tion and the pH of the solution (Fig. 2). Exflagellation increased sharply from 0 to 100 percent of the control value between pH 7.7 and 8.0 and declined more slowly at higher pH values until no exflagellation occurred above pH 8.6. Thus, regardless of the pCO_2, whether physiological or atmospheric, the level of exflagellation was determined solely by the pH of the medium bathing the cells.

Within the range of values studied the bicarbonate concentration was without marked effect on exflagellation (Fig. 1). In the complete absence of bicarbonate, however, exflagellation is totally suppressed at all pH values (*9*). The relationship between exflagellation and pH is, therefore, entirely dependent upon the presence of bicarbonate. We have, at present, no hypothesis to explain how the bicarbonate-dependent pH control of exflagellation operates at the cellular level.

Our results show that pCO_2 influences the amount of exflagellation in our in vitro system only by its effect on the pH of the medium; similarly above an undetermined minimum concentration bicarbonate ion affects the amount of exflagellation primarily by its control of the pH of the medium. In the light of these findings the natural control of exflagellation could be interpreted as follows. In venous blood taken from the circulation and equilibrated with air the bicarbonate concentration (approximately 25 m*M*) does not change significantly. As the blood passes into the gut of an engorging mosquito through the fascicle, the hairlike food channel in the proboscis, the pCO_2 probably falls to equilibrate with that of the surrounding atmosphere. Such a fall in pCO_2 mediates a rise in pH from that of the circulating blood to one at which exflagellation is initiated. Measurements on the pH of blood in the stomach of mosquitoes taken between 5 and 10 minutes after engorgement (*4*) confirm that these pH values overlap the range at which exflagellation is initiated in the in vitro system. Nevertheless, further experimentation is necessary before it will be possible to say whether the bicarbonate-dependent pH control is alone responsible for initiating exflagellation in the mosquito.

Whether or not factors within the insect vector are involved in controlling the events of exflagellation, our results explain how equilibration with atmospheric pCO_2 is, in the case of *P. gallinaceum*, sufficient to initiate them. Like the malaria parasites and other Haemosporidia, parasitic protozoa such as the leishmanias and African and American trypanosomes generally transform into forms similar or identical to those found in the insect vector when cultured under atmospheric conditions (*1*). The possibility arises, therefore, that atmospheric equilibration may be an important factor for the initial transformation to the vector form in other arthropod-transmitted parasites.

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Coronary Tone Modulation: Formation and Actions of Prostaglandins, Endoperoxides, and Thromboxanes

Abstract. *Exogenous prostaglandin (PGE_2) contracts bovine and human coronary arteries but its precursor, arachidonic acid, relaxes them. The endoperoxides PGH_2 and PGH_3 relax bovine coronary strips, but PGH_1 produces contraction. The primary prostaglandins exert opposite effects to their own endoperoxide precursors, thus, PGE_2 and PGE_3 contract, and PGE_1 relaxes the bovine coronary arteries. The paradoxical coronary dilation produced by the arachidonate or the PGH_2 suggest that little if any coronary isomerase which converts endoperoxide into PGE_2 exists, or that a novel, potent, PG-like substance is produced by the isolated coronary arteries. Although the coronaries do not possess thromboxane A_2 synthetase activity, the vessels are profoundly contracted by exogenous thromboxane A_2. Thromboxane A_2 can be synthesized and released by circulating platelets when they are aggregated by endothelial injury or thrombin. Thus, coronary tone, and possible spasm, in ischemic myocardial zones may be influenced markedly by interplay between prostaglandins, endoperoxides, and thromboxane formed by platelets on the one hand, and endoperoxide products synthesized endogenously in the coronary arteries on the other.*

Isolated or cultured vascular smooth muscles synthesize prostaglandins which may be involved in the regulation of blood vessel tone (*1*, *2*). Isolated bovine (*3*) and human coronary arteries exhibit dose-dependent contractions to prostaglandin (PG) E_2 and $PGF_{2\alpha}$ (*2*). Paradoxically, administration of arachidonic acid, precursor of PG's of the 2 series, caused relaxation of the bovine and human coronary arteries, which was abolished by cyclooxygenase inhibitors (*2*). These results suggest that arachidonate was converted by coronary PG-synthetase to a substance which has a vasodilating effect, and therefore is not PGE_2 or $PGF_{2\alpha}$. The unstable endoperoxides, PGG_2 and PGH_2, are candidates for vasodilating substances produced from arachidonate. However, the existing evidence indicates that the endoperoxides contract vascular smooth muscle including rabbit thoracic aorta (*4*), and isolated pig coronary artery strips (*5*). A further understanding of endogenous regulators of coronary resistance and their potential impact in such conditions as myocardial ischemia, coronary vasospasms, and coronary thrombosis seems of critical importance.

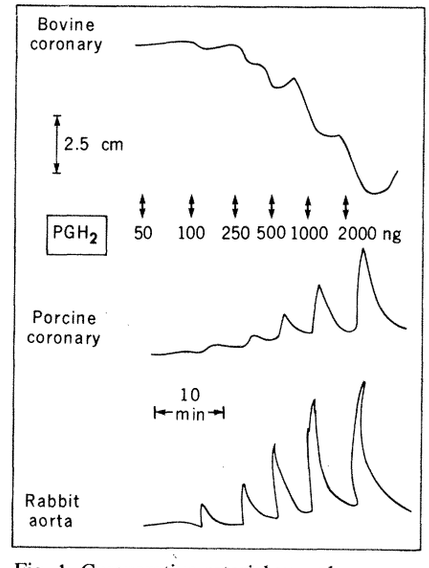

Fig. 1. Comparative arterial vascular responsiveness to the endoperoxide PGH_2.

Bovine and porcine coronary artery and rabbit aorta were excised and handled from freshly removed hearts as previously described (*2*), and spiral strips were prepared (*6*). In experiments designed to compare simultaneously the re-

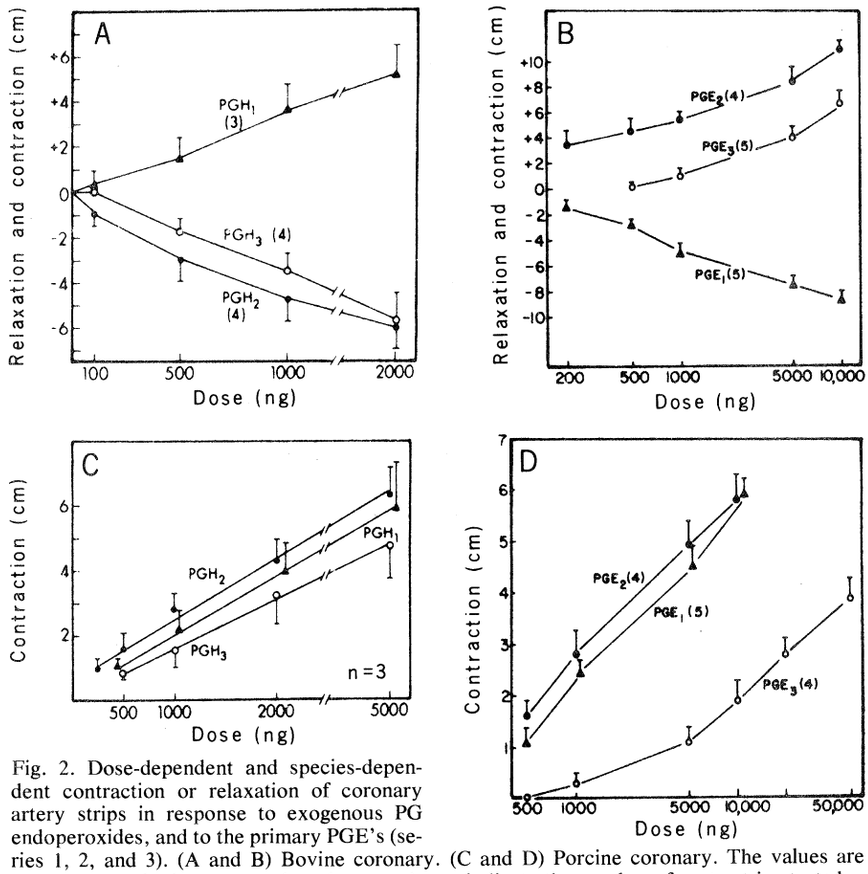

Fig. 2. Dose-dependent and species-dependent contraction or relaxation of coronary artery strips in response to exogenous PG endoperoxides, and to the primary PGE's (series 1, 2, and 3). (A and B) Bovine coronary. (C and D) Porcine coronary. The values are means ± standard error; numbers in parentheses indicate the number of aorta strips tested.

sponses to endoperoxides in blood vessels removed from various species, a superfusion cascade was employed. The spiral strips of blood vessels were continuously perfused (10 ml/min) with Krebs-Henseleit (O_2 : CO_2, 95 : 5 percent) which contained a mixture of antagonists that rendered the tissues insensitive to catecholamines, histamine, acetylcholine, and serotonin. The prostaglandin endoperoxides PGG_1–PGH_1; PGG_2–PGH_2; and PGG_3–PGH_3 were synthesized from ^{14}C-labeled precursor fatty acids and purified as previously described (*7, 8*). The concentrations of the isolated endoperoxides were calculated from the radioactivity measured in the final product, since the purified endoperoxide would have the same specific activity as its labeled precursor, [1-^{14}C]arachidonic acid. Thromboxane A_2 and A_3 (TA_2 and TA_3) were enzymatically generated when PGH_2 and PGH_3 were incubated for 2 minutes at 0°C with human platelet microsomes according to the method previously described (*7, 9*).

The endoperoxide PGH_2 (the PG-intermediate between arachidonic acid and PGE_2) was administered directly across a superfusion cascade which contained a bovine and porcine coronary artery (left anterior descending) and a rabbit thoracic aorta strip (Fig. 1). Surprisingly, PGH_2 (or PGG_2) produced a concentration-dependent relaxation of the bovine coronary artery strips (Figs. 1 and 2A), while PGH_2 (or PGG_2) produced a concentration-dependent contraction of the pig coronary artery and rabbit aorta (Figs. 1 and 2C). Comparable results in both bovine (that is, relaxation) and porcine (that is, contraction) coronaries were obtained with the endoperoxide PGH_3 (the PG-intermediate between eicosapentanoic acid and PGE_3) (Fig. 2, A and C). In sharp contrast, the endoperoxide PGH_1 (the PG intermediate between dihomo-γ-linolenate and PGE_1) caused a dose-dependent contraction of all three vascular smooth muscle preparations (Fig. 2, A and C). The dose-response relationship indicates that in bovine coronary arteries the PGH_2 and PGH_3 are vasodilators of comparable potency; whereas PGH_1 exhibits similar, but directionally opposite, potency as a constrictor (Fig. 2A). On the other hand, in pig coronary artery spiral strips all three endoperoxides, PGH_1, PGH_2, and PGH_3, were constrictors and were of comparable potency (Fig. 2C), which agrees with the report that PGH_2 contracts pig coronary artery (*5*). The three endoperoxides also contract the rabbit thoracic aorta strips at all doses tested, with PGH_2 being about five times more potent a constrictor than PGH_1 and PGH_3 (*7*). The G endoperoxides gave the same results as their respective PGH's.

It is remarkable that in the bovine coronary arteries, the primary prostaglandins produce directionally opposite contractile responses from the endoperoxide intermediates. Thus, PGE_2 and PGE_3 constrict the bovine coronary artery, while PGE_1 relaxes it (Fig. 2B). The fact that both arachidonate and PGH_2 (or PGG_2, not shown) produced bovine coronary artery relaxation strongly supports the hypothesis that the active modulator of coronary vascular tone by arachidonate is the PGH_2 endoperoxide. In contrast, the primary prostaglandins, as well as the precursor arachidonic acid, induce contractions of the porcine pig coronary arteries (Fig. 2D) and rabbit thoracic aorta strips (data not shown). Thus, in porcine coronary artery, and the rabbit thoracic aorta (the traditionally employed example of peripheral arterial vasculature), the fatty acid precursors, the endoperoxide intermediates, and the primary prostaglandins all produce a contractile response.

The recent demonstration that endoperoxides can be converted in certain tissues to a potent vasoactive product, TA_2 (*10*), suggests the possibility that TA_2 is favored in the coronary vasculature. To test this possibility, we incubated blood vessel mirosomes (100,000*g* pellet) prepared from beef or pig coronary arteries or rabbit aorta with PGH_2. We were unable to demonstrate the formation of TA_2; as a positive control we were able to produce TA_2 [determined by bioassay (*9*)] by incubating human umbilical artery with the endoperoxide (data not shown). We enzymatically generated PG endoperoxide, TA_2, and PGE_2 as previously described (*7*) and compared their effects on a superfusion of the bovine coronary artery and thoracic aorta (Fig. 3). The endoperoxide [sheep seminal vesicle microsomes (SSV) plus arachidonic acid (AA)] relaxed the bovine coronary, but the TA_2 generated from the endoperoxide by further incubation with human platelet microsomes (SSV + AA + IPM) was an extremely potent coronary vasoconstrictor. Indeed, profound contractions were produced by the addition of TA_2 [median effective dose (ED_{50}) approximately 50 ng expressed in terms of the endoperoxide originally incubated with platelet microsomes] or TA_3 (ED_{50} ~ 250 ng) to a superfusion cascade of the bovine and porcine coronary arter-

ies and the rabbit aorta. The rabbit aorta and porcine coronary were contracted by all the arachidonate products (that is, endoperoxides, primary PG's, and thromboxanes).

Bovine and human coronary arteries are qualitatively similar in several of their responses, both being contracted by PGE_2, $PGF_{2\alpha}$, and indomethacin; relaxed by arachidonic acid; and relaxed by PGE_1 (*2, 3*). The bovine coronary artery exhibits remarkable receptor specificity for the degree of unsaturation of the prostaglandin molecule as well as the configuration of the prostanoate ring. The endoperoxide ring structure alone does not determine the vascular response, since PGH_1 (lacking the C-5 double bond) contracts, while PGH_2 and PGH_3 (the latter having an additional double bond at C-17) relax the bovine coronary. The critical role of the C-5 double bond in prostaglandins is again emphasized by the fact that PGE_1 dilates, while PGE_2 and PGE_3 constrict the bovine coronary. Equally as striking is that the isomerization of all three endoperoxides into their primary PGE products produces a qualitatively opposite biological response in the bovine coronary artery. The importance of changes in the ring structure is also demonstrated by the fact that isomerization of the endoperoxide into an oxane ring converts the compound from a dilator (PGH_2, PGH_3) to a constrictor substance (TA_2, TA_3) of the bovine coronary.

Our experiments suggest that the endoperoxides should not just be regarded as intermediates but as the major active species generated by the oxidation of the precursor fatty acid, with the primary PG's being the metabolites. Such a situation has been demonstrated in platelets where the endoperoxides and possibly the thromboxanes are critical for aggregation, whereas the primary PG's represent minor degradation products (*11*). The observation that arachidonate relaxes human and bovine coronary arteries suggests at least two possibilities. (i) the coronary might possess a cyclooxygenase but lack the isomerase that converts PGH_2 to PGE_2, thus in the absence of enzymatic generation of primary PG's, the dilator endoperoxides PGH_2 and PGG_2 may accumulate. Similarly, if PGE_2 were generated slowly at a very low concentration it would not exert enough of a contractile response physiologically to antagonize the endoperoxide-induced vasodilation. (ii) Another possibility is that the bovine coronary artery is producing a novel PG-like substance (from the arachidonate or the endoperoxide) which is highly potent but only present in low concentrations.

These observations have physiological and pathological implications. In investigations in open-chested dogs, intracoronary injection of arachidonate produced coronary dilation which was abolished by indomethacin (*12*), thereby indicating a correlate to our experiments. Potentially noxious stimuli, such as low oxygen tension, could result in the local biosynthesis of a PG-endoperoxide product which would enhance regional coronary blood flow, and thereby overcome the local cardiac oxygen and metabolic deficit. On the other hand, although we could not demonstrate thromboxane synthesis in the coronary artery, the potent vasoconstrictor TA_2 can be synthesized by circulating platelets during thrombosis and aggregation. Thus, coronary tone, and possibly spasms, in ischemic zones of the myocardium may be influenced markedly by interplay between thromboxanes from platelets on the one hand and endoperoxide intermediates synthesized endogenously on the other.

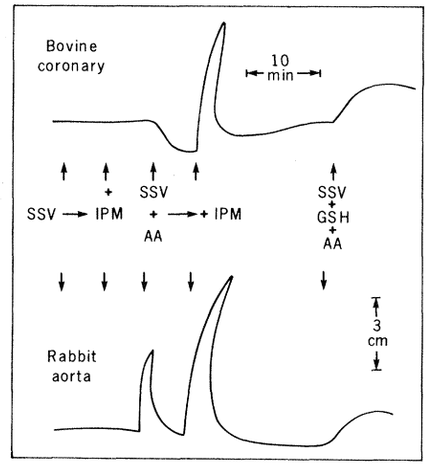

Fig. 3. Comparative contractile response to enzymatically generated PG endoperoxide, thromboxane A_2, and PGE_2 on superfused bovine coronary artery and rabbit aorta strips. The incubation of sheep seminal vesicle microsomes (*SSV*, source of cyclooxygenase) plus 500 ng of arachidonate (*AA*) in 50 m*M* phosphate buffer at *p*H 7.4 for 1 minute at room temperature quantitatively generates a mixture of PGH_2 and PGG_2 (*7, 8*) which relaxes the bovine coronary but contracts the aorta. If this mixture is then incubated with indomethacin platelet microsomes (*IPM*, the source of thromboxane synthetase) at 0°C for 2 minutes, then 25 to 30 percent of the endoperoxides are converted to the potent vasoconstrictor thromboxane A_2 (*7–9*). When arachidonate is incubated in the presence of reduced glutathione it is quantitatively converted to PGE_2 (*7*), which contracts both the bovine coronary and the rabbit aorta. Incubation of the enzymes (*SSV* and *IPM*) without substrate (*AA*) produced no response on the blood vessels.

We recently found that when PGH_2 is incubated with bovine coronary microsomes, the PGH_2 is completely metabolized (that is, there is a loss of rabbit aorta contraction) to a labile compound which is a much more potent coronary relaxant (*13*). We also found that a primary product generated by bovine coronaries incubated with ^{14}C-arachidonate, is 6-keto-$PGF_{1\alpha}$ (*13*). This end product was inactive as a coronary relaxant. Thus, the coronaries possess a major novel arachidonate metabolic pathway which generates a labile substance apparently intermediate between PGH_2 and the final 6-keto-$PGF_{1\alpha}$-like product, which is a potent coronary vasodilator. On the other hand, PGH_1 was not metabolized by bovine coronary microsomes, and thus exhibited no loss of rabbit aorta or coronary contraction. Formation of 6-keto-$PGF_{1\alpha}$ requires the presence of a double bond between C-5 and C-6 which is lacking in PGH_1 but not in PGH_2 or PGH_3. The major arachidonate end product produced by isolated perfused rabbit hearts was a 6-keto-$PGF_{1\alpha}$-like product, whereas a compound comparable to the novel prostaglandins is not produced from dihomo-γ-linolenic acid [C_{20} : 3 (n − 6), where n is the position of the first double bond], the fatty acid (precursor of PGH_1) which lacks the double bond between C-5 and C-6 (*14*). Thus, vascular resistance in the intact heart and in isolated coronary arteries appears to be largely determined by this major novel metabolic pathway.

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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-

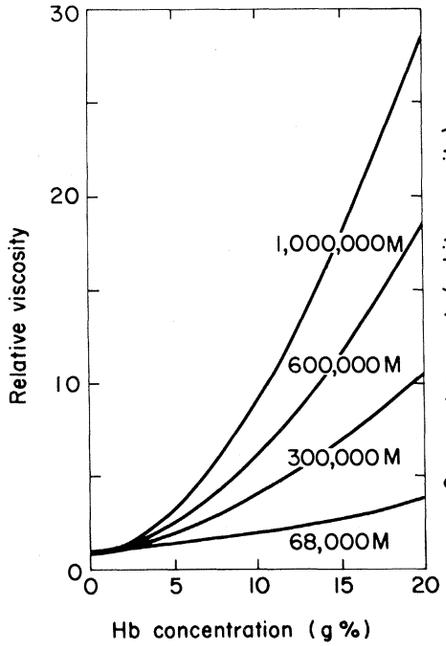

Fig. 1. Effects of hemoglobin concentration and molecular weight on the viscosities of hemoglobin solutions.

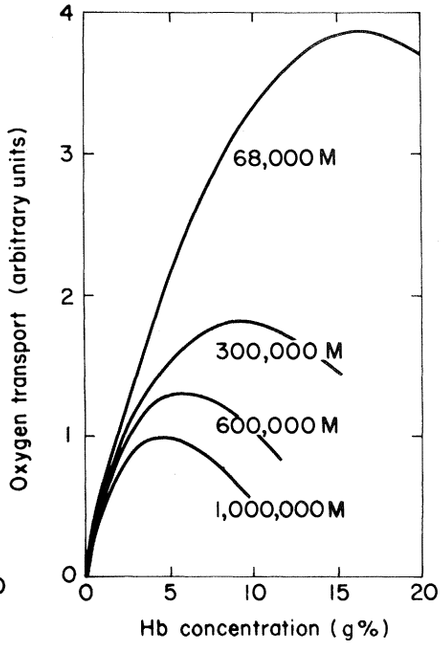

Fig. 2. Oxygen transport as a function of hemoglobin concentration and molecular weight.