brane there is an "(oxy)hemoglobin shuttle" which, by diffusion and continuous unloading, transports the O_2 the rest of the way. In the middle portion of the membrane, one finds a gradual transition in the nature of the oxygen transported—from primarily free oxygen to HbO_2.

The proposed model easily explains the observation (*2*) that a slight backpressure of oxygen at the exit of the membrane diminishes the enhancement by hemoglobin of the oxygen flux. Any factor interfering with the unloading of hemoglobin would, of course, diminish the gradient for oxyhemoglobin in that part of the membrane in which the hemoglobin shuttle operates, and thereby alter also the gradient of free oxygen in the first portion of the membrane.

It is possible that, in physiological systems exhibiting material transport over and above that which can be explained by simple diffusion of solute, a facilitated diffusion analogous to that described here may occur. Such diffusion requires only the presence of a carrier molecule that combines reversibly with solute and which itself is subject to diffusion gradients.

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References and Notes

1. P. F. Scholander, *Science* **131**, 585 (1960).
2. E. Hemmingsen and P. F. Scholander, *ibid.* **132**, 1379 (1960).
3. J. H. Wang, *ibid.* **133**, 1770 (1961); R. E. Collins, *ibid.*, p. 1593; J. H. Wang, *J. Theoret. Biol.* **4**, 175 (1963).
4. I. Fatt and R. C. LaForce, *Science* **133**, 1919 (1961).
5. T. Enns, *Proc. Nat. Acad. Sci. U.S.* **51**, 247 (1964).
6. The correctness of this assumption has been questioned as to the diffusion of CO in hemoglobin solutions [M. Mochizuki and R. E. Forster, *Science* **138**, 897 (1962)]. However, the rate-constants for the O_2-hemoglobin system are much larger than those for CO; see table XVIII in A. Ross Fanelli, E. Antonini, A. Caputo, *Advan. Protein Chem.* **19**, 73 (1964). For example, it can be shown that at an O_2 pressure of 50 mm-Hg at the inlet the exchange rate of the fourth oxygen bound to hemoglobin is about 2000 times as large as the total oxygen transport in the membrane.
7. A. Polson, *Kolloid-Z.* **87**, 149 (1939).
8. E. Hemmingsen, *Science* **135**, 733 (1962).
9. The theoretical values in Fig. 1 are based on the diffusion coefficients of oxygen and hemoglobin in water rather than in the more viscous 15 percent hemoglobin solutions; the diffusion path length is assumed to equal the membrane thickness. Tortuous channels in the filter would materially lengthen the path. No account is taken of the presence of solids in the filter or of the screen supporting the filter. All these factors would lower the calculated absolute values (Fig. 1) but would not materially affect the relative positions of the two curves in Fig. 1 or the values in Table 1.
10. Supported by PHS grant HE-01238 and a Career Investigatorship of the American Heart Association. I thank P. F. Scholander and T. Enns for stimulating discussions.

9 April 1965

Tension Gradients Accompanying Accelerated Oxygen Transport in a Membrane

Abstract. *Facilitated transport of oxygen through hemoglobin has been studied by use of a membrane sandwich of hemoglobin, Teflon, and water. Transport is enhanced only when there is a hemoglobin-bound-O_2 gradient in the pigment. When passing through a fully oxygenated layer, the accelerated transport is carried by an increased diffusion gradient of oxygen pressure through the solvent. The results substantiate experimentally the diffusion gradients proposed by Zilversmit.*

The absolute rate of enhancement of oxygen transport through a membrane containing hemoglobin reaches a maximum when the input side is saturated with oxygen and the output side is fully reduced; it makes no difference if the oxygen pressure is considerably beyond saturation on the input side. At an oxygen tension of 450 mm-Hg on the input side in one experiment some 95 percent of the membrane was fully oxygenated; it seemed clear, therefore, that the additional oxygen must be able to pass through a layer of fully saturated oxyhemoglobin (*1*, *2*). If oxygen pressure on the output side was allowed to increase, it appeared that enhancement vanished when full saturation was reached (*3*).

The last point has been studied with a more direct approach: optical oxygen saturation has been compared with gasometric enhancement; the same hemoglobin solution was used. For these measurements specially designed membranes each incorporated two Millipore

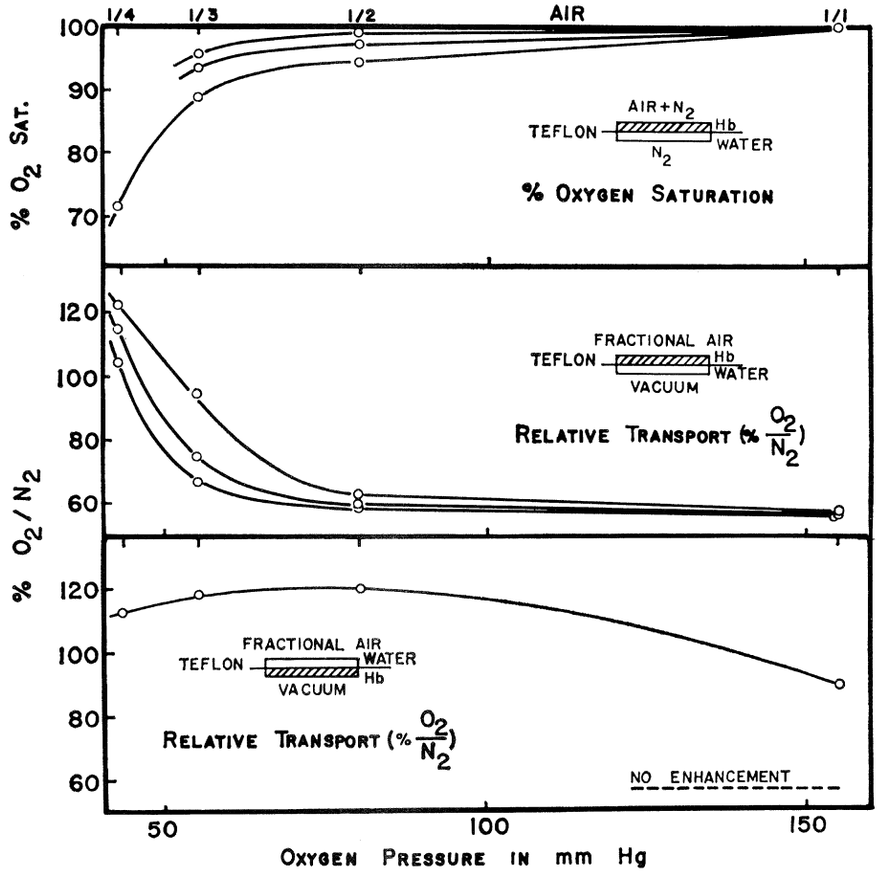

Fig. 1. Hemoglobin oxygenation and relative enhancement of oxygen transport in sandwich membranes. Each curve represents a separate experiment. The same hemoglobin solution was used in all experiments; it was prepared from human blood (*1*) having an oxygen capacity near 21 percent by volume. (Top) Oxygenation determined spectrophotometrically at a wavelength of 562 mμ; total gas pressure on each side of membrane sandwich was 1 atm. (Center) Relative enhancement determined gasometrically (*1*); value of no enhancement (methemoglobin or water), $O_2:N_2 = 56$ percent. Air at various pressures on input side. (Bottom) Relative enhancement when the hemoglobin membrane faced vacuum.

filters separated by a 10-μ Teflon film. One membrane was saturated with the hemoglobin solution; the other, with water. By diffusing air at various pressures through such a system one determines the relative enhancement in transport from the oxygen-nitrogen ratio, and by placing such assemblies in the light path of a spectrophotometer (*2*, *4*) one measures the integrated oxygenation. The optical density for full oxygenation and full reduction was determined by having, respectively, air and nitrogen on either side.

In one series (Fig. 1, top) the hemoglobin membrane was on the input side. With full pressure of air (155 mm-Hg O_2) the ratio of O_2 to N_2 diffusing through was close to 56 percent, which is the ratio for zero enhancement given by methemoglobin or water. As soon as reduction appeared optically, when the air pressure was lowered, enhancement in transport developed (Fig. 1, center). In a second series the input membrane contained water, while the hemoglobin membrane faced the vacuum. Under such conditions there is always an oxygenation gradient in the hemoglobin, and transport was always enhanced (Fig. 1, bottom).

These experiments, like the back-pressure experiments (*3*), show that the hemoglobin does not aid the net transport of oxygen unless there is a hemoglobin-bound-O_2 gradient through the membrane. The results lend experimental support to the explanation formulated by Zilversmit (*5*) that the enhancement in the fully oxygenated part of a membrane is carried entirely by an increased oxygen-pressure gradient through the solvent. The reason for this steep gradient is, of course, the facilitated flux through the oxyhemoglobin gradient at the outlet. To what extent this "hemoglobin shuttle" may numerically fit the expedient but poorly defined concept of oxyhemoglobin diffusion must await determination of the diffusion characteristics of hemoglobin through a Millipore membrane.

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References and Notes

1. P. F. Scholander, *Science* **131**, 585 (1960).
2. T. Enns, *Proc. Nat. Acad. Sci. U.S.* **51**, 247 (1964).
3. E. Hemmingsen and P. F. Scholander, *Science* **132**, 1379 (1960).
4. E. Hemmingsen, *Science* **135**, 733 (1962).
5. D. B. Zilversmit, *Science*, this issue.
6. Supported by NIH grant GM 10521. I thank T. Enns and D. Zilversmit for stimulating discussions, and Georgina Bien and Edda Bradstreet for the analyses.

3 May 1965

Temperature-Sensitive Repression of Staphylococcal Penicillinase

Abstract. *In eight highly inducible strains of* Staphylococcus aureus *repression of the formation of penicillinase was temperature-sensitive under conditions suggesting direct thermal inactivation of the repressor. Restoration of repression required protein synthesis. These strains were resistant to benzylpenicillin and to many other antibiotics. One auxotrophic mutant had greatly reduced temperature sensitivity but was still normally inducible. Six strains were relatively poorly inducible, exhibited a proportionately smaller increase in enzyme after exposure to elevated temperature, and were sensitive to antibiotics other than benzylpenicillin. Temperature sensitivity may be a useful character in studies of the physiology and genetics of the repression of staphylococcal penicillinase.*

Penicillinase, the enzyme which hydrolyzes the β-lactam bond of the penicillins, is found in all clinically pathogenic penicillin-resistant staphylococci in which it mediates resistance to penicillin treatment. Its usually low basal rate of formation can be increased manyfold by induction with penicillins, cephalosporins, or certain peptides (*1*). Increased synthesis, without specific inducers, may be achieved by cultivation of some inducible strains in highly acidic media (*2*).

In a search for other methods to analyze control mechanisms for synthesis of penicillinase in staphylococci we have investigated the property of temperature sensitivity, that is, the selective inactivation of biologically important macromolecules at moderately elevated temperature, an increasingly useful tool in the analysis of microbial biosynthetic mechanisms (*3*). In certain mutants of *Escherichia coli*, but not in wild-type organisms, the normal repression of synthesis of β-galactosidase or alkaline phosphatase is temperature-sensitive by either of two mechanisms. In one the proposed repressor itself appears to be inactivated at 43° to 45°C (*4*). In the other, the synthesis of repressor, but not its function, is progressively inhibited with rising temperatures of bacterial growth (*5*). In either case enzyme formation is repressed (inducible) during growth at low temperatures but is derepressed (constitutive) at higher temperatures.

We have found that repression of the formation of penicillinase is temperature-sensitive, apparently through the inactivation of preformed repressor, in many strains of wild-type *Staphylococcus aureus* and in one strain of wild-type *S. epidermidis*. In one auxotrophic mutant the repression system is relatively stable to elevated temperatures.

In typical experiments, cells of *S. aureus* strain 55C1, in the log phase of growth, grown at 37°C in tryptic digest broth (Baltimore Biological Laboratory, Inc.) with added 50 m*M* sodium phosphate buffer (*p*H 7.2), were washed once with buffer [11 m*M* KCl, 4 m*M* phosphate (Na), 1.4 m*M* $MgSO_4$, *p*H 7.2]. The cells were resuspended in the same buffer to a dilution where the optical density was 0.5, heated to 42°C for 10 minutes, and then diluted fourfold into broth or synthetic medium (*2*) at 37°C and grown while being shaken. Samples of cultures were collected into cold tubes with chloramphenicol (final concentration 100 μg/ml) and assayed iodometrically for penicillinase (*6*). A unit of penicillinase hydrolyzed 1 μmole of benzylpenicillin per hour at 30°C and *p*H 5.8. Specific activities are given in units per unit of optical density mea-

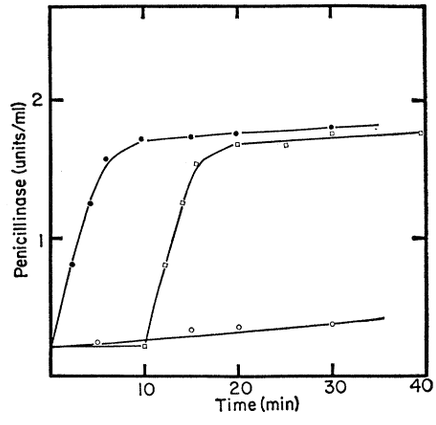

Fig. 1. Temperature-sensitive repression of staphylococcal penicillinase. Strain 55C1 was grown in broth, heated in buffer (with Mg^{++} reduced to 0.28 m*M*) at 42°C for 10 minutes; at zero time, the culture was added at 37°C to synthetic medium (closed circles), and to synthetic medium containing 500 μg/ml of puromycin (open squares). The culture with puromycin failed to grow, and, in separate experiments, its cells incorporated little or no carbon-14–labeled valine into protein. After 10 minutes, puromycin was removed by membrane filtration and washing. The cells, resuspended in synthetic medium, grew normally without lag. Open circles give results with an unheated culture.