unexpected because they should have been removed during the preparation of myelinated axons. Thus, the origin of these dense profiles is still open to question. No axolemma or neurotubules are evident in either type of fiber. The absence of an axolemma may be an artifact of the preparation procedure for electron microscopy (*8*). Alternatively, this membrane may be lost through mechanical disruption, or it may separate with the myelin when the axons are suspended in the hypotonic medium. The absence of neurotubules is not unexpected since these are known to be labile structures and may depolymerize under the isolation conditions (*12*).

The axonal lipid content is 13.4 ± 1.3 percent of the dry weight and contains 60.0 ± 2.7 percent phospholipid, 20.1 ± 1.1 percent cholesterol and 20.1 ± 2.6 percent galactolipid. The galactolipid is composed of both cerebrosides and sulfatides in a molar ratio of approximately 2 to 1. The finding of significant amounts of cerebroside and sulfatide made it essential to ascertain that these lipids were not the result of myelin contamination since we calculated that 14 percent myelin contamination could account for all of the galactolipid.

Myelin fragments were seldom seen in the electron micrographs. The specific activity of the enzyme 2′,3′-cyclic nucleotide-3′-phosphohydrolase in the axon preparation was 0.1 unit per milligram of protein—less than 2 percent of the specific activity of 7.70 units per milligram of protein determined for bovine myelin. Disc-gel electrophoresis of 200 μg of total axonal protein indicated mostly proteins of high molecular weight with a prominent band corresponding to neurofilament protein (*13*). Electrophoresis of myelin proteins mixed with axonal proteins showed that we could detect a 2 percent contamination of myelin protein, but no specific myelin proteins were detected.

This indicates that myelin contamination of the axon fraction is negligible. We therefore conclude that these galactolipids are intrinsic axonal constituents, a finding consistent with previous reports showing an extramyelin compartment for these compounds (*4, 14*).

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Oxygen Affinity in Red Cells: Changes Induced in vivo by Propranolol

Abstract. *Propranolol, a blocking agent for the beta adrenergic receptor, produces a redistribution of 2,3-diphosphoglycerate in the red cell. At concentrations of 3.3 × 10⁻⁵M, 2,3-diphosphoglycerate in the red cell membrane becomes unbound in vitro. The administration of propranolol to humans produces similar changes and results in a decrease in the affinity of hemoglobin for oxygen.*

The concentration of 2,3-diphosphoglycerate (DPG) in the human erythrocyte plays a central role in regulating the affinity of hemoglobin for oxygen (*1*). As the concentration of DPG increases, the affinity of hemoglobin for oxygen decreases. In a wide variety of clinical disorders, the position of the oxygen-hemoglobin dissociation curve, as reflected in the P_{50} (the partial pressure of oxygen at which the hemoglobin is 50 percent saturated), bears a direct relation to the concentration of DPG in the red cell (*2*); however, exceptions to this rule have been described (*3*). Recently, Pendleton and his co-workers (*4*) observed that when propranolol, a blocking agent for the beta adrenergic receptor, was added to a suspension of intact human erythrocytes, it produced a shift to the right in the position of the oxygen-hemoglobin dissociation curve without producing any change in the concentration of DPG in the red cell. This effect was not observed when propranolol was added to either hemoglobin in solution or to suspensions of erythrocytes depleted of DPG. We have found that propranolol produces a release of DPG that is bound to the red cell membrane, both in vitro and in vivo, and that its administration to humans results in a decrease in the affinity of hemoglobin for oxygen.

Freshly drawn blood was obtained from healthy adult volunteers, was mixed with heparin, and was divided into portions to which was added varying concentrations of propranolol hydrochloride. The whole blood was then allowed to remain at room temperature for 10 minutes and a portion was re-

Table 1. The effects of propranolol ($3.3 \times 10^{-5}M$) in vitro on distribution of 2,3-diphosphoglycerate (DPG) in the red cell, and the effects of epinephrine ($3.3 \times 10^{-5}M$) on the interaction. Results are expressed as mean ± 1 S.D., for 20 experiments.

Incubation condition	DPG in red cell (μmole per gram of hemoglobin) in:		"Unbound" (%)
	Whole lysate	Stroma-free supernatant	
Whole blood	15.7 ± 1.4	10.9 ± 1.1	69.7 ± 4.6
Whole blood and propranolol	15.6 ± 1.2	15.8 ± 1.3	100 ± 0
Whole blood with propranolol and epinephrine	15.8 ± 1.3	10.0 ± 0.7	64.2 ± 5.3

moved for analysis of DPG and for determination of hemoglobin. The samples were then centrifuged for 10 minutes at 3000 rev/min in a PR-2 centrifuge at room temperature. The plasma was removed, and a hemolyzate was prepared by the addition of equal volumes of distilled, deionized water. After thorough mixing, the hemolyzate was allowed to stand for an additional 10 minutes, and another portion was removed for subsequent determination of DPG content. The lysate was then centrifuged at 3500 rev/min at room temperature for 10 minutes; the clear hemoglobin supernatant was removed from the lower layers that contained stroma. The supernatant was then passed through a capillary tube (0.5 by 15 cm) containing a Pyrex wool plug to remove all residual red cell stroma. The effluent was centrifuged for 15 minutes at 3500 rev/min, and the clear hemoglobin solution was again sampled for its content of both DPG and hemoglobin.

Propranolol was administered to nonsmoking human volunteers in a dosage schedule of 10 mg every 4 hours for a total dose of 40 mg. Blood samples were obtained before the drug was administered, 2 hours after, and 24 hours after the initial dose. The samples were studied for the measurement of P_{50}, the total content of DPG in red cells, and the fraction of bound and unbound DPG in the red cell. The determination of DPG and P_{50} were performed by techniques previously described (*5*).

It was found, in 20 studies in vitro, that 69.7 ± 4.9 percent of DPG in the red cell was present in the stroma-free hemoglobin ("unbound") (Table 1). In the presence of propranolol with a final concentration of $3.3 \times 10^{-5} M$, all of the DPG was recoverable in the stroma-free hemoglobin. Changes in the ratio of "bound" to "unbound" DPG were observable at propranolol concentrations of $1.0 \times 10^{-5} M$. Incubation of red cells with propranolol in the concentrations tested produced no change in the total content of DPG in the red cell. The prior addition of epinephrine in equimolar concentrations completely prevented the "unbinding" effect of propranolol.

The administration of propranolol to the human volunteers produced observable effects within 2 hours of its initial administration (Table 2). By 24 hours after the start of drug administration, virtually all of the DPG in the red cell was unbound and the P_{50} had risen by a mean of 2.5 mm-Hg. A shift to the right of the P_{50} curve was observed in blood from each individual. The P_{50} increases ranged from 1.5 to 4.0 mm-Hg.

Table 2. The effects of propranolol administration to humans on red cell P_{50} and the percentage of "unbound" 2,3-diphosphoglycerate in the red cell. Results are expressed as the mean of the determinations obtained from blood samples of seven nonsmoking adults.

Time (hr)	DPG in the red cell (μmole per gram of hemoglobin)	P_{50} (mm-Hg)	"Unbound" (%)
0	15.8	28.5	76.1
2	15.6	30.2	96.2
24	15.7	31.0	97.5

Propranolol has been reported to be actively incorporated into the human erythrocyte (*6*). Our findings suggest that it produces conformational changes in the red cell membrane, resulting in the release of bound organic phosphate. The presence of adenosine triphosphate that is bound to the red cell membrane has been demonstrated (*7*). It would appear that a certain fraction of DPG in the red cell may also be normally bound to the membrane and does not interact with hemoglobin to alter the hemoglobin affinity for oxygen. If approximately 30 percent of the normal concentration of DPG in the red cell (5.0 μmole per milliliter of red cells) is not normally interacting with hemoglobin, the red cell has a potential reserve of DPG that, when "unbound," could increase the P_{50} by approximately 3.0 mm-Hg (*2*). The observation that epinephrine prevents the effect of propranolol on the red cell membrane strengthens the argument that the red cell is responsive to vasoactive agents (*8*), and suggests that the red cell may contain adrenergic receptors. The efficacy of propranolol in relieving the symptoms of angina pectoris may be mediated, in part, by the ability of the drug to cause more oxygen to be delivered to the myocardium as a consequence of the altered hemoglobin-oxygen affinity. These data represent the first observations, in humans, of a pharmacologic agent that can be used to alter the position of the oxygen-hemoglobin curve for potential therapeutic benefit.

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Acetylcholine: Possible Neuromuscular Transmitter in Crustacea

Abstract. *The tonic flexor muscles of the crayfish abdomen respond with a large depolarizing potential to acetylcholine iontophoresed onto a neuromuscular junction, but not to glutamate. Excitatory junctional potentials are abolished by* d-*tubocurarine and enhanced by a cholinesterase inhibitor. The membrane is depolarized and the junctional potentials are desensitized by excess acetylcholine. Thus acetylcholine is thought to be the neuromuscular transmitter.*

Although the presence of acetylcholine (ACh) and cholinesterase has been reported in crustacean nerves and muscles (*1*), respectively, crustacean neuromuscular junctions have previously not been thought to be cholinergic (*2*, *3*). Instead, glutamate has been proposed as the excitatory transmitter at these junctions (*4*). I now present evidence that ACh may be the transmitter in at least one neuromuscular system of the crayfish.

The tonic postural flexor muscles in each hemisegment of the abdomen of the crayfish *Procambarus clarkii* are innervated by five excitatory neurons and one inhibitor (*5*). In most experiments the largest excitatory axon was selectively stimulated with trains of brief pulses delivered through a suction