Role of Water in the Stability of Oxyhemoglobin

Abstract. Electron exchange involving the valence states of iron occurs via water bridges. Molecular oxygen reversibly displaces the sole coordinated water of the ferrous iron in hemoglobin, and, in the absence of this ready path for electron transfer, the oxygen is transported without oxidation of the ferrous iron of hemoglobin.

The heme in hemoglobin combines reversibly with molecular oxygen without oxidation of the ferrous iron (*1*)

$$Fe_p^{II}(H_2O) + O_2 \rightleftharpoons Fe_p^{II}(O_2) + H_2O \quad (1)$$

while the ferrous iron of free heme is readily oxidized by oxygen. Because of the physiological importance of oxygen transport by hemoglobin, these reactions have attracted considerable attention, yet the causes of these differences in behavior have not yet been explained in an entirely satisfactory manner.

The hemes in hemoglobin are evidently buried within the protein molecule, and, as a consequence, the situation is complicated by the details of protein structure. Wang *et al.* (*2*) have suggested that the globin in hemoglobin partially screens the hemes with hydrophobic groups, thus tending to exclude water and altering the effective dielectric constant at the bonding sites. A hemoglobin model has been synthesized, and its properties have been studied in order to substantiate these views.

I have pointed out that processes in solution involving iron(III), and, by implication, iron(II), may be more dependent on specific solvation effects than on changes in the macroscopic dielectric constant (*3*). Furthermore, explanations which invoke the notion of the microscopic dielectric constant are inherently ambiguous since this concept is not amenable to direct experimental investigation.

Studies of the kinetics of the iron (II)-iron(III) electron-exchange reaction (*4*), especially in mixed solvent media (*5*), have yielded one conclusion relevant to the problem of the stability of oxyhemoglobin: that the 9- to 10-kcal/mole iron(II)-iron(III) oxidation-reduction process involves transfer of a single electron through waters of solvation. Electron transfer by alternative mechanisms is less favored energetically. This conclusion is supported by the observations that the iron(II)-iron(III) exchange does not occur in the absence of water (*5*), that the specific reaction rate constant goes through a maximum with increasing concentration of catalyzing, strongly-complexing anions (*6, 7*), that there is a pronounced deuterium effect (*8*), and that a cyanide ligand must be replaced by water in $Fe(CN)_6^{-4}$ before that species can be oxidized by hydroperoxides (*9*). Also, work on the iron(II)-iron(III) exchange, especially that of Hudis and Wahl (*6*) together with work on a multitude of other electron-exchange reactions, has clearly shown the absence of any systematic reactant charge-product dependency, and thus, presumably, the comparative unimportance of dielectric constant effects.

Now, in hemoglobin, four of the six coordination positions in iron(II) are occupied with the near-planar porphyrin system, one is occupied by the globin, and the sixth only is occupied by a water molecule (*1, 10*). This single water molecule, on the basis of the above arguments, must play a crucial role in oxidation processes, and when it is displaced, even by oxygen, no path will remain for the ready oxidation of iron(II). Oxyhemoglobin, therefore, should be quite resistant to oxidation. Any ligand, such as CO, F^-, CN^-, or even alcohols [assuming behavior similar to that of iron(III) (*3*)] which can displace the water of solvation of iron(II) should impair oxygen transport by hemoglobin and produce unfortunate physiological effects. In the instance of free heme, on the other hand, with presumably two waters of solvation, the situation is less critical, and even when one of the water molecules is replaced by oxygen or some ligand the remaining water will still provide a path for electron transfer.

Oxygenated heme ("oxyheme"), if formed, would be unstable in aqueous solution and would be oxidized immediately to the ferric state, just as certain synthetic, reversible oxygen-addition complexes of iron(II) that have been reported (*11*) are unstable in the presence of water.

$Mo(CN)_8^{-3}$ and $IrCl_6^{-2}$ oxidize hemoglobin and its derivatives (*12*). Possibly the species $Mo(H_2O)(CN)_7^{-2}$ and $Ir(H_2O)Cl_5^-$, carrying their own water bridges, are responsible for the oxidation. Dissociation of the derivative, as in the case of the oxidation of carbonmonoxyhemoglobin by $Fe(CN)_6^{-3}$, or displacement of the ligand of iron(II) by the anionic oxidant, may be a preliminary step. It is significant to notice that cationic oxidizing agents, such as $Fe(phenanthroline)_3^{+3}$ and $Ru(dipyridyl)_3^{+3}$, of equal or even greater oxidizing power, are ineffective (*12*), although size rather than coulombic repulsion may be critical in these cases.

Consideration of the role of water, together with specific protein influences, may well provide an explanation for the behavior of hemoglobin and related compounds without recourse to speculation concerning the microscopic dielectric constant at reaction sites.

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Inheritance of Diego Blood Group in Mexican Indians

Abstract. Diego blood factor is characteristic of Mongoloid populations. A study of 152 samples from Mexican Indians (Tlaxcaltecans) revealed this factor in 20.39 percent of the cases. Analysis of 30 Indian matings with 62 children support the hypothesis that the Diego antigen is transmitted through a gene capable of expressing itself in a single or double dose.

The studies of the Diego blood factor (Di^a) were begun in 1954, when Levine, Koch, McGee, and Hill (*1*) demonstrated its presence in the serum of a Venezuelan mother whose baby had hemolytic disease. This antibody, besides having pathological importance, has proved to be of considerable anthropological interest, for it exists only exceptionally in the blood of the Caucasoid (*2*) and has not been found in Negroes (*3*), in Australian aborigines, or in Polynesians (*4*), while it has been detected in Mongoloid bloods in percentages ranging from 5 (in Chinese from Canton) (*5*) to 45.8 (in Kainganges Indians, in Brazil). According to our studies of a sample of 152 bloods taken from not-closely-related Tlaxcaltecan Indians with some slight Spanish mix-

Table 1. Inheritance of Diego blood factor (Exp., expected; Obs., observed).

Matings (Obs.)	Children			
	Di(a+)		Di(a–)	
	Exp.	Obs.	Exp.	Obs.
D × D (0)	0	0	0	0
D × d (11)	11.1	8	9.9	13
d × d (19)	0	0	41	41

ture, the character Di^a (+) is present in 20.39 percent.

Inheritance of the Di^a (+) factor was studied in four families by Layrisse, Arenas, and Dominguez Sisco in 1955 (*6*), and later, in 1957, Lewis, Kaita, and Chown (*7*) gave a large pedigree formed by 50 Japanese families. The present study deals with the testing with anti-Di^a serum of 30 Indian matings with 62 children. The results are shown in Table 1 (*8*).

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Experimental Infestation of Peromyscus leucopus with Larvae of Cuterebra angustifrons

Abstract. Four newly hatched larvae of the warble fly, *Cuterebra angustifrons*, were successfully introduced into the mouse host, *Peromyscus leucopus*, by application to the belly skin, the mouth, and the nose. Regardless of the site of entry, each larva migrated to the inguinal region of the host and completed its development normally.

Cuterebrid flies are widely distributed in North and South America. Their larvae are found encapsulated in the subcutaneous tissues of many rodents (chipmunks, deer mice, pack rats, and so on), rabbits, and occasionally dogs, cats, and other mammals. Larvae of two species of the genus *Cuterebra* (*C. angustifrons* and *C. grisea*) have been found infesting the common deer mouse of wood lots in southern Ontario, *Peromyscus leucopus noveboracensis* (*1, 2*). Maturing larvae are usually observed beneath the skin of the inguinal region but have been seen elsewhere on the body of the hosts. After gaining entry to the host, larvae wander beneath the skin for the first 7 to 8 days and are not visible externally during this period. They then make a small hole in the skin, to which they apply their posterior ends for respiration and excretion. At this time they are in the second instar (recognizable by the characteristic stigmal plate pattern) and may remain in this stage for up to 8 days. After molting to the third instar (recognizable by the change in the stigmal plate pattern) they begin the final phase of larval development, which lasts an average of 12 to 13 days but may vary from 8 to 17 days. When mature, the third instar larva drops from its host, burrows into the ground, and pupates. The time for total development of the larva in its host varies from 19 to 44 days, but probably averages between 25 and 30 days (*2*).

Numerous adult flies have been reared over two winters, but attempts to mate flies in the laboratory have been unsuccessful to date (*2, 3*). Adult flies were not seen in four summers of observation in wood lots from which infested deer mice were trapped. No eggs were seen on the hair or skin of some 1000 deer mice, chipmunks, and other small mammals live-trapped during four summers (*3*). Beamer *et al.* (*4*) found eggs of *Cuterebra beameri* affixed to brush in and around the entrances to burrows of the pack-rat host. Presumably, eggs of cuterebrids are not laid on the bodies of their mammalian hosts. It has been demonstrated that larvae of *C. tenebrosa* may enter the host by mouth (*5*) or by penetration through the skin (*6*); those of *C. angustifrons*, by nose (*7*).

We were fortunate in capturing a female *C. angustifrons* in a cabin at Sparrow Lake, Ontario, 5 Sept. 1958. Since obtaining fertile females for experimental infestations is difficult, in that these flies have not as yet been mated in captivity and fertile females are only rarely taken in the field, we think the following observations worth reporting.

The fly began to oviposit within a few minutes on moistened tissue paper in a glass tumbler and laid a total of 86 eggs within 24 hours. She died 24 hours later. The eggs were placed in a humidor above a saturated solution of sodium chloride (relative humidity approximately 83 percent). Several eggs were examined on the sixth and seventh days after oviposition. Fully formed larvae were found in five eggs; three others showed no development and may have been infertile. One of the larvae, when exposed by removal of the operculum, exhibited feeble movements but died without escaping from the egg.

On the 12th day after oviposition, a larva was seen to have hatched. A closer check revealed three more larvae, one of which was observed in the act of pushing off the operculum and escaping from the egg. Two other larvae had hatched but died before they were found. An examination of the remaining eggs showed that many contained fully formed embryos, but all of these appeared to be dead.

Laboratory-raised *Peromyscus* juveniles were used as hosts for available larvae. Mouse No. 441-48 was prepared by clipping its belly hair and lightly anesthetizing it with ether. A larva was transferred on the moistened tip of a dissecting needle and placed on the dry clipped area. It seemed to be unable to navigate and struggled helplessly among the short clipped hairs. Moistening the hair with water seemed to help, but it still made no attempt to penetrate the skin. A slight abrasion was made in the belly skin. The larva was placed close to but not in the wound. However, fluid from the wound bathed it and it began to burrow into the unbroken skin. When about half buried it remained inactive for a minute or more, and during this time the mouse moved out of the field of the dissecting microscope. When it was again brought into view, the larva was no longer seen. It was not certain at this time whether the larva had been dislodged or had gained entry to the host.

A second larva was placed between the nostrils of mouse No. 431-58. It appeared to be quite active on the moist tip of the nose and moved quickly toward one of the nostrils. The mouse moved out of the field of observation, and the larva had disappeared from view when observations were resumed.

The third larva was placed in the mouth against the lip of mouse No. 432-58. It seemed to be stimulated by the moist environment and was observed to move out of sight along the side of the tongue towards the back of the throat.

With the last larva available, a second attempt at nasal transplant was made. The larva was seen to disappear actively down the nasal passage, but the mouse failed to revive from the anesthetic, even though oxygen was applied. About 20 minutes after the attempt, the dead mouse was necropsied, and the larva was found embedded in the tissue of the nasal septum about ¼ in. from the naris. It was motionless and appeared to have a gas bubble in its alimentary tract. After being removed from the nasal tissues, the larva revived somewhat and was transferred to the tongue of mouse No. 435-58. In this case no anesthetic was used, and the mouse appeared to swallow the slowly moving larva.

Larvae from the first three transfers reappeared in the inguinal region 8 days after entry via skin, nostril, and mouth; in the fourth instance, 12 days elapsed before the larva reappeared. In the first three cases, the molt from second to third instar was observed on the 11th day after experimental infestation; in the fourth case, the molt occurred on the 15th day. There was no significant difference in the length of the third instar —13 days for the first three larvae, 14 days for the fourth. Three of the four larvae dropped on the 24th day, the fourth on the 29th day, after infestation. Three of the four successfully pupated,