other properties is necesary to provide a more detailed picture of the liquid. Measurements of the viscosity and thermal conductivity at very low temperatures would provide useful information. The predicted unusual behavior of sound propagation in the liquid at very low temperatures is of particular interest, and measurements of high-frequency sound propagation and attenuation at extremely low temperatures would be of great value, together with possible experiments on scattering of light. The fact that many of the critical experiments involve measurements at temperatures reached only by magnetic cooling provides a technical challenge which is being taken up today in many institutions specializing in low-temperature physics.

References and Notes

1. L. W. Alvarez and R. Cornog, *Phys. Rev.* **56**, 379, 613 (1939).
2. S. G. Sydoriak, E. R. Grilly, E. F. Hammel, *ibid.* **75**, 303 (1949).
3. J. G. Daunt, *Advances in Phys.* **1**, 209 (1952).
4. See for example: J. Wilks, *Nuovo cimento* **10**, suppl., 509 (1953); B. M. Abraham, D. W. Osborne, B. Weinstock, *Science* **117**, 121 (1953); E. F. Hammel, in *Progress in Low-Temperature Physics*, C. J. Gorter, Ed. (North Holland, Amsterdam, 1955), p. 78; R. A. Chentsov, *Uspekhi Fiz. Nauk* **55**, 49 (1955); J. G. Daunt, Ed., *Symposium on Liquid and Solid He*^{3} (Ohio State Univ. Press, Columbus, 1957); V. P. Peshkov and K. N. Zinov'eva, *Repts. Progr. Phys.* **22**, 504 (1959).
5. F. London, *Superfluids* (Wiley, New York, 1954), vol. 2.
6. A. A. Abrikosov and I. M. Khalatnikov, *Repts. Progr. Phys.* **22**, 329 (1959).
7. K. R. Atkins, *Liquid Helium* (Cambridge Univ. Press, New York, 1959).
8. H. L. Anderson, *Phys. Rev.* **76**, 1460 (1949); H. L. Anderson and A. Novick, *ibid.* **73**, 919 (1948).
9. F. London, *Nature* **163**, 694 (1949); see also E. Pollard and W. L. Davidson, *Applied Nuclear Physics* (Wiley, New York, 1942), p. 183; J. Franck, *Phys. Rev.* **70**, 561 (1946).
10. J. de Boer, *Physica* **14**, 139 (1948); ———, *Repts. Progr. Phys.* **12**, 305 (1949); ———, and B. S. Blaisse, *Physica* **14**, 149 (1948); J. de Boer and R. J. Lunbeck, *ibid.* **14**, 318, 510, 520 (1948).
11. W. H. Keesom, *Helium* (Elsevier, Amsterdam, 1942).
12. J. G. Daunt and R. S. Smith, *Revs. Modern Phys.* **26**, 172 (1954); K. Mendelssohn in *Handbuch der Physik* (Springer, Berlin, 1956), vol. 25, p. 370.
13. L. N. Cooper, R. L. Mills, A. M. Sessler, *Phys. Rev.* **114**, 1377 (1959). See also R. L. Mills, A. M. Sessler, S. A. Moszkowski, D. G. Shankland, *Phys. Rev. Letters* **3**, 381 (1959).
14. L. Landau, *J. Phys. U.S.S.R.* **5**, 71 (1941); *ibid.* **8**, 1 (1944).
15. R. P. Feynman, *Phys. Rev.* **90**, 116 (1953); *ibid.* **91**, 1291, 1301 (1953); *ibid.* **94**, 262 (1954); ———, in *Progress in Low-Temperature Physics*, C. J. Gorter, Ed. (North Holland, Amsterdam, 1955).
16. H. C. Kramers, J. D. Wasscher, C. J. Gorter, *Physica* **18**, 329 (1952).
17. M. Cohen and R. P. Feynman, *Phys. Rev.* **101**, 13 (1957).
18. Palevsky, Otnes, Larsson, Pauli, Stedman, *ibid.* **108**, 1346, (1957); Palevsky, Otnes, Larson, *ibid.* **112**, 11 (1958).
19. Yarnell, Arnold, Bendt, Kerr, *Phys. Rev. Letters* **1**, 9 (1958); *Phys. Rev.* **113**, 1379 (1959).
20. D. G. Henshaw, *Phys. Rev. Letters* **1**, 127 (1958).
21. P. L. Kapitza, *Nature* **141**, 74 (1938).
22. J. F. Allen and A. D. Misener, *ibid.* **141**, 75 (1938).
23. L. D. P. King and L. Goldstein, *Phys. Rev.* **75**, 1366 (1949).
24. G. de Vries and J. G. Daunt, *ibid.* **92**, 1572 (1953); *ibid.* **93**, 631 (1954).
25. T. R. Roberts and S. G. Sydoriak, *ibid.* **93**, 1418 (1954); *ibid.* **98**, 1672 (1955).
26. D. W. Osborne, B. M. Abraham, B. Weinstock, *ibid.* **94**, 202 (1954); B. M. Abraham, D. W. Osborne, B. Weinstock, *ibid.* **98**, 551 (1958).
27. D. W. Osborne, B. Weinstock, B. M. Abraham, *ibid.* **75**, 988 (1949).
28. J. G. Daunt and C. V. Heer, *ibid.* **79**, 46 (1950). For more recent evaluations of the lambda temperature as a function of He3 composition, see, for example, T. R. Roberts and S. G. Sydoriak, *Low Temperature Physics and Chemistry* (Univ. of Wisconsin Press, Madison, 1958), p. 170; S. D. Elliott, Jr., and H. A. Fairbank, *ibid.* p. 180.
29. D. F. Brewer, J. G. Daunt, and A. K. Sreedhar [*Phys. Rev.* **115**, 836 (1959)] give references to and discussions of this question.
30. D. F. Brewer, A. K. Sreedhar, H. C. Kramers, J. G. Daunt, *Phys. Rev.* **110**, 282 (1958).
31. K. A. Brueckner and J. L. Gammel, in *Symposium on Liquid and Solid He*^{3} (Ohio State Univ. Press, Columbus, 1957), p. 186; *Phys. Rev.* **109**, 1040 (1958).
32. L. Landau, *Zhur, Eksptl. i Teoret. Fiz.* **30**, 1058 (1956) [translation, *Soviet Phys. JETP* **3**, 920 (1957)]; *Zhur. Eksptl. i Teoret. Fiz.* **32**, 59 (1957) [translation, *Soviet Phys. JETP* **5**, 101 (1957)]; *Zhur Eksptl. i Teoret Fiz.* **35**, 97 (1958) [translation, *Soviet Phys. JETP* **35**, 70 (1959)].
33. A. A. Abrikosov and I. M. Khalatnikov, *Zhur. Eksptl. i Teoret. Fiz.* **32**, 915 (1957) [translation, *Soviet Phys. JETP* **5**, 745 (1957)].
34. W. M. Fairbank, W. B. Ard, H. G. Dehmelt, W. Gordy, S. R. Williams, *Phys. Rev.* **92**, 208 (1953); W. M. Fairbank, W. B. Ard, G. K. Walters, *ibid.* **95**, 566 (1954); G. K. Walters and W. M. Fairbank, *ibid.* **103**, 263 (1956); W. M. Fairbank and G. K. Walters, in *Symposium on Liquid and Solid He*^{3} (Ohio State Univ. Press, Columbus, 1957), p. 205.
35. A. A. Abrikosov and I. M. Khalatnikov, *Zhur. Eksptl. i Teoret. Fiz.* **32**, 1084 (1957) [translation, *Soviet Phys. JETP* **5**, 887 (1957)].
36. Similar conclusions were arrived at earlier also by R. A. Buckingham and H. N. V. Temperley [*Phys. Rev.* **78**, 482 (1950)] and by I. Pomeranchuk, [*Zhur. Eksptl. i Teoret. Fiz.* **20**, 919 (1950)].
37. K. N. Zinov'eva, *Zhur. Eksptl. i Teoret. Fiz.* **34**, 609 (1958) [translation, *Soviet Phys. JETP* **7**, 421 (1958)].
38. H. A. Fairbank and D. M. Lee, in *Symposium on Liquid and Solid He*^{3} (Ohio State Univ. Press, Columbus, 1957), p. 26.
39. A. A. Abrikosov and I. M. Khalatnikov, *Zhur. Eksptl. i Teoret. Fiz.* **33**, 110 (1957) [translation, *Soviet Phys. JETP* **6**, 84 (1958)].
40. H. L. Laquer, S. G. Sydoriak, T. R. Roberts, *Phys. Rev.* **113**, 417 (1959).
41. K. R. Atkins and H. Flicker, *ibid.* **113**, 959 (1959).
42. A. A. Abrikosov and I. M. Khalatnikov, *Zhur. Eksptl. i Teoret Fiz.* **34**, 198 (1958) [translation, *Soviet Phys. JETP* **7**, 135 (1958)].
43. D. F. Brewer and J. G. Daunt, *Phys. Rev.* **115**, 843 (1959).
44. L. Goldstein, *Ann. phys.* **8**, 390 (1959).

Oxygen Transport through Hemoglobin Solutions

How does the presence of hemoglobin in a wet membrane mediate an eightfold increase in oxygen passage?

P. F. Scholander

Evolution from single cells to organisms is linked intimately with the development of a circulatory system. Without this, both size and activity would be severely limited by the slowness of diffusion. But even with a circulatory system, oxygen transport would be hampered by still another "unfitness of the environment"—namely, the very low solubility of oxygen in water. This difficulty was overcome by the evolution of oxygen-carrying pigments, which when circulated to the tissues could carry many times more oxygen than can water alone.

Oxygen-carrying pigments appeared not only in blood, however, but also in a vast area where visible means for transport of the pigment is lacking—namely, as myoglobin in the muscle system. Here it is found within the muscle cells, providing, so to speak, the last leg of the supply line to the oxygen-needy contractile machinery. But how could this myoglobin enhance oxygen transport unless it were circulated within the cell? Simple diffusion could hardly be aided by the pigment. It is true enough that the increased oxygen capacity could help to smooth out a fluctuating demand, as demonstrated by Millikan (*1*), but could it possibly also be that the pigment might

The author is professor of physiology at Scripps Institution of Oceanography, University of California, La Jolla.

serve as a specific conveyor belt for oxygen, enhancing its rate of delivery?

If we turn from a normally aerated environment to habitats of low oxygen tension we find that many animals develop high concentrations of oxygen-carrying pigments. One may mention mud-dwelling worms or insect larvae (chironomids), certain crustaceans in stagnant pools (*Daphnia*), intestinal nematodes and maggots (*Ascaris* and *Gastrophilus*), and many mammals exposed naturally or experimentally to high altitudes. And again we are drawn to the fundamental question: Is it possible that an oxygen-carrying pigment can enhance oxygen transfer through a stationary solution?

A great deal of penetrating experimental and theoretical work has been done to elucidate the kinetics of situations where oxygen, carbon monoxide, and other gases load and unload hemoglobin solutions (*2*). Unfortunately, however, this approach is not so suitable if one wishes specifically to study what happens in a pure steady-state situation, since the capacity factor can easily obscure the steady-state events.

The primary aim of the investigation under discussion has been to find out what happens when air diffuses through human hemoglobin solutions. Complications from oxygen capacity or storage have been eliminated by adhering strictly to steady-state conditions. It is shown below that the rate of oxygen transport may indeed be enhanced by the presence of hemoglobin, and a few preliminary experiments on myoglobin and other pigments indicate that the phenomenon may have wider applications (*3*).

Methods

Principle. Moist air at various pressures is diffused through a Millipore membrane charged with a hemoglobin solution; on the other side of the membrane a moist vacuum is maintained. When a steady state is reached, the amounts of oxygen and nitrogen gas coming through the membrane in a measured time interval are analyzed. With the nitrogen values as a base line, the oxygen transport can be calculated.

Instrumentation. The diffusion apparatus (Fig. 1) consists of a diffusion chamber which may be divided into two compartments by a wet Millipore filter. The membrane rests on a stainless-steel grid. The upper compartment is connected to a vacuum gauge and a glass-stoppered vent tube (*D*); the lower compartment, to the vacuum chamber by means of a taper joint. Each compartment is kept moist by a piece of wet filter paper.

The vacuum chamber is a 300-ml mercury reservoir connected through a gas trap and stopcock to the leveling bulb. The chamber has one wide-bore (5-mm) stopcock (*A*) to the diffusion chamber and a regular one of narrower bore (*B*) to the vacuum line. The upper end of the vacuum chamber terminates in a 1-mm-bore capillary and cup which can be closed under mercury by inserting into it a polyethylene-tipped wire plug. The capillary carries a movable millimeter scale to measure the total amount of gas.

Gas is transferred to the micro gas analyzer by means of a micro syringe. This is made from a glass tube of 2 mm inside diameter fitted with a fine polyethylene tip and a wire plunger. The latter is made airtight by means of a piece of polyethylene tubing which is flared at the end by heating slightly. This micro syringe is filled with mercury, and the gas is always protected by mercury during handling. From this pipette the gas is introduced into the micro analyzer (*4*).

The Millipore membrane (HA grade) holding the hemoglobin solution has a porosity fine enough so that air pressure of 1 atm will not break the capillarity. This filter, which is 0.15 mm thick, has about 80 percent space for the liquid.

Procedure. The basic data were obtained from heparinized human blood. The cells were washed twice in 0.9 percent saline and hemolyzed by repeated freezing and thawing. The oxygen capacity of the hemoglobin solutions was determined by the syringe method (*5*).

To saturate the dry Millipore membrane with the hemolyzed cell solution, it is placed on top of the solution until it is soaked through, whereupon it is submerged. It is then blotted on both sides and placed on the grid of the lower half of the diffusion chamber, which is kept moist by a ring of wet filter paper. The upper half, also carrying a wet filter paper, is screwed vacuum-tight onto the lower half with the wet membrane in between. A wide rubber band prevents evaporation from the crack. The chamber is put on the taper joint of the vacuum chamber, which is stoppered by the plug, and evacuated through *B*. *B* is closed, *A* is opened momentarily, and the chamber is again evacuated through *B*. This process is repeated twice, and the air pressure on the membrane is adjusted to the desired level through screw clamp *C* and vent *D*.

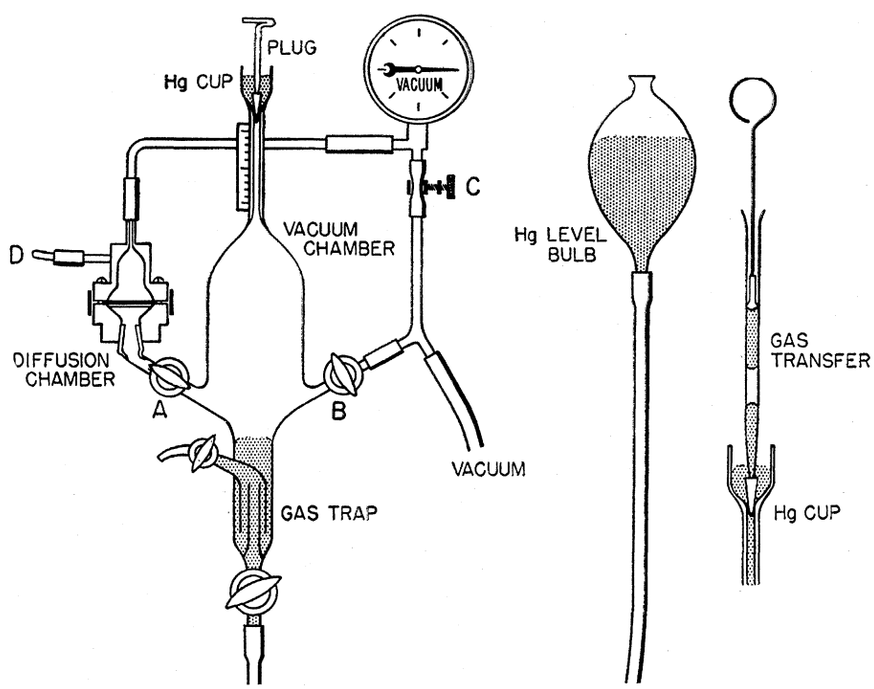

Fig. 1. Apparatus for steady-state diffusion.

Diffusion of gases now proceeds from the upper air compartment through the membrane and into the wide-bore channel to the vacuum chamber. The time of diffusion is clocked by means of a stop watch, and the amount of gas which has moved through the membrane is periodically checked by closing off the diffusion chamber at stopcock *A*, letting the mercury rise, and measuring the volume of the gas bubble in the capillary. Twenty cubic millimeters of gas suffice for an accurate determination of the quantity as well as the composition.

The gas transfer is executed with the gas pressure in the capillary slightly below atmospheric pressure. The plug is removed and quickly replaced by the mercury-filled transfer pipette. The gas is drawn up into this and is followed by mercury, whereupon the pipette is removed and replaced by the plug. The gas sample is now transferred to the cup of the analyzer containing alkaline citrate, and the oxygen and nitrogen are determined by absorption in the conventional way (*4*).

Successive samples are analyzed until the O_2/N_2 ratio becomes constant—that is, until the oxygen content of the filter is constant. Under the least favorable conditions (high pressure and high hemoglobin concentration) the membrane contains initially, at most, some 14 to 20 mm^3 of oxygen, but diffusion then proceeds so quickly that some 6 to 8 times this amount goes through before final sampling. At low pressures the membrane holds less than 1/20 of the amount analyzed.

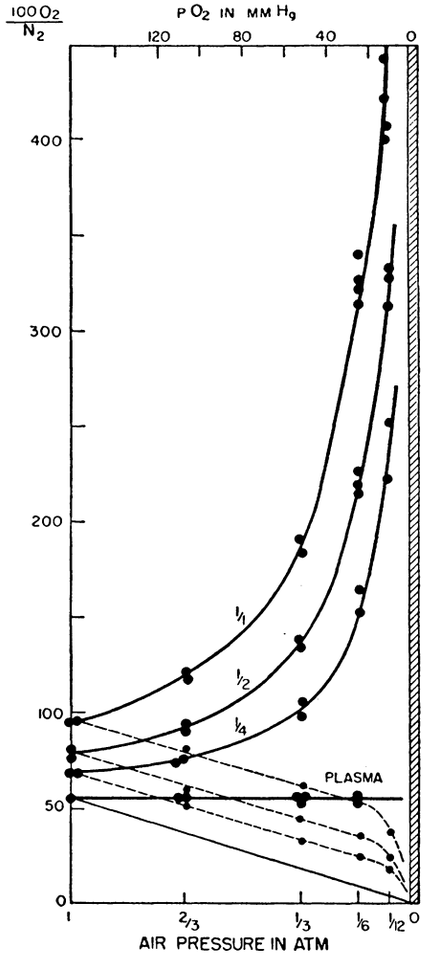

Fig. 2. Diffusion of air through hemoglobin solutions of three concentrations. Curve 1/1 represents oxygen capacity of 22.9 volumes percent; the other curves represent oxygen capacity of ½ and ¼ this amount, respectively. Horizontal line, O_2/N_2 ratio through plasma; lower diagonal line, rate of oxygen diffusion through plasma; three dashed parallel lines, relative rates of oxygen transport calculated from the corresponding O_2/N_2 data; shaded area, water-vapor tension.

Results

Diffusion of air through a membrane charged with water. The rate of simple gas diffusion through a membrane is proportional to the pressure difference and solubility of the gas and is inversely proportional to the square root of the molecular weight. When air diffuses through a layer of water, the steady-state ratio of oxygen to nitrogen should accordingly be 49.0 percent at 24°C and 48.7 percent at 26°C. In actual fact, our figures for air diffusing through water held in a Millipore filter or a dialyzing membrane come out as 56 ± 1 percent at all pressures. The reason for this quite substantial deviation from the theoretical value is not known, but it may be that a slight diffusion through the membrane substance itself and its 30 percent or so of bound water would favor the oxygen. The oxygen-nitrogen ratio was unchanged by charging the filter with a lampblack suspension or by using the VF grade Millipore filter; both of these procedures give a higher filter-to-water ratio. The same figure was also obtained by diffusing air through blood plasma and methemoglobin solutions (see Figs. 2–4).

In all cases, the total (and partial) amount of the gases diffusing through was proportional to the pressure (Fig. 5).

Oxygen transport through hemoglobin solutions. Typical curves for oxygen transport through hemoglobin solutions are given in Fig. 2. The basic curve (1/1) was obtained from a hemoglobin solution with an oxygen capacity of 22.9 volumes percent. It will be seen that the O_2/N_2 ratio at air pressure of 1 atm is 95 percent instead of 56 percent as it is in water or plasma. The ratio increases rapidly with lowering of the air pressure, reaching over 400 percent at 1/12 atm. But when the rate of nitrogen diffusion is calculated from

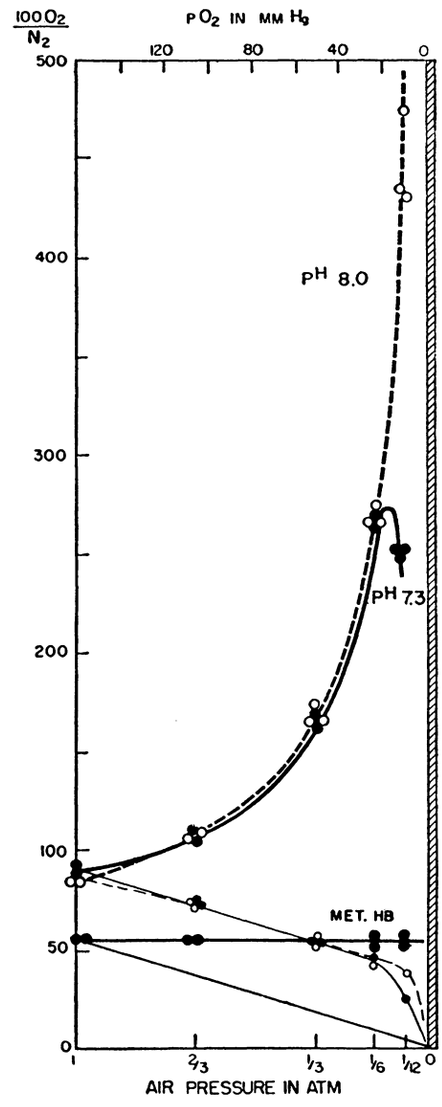

Fig. 3. Diffusion of air through two hemoglobin solutions of the same concentrations (O_2 capacity, 15.8 volumes percent) but with different *p*H's. Horizontal line, O_2/N_2 ratio of diffusion through a methemoglobin solution; lower diagonal line, rate of oxygen diffusion through water; two parallel curves, relative rates of oxygen transport calculated from the O_2/N_2 values; shaded area, water-vapor tension.

the total gas volume and the O_2/N_2 ratio, we find that it is always proportional to the pressure (Fig. 5). Two things follow: (i) the increased O_2/N_2 ratio is exclusively caused by an increased rate of oxygen transport, and (ii) the oxygen transport is proportional to the product of O_2/N_2 and Δp, where Δp is the diffusion pressure.

This enables us to calculate the relative rate curves for oxygen transport in Fig. 2. It will be seen that the transport rates through hemoglobin in all three dilutions are parallel to the diffusion rate through water—that is, numerically the oxygen transport is the result of two additive processes, the one, plain diffusion through the water, the other, a specific transport mediated by the hemoglobin. The latter rate remains constant over a large gradient of oxygen pressures. This simple relation holds quite precisely for all cases investigated, with various concentrations of hemoglobin or diffusing through intact red cells (Figs. 2–4).

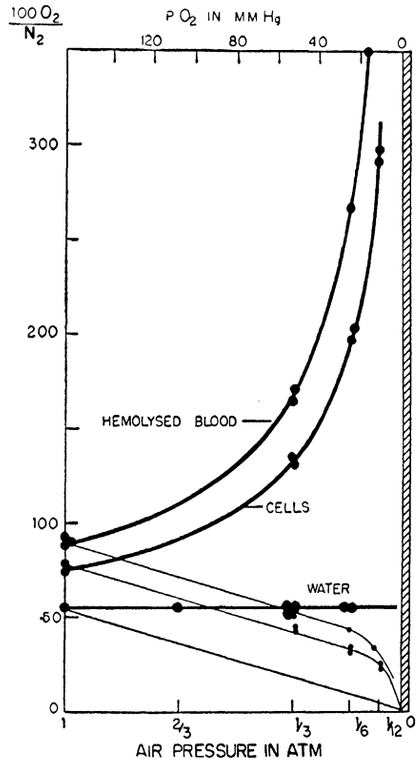

Fig. 4. Diffusion of air through (i) whole blood (O_2 capacity, 21 volumes percent) hemolyzed by HA membrane, and (ii) washed cells smeared on the under side of a dry VF Millipore filter. Horizontal line, O_2/N_2 ratio through water; lower diagonal curve, rate of oxygen diffusion through water; parallel curves above this, relative rates of oxygen transport calculated from the O_2/N_2 data; shaded area, water-vapor tension.

Figure 3 shows the effect of *p*H on the same hemoglobin solution. The only significant difference appears at the very lowest pressure—namely, 1/12 atm where the transport drops off in the acid sample but keeps on undiminished in the alkaline sample.

As would be expected, the hemoglobin concentration has a marked effect on the oxygen transport. In Fig. 2 the decrease in specific transport was proportionately less than the dilution of the hemoglobin. At half strength the rate of transport was reduced to 62 percent and at quarter strength, to 39 percent. When the concentration was doubled there was a marked increase in viscosity and a decrease in the rate of oxygen transport. The rate of nitrogen diffusion through this solution also dropped, and this finding implicates the high viscosity as the cause (*2*, *6*).

The retarding effect of increased viscosity was demonstrated by adding 10 percent of gelatine to the hemoglobin solution, which was enough to make it solidify. This almost halved the specific rate of oxygen transport by the hemoglobin but hardly affected the nitrogen diffusion.

Oxygen transport through red cells. In these experiments the washed red cells were smeared on either the upper or the lower side of the finest Millipore filter (VF grade). It will be seen from Fig. 4 that the oxygen transport was almost doubled at pressure of ⅓ atm. When this procedure was tried on a regular HA filter, the capillary forces in the filter ruptured the blood cells and the hemoglobin solution came through on the other side. This did not happen on a VF filter, and when the filter was soaked afterward in isotonic saline there was little, if any, evidence that hemolysis had taken place. It is therefore indicated that the transport effect displayed by hemoglobin solutions is also operative in the intact red cell.

Oxygen transport through myoglobin and other pigments. A preliminary study was made on oxygen transport through a myoglobin solution. This was prepared from the very dark pectorals of a California sea lion. Thin slices cut across the fibers were repeatedly washed in isotonic saline solution and wrung in a dry towel. The amount of blood left after this procedure is very small compared to the high concentration of myoglobin. The slices were passed through a meat grinder and repeatedly frozen and thawed, and the macerate was ground under a few milliliters of water in a mortar and centrifuged. The fluid was passed through an HA Millipore filter.

This solution at an air pressure of ⅓ atm gave initial O_2/N_2 ratios of 100 percent and 87 percent—that is, there was a substantial oxygen enhancement. After a few hours, and contrary to the finding for hemoglobin solutions, the ratio dropped to that of water. Although this is an unstable preparation, it demonstrates that myoglobin in vitro is capable of implementing a potent steady-state oxygen transport.

A marine sand-dwelling worm, *Thoracophelia,* when cut into pieces and centrifuged, yielded a deep red solution with an oxygen capacity of 5.3 volumes percent. This mixture of blood and body liquid yielded large amounts of CO_2, together with oxygen and nitrogen, but nevertheless showed some 12- to 15-percent enhancement of the oxygen transport when measured at air pressures of 1/6 or 1/12 atm. In view of the loss of oxygen in the membrane, these are clearly minimum figures.

Hemolyzed red cells from fish blood, —mackerel, with an O_2 capacity of 12.1 percent and yellowtail (*Seriola dorsalis*), with a higher O_2 capacity—showed an enhancement of 100 percent at air pressure of ⅓ atm in the mackerel and 110 percent in the yellowtail.

Discussion

We have observed a steady-state rate of oxygen transport which can be attributed to the added effects of two simultaneous processes: one, a diffusion through the solvent; the other, a transport specifically dependent upon hemoglobin. The latter proceeds at a constant rate over a wide range of pressures and depends upon the kinetics and oxygen-binding properties of the hemoglobin molecule.

It therefore seems that when a tension gradient of oxygen is imposed upon a hemoglobin solution, oxygen molecules are handed down from one hemoglobin molecule to the next in a chain or "bucket-brigade" fashion. Provided the

"buckets" are emptied at one end and filled at the other, a steady-state system is set up which results in facilitation of oxygen transport through the chain (see Fig. 6).

The steady-state situation requires that the oxygen tension at any level between the upper and lower surfaces of the membrane remain constant. Above, it is near the tension of the gas phase; below, it is near zero. The oxygen saturation at various levels is determined by the oxygen dissociation curve, but the exact relations are here immaterial as long as conditions remain constant. One may tentatively assume that a linear tension gradient exists and that the hemoglobin loses is first oxygen at air pressure of approximately ⅓ atm, its second at ⅙ atm, and its third at 1/12 atm. Near the lower surface only the fourth molecule remains attached to the hemoglobin (Fig. 6). This is the one which jumps off into the vacuum and which is then replenished from the hemoglobin chain above. The maximum rate at which the chain can keep the oxygen moving evidently depends upon the constant kinetic motion of the hemoglobin molecules, for the rate of oxygen transport via this route is constant and independent of the oxygen gradient.

That a rate-limiting factor is located at the vacuum end of the chain is indicated by the *p*H effect. It was found that an increased affinity for oxygen—that is, a high *p*H—is able to maintain the transport unimpaired at very low pressures, whereas lowering of the *p*H slowed it down. A glance at the last column of Fig. 6 will show that at low *p*H and a pressure head of ⅙ atm, the lower half of the chain gets increasingly disrupted by completely reduced hemoglobin molecules, and we may assume that as a consequence of this the delivery slows down.

If the head of pressure is only 1/12 atm to begin with, one would expect a great number of void (unoxygenated) molecules in the lower part of the chain, even at high *p*H; nevertheless, oxygen was delivered at full rate. This seems to weaken the explanation of the *p*H effect just mentioned, unless possibly another factor entered—namely, back pressure of oxygen. From the lower surface of the membrane into the vacuum chamber is a long way for oxygen to go, and this simple diffusion depends, of course, upon a concentration gradient. It is possible that the oxygen back pressure built up sufficiently to keep the chain saturated to the very end. This raises a fundamental question: If the low-pressure side were raised deliberately, would the transport system still work? The answer can be obtained only by the use of a different technical approach.

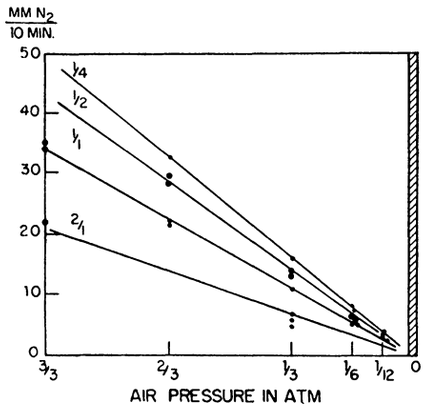

Fig. 5. Rate of nitrogen diffusion through various concentrations of hemoglobin. Curve 1/1 represents oxygen capacity of 22.9 volumes percent; the other curves represent fractions of this amount. Ordinate = vol/min.

What then, one may wonder, is the possible biological significance of this oxygen-specific, steady-state, enhanced rate of oxygen transport?

It will be seen from the various experiments in vitro that the oxygen transport mediated by human hemoglobin may dominate straight diffusion by a factor of 8 or more at low pressures and can at full atmospheric pressure almost double the rate. The effect has also been demonstrated in intact red cells, and it is possible, although this has not been demonstrated, that it plays a role in their normal gas exchange, even when the low-pressure side is not zero.

More relevant, however, is the physiological implication of the role of enhanced rate of oxygen transport in the case of myoglobin. One may visualize a system which in addition to its storage function would grease, so to speak, the oxygen transfer from the blood in the capillaries into the metabolic machinery of the muscle fiber. Such a transport would be enhanced even more if the myoglobin were mobile within the muscle cells, somewhat like water in a sponge, rather than held in a viscous medium. But whatever is the case, muscle contractions would undoubtedly favor the transport.

It was found that sea lion myoglobin, indeed, enhances oxygen transport in vitro. The increase in hemoglobin and sometimes in myoglobin observed in many high-altitude mammals fits well into this same line of argument, and the same holds true also for those larvae, worms, intestinal parasites, and other animals which live under low oxygen tension and harbor red hemoglobin-like pigments. A steady-state transport effect of the blood pigment from one such worm has indeed been demonstrated.

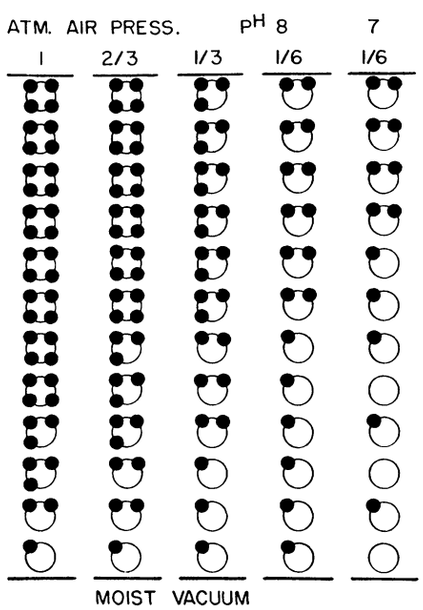

Fig. 6. Schematic presentation of possible mode of oxygen transport via hemoglobin molecules. Solid circles, oxygen molecules; open circles, hemoglobin molecules.

Summary

A study has been made of steady-state diffusion of air at various pressures through hemoglobin solutions. Whereas nitrogen diffused in proportion to the pressure, the rate of oxygen transport was greatly enhanced and seemingly proceeded by means of two processes which are additive. One is a regular diffusion through the solvent (water), which is proportional to the pressure; the other is a specific transport mediated by the hemoglobin molecules. The rate of the latter is constant over a wide pressure range, and the process may at low tensions transport over eight times more oxygen than does straight diffusion. Preliminary studies

have established that myoglobin and a few other pigments in vitro have the same property.

References and Notes

1. G. A. Millikan, *Proc. Roy. Soc. (London)* **B123**, 218 (1937); *Physiol. Revs.* **19**, 503 (1939).
2. I. S. Longmuir and F. J. W. Roughton, *J. Physiol. (London)* **118**, 264 (1952); F. Kreuzer, *Helv. Physiol. et Pharmacol. Acta.* **9**, 379 (1951); ———, *ibid.* **11**, suppl. 9 (1953).

3. This investigation was supported by a research grant (No. RG-5979) from the U. S. Department of Health, Education, and Welfare, Public Health Service, and by the Norwegian Research Council. I wish to thank A. Baird Hastings, Denis L. Fox, and Y. Zotterman for their stimulating interest and advice, and I gratefully acknowledge the excellent laboratory facilities extended to me by Edmund L. Keeney and Hastings at the Scripps Clinic and Research Foundation. The effect described here was found in 1956 at the Institute of Zoophysiology, University of Olso, where valuable assistance was rendered by G. Sundnes and H. Leivestad.

4. P. F. Scholander, L. van Dam, C. L. Claff, J. W. Kanwisher, *Biol. Bull.* **109**, 328 (1955).
5. F. J. W. Roughton and P. F. Scholander, *J. Biol. Chem.* **148**, 541 (1943).
6. A. Klug, F. Kreuzer, and F. J. W. Roughton [*Helv. Physiol. et Pharmacol. Acta* **14**, 121 (1956)] exposed thin films (0.1 by 45 mm) of reduced hemoglobin solutions to oxygen pressure of 0.9 atm and studied optically the oxygenation rate. On comparing their findings with earlier determinations of nitrogen diffusion (diffusion into a system much less sensitive to errors from surface disturbance and convection), they estimated an oxygen enhancement of 100 percent at low viscosity vanishing at high hemoglobin concentrations. This effect, attributed to diffusion of oxyhemoglobin, would at a pressure of 0.2 atm amount to 450 percent. This is totally out of the range of our findings and the effect is seemingly, therefore, of a different nature.

Changing Environment of Zoological Research

The era of abundance in funds and transportation opens new vistas for research and exploration.

Herbert Friedmann

When an ecologist studies the relations between the species of animals and plants in a given type of environment, he tries to measure, as far as he can, the various external factors, but at the same time he is aware of the fact that the degree or the speed of reaction to these factors varies individually within more or less specific limits for each species. Obviously, the more responsive individuals will be either helped or harmed, by any external change, to a greater degree than the less responsive ones, but, by and large, the ecologist comes to assume a mean response for each species and then proceeds to treat all the individuals of that species as essentially alike. Similarly, in this attempt to survey the present environment in which we work, I assume an average zoologist, if there be such a person, and pay attention to the environment in which he goes through the motions and activities inherent in being a zoologist.

The old, traditional concept of the scholar or the research worker is that of a relatively solitary individual, plodding along with his studies and only too glad to be left alone to pursue them. For many years, for centuries even, in the early history of science, the scholar was considered to be possibly quite interesting, but neither a direct contributor to the welfare of mankind, nor actually harmful. It was only when it began to be realized generally that no knowledge was without potential value that society began to pay an increasing amount of attention to the individuals engaged in extending the limits of knowledge. There is no need to trace the details of this historical change of attitude from the time of the Renaissance, when scholars were still the protégés of powerful and enlightened princes and dukes and even of lesser, local VIP's, so we may jump to the situation as it was in our own experience a generation or two ago, limiting the picture to the zoological portion of the scientific panorama, and comparing that situation with the present one.

The situation as it was then was far more favorable for the prosecution of zoological work than it had been at any previous time. There were literally hundreds of laboratories in our colleges and universities, not all equally well equipped or of equal coverage, but still capable of training future zoologists and of providing, within very variable limits, the opportunity for research. Most of our major research museums were in existence, although their collections were far less complete than they are now, and library facilities, which have since increased greatly in scope and in completeness, were generally adequate. There were some, but not many, organizations from which research grants might be obtained, although most of these organizations had established fairly definite perimeters to their interests.

Within this setting the hypothetical average zoologist went about his work, and the results, seen in the perspective of 30 years, were good. There was little need to do anything other than enlarge the facilities and provide opportunities for an increasing number of people in the zoological field. To judge by present conditions there was a more leisurely atmosphere then than now.

Urgency and Research

At the present time we are faced with a tension in the political atmosphere that has unfortunately invaded the scientific environment to a dangerous degree. We are expected to do research, but with an urgency that has no place in research; we must do things without delay, lest the Russians anticipate us; we are told that our survival depends on our intensity and application in scientific work. While this unfortunate trend may or may not have some validity in matters of missiles and other defense developments, it has no place in many areas of science, including zoology. The preachers of urgency forget that research is one of the most ennobling forms of human endeavor and

The author is head curator of the department of zoology, Smithsonian Institution, Washington, D.C. This article is adapted from his vice presidential address, delivered before Section F (Zoological Sciences), during the Chicago meeting of the AAAS, 26–31 Dec. 1959.