collagenous protein. No correction is made here for the differential incorporation of proline into collagenous and noncollagenous protein [H. Green and B. Goldberg, *Nature* **200**, 1097 (1963)].
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Oxygen Affinity and Electrolyte Distribution of Human Blood: Changes Induced by Propranolol

Abstract. *Propranolol causes a massive leakage of potassium ions from red cells, which results in an alteration of the Gibbs-Donnan equilibrium across the red cell membrane. According to such a mechanism, the presence of propranolol significantly increases the hydrogen ion activity of the interior of the red cell, causing a decreased oxygen affinity of hemoglobin according to the classical Bohr effect. No release of 2,3-diphosphoglycerate which may be bound to the membrane is thus necessary to explain the effect of propranolol on the oxygen dissociation curve of blood.*

Pendleton *et al.* (*1*) showed that propranolol, added to washed human erythrocytes at a concentration of (1 to 5) $\times 10^{-4}M$, is able to shift the hemoglobin-oxygen dissociation curve. No effect of the drug was found for dialyzed hemoglobin solutions. Pendleton *et al.* could not determine the mechanism of action of propranolol, but suggested that it may be mediated by an action of the drug on the cell membrane.

The mechanism of action of propranolol has been further investigated by Oski *et al.* (*2*), who found that incubation of red cells with this drug produced no change in the total 2,3-diphosphoglycerate (DPG) content. According to Oski *et al.*, however, a certain fraction of 2,3-DPG in the red cell may usually be bound to the membrane and thus cannot interact with hemoglobin to affect its oxygen affinity. They suggested that propranolol might affect the oxygen affinity of blood by interacting with the erythrocyte membrane and releasing the bound 2,3-DPG in the interior of the red cell. Brann and Newman (*3*), however, could not demonstrate significant binding of 2,3-DPG to the red cell membrane.

It is relevant to ask whether the experiments reported by Pendleton *et al.* and Oski *et al.* were carried out, in the presence and in the absence of propranolol, at constant activity of all the ligands known to affect oxygen affinity in human blood. So far four allosteric ligands have been found to affect the oxygen affinity of hemoglobin solutions or whole blood; these are protons (*4*), carbon dioxide (*5*), organic phosphates [such as 2,3-DPG and adenosine triphosphate (*6*)], and the anions of various salts and acids (*7*). Intracellular *p*H was not measured in the experiments of Pendleton *et al.* nor those of Oski *et al.* They reported, moreover, that propranolol has a marked effect in decreasing the hematocrit value of red blood cell suspensions, which indicates substantial changes in the electrolyte distribution across the red cell membrane. Alterations in the distribution of sodium and potassium ions in red cells treated with propranolol have also been described and systematically investigated by Manninen (*8*).

We performed a series of experiments to determine whether the effect of propranolol on the hemoglobin-oxygen dissociation curve is mediated by changes in the distribution of electrolytes and hydrogen ions across the red cell membrane. Fresh human blood (about 400 ml) was collected under sterile conditions in bottles treated with heparin and was stored for no more than 8 hours at 2° to 4°C. During this time no significant changes in the distribution of 2,3-DPG, potassium, or sodium were found to occur. Values of P_{50} (the partial pressure of oxygen at which hemoglobin is 50 percent saturated) were obtained according to the technique of Brenna *et al.* (*9*).

Either sodium bicarbonate or hydrochloric acid was used to adjust the blood *p*H to the desired value. Hemolysis was routinely checked by measuring the hemoglobin content of the plasma. The sodium and potassium concentrations were determined by flame photometry both in the plasma and in total blood. All the measurements were made 30 minutes after the addition of propranolol. A mixture of *d*- and *l*-propranolol was added to the blood to give a final propranolol concentration of $5 \times 10^{-4}M$. Control experiments (without propranolol) were done by diluting the blood with an equivalent amount of saline.

Figure 1 shows the effect of propranolol on log P_{50} at various plasma *p*H values. The data agree with the results of Pendleton *et al.* (*1*) and Oski *et al.* (*2*) in showing that propranolol lowers the oxygen affinity of whole blood (at constant plasma *p*H). However, propranolol affects the oxygen affinity more at alkaline than at acid *p*H. The influence of *p*H on the effect of propranolol on oxygen affinity does

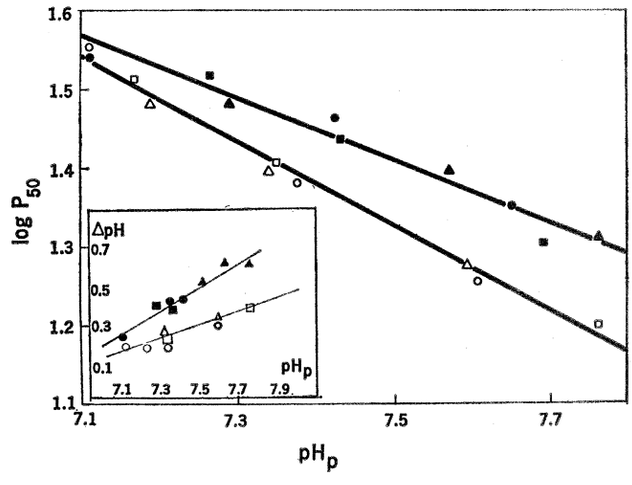

Fig. 1. Effect of propranolol on the oxygen affinity of human blood at 37°C. The different symbols refer to different subjects; (closed symbols) experiments with propranolol; (open symbols) experiments without propranolol; pH_p refers to plasma *p*H. The inset shows values of $pH_p - pH_c$ (the intracellular *p*H) as a function of pH_p.

not support a mechanism of action mediated by 2,3-DPG. In hemoglobin solutions, the effect of 2,3-DPG on the oxygen affinity at alkaline *p*H is considerably smaller than at acid *p*H, the association constant between 2,3-DPG and hemoglobin falling by a factor of 10 between *p*H 7.0 and *p*H 7.8 (*10*).

A second series of experiments was then carried out to measure the effect of propranolol on P_{50} at constant intracellular *p*H. Fresh human blood of normal 2,3-DPG content was equilibrated with a suitable partial pressure of oxygen in the presence and absence of propranolol, as described above. After equilibration the blood was collected and the plasma and red cells were separated anaerobically (in about 30 seconds) in an Eppendorf microcentrifuge (model 3200). The red cells were hemolyzed by freezing and thawing, and the hemolyzed solution was then reequilibrated with the same gas phase as before. Oxygen saturation and *p*H were measured on the hemolyzed red cell content.

The inset of Fig. 1 shows that propranolol produces (in 16 experiments with the plasma *p*H varying between 7.1 and 7.7) a significant increase of the difference in *p*H between the plasma and the interior of the red cell.

We also found in the blood of six normally healthy persons, in vitro, that the plasma potassium concentration increases from 4.01 meq/liter (standard error, ± 0.71) in the absence of propranolol to 26.19 ± 1.28 meq/liter in the presence of $5 \times 10^{-4} M$ propranolol.

These results agree with the data of Manninen (*8*) on washed human erythrocytes. As Glynn and Warner (*11*) pointed out, propranolol makes the cells more permeable to potassium than to chloride ions, so that the membrane potential (and therefore the *p*H gradient) is controlled by the concentration ratio of cell potassium to plasma potassium, K_c/K_p. Assuming that, when the blood is treated with propranolol, the plasma potassium concentration represents a true equilibrium value, we calculate that the potassium concentration in the red cells should be about 86 meq per liter of cell water. In these calculations we assume a cell water content of 60 percent, a hematocrit value of 40, and an original cell potassium concentration of 140 meq per liter of cell water. The *p*H gradient (ΔpH) must therefore equal log (H_c/H_p), which equals log (K_c/K_p) = log (86/26) = 0.51, which is in agreement with the value in the inset of Fig. 1 at *p*H 7.5. This ΔpH is much higher than the value for untreated cells, in which Cl_p/Cl_c, and therefore H_c/H_p, should be about 1.4 ($\Delta pH = 0.15$).

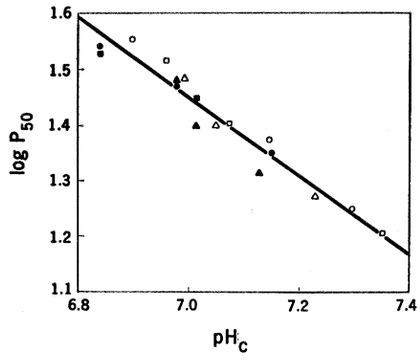

Fig. 2. Log P_{50} as a function of intracellular *p*H (pH_c) in the presence (closed symbols) or absence (open symbols) of propranolol.

Figure 2 shows values of log P_{50} in the absence and in the presence of propranolol as a function of the internal *p*H of the red cells. No significant differences beyond experimental error are now apparent.

Thus, although our experimental results do not bear directly on the debated problem of the effect of propranolol on the release of 2,3-DPG which may be bound to the red cell membrane, they show that the effect of propranolol on the hemoglobin-oxygen dissociation curve of whole blood is simply due to the classical Bohr effect, that is, to an increased acidity of the interior of the red cell.

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Crowding among Hunter-Gatherers: The !Kung Bushmen

Abstract. *Highly crowded living conditions exist among the !Kung Bushmen, hunter-gatherers who live on the edges of the Kalahari Desert in Botswana and South-West Africa. The !Kung appear to be crowded by choice, and biological indicators of stress are absent. Data indicate that residential crowding alone does not produce symptoms of pathological stress.*

Recent studies of crowding among nonhuman primates and other mammals suggest that various biological and social pathologies are associated with abnormally increased population density (*1*). The effects of density, however, vary among different species; while speculations are increasing in the popular literature about the deleterious effects of crowding on humans (*2*), in fact, the evidence for the effect of crowding is equivocal. An article by Galle *et al.* (*3*) reviewed the problem of interpreting the possible significance to man of density effects observed in other mammals and suggest refinements of the concept of density with reference to human (particularly urban) populations. The authors recommended specifying separate components of density (the number of persons per room, the number of rooms per housing unit, the number of housing units per structure, the number of residential structures per acre) instead of relying on an overall measure of population density in terms of numbers of people per unit area. The authors suggested that for certain pathologies the most important components of density are those related to "inter-