

THE RATE OF ESCAPE OF HEMOGLOBIN FROM THE HEMOLYZED RED CORPUSCLE

By HUGO FRICKE

(From the Walter B. James Laboratory for Biophysics, The Biological Laboratory, Cold Spring Harbor, Long Island)

(Accepted for publication, March 27, 1934)

In a recent note¹ Ponder has presented some preliminary results of an experimental study of the rate of escape of hemoglobin from hemolyzed red corpuscles. These experiments are of interest in furnishing a means of determining to what extent the hemoglobin is free to leave the corpuscle. In particular, assuming that the resistance is at the surface of the corpuscle, the permeability of the surface of the hemolyzed corpuscle may be calculated.

In the following discussion the assumption is made that the escape of the hemoglobin is due solely to its diffusion, neglecting any influence of gravity or convection currents, which appears wholly justified in view of the small size of the corpuscle and of the experimental conditions under which the escape is measured.

We may start by assuming that the surface of the corpuscle becomes completely permeable, and shall calculate the change of concentration of hemoglobin as a function of time, at any particular point, which takes place as a result of the diffusion. The corpuscle may be assumed to be spherical, since it appears² that a change to this form always takes place before hemolysis occurs. We shall furthermore assume that Fick's law holds, with a constant value (D) of the coefficient of diffusion. The diffusion of hemoglobin does decrease with increasing concentration,³ but to too slight an extent to require its consideration for the present purpose.

The increase of hemoglobin inside a spherical shell, thickness dr ,

¹ Ponder, E., *Proc. Soc. Exp. Biol. and Med.*, 1934, **31**, 562.

² Ponder, E., *The mammalian red cell and the properties of haemolytic systems*, Protoplasma-Monographien, Berlin, Gebrüder Borntraeger, 1934, **6**.

³ Zeile, K., *Biochem. Z.*, Berlin, 1933, **258**, 347.

during the time dt , is equal to the difference between the amount of pigment entering and leaving the shell. The latter quantities are obtained from Fick's law. The following differential equation expresses this equality:

$$4\pi r^2 \frac{dc}{dt} = D \frac{d \left[4\pi r^2 \frac{dc}{dr} \right]}{dr}$$

from which

$$\frac{d(rc)}{dt} = D \frac{d^2(rc)}{dr^2}$$

with the boundary conditions as follows:

$t = 0$, $c = c_0$ from $r = 0$ to $r = \rho$, and $c = 0$ from $r = \rho$ to $r = \infty$, where c is the concentration of hemoglobin at a distance r from the center of the corpuscle and at a time t , and c_0 is the initial value of c . The radius of the corpuscle is ρ . The solution is:

$$c = \frac{c_0 \sqrt{Dt}}{\sqrt{\pi \cdot r}} \left[e^{-\left(\frac{\rho+r}{2\sqrt{Dt}}\right)^2} - e^{-\left(\frac{\rho-r}{2\sqrt{Dt}}\right)^2} \right] + \frac{c_0}{2} \left[\Phi\left(\frac{\rho+r}{2\sqrt{Dt}}\right) + \Phi\left(\frac{\rho-r}{2\sqrt{Dt}}\right) \right]$$

Φ represents Gauss' function.

Introducing $\frac{c}{c_0} = C$, also $\frac{r}{\rho} = R$, and $\left[\frac{2\sqrt{Dt}}{\rho} \right]^2 = T$, (1) we have

$$C = \frac{1}{2\sqrt{\pi}} \frac{\sqrt{T}}{R} \left[e^{-\frac{(1+R)^2}{T}} - e^{-\frac{(1-R)^2}{T}} \right] + \frac{1}{2} \left[\Phi\left(\frac{1+R}{\sqrt{T}}\right) + \Phi\left(\frac{1-R}{\sqrt{T}}\right) \right] \quad (2)$$

The average concentration of hemoglobin, along a radial line, is:

$$C_m = \int_0^\infty C \cdot dR \quad (3)$$

Equations (2) and (3) are solved by numerical calculation. Fig. 1 shows C_m as a function of T .

Ponder's observations are for the corpuscles of man. Taking the volume of this corpuscle as $89\mu^3$, the value of ρ is $\rho = 2.8 \times 10^{-4}$ cm. Measurements of the coefficient of diffusion of hemoglobin by Sved-

berg and Nichols,⁴ by Northrop and Anson,⁵ and by Zeile³ are in substantial agreement, giving as an average, over the range of concentrations of hemoglobin from 0 to 30 per cent: $D = 7 \times 10^{-7}$ cm.²/sec. (20°C.). For a value of $C = 0.10$, we obtain from Fig. 1: $T = 6$. Therefore, from (1): $t = 0.16$ sec.

The much longer times of 2 to 6 seconds, found by Ponder¹ for hemolysis with dilute saponin, indicates a considerable impermeability of the surface of the hemolyzing corpuscle.

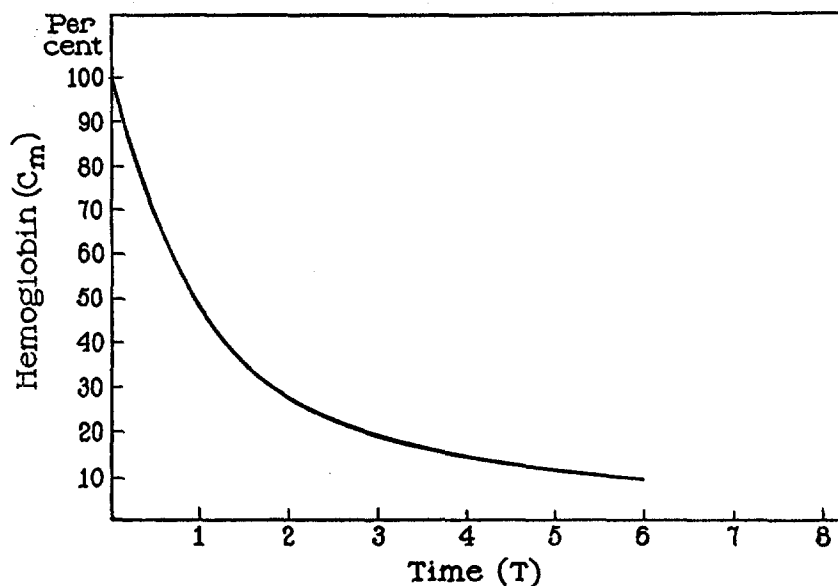


FIG. 1. The escape of hemoglobin, as a function of time, from a completely permeable erythrocyte.

For stronger saponin, the method used by Ponder cannot be used, because it requires a fairly long latent period. That the escape becomes more rapid as the concentration of the lysin is increased, may be concluded by observing the speed with which a suspension of cells hemolyzes when strong saponin is added. It is quite possible that a time of escape as short as the theoretical value may be at-

⁴ Svedberg, T., and Nichols, J. B., *J. Am. Chem. Soc.*, 1927, **49**, 2920.

⁵ Northrop, J. H., and Anson, M. L., *J. Gen. Physiol.*, 1929, **12**, 543.

tained, indicating complete permeability of the surface of the hemolyzing corpuscle. Measurements of the electric conductance⁶ lead to a similar view. Cells hemolyzed with mild lysins (water, complement-amboceptor, saponin in low concentration) were found to have a conductance too low to measure, while with strong saponin a complete permeability of the cells to the electric current is produced.

When the time of escape is as long as that found by Ponder, the concentration gradient of the pigment is practically all at the membrane of the corpuscle. The permeability μ_H of the membrane to hemoglobin is defined as the amount of pigment passing through unit area of membrane, in unit time, per unit difference of concentration. The decrease of hemoglobin inside the corpuscle is equal to the amount which has diffused through the membrane. The latter quantity is obtained from Fick's law. The following equation expresses this equality:

$$\frac{4}{3} \pi \rho^3 dc = \mu_H \times c \times 4\pi \rho^2 dt$$

from which

$$c = c_0 e^{-\frac{3\mu_H t}{\rho}}$$

$$\mu_H = \frac{\rho}{3t} \log_e \frac{c_0}{c} \text{ cm./sec.} \quad (4)$$

Using Ponder's average time of 4 seconds for $\frac{c}{c_0} = 0.10$ with $\rho = 2.8 \times 10^{-4}$ cm., we obtain $\mu_H = 5 \times 10^{-5}$ cm./sec. This value of μ_H is for a concentration of saponin which produces complete lysis of human cells (in 1 per cent NaCl) in about 3 minutes.

Claim has been made⁷ that the hemoglobin leaves through one or more holes in the membrane. It may be of interest to calculate, for any particular number of holes, how large each hole must be to give a

⁶ Fricke, H., and Curtis, H. J., The electric impedance of suspensions of biological cells, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1933, 1, 117.

⁷ Ponder,² p. 85.

certain rate of escape. With N holes (diameter d) placed so far from each other that there is no appreciable interference, the value of μ_H is:

$$\mu_H = \frac{0.96 \times d \times D \times N}{4\pi\rho^2}. \quad (5)$$

This expression may be obtained from a formula giving the solution of a parallel problem in electric conductance.⁸ Using $\mu_H = 5 \times 10^{-5}$ cm./sec., $D = 7 \times 10^{-7}$ cm.²/sec., and $\rho = 2.8 \times 10^{-4}$ cm., we obtain

$$d = \frac{0.7}{N} 10^{-4} \text{ cm.}$$

CONCLUSIONS

A theoretical treatment is given of the rate of escape of hemoglobin from the hemolyzed red corpuscle. For complete permeability of the surface, as may perhaps be produced by strong lysins, the time taken for the hemoglobin to decrease to 10 per cent of its original concentration is calculated to be 0.16 seconds (for the human cell). For dilute saponin, giving complete lysis of human cells in 3 minutes, Ponder found a time of escape of 4 seconds, from which the permeability of the membrane to the pigment is calculated to be $\mu_H = 5 \times 10^{-5}$ cm./sec.

⁸ Jeans, J. H., Electricity and magnetism, Cambridge University Press, 1915, 356.