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FACILITATED DIFFUSION IN HUMAN ERYTHROCYTES

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SUMMARY

1. A densimeter technique was used to make a kinetic analysis of the swelling of human erythrocytes in the presence of glycerol, ethylene glycol and urea.

2. Saturation was observed in all three cases.

3. A half-saturation value of approximately one isotone or less was estimated for each of the carriers.

4. These data give additional support to the suggestion that molecules which cross the erythrocyte membrane by facilitated diffusion will have their permeability decreased by butanol.

INTRODUCTION

Various authors have commented on the action of narcotics on the permeability of erythrocytes[1–4]. I have suggested that the action of butanol can be used to distinguish between those molecules which cross the membrane by simple diffusion and those which cross by facilitated diffusion[5,6]. I have been making kinetic studies of the permeability of erythrocytes to various substances in an attempt to test this suggestion. In the present report I shall present data obtained from a kinetic study of the penetration of glycerol, ethylene glycol and urea into human erythrocytes.

MATERIALS AND METHODS

Theoretical

The method of analysis has been previously described in some detail[7,8]. It is based on the fundamental equation,

$$\frac{dS}{dt} = K\left\{\frac{C}{C+\phi} - \frac{S/V}{(S/V+\phi)}\right\} \qquad (1)$$

in which S is the amount of penetrant within the cell, t is time, C is external concentration of penetrant, V is volume of cell water (isotonic volume = 1), ϕ is the value for half-saturation of the carrier and K is a constant. Concentrations and ϕ are expressed in isotones[9] (an isotonic solution contains 1 isotone). LEFEVRE[10] has shown that this basic equation can take the form:

$$F^0(C,V) = k^0 t = (C+\phi)\left\{(C'+1)(C+\phi+1) - (C+1)(C+\phi+1)V + (C+1)(C+\phi)\ln\frac{C-C'}{(1-V)(C+1)}\right\} \qquad (2)$$

in which C' is the concentration of penetrant with which the cells have previously been equilibrated.

Experimentally, several additions of a penetrating nonelectrolyte in 1 % NaCl are added to a cell suspension in 1 % NaCl and a record of the swelling of the cells is obtained for each addition. The values of C' and C are known for each addition. By substituting these values and selected values of V in Eqn. 2 it assumes the form:

$$F^0(C,V) = k^0 t = A + B\phi + C\phi^2 \quad (3)$$

TABLE I

CALCULATED VALUES OF $F(C,V)$, $F'(C,V)$ AND $F^0(C,V)$ FOR THE FOLLOWING SYSTEM

Cells are added to 8.5 ml of 1 % NaCl. Eight additions of 0.5 ml each of 8.1 M nonelectrolyte in 1 % NaCl are made. Time is allowed following each addition of penetrant for equilibrium to be reached before next addition.

Initial concn. of penetrant (isotonic units), C'	*Final concn. of penetrant (isotonic units), C*	*Relative volume of cell water, V*	$F^0(C,V)$		
			A $As\ \phi \to 0$; $F'(C,V)$	*B*	*C* $As\ \phi \to \infty$; $F(C,V)$
0	1.50	0.90	5.39	8.44	3.23
		0.92	6.44	9.89	3.73
		0.94	7.88	11.85	4.40
		0.96	9.99	14.73	5.38
1.50	2.84	0.90	28.20	20.81	3.83
		0.92	34.25	25.15	4.61
		0.94	42.40	30.98	5.65
		0.96	54.27	39.39	7.14
2.84	4.05	0.90	57.71	29.19	3.69
		0.92	74.64	37.67	4.75
		0.95	110.20	55.36	6.95
		0.97	150.62	75.42	9.44
4.05	5.14	0.90	77.61	30.67	3.03
		0.92	110.56	43.61	4.30
		0.95	180.83	71.16	7.00
		0.97	260.19	102.12	10.02
5.14	6.14	0.90	77.92	25.65	2.11
		0.92	131.95	43.41	3.57
		0.95	249.04	81.76	6.71
		0.98	487.33	93.25	13.06
6.14	7.04	0.90	39.64	11.33	0.81
		0.92	119.40	34.14	2.44
		0.95	293.00	83.72	5.98
		0.98	643.53	183.56	13.09
7.04	7.88	0.92	82.50	21.03	1.34
		0.94	230.57	58.81	3.75
		0.96	439.78	112.15	7.15
		0.98	809.99	206.25	13.13
7.88	8.64	0.94	179.19	41.65	2.42
		0.96	456.71	106.17	6.17
		0.98	939.34	218.02	12.65

A table of calculated values of A, B and C for different concentrations and volumes can be constructed (Table I). When $\phi \to \infty$, only the C-term need be considered. This is equivalent to the $F(C,V)$ of WIDDAS[9] which describes the kinetics of simple diffusion. When $\phi \to 0$, only the A-term is left, which is equivalent to the $F'(C,V)$ of WIDDAS and describes the kinetics of a near-saturated carrier. With values of ϕ intermediate between 0 and ∞, the three terms, A, B, multiplied by ϕ, and C, multiplied by ϕ^2, can be added to calculate $F^0(C,V)$. Times to reach the selected volumes that were used in the calculations can be measured on the experimental curves. These data can then be plotted against the three functions of concentration and volume to determine which function best describes the kinetics of the experimental system as indicated by a fit of the experimental points to a single straight line.

The response of the densimeter was not linear over the complete range of volume changes. In order to mark specific volumes on the experimental curves, a calibration curve similar to that shown in Fig. 2 was constructed for each experiment in the following manner. The total deflection of each individual swelling curve was measured and this distance in mm was plotted against the calculated value of the minimum volume, which is numerically equal to $(1+C')/(1+C)$. Only volumes in the 1.0–0.9 range were used in the present analysis.

Experimental

The methods used in obtaining the blood, preparing the solutions and obtaining swelling records with a densimeter have been described[7,8]. Eight additions of 0.5 ml each of an 8.1 M nonelectrolyte in 1 % NaCl solution were made to the cell suspension. The penetration of glycerol, ethylene glycol and urea was studied. The temperature was maintained at $5 \pm 0.5°$.

RESULTS

Typical swelling curves are presented in Fig. 1. At the end of Curves 2–7 the setting of the amplifier was changed in order to keep the record on the paper. This

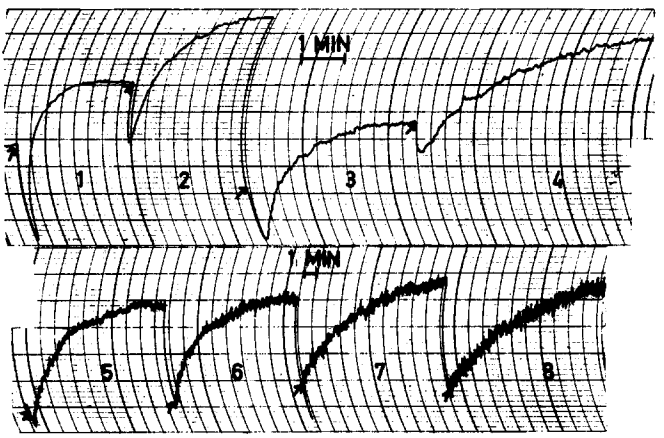


Fig. 1. Swelling of human erythrocytes suspended in 1 % NaCl following eight successive additions of 0.5 ml each of a solution of 8.1 M glycerol in 1 % NaCl. The setting of the amplifier was changed following Curves 2–7. The arrow at the beginning of each curve indicates where the glycerol was added in each instance.

accounts for the large downward deflection of the pen following the completion of each of these curves. The arrow at the beginning of each curve indicates the point at which the glycerol solution was added. The downward deflection of the pen following each addition results from the rapid exit of water. Since the osmotic gradient decreases with each addition of penetrant, this initial volume change becomes less and less. The increase in the "noise" level in Curves 5–8 results from two factors. A decrease in the speed with which the paper moves in the pen recorder, as occurred between Curves 4 and 5, always increases the apparent noise. A second factor which is always encountered when the cells contain a high concentration of penetrant is the increased "silkiness" of the suspension. The increased noise makes it more difficult to estimate accurately the position of specific volumes on the curves, but the magnitude of this error is not sufficient to change the interpretation of the present data.

In Fig. 3 one can see the family of straight lines that is obtained when average experimental times to reach specific volumes as glycerol entered the cells are plotted against $F(C,V)$. This suggests that the kinetics are not those of simple diffusion. These same experimental times plotted against $F'(C,V)$ can be seen in Fig. 4. The data obtained with the two lowest concentrations deviate slightly, but all of the remaining data fall on a single straight line. This is characteristic of a near-saturated carrier. As can be seen from looking at Table I, with small values of ϕ the A-term is predominant, so all plots of $F^0(C,V)$ with a value of ϕ equal to or less than one will be very similar to the F'-plot. In Fig. 5 the data are plotted against $F^0(C,V)$ with a value of $\phi = 1.0$.

Average experimental times obtained with ethylene glycol when plotted against

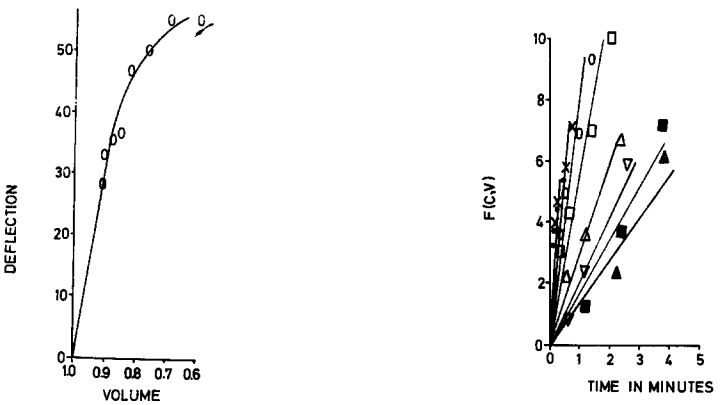
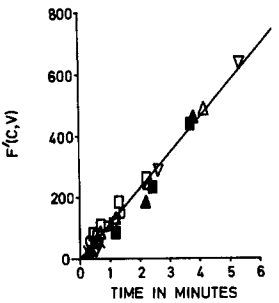


Fig. 2. Calibration curve for a single experiment relating volume change of the cells when ethylene glycol was added and deflection of the pen recorder. Abscissa, calculated volume of cell water. Ordinate, deflection of the pen recorder in mm. The points are in the reverse order of the additions, *i.e.* the last point on the curve was obtained with the first addition of ethylene glycol.

Fig. 3. Average values of times to reach specified volumes as measured from experimental records plotted against calculated values of $F(C,V)$. Eight successive additions of 0.5 ml each of 8.1 M glycerol in 1% NaCl were made to 8.5 ml of a suspension of human erythrocytes in 1% NaCl. ●, $C' = 0, C = 1.50$; ×, $C' = 1.50, C = 2.84$; ○, $C' = 2.84, C = 4.05$; □, $C' = 4.05, C = 5.14$; △, $C' = 5.14, C = 6.14$; ▽, $C' = 6.14, C = 7.04$; ■, $C' = 7.04, C = 7.88$; ▲, $C' = 7.88, C = 8.64$ isotones. The symbols have the same significance in all subsequent figures. It should be remembered that the ordinate in this and all subsequent figures is a calculated function which takes into consideration the concentrations of penetrant on the two sides of the cell membrane and selected volumes which the cells attain as additional penetrant enters and the cells swell from their minimum to their original volumes.

$F(C,V)$ give a family of straight lines very similar to Fig. 3. When these same data are plotted against $F'(C,V)$, the points for the three lowest concentrations give a family of straight lines but all of the other data fall on a single straight line (Fig. 6).



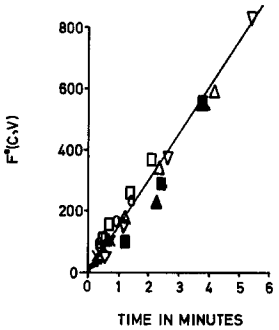
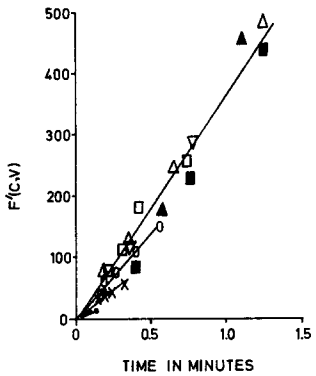


Fig. 4. Same data as in Fig. 3 plotted against $F'(C,V)$.

Fig. 5. Same data as in Fig. 3 plotted against $F^0(C,V)$, $\phi = 1.0$.



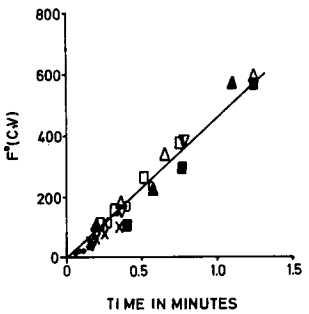


Fig. 6. Average values of times to reach specified volumes as measured from experimental records plotted against calculated values of $F'(C,V)$. Eight successive additions of 0.5 ml each of 8.1 M ethylene glycol in 1 % NaCl were made to 8.5 ml of a suspension of human erythrocytes in 1 % NaCl.

Fig. 7. Same data as in Fig. 6 plotted against $F^0(C,V)$, $\phi = 1.0$.

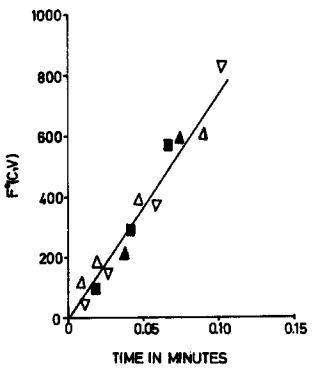


Fig. 8. Average values of times to reach specified volumes as measured from experimental records plotted against calculated values of $F^0(C,V)$, $\phi = 1.0$. Eight successive additions of 0.5 ml each of 8.1 M urea in 1 % NaCl were made to 8.5 ml of a suspension of human erythrocytes in 1 % NaCl. Only data obtained from the last four additions are included in the figure.

The reasonably good fit to a single straight line when these data are plotted against $F^0(C,V)$ with $\phi = 1.0$ can be seen in Fig. 7. Once again, these data suggest a carrier mechanism with a relatively small value for its half-saturation.

Urea penetrates human erythrocytes so rapidly even at 5° that it was not possible to obtain data following the first four additions. When average times for the last four additions are plotted against both F' and $F^0(C,V)$ with a value of $\phi = 1.0$, reasonably good fits to a single straight line are obtained (Fig. 8). These same data plotted against $F(C,V)$ give a family of straight lines. As was true in the case of glycerol and ethylene glycol, the urea data suggest the presence of a carrier with a small value for half-saturation.

DISCUSSION

A number of studies of the permeability of human erythrocytes to glycerol have suggested that more than simple diffusion is involved in this system. Inhibition by copper[11], by sulfhydryl reagents[12] and by narcotics[3] has been observed. Saturation with this system has been reported[2,13]. STEIN[14,15] proposed a dimer hypothesis, but this has subsequently been questioned[16,17]. The present kinetic analysis is consistent with the suggestion that glycerol penetrates these cells by a carrier mechanism.

JACOBS[18] observed competition between ethylene glycol and glycerol which is suggestive of a carrier mechanism, as is the present kinetic analysis which shows saturation with this system.

The very high permeability of mammalian erythrocytes to urea is a well-known feature of these cells[19,20]. The present data suggest saturation for this substance also. In the case of all three of these substances, the fit to a single straight line with the $F^0(C,V)$ plot is equally good with all values of ϕ equal to one or less. The possibility exists that cells equilibrated with the quantity of urea used in the present experiments might be abnormal. It has previously been demonstrated[8] that such cells retain an apparently normal permeability to other substances.

Previously it has been shown that the permeability of human erythrocytes to glycerol, ethylene glycol and urea is decreased by butanol[1,6]. The present data, then, include three more examples of molecules which show carrier kinetics and whose rate of penetration is decreased by butanol.

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