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Blood Corpuscles and Blood Hemoglobins: A Possible Example of Coevolution

Abstract. *A model which equates oxygen transport to hemoglobin concentration and molecular weight is used to demonstrate that high concentrations of hemoglobin will augment oxygen transport only if the molecular weight of the hemoglobin is low. The evolution of corpuscles is a necessary counterpart to having high concentrations of the low molecular weight hemoglobins; corpuscles prevent loss of the small molecules by way of excretory filters and prevent the development of exceedingly high plasma osmotic pressures.*

Some animals have hemoglobins (Hb's) which are carried in solution, while others have Hb's which are located in blood corpuscles. Animals with high Hb concentrations always have blood corpuscles (*1*), and it has been suggested that the blood corpuscles have evolved because a solution of Hb of the same oxygen capacity would be highly viscous, although recent evidence has indicated that this is not the case (*2*). A much overlooked observation is that the Hb's which are carried in solution have large molecular weights ($> 5 \times 10^5$) while the Hb's located in corpuscles have low molecular weights ($< 7 \times 10^4$). In this report, I show that it is the low molecular weight of the Hb that is critical to obtaining blood with a high oxygen capacity. The packaging of the Hb in corpuscles is a necessary counterpart to having high concentrations of the low molecular weight Hb; such packaging prevents loss of the molecules of low molecular weight by way of excretory filters and prevents marked increases in plasma osmotic pressure.

The significance of molecular weight can be demonstrated with a simple model which equates oxygen transport (O_t), that is, the volume of oxygen flowing per minute in the arterial blood, to molecular weight, M, in solution. It is well established that O_t is equal to the product of blood flow ($\dot{V}_B$) and the amount of oxygen bound to the Hb. Thus

$$O_t = \dot{V}_B(k_1 C_{Hb}) \quad (1)$$

where k_1 is a constant for the binding capacity of Hb (*3*) and C_{Hb} is the concentration of Hb. However, C_{Hb} will also affect $\dot{V}_B$ by changing blood viscosity (η). For example, from the Poiseuille equation (*4*), one can approximate blood flow as

$$\dot{V}_B = k_2/\eta \quad (2)$$

where k_2 is a constant that embodies the vascular dimensions of the vessel system and the driving pressure, for example. For any given k_2, blood flow will vary inversely with blood viscosity. In turn, blood viscosity is directly related to the intrinsic viscosity $[\eta]$ of Hb and C_{Hb} or

$$\eta = 1 + [\eta]C_{Hb} + ([\eta]C_{Hb})^2 \quad (3)$$

and the intrinsic viscosity of the Hb is directly related to M

$$[\eta] = k_3 M^a \quad (4)$$

where k_3 and a are constants (*5*). By substituting Eqs. 4, 3, and 2 into Eq. 1, then

$$O_t = k_4 C_{Hb}/[1 + k_3 M^a C_{Hb} + (k_3 M^a C_{Hb})^2] \quad (5)$$

where k_4 combines k_1 and k_2.

From Eq. 5, it is evident that the relation between O_t and Hb is a complex one. The two components that are important are the direct relation between O_t and Hb, which is a consequence of the oxygen-binding properties of the carrier molecule, and an inverse relation between the two which depends on the effects of Hb on η. Of particular interest is the fact that the influence of Hb on η is dependent on both concentration of Hb and on M. Although a variety of carrier pigments have been identified, they are all polymers or aggregates of molecules with low molecular weights. If the large Hb's are split into smaller units, η will be reduced without the oxygen capacity of the blood being changed.

The influence of M on η and O_t are illustrated in Figs. 1 and 2. In Fig. 1 are shown the results obtained after substituting Eq. 4 into Eq. 3. The $[\eta]$ of the Hb's, based on known values for myoglobin and various polypeptides, is approximated from

$$[\eta]_{Hb} = 0.0238 \times M^{0.5} \quad (6)$$

which accurately predicts the known $[\eta]$ for myoglobin, 3.1 cm^3/g (*6*). The accu-

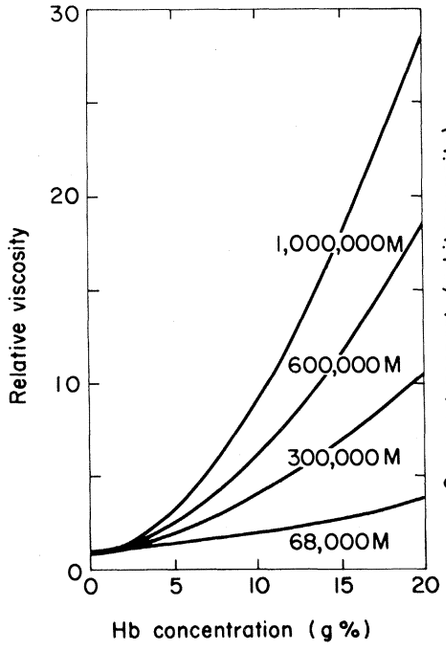

Fig. 1. Effects of hemoglobin concentration and molecular weight on the viscosities of hemoglobin solutions.

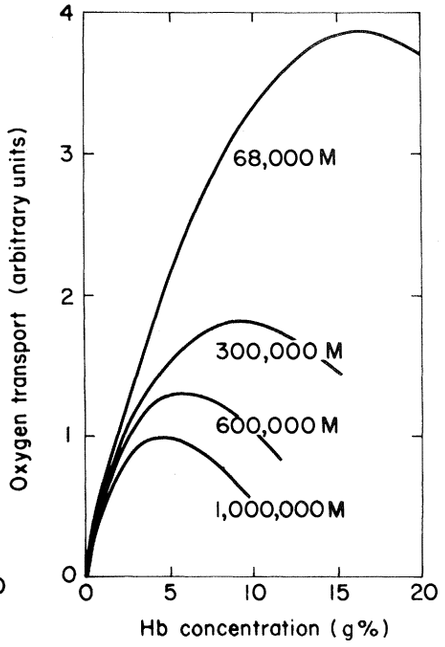

Fig. 2. Oxygen transport as a function of hemoglobin concentration and molecular weight.

racy of Eq. 6 is also strengthened by the fact that, after substituting Eq. 6 into Eq. 3, I was able to approximate the η of Hb solutions prepared from dog and goat bloods; 2.8 for the predicted compared to 3.3 for the observed (*7*).

For all values of M, at the low Hb concentrations, an increment in C_{Hb} results in an increase in O_t (Fig. 2). However, at some point, which is peculiar to the molecular weight of the Hb, O_t reaches a maximum value and then decreases with further increments in C_{Hb}. The shape of the curve for O_t lends itself to analysis to determine the components of Eq. 5 that affect the point of $O_{t,max}$; that is, where the slope of the O_t line is zero (*3*). By taking the first derivative of Eq. 5 with respect to Hb and setting the derivative to zero one obtains the conditions at $O_{t.max}$:

$$\delta O_t/\delta C_{Hb} = 0 = 1/k_3 M^a \quad (7)$$

In other words, $O_{t,max}$ is determined by the inverse of M (*3*). Thus, for hemoglobins of lower molecular weight, not only is O_t higher at any given concentration of Hb, but $O_{t,max}$ is also further to the right (Fig. 2). These combined effects of M on O_t result in a fourfold increase in $O_{t,max}$ when M is reduced from 1×10^6 to 7×10^4 (Fig. 2).

The enclosing of the Hb in corpuscles appears to be requisite for a reduction in M. Freely dissolved molecules in the mammalian circulatory system must have a large molecular weight if they are not to be lost via excretory filters. For example, mammalian Hb in solution will pass the glomerular filter. Animals with Hb's of low molecular weight in solution do exist (*1*), but these animals have no excretory filters (*8*). Where fluid filtration does occur, a mechanism that would avoid loss of Hb would be packaging the molecules in corpuscles. An additional problem is that if the Hb in mammalian blood were in solution, the plasma osmotic pressure would be increased approximately threefold (*9*). The effects of such a high osmotic pressure would be profound, because normal fluid distribution and flow could only be achieved by a comparable increase in blood pressure. Through cation impermeability, the corpuscle membrane serves not only to localize the Hb, but to remove it from the plasma osmotic space as well (*9, 10*). Thus, corpuscles appear to be an evolutionary step in obtaining high concentrations of Hb. However, this is not necessarily because of the effects of the corpuscles on η. In fact, it has been suggested that corpuscle suspensions have greater viscosities than do Hb solutions of comparable oxygen capacity (*2*). However, the viscosity of a corpuscle suspension is markedly reduced when flow occurs through tubes of small radial dimensions (*11*). Because in the circulatory system, this is the area where resistance to flow is the greatest, the anticipated high viscosities of corpuscle suspensions are not realized (*12*).

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Congenital Anomalies Induced in Hamster Embryos with Ribavirin

Abstract. *Ribavirin, when given to pregnant hamsters in relatively small single doses, induces congenital anomalies of limbs, ribs, eyes, and central nervous system, as well as fetal deaths, On the basis of these findings, caution should be used in giving ribavirin to women of child-bearing age.*

As recently summarized by Maugh (*1*) ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) has a wide spectrum of antiviral activity. Maugh points out that teratogenicity is a characteristic of antiviral agents that are nucleoside analogs and that ribavirin causes defects after it is ingested by female rodents. Nothing, however, has been published on this subject as far as we are aware. In this report we show that ribavirin (Virazole, ICN Pharmaceuticals) is an extremely effective teratogen when given to pregnant hamsters.

Hamsters of the LVG strain (Lakeview) were purchased from Charles River and injected intraperitoneally on gestation day 8. The single doses used (diluted in 0.5 to 1 ml of buffered saline; 1.25 to 4.2 mg of ribavirin per kilogram of body

Table 1. Effects of ribavirin on fetal development when given to pregnant hamsters in a single dose intraperitoneally on gestation day 8.

Ribavirin (mg/kg)	Number of gestation sacs (22 mothers)	Resorptions		Normal fetuses		Abnormal fetuses	
		Number	Percent	Number	Percent	Number	Percent
1.25	35	4	11	29	83	2	6
2.1	30	1	3	15	50	14	47
2.5	62	13	21	15	24	34	55
3.1	67	9	13	35	52	23	34
4.2	64	24	37	3	4	37	57
6.25	23	23	100				

*Percentages refer to numbers of gestational sacs.

Table 2. Frequency and distribution of malformations in 106 abnormal fetuses of mother hamsters that received ribavirin. CNS, central nervous system.

Parameters	Defects*				
	Limb	Eye	CNS	Rib	Other
Fetuses with malformations	85	32	18	45	27
Frequency (%)	80.1	30.1	17	42.5	25.4

*For details of types, see text.