16064

Certain Mathematical Aspects of the Susceptibility of Erythrocytes to Lysis.*

C. W. HIATT. (Introduced by E. E. Ecker.)

From the Institute of Pathology, Western Reserve University, and the University Hospitals, Cleveland, Ohio.

Variation in the value of the parametric constant 1/n in the von Krogh alternation formula[1] has been used by several authors[2-4] as a convenient index of the susceptibility of red cells to lysis in the presence of complement and a hemolytic antiserum. It is of interest to consider in some detail the relationship between the value of the constant and the distribution of susceptibility to lysis among individual red cells.

As ordinarily expressed, von Krogh's formula is:

$$x = k\left(\frac{y}{1-y}\right)^{\frac{1}{n}} \qquad (1)$$

where x = lysin concentration
y = fraction of cells hemolyzed
k, 1/n are constants for a given set of reagents.

The plot of y versus x (Fig. 1) is a sigmoid curve which provides an excellent fit for experimental points between the limits y = 0.1 and y = 0.9. The lack of fit beyond these limits has been attributed to the rapid rate of change of y/1—y as y approaches zero or unity.

Solving Equation 1 for y gives:

$$y = \frac{x^n}{k^n + x^n} \qquad (2)$$

and, differentiating with respect to x,

$$\frac{dy}{dx} = \frac{nk^n x^{n-1}}{(k^n + x^n)^2} \qquad (3)$$

If x is expressed in terms of 50% hemolytic units, then it may be seen from Equation 1 that, at 50% hemolysis (y = 0.5), the fraction y/1—y is unity and k = 1. This choice of the units of x allows Equation 3 to be simplified to:

$$\frac{dy}{dx} = \frac{nx^{n-1}}{(1 + x^n)^2} \qquad (4)$$

The derivative dy/dx represents the rate of change of the degree of hemolysis with respect to lysin concentration. The value of the derivative at any point x is then proportional to the number of cells that require that concentration of lysin for hemolysis. Thus the curve of dy/dx versus x will provide a graphic representation of the distribution of susceptibility among the red cells of a given population.

The shape of this curve for 3 commonly encountered values of 1/n is shown in Fig. 2. The asymmetry of the curve, which suggests that the lysin is proportionally less effective in the higher concentrations, conforms with Ponder's view,[5] based on experimental data, that the accumulated products of hemolysis have an inhibiting effect on the lysin.

The respective curves for the 3 values of 1/n disclose that there is an inverse relationship between the value of 1/n and the uniformity of a given cell population with respect to susceptibility to lysis. It is suggested that any condition which leads to a change in uniformity will lead to an inverse change in the value of 1/n. This supposition is consistent with the observation of Kent[4] that the optimal degree of cell sensitization with an homologous antiserum occurs at a minimum value of 1/n for the system, and with the disclosure of Morse[2] that 1/n tends to increase either with aging of the cells, or on the introduction of certain impurities.

* Aided by a grant from the Commonwealth Fund.

1 von Krogh, M., *J. Infect. Dis.*, 1916, **19**, 452.

2 Morse, S., PROC. SOC. EXP. BIOL. AND MED., 1922, **19**, 17.

3 Wadsworth A., Maltaner, E., and Maltaner, F., *J. Immunol.*, 1931, **21**, 313.

4 Kent, J. F., *Science*, 1947, **105**, 316.

5 Ponder, E., *Proc. Roy. Soc.*, B, 1923, **95**, 382.

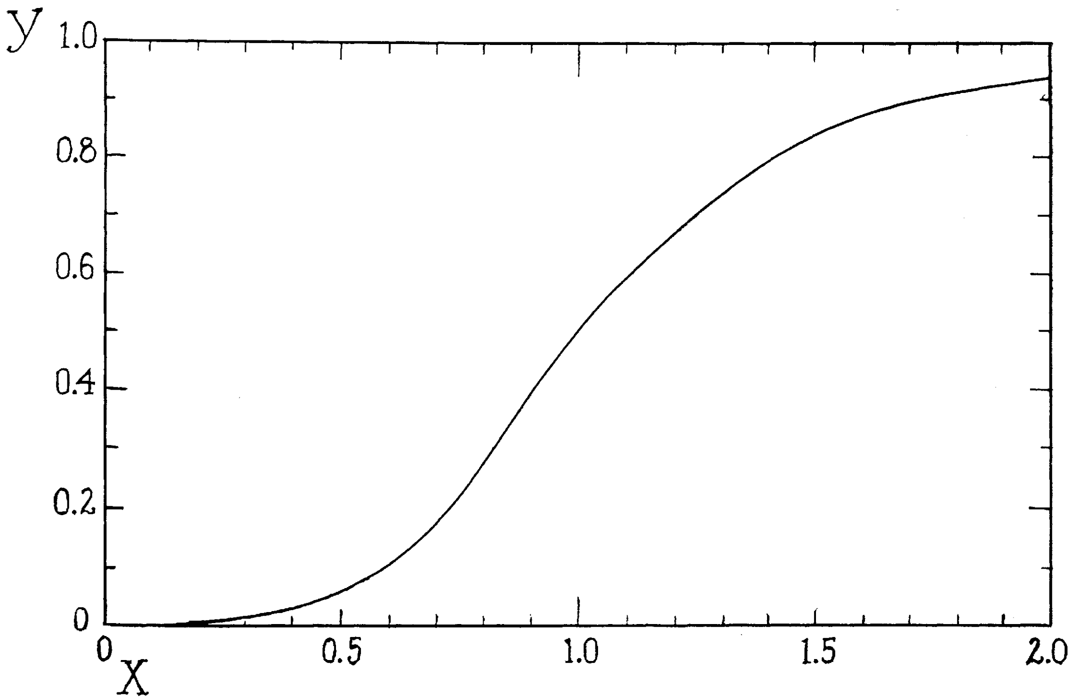
y
1.0
0.8
0.6
0.4
0.2
0
0
0.5
1.0
1.5
2.0
X

Fig. 1.

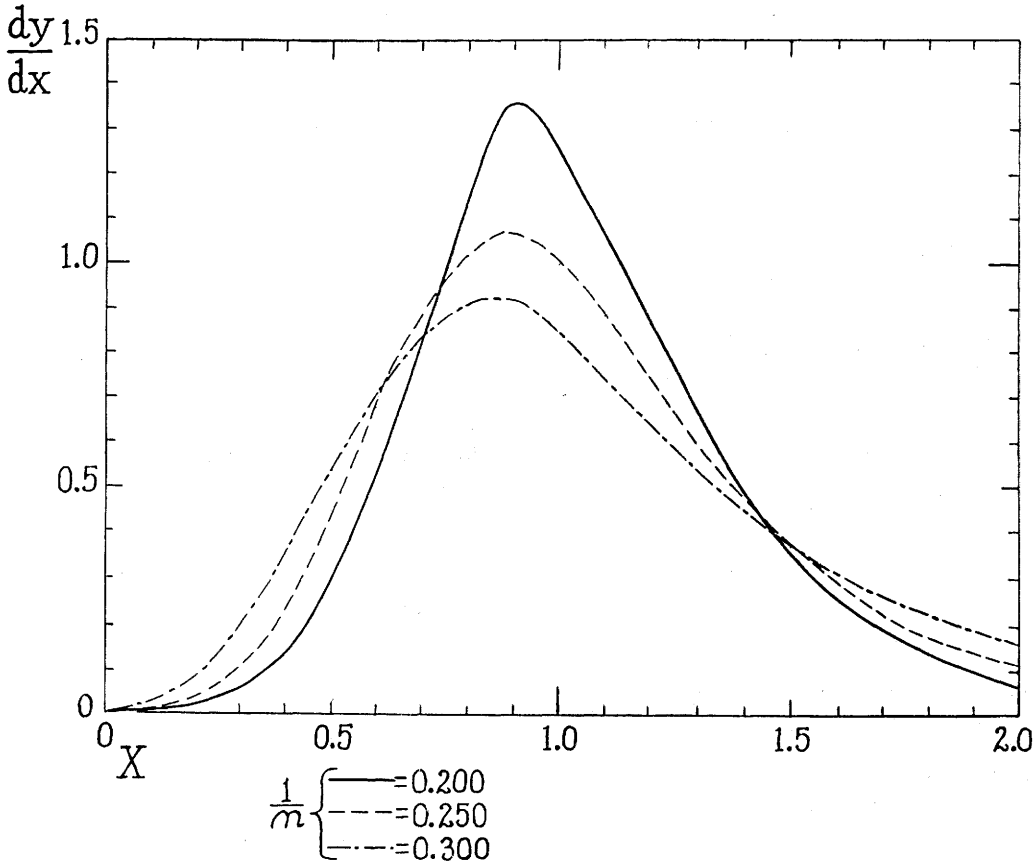
dy/dx
1.5
1.0
0.5
0
0
0.5
1.0
1.5
2.0
X
1/m
=0.200
=0.250
=0.300

Fig. 2.

Summary. An approximate curve representing the distribution of susceptibility to hemolysis among the erythrocytes of a given population is obtained by differentiation of the von Krogh equation. The von Krogh constant 1/n is shown to be an inverse measure of the uniformity of distribution. Variations in the value of 1/n are concluded to be indicative of variations in the uniformity of the cell suspension with respect to its susceptibility to hemolysis.

The assistance of Dr. E. E. Ecker in the initiation and interpretation of this work is gratefully acknowledged by the author.

16065

Bacteria and Cellular Activities. I. Effect of *Streptococcus beta-hemolyticus* on Permeability of Chicken Erythrocytes.*

F. R. HUNTER AND HOWARD W. LARSH.

From the Departments of Zoological Sciences and of Plant Sciences, University of Oklahoma, Norman.

In order to obtain a better understanding of some of the changes which may take place in cells under the influence of bacteria and their products, a series of experiments is being undertaken. The initial studies involved changes in the permeability of erythrocytes in the presence of a hemolytic bacterium.

Ponder[1] has reviewed the older literature on this general subject. Many similar experiments have been performed since (Maizels[2]). In all of the work cited interest was centered on the actual hemolysis of the erythrocytes. In the present investigation the permeability of the cells prior to hemolysis was considered.

The following data then are of interest not only with reference to the general problems mentioned, but also emphasize the care which must be exercised to eliminate the possible effect of bacterial contamination in permeability studies of erythrocytes.

Procedure. The test organism, *Streptococcus beta-hemolyticus* was secured from the American Type Culture Collection. It was seeded on proteose No. 3 agar and incubated for 24 hours at 37.5°C. An aliquot of the inoculum removed from the medium by washing with sterile Ringer-Locke solution was added to an equal volume of heparinized chicken erythrocytes obtained by cardiac puncture. This suspension of erythrocytes and bacteria was incubated at 37.5°C. A control suspension was made up in a similar manner except that the organisms were omitted. Aliquots were removed at varying intervals of time, and the permeability of the erythrocytes to glycerol was measured by the photronic cell technique employed by one of the authors (Hunter[3]). Change in volume of the erythrocytes alters the amount of light transmitted to the photocell, hence the current induced and the "scale reading" of the galvanometer. The volumes of the erythrocytes may be routinely varied (1) by placing them in an isotonic solution of a penetrating substance (0.3 M glycerol) which ultimately results in hemolysis or (2) by placing them in a hypertonic solution of a penetrating substance in Ringer-Locke solution (0.3 M glycerol in Ringer-Locke solution), which produces a rapid shrinkage and a subsequent swelling. The light transmission is increased both by swelling and by hemolysis. In the hypertonic solution swelling alone is meas-

* The authors are indebted to the Faculty Research Fund of the University of Oklahoma for grants-in-aid.

1 Ponder, E., *The Mammalian Red Cell and the Properties of Haemolytic Systems*, Berlin, 1934.

2 Maizels, M., *Quart. J. Exp. Physiol.*, 1946, **33**, 183.

3 Hunter, F. R., *J. Cell. and Comp. Physiol.*, 1936, **9**, 15.