenize the blood, the sample was poured slowly into a syringe and allowed to drain by gravity flow through flexible tubing into the central region between disks. This procedure, advancing the blood radially outward between disks, prevented gas entrapment. The fully loaded sample was allowed to deoxygenate for about 10 min before shear testing was begun.

Blood samples were taken during shear through a sampling tube in the reservoir floor. Samples were immediately centrifuged in microhematocrit tubes and the hematocrit, H, determined. Hemoglobin concentration, C, was determined by a colorimetric method (Lijana, 1978). All test runs on a given day employed the same blood, with and without D70. Hemolysis data were evaluated as incremental hemoglobin release due to shear:

$$\Delta C \equiv C(t) - C(0) \qquad (1)$$

Curves of ΔC vs. t are designated as kinetic hemolysis curves (KHC).

Results

The KHC for two of the D70 comparisons are shown in Figure 1. These KHC display the difference in hemolysis between blood with no added D70 and blood with 8.5 or 16% wt./vol. D70. Dramatic reductions in ΔC level and $d\Delta C/dt$ are evident, with the rate becoming zero after about 2,000 s. The third blood (AH844), not shown, behaved similarly in two tests (four days apart), but did not quite achieve $d\Delta C/dt = 0$ at $t = 5{,}000$ s. Collectively, these hemolysis suppressions are unmatched by tests

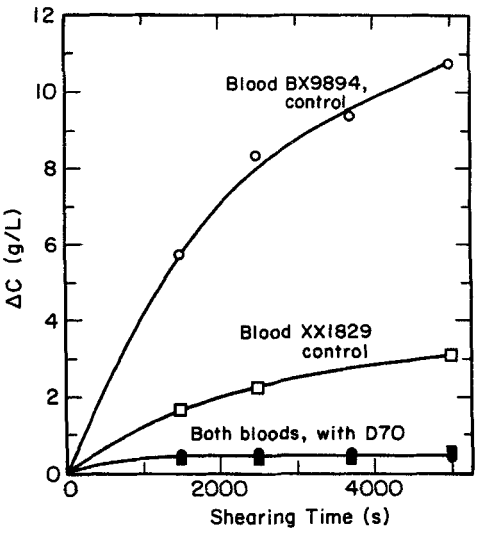

Figure 1. Kinetic hemolysis curves for effects of dextran 70 on preserved whole blood.

Blood storage age, 23–24 days
Additive concentration and resulting plasma viscosity:
○ control (0% D70), 1.4 mPa · s; □ control, 1.5 mPa · s
● 8.5% D70, 5.3 mPa · s; ■ 16% D70, 9.2 mPa · s

with any other additive. The fact that plateau levels in Figure 1 are the same for different D70 concentrations and for bloods from different donors is unexpected; the performance of all other additives has varied with concentration, and additive-free hemolysis is strongly donor-dependent: note the differences in the two control curves.

Although viscosities of the whole-blood samples were not measured, they presumably varied in proportion to the measured plasma viscosities, Table 1. Thus, bloods with D70 possessed enhanced viscosities, but at most by a factor of about four; stress magnitudes still remained well within the low-stress regime.

Discussion

While the present data are not extensive enough to define the whole phenomenon of D70 protection of flowing blood, they are convincing that a major beneficial effect is occurring. To place this in perspective, we review briefly some related reports of dextran behavior.

Some beneficial effects due to dextran arise in nonflow situations. Erythrocytes suspended in a low-salt solution containing D50 did not release as much hemoglobin (osmotic hemolysis) as cells suspended without dextran (Hjelm et al., 1966; Marsden et al., 1957). Those tests also showed that the protective effect became more pronounced as dextran concentration increased;

Table 1. Blood Plasma Viscosities

Blood I.D. No.	Storage Age Days	Plasma Concentration Dextran 70, %	Plasma Viscosity at 20°C, mPa · s	
			Control	Dextran
XX1829	23	8.5	1.5	5.3
AH844	23	14	1.3	5.2
AH844	27	14	1.3	5.3
BX9894	24	16	1.4	9.2

80% inhibition of lysis occurred at a 9% dextran concentration in the buffer. Davies et al. (1968) noted that D150 also inhibited osmotic hemolysis as it was taken up by the erythrocytes. Seeman (1974) found that many kinds of macromolecules (including albumin) can inhibit osmotic hemolysis. Explanations for this phenomenon have been proposed in terms of the polymer effect on solution osmotic pressure (Davies et al., 1968) and, for dextran, in terms of hydration effects (Seeman, 1973); however, universal agreement is lacking.

Only a small amount of work has been directed toward the protective effect of dextran in flow situations, and none of it quantitative with respect to low-stress hemolysis. Feo and Phillips (1982) noted hemolysis protection due to D40 under concentric cylinder shear even when substantial shear-induced changes in erythrocyte dimensions were observed (but still in the low-stress regime). Coakley et al. (1977) studied the capillary flow of suspended erythrocytes using D500 to produce suspending medium viscosities of 2–33 mPa · s. They found that a critical shear stress of around 350 N/m^2 was needed for high-stress hemolysis to commence in the presence of the dextran, regardless of medium viscosity; note the analogy here to the results in Figure 1. Champion et al. (1971) obtained similar findings with D500, using a cone-and-plate viscometer, the critical shear stress being around 220 N/m^2. All together, these observations report qualitatively that dextrans suppress low-stress hemolysis and delay the onset of high-stress hemolysis, but quantitative data and kinetics have not been available until now.

With this background, and with results presented here, we will discuss some of the mechanisms that might be involved in the hemolysis reduction.

Cell Properties

Surely dextrans of all sizes interact strongly with erythrocyte membranes, and we can speculate that some penetration of the bilayer can occur. It is tempting to propose that such adsorption/penetration is at the root of the basic phenomenon in both static (osmotic) and flow situations. One potential consequence of this is reduced cell deformability of the sort that occurred with antibiotics and theophylline; this argument implies that stiffening of the membrane should make more difficult any sort of membrane separation or breach that would allow hemoglobin to escape. However, since the latest deformability study (Nash and Meiselman, 1983) indicates that the magnitude of its change is small, while the reduction of hemolysis is enormous, some doubt is cast upon this explanation with dextran.

Alternatively, the D70 membrane presence could provide a simple sealing effect. By establishing a strong exterior layer that would prevent the penetration of hemoglobin, an external cell coating could suppress hemolysis without modifying cell membrane deformability. Because of its electroneutrality, the dextran molecule might also be capable of entering membrane pores rather easily, thus "plugging" various channels by which hemoglobin could escape. Both explanations are consistent with the data in Figure 1 showing $d\Delta C/dt = 0$ at long times, for bloods with greatly different dextran content—i.e., protection would increase with concentration only until all cell surfaces were coated (or all membrane pores plugged), and additional dextran increase would have little effect.

Dextran influences on the state of cell aggregation appear to be irrelevant in the present work. Since aggregates are almost completely dispersed at stresses near 1 N/m^2 in whole blood, and dextran does not change this much in the normal presence of blood proteins, we expect that all the data in Figure 1 represent the fully dispersed case.

Plasma Viscosity

Studies of cell deformability (Mohandas et al., 1980; Pfafferott et al., 1985) have shown that even compliant cells do not deform greatly when the external medium viscosity is at normally low levels. Cells rotate in such a shear field, rather than deforming, because their internal cytoplasm viscosity—5 to 10 mPa · s—is much higher than that of the plasma. However, large cell deformations can be induced by increasing plasma viscosity beyond intracellular levels, and a viscosity-matching condition appears near optimum for a tank-treading membrane motion (Schmid-Schonbein, 1975). In the present experiments, plasma viscosity was close to the matching range.

This argument contends that hemolysis is minimized if the cell membrane can tank-tread, due to minimization of forces actually being felt by the membrane (hence, less breaching) as external field stresses are transmitted into the cell. However, there is as yet no micromechanical model analysis of cell stress distribution to support this argument. Moreover, if such a mechanism were involved, it seems unlikely that the two bloods shown in Figure 1 would have responded with identical KHC after dextran exposure when their plasma viscosity enhancements were quite different (3.5 vs. 6.6 for enhancement ratios). Finally, the concept is entirely irrelevant to the protection afforded cells by dextran in osmotic hemolysis.

Device Surface

Precoating solid surfaces with plasma proteins is known to reduce cell damage when resuspended cells flow past those surfaces (Nichols and Williams, 1976), and even when whole blood is flowing there is some cell protection afforded by albumin precoating (Bernstein, 1971). No such study has apparently been made of dextran effects as a protective layer on otherwise damaging solid surfaces, and almost certainly it would deposit from the plasma and adsorb on the polyethylene disks (Horbett et al., 1977) used for shearing here. However, unless the special combination of adsorbed dextran and proteins conveys unusual properties to the surface, it is difficult to understand why the improvement should be so great over the proteins alone. Furthermore, like the viscosity argument, this does not accommodate the osmotic benefits.

Conclusions

A remarkably large suppression of hemoglobin release in flowing blood occurred in the presence of dextran D70. The phenomenon was manifested by near-zero release rates very quickly after the inception of shear, with stable hemoglobin levels about 0.4 g/L above background for three different bloods (four tests) whose plasma contained 8 to 16% D70. Explanations are not yet available, but the best candidate seems to be a mechanism of dextran adsorption on the cell membrane with subsequent external sealing or membrane pore plugging. If so, very much lower dextran concentrations should be equally effective, as the membrane saturation condition should be achievable with only trace amounts present in the plasma, and the additive effectiveness should be essentially independent of its molecular weight. These latter speculations have yet to be tested.

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Notation

C = concentration of hemoglobin in blood plasma, g/L
KHC = kinetic hemolysis curve, ΔC vs. t
t = time, s

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