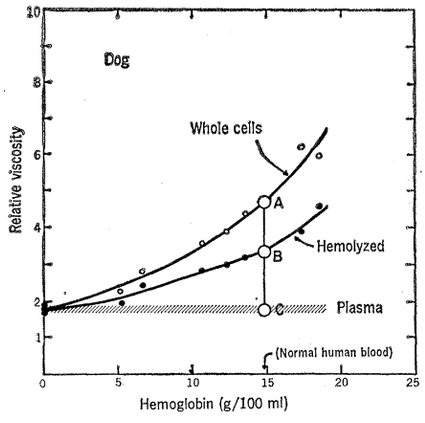

Fig. 1. Relative viscosity (water = 1.00) of dog blood (four animals) with whole cells (upper curve) and of the hemoglobin solution obtained by disrupting the corpuscles of the same samples with ultrasound (lower curve) at various hemoglobin concentrations. The horizontal, hatched area refers to the range of observed plasma viscosity of the blood samples. Points *A* and *B* denote the viscosities at the hemoglobin concentration of normal dog or human blood, 14.8 g/100 ml.

much less than that of the red cell suspension (relative viscosity, 30.1).

In goat blood, at 14.8 g of hemoglobin per 100 ml, the contribution of the hemoglobin to blood viscosity was reduced by 57 percent when the hemoglobin was carried in solution rather than in cells. The relative reduction is approximately the same at the normal concentration of hemoglobin in goat blood (about 12.6 g/100 ml).

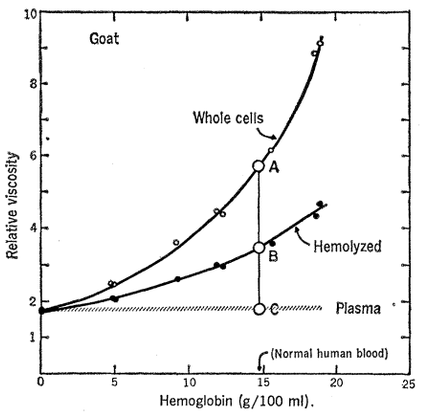

Fig. 2. Relative viscosity (water = 1.00) of goat blood (two animals) with whole cells (upper curve) and the hemoglobin solution obtained by disrupting the corpuscles of the same samples with ultrasound (lower curve) at various hemoglobin concentrations. The horizontal cross-hatched area refers to the range of observed plasma viscosity of the same blood samples. Points *A* and *B* denote the viscosities at a hemoglobin concentration of 14.8 g/100 ml.

The conspicuous reduction in relative viscosity which we found was measured in a glass capillary 0.63 mm in diameter and 70 mm long. In narrower tubes (less than about 0.5 mm), however, the viscosity of blood decreases relative to that of water in the same tube (the Fåhraeus-Lindquist effect) (*6*). If the relative viscosity of blood is measured by perfusion of the hind limb of a dog, it is lower than the relative viscosity measured in an Ostwald viscometer (*7*), probably due to the Fåhraeus-Lindquist effect. It is therefore desirable to extend our observations with determinations of the viscosity in small tubes with a diameter similar to the capillaries of the living organism.

If the red cell contributes a greater increase in blood viscosity than a hemoglobin solution of the same oxygen carrying capacity, what is the rationale for the existence of red blood cells? Perhaps the most convincing argument is that without the red cells the dissolved hemoglobin would cause the colloidal osmotic pressure of mammalian blood to be about three times as high as that caused by plasma proteins alone. This would have profound effects on movement of fluid through capillary walls, and, in particular, on the filtration process in the renal glomerulus, which would require a correspondingly higher filtration pressure and therefore also blood pressure. There is, however, a way out of this dilemma. The contribution of respiratory pigments to the colloidal osmotic pressure could be reduced by carrying the proteins as large-size molecular aggregates. This is the situation in many invertebrates which carry their respiratory proteins in aggregates with molecular weights of several million (*1*).

One possible function of red cells may be related to the fact that they cause the liquid flow in the capillary to change from a laminar to a bolus flow. This may have advantages for the gas exchange in the capillary because with bolus flow there is no stagnant layer of fluid along the capillary wall.

Another consequence of the existence of red blood cells is that various blood components, such as enzyme systems, are kept localized and in high concentration within the cell in close proximity to the hemoglobin. For example, the action of the enzyme glutathion reductase (which normally reduces the respiratory inactive methemoglobin to normal oxygen-carrying hemoglobin) is facilitated when both reactants are present in high concentration within the confined space of the red cell. Whatever reason there may be for the existence of red blood cells, our conclusion must be that experimental evidence does not support the assumption that their existence contributes to a reduced blood viscosity.

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14 June 1968

Rheological Comparison of Hemoglobin Solutions and Erythrocyte Suspensions

Abstract. *Hemoglobin solutions prepared from hemolyzed human erythrocyte packs have Newtonian flow properties. Diluted solutions are also Newtonian. All solutions have a viscosity lower than the apparent viscosity of erythrocyte suspensions of equal oxygen-carrying capacity. The presence of cell debris in hemoglobin solutions causes non-Newtonian (pseudoplastic or rheopectic) flow behavior.*

It has been reported (*1*) that the viscosity of blood is considerably less than that of a hemoglobin solution of comparable oxygen-carrying capacity. It has been argued from this that hemoglobin packaged in red blood cells has evolved as a means of reducing the heart work

Table 1. Characteristics of fluids described in Fig. 1. Viscosity data were taken at 37.0°C. Solution A′ is undiluted RBC contents. Isotonic saline viscosity at 37.0°C equals 0.800 centipoise (cp).

Measurement	RBC suspensions			Solutions of RBC contents		
	A	B	C	A′	B′	C′
Hematocrit (%)	95.5	63.9	30.8			
Hemoglobin (wt. %)	33.2	21.5	10.8	31.5	21.0	10.2
Total solids (wt. %)				32.1	22.6	12.3
Viscosity (cp)				5.91	2.07	1.12

of pumping blood around the circulatory system. We now report data which indicate that the apparent viscosity of blood is not less than that of a hemoglobin solution of comparable oxygen-carrying capacity.

A smooth-surfaced concentric-cylinder viscometer [a modified GDM viscometer (*2*)] was used to obtain flow data from which the relationships of shear stress and shear rate for a sample were calculated. A standard technique in which two bobs of equal diameters but different lengths are used was employed to eliminate end and edge effects, and a guard ring was used to eliminate effects of the liquid-gas interface. The Krieger-Elrod equation (*3*) was used to calculate shear rates; corresponding shear stresses were determined by use of the equation for the conservation of angular momentum. In all cases, the temperature was 37.00°C; it did not vary during experiments by more than 0.05°C. The samples were either washed red blood cells suspended in isotonic, buffered saline (*4*), or the contents of red blood cells dispersed in the same saline.

Human red blood cells (RBC's) were obtained from blood drawn into a standard solution of citric acid, sodium citrate, and dextrose by normal blood bank procedure. Fresh blood or blood stored less than 7 days at 4°C was used. A blood sample was centrifuged, and the plasma and buffy coat were removed. The RBC's were washed three times with saline, and finally centrifuged to make a cell pack. For RBC suspensions, appropriate amounts of RBC pack and saline were combined, and the sample hematocrit was determined by the microhematocrit method. For hemoglobin solutions, the RBC pack in a tube, was frozen in a mixture of dry ice and ethanol for 7 minutes and then melted in a 37°C bath. Approximately 30 ml of this solution was combined with 15 ml of CCl_4 (J. T. Baker Chemical Co., reagent grade), and the immiscible mixture was shaken and then centrifuged at 27,000*g* for 1 hour at 3° to 5°C. The aqueous solution of the RBC contents was removed with a syringe; microscopic examinations showed it to be free of cells and stroma. As far as is known, this method of obtaining RBC contents does not alter hemoglobin (*5*). Hemoglobin solutions were prepared by combining various amounts of this solution of RBC contents with saline. The total amounts of solids of such solutions were determined gravimetrically on samples dried at 110°C until no weight change could be detected with further drying. Hemoglobin concentrations of the cell suspensions and the solutions of RBC contents were determined spectrophotometrically by a cyanmethemoglobin method (*6*).

The solutions of RBC contents always showed Newtonian flow behavior, even when not diluted with saline. The fact that the RBC suspensions were non-Newtonian confirms earlier work by Gregersen *et al.* (*7*) and others. Data obtained at 37.0°C with solutions and suspensions prepared from one blood sample are shown in Fig. 1. The characteristics of these samples are given in Table 1. In preparing the samples, we tried to make the hemoglobin content of a suspension of RBC's equal to that of a solution of RBC contents.

Over the range of shear rates investigated, the apparent viscosity of a RBC suspension is always larger than that of the solution of RBC contents of the same hemoglobin concentration. The RBC suspensions approach Newtonian behavior at high shear rates, and these limiting viscosities can be estimated. Since blood with a hematocrit of 40 percent becomes Newtonian at shear rates above about 100 per second (*8*), the RBC suspension with 30.8 percent hematocrit will be Newtonian above 100 per second, and will have an apparent viscosity of close to 2.1 centipoise. The asymptotic apparent viscosities of the other RBC suspensions can then be estimated from the empirical observation (*9*) that the logarithm of the limiting viscosity of RBC suspensions in saline is a linear function of the hematocrit. From this relationship, the minimum apparent viscosities of the other RBC suspensions were estimated (Fig. 1). Even with these asymptotic viscosity values, the RBC suspension is always more viscous than the solution of RBC contents of comparable hemoglobin concentration.

The relative viscosities of the hemoglobin solutions shown in Fig. 1 are plotted in Fig. 2 as a function of hemoglobin concentration. The Huggins' equation was used, values for the intrinsic viscosity of hemoglobin in saline and Huggins' constant being taken from the literature (*10*), to calculate the dashed curve in Fig. 2. The agreement of our data is good for low concentra-

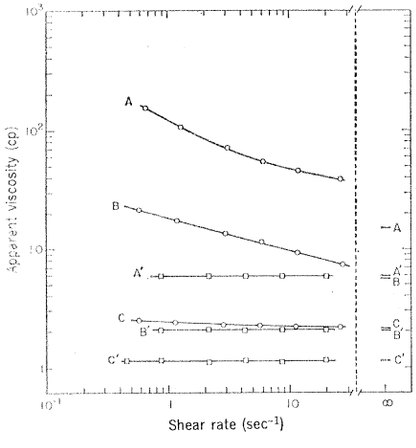

Fig. 1. Experimental apparent viscosities of erythrocyte suspensions and hemoglobin solutions (see Table 1 for fluid identification). Estimated asymptotic viscosities are shown at the right. Temperature is 37.0°C; cp, centipoise.

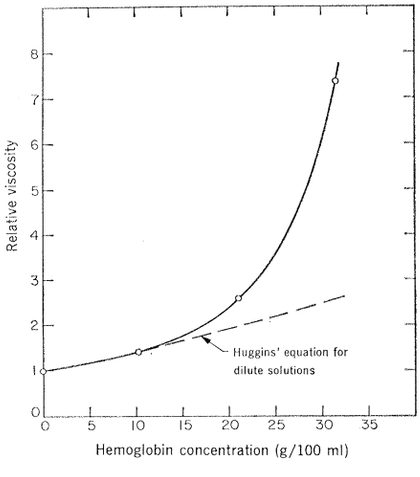

Fig. 2. Experimental relative viscosities of hemoglobin solutions at various concentrations and comparison with dilute solution behavior. Temperature is 37.0°C.

tions but deviates when the concentration is above 10 g/100 ml; this is expected since Huggins' equation is valid only at low concentration.

It is very important to remove all cell debris from solutions of RBC contents before attempting to measure the flow properties of the solutions. Solutions prepared with cell debris in them were non-Newtonian and showed apparent viscosities (at 20 per second shear rate) 50 to 100 times that of the debris-free solutions of the same hemoglobin concentration. In some cases, solutions containing debris showed rheopectic flow behavior due to the flow-induced aggregation of debris with time.

These experiments were performed with a saline solution as the continuous medium rather than with plasma. Erythrocytes suspended in plasma aggregate at low shear rates. Except for cases where the hematocrit is very close to zero or 100 percent, this causes the apparent viscosity of human blood at a given shear rate to be much larger than the apparent viscosity of the same RBC's suspended in isotonic saline at the same hematocrits (*7*, *11*). Also, experiments performed with crystallized human hemoglobin (Nutritional Biochemicals Corporation) dissolved in plasma showed such solutions to be Newtonian with relative viscosities close to the relative viscosities of the hemoglobin solutions described here (*11*). Consequently, at low shear rates, the ratio of the apparent viscosity of blood at a given shear rate to the viscosity of a solution of hemoglobin in plasma will be equal to or larger than (depending on hematocrit) that shown for the same fluids with isotonic saline as the continuous phase. The discrepancy will be largest at lower shear rates.

It should also be noted that the use of smooth viscometer walls probably results in the RBC suspensions with very high hematocrits showing lower shear stresses at a given shear rate than is correct (*12*). If true, this would only reinforce the main conclusion of this work.

With these findings, one cannot tell if the heart work is lower with the hemoglobin in RBC's than it would be if the hemoglobin were dispersed in solution. While our data indicate that the contribution to the heart workload from blood flow in the larger blood vessels is lower when the hemoglobin is in solution, this cannot be said about the contribution from flow in the smaller vessels. The continuum model for blood flow fails in the smallest vessels, but the size of the vessels where the failure is first noticeable is not known. Since the major drop in pressure in the circulatory system occurs in such small vessels, further discussion on this question must await data on blood flow in vessels ranging in size from 5 to 100 μm.

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Brain Serotonin Concentration: Elevation Following Intraperitoneal Administration of Melatonin

Abstract. *The intraperitoneal administration of melatonin to rats caused an increase in brain serotonin concentration, especially in the midbrain. This effect could be demonstrated within 20 minutes of melatonin administration and was not associated with changes in norepinephrine concentration.*

Melatonin (5-methoxy-*N*-acetyltryptamine) is produced in mammals only in the pineal gland (*1*, *2*). The synthesis of this compound is inhibited by environmental lighting and stimulated in darkness (*3*); information about the state of lighting is transmitted to the pineal by a special pathway involving the eyes, the inferior accessory optic tracts, the superior cervical ganglia, and the sympathetic innervation of pineal parenchymal cells (*2*, *4*). Because the rate of melatonin synthesis varies with a 24-hour rhythm in response to the light-dark cycle (*5*), it has been suggested that the pineal functions in mammals as a "biologic clock" or timing apparatus which emits an endocrine signal whose amplitude varies as a function of time of day (*6*).

The administration of melatonin to experimental animals produces both endocrine and neural effects (*2*). Melatonin injections cause a rapid fall in the concentration of the melanocyte-stimulating hormone (MSH) in the pituitary gland (*7*); they have also been reported to modify pituitary and serum gonadotropin levels (*8*) and the size and functional activity of rat gonads (*9*). Effects of melatonin administration on neural function include (i) the induction of sleep and of electroencephalographic changes in cats and (ii) the potentiation of hexobarbital sleeping time in mice (*10*). Since circulating melatonin enters the brain with little difficulty (*11*) and melatonin implants in the midbrain or median eminence also modify pituitary-gonadal function (*12*), it has been suggested that neuroendocrine centers in the brain constitute loci at which melatonin acts to produce its endocrine effects (*11*, *12*). We now report that intraperitoneal injections of melatonin are followed by a rapid rise in the concentration of brain serotonin. The greatest effects of melatonin administration may be seen in the midbrain, that portion of the brain which contains most of the cell bodies of the central serotoninergic neurons (*13*).

Female Sprague-Dawley rats (180 g) received intraperitoneal injections of melatonin dissolved in dilute (2 percent) ethanol in a final volume of 0.1 ml. Control animals received only the ethanol solution. Animals were caged individually and kept under standard laboratory lighting conditions (lights on from 6:00 a.m. to 6:00 p.m.; light provided by cool white fluorescent bulbs yielding approximately 27 to 54 mphot at the level of the animals); they were