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The thiourea carrier in human erythrocytes

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THE THIOUREA CARRIER IN HUMAN ERYTHROCYTES

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INTRODUCTION

The human-glycerol and human-glucose systems were investigated by LeFevre (1948). He found that the kinetics of the volume changes in glucose-saline solutions suggested a mechanism for transport of glucose into the cell other than simple diffusion. He also found that mercury, iodine and phlorizin depressed glucose penetration, which led him to suggest that the transport of glucose into human erythrocytes was effected by an active metabolic system in which at least one essential link involved a sulfhydryl group.

In subsequent investigations concerning the transport of hexoses LeFevre and LeFevre (1952) and Widdas (1952), suggested that the kinetic treatments of the facilitated transfer of sugars across cell membranes were based on a symmetrical carrier transfer mechanism. The carrier system of Widdas (1952) postulated the presence of carriers in the membrane which pass backwards and forwards across the membrane owing to thermal agitation, irrespective of whether they are saturated with hexoses or not.

LeFevre (1954) suggested that facilitated diffusion involves the following steps:

1. The formation of the substrate carrier complex on the outside of the membrane.

2. The penetration of the complex through the membrane.

3. The uncoupling of the substance from the carrier upon arrival at the inside face of the membrane.

Rosenberg and Wilbrandt (1955) proposed a carrier system similar to Widdas' except that the formation and dissociation of the carrier complex was catalyzed by an enzyme present in the membrane.

Bowyer and Widdas (1956) and Stein and Danielli (1956) suggested a polar pore system where molecules pass through pores which extend from the outside to the inside of the membrane.

Stein (1961) proposed neither the existance of pores nor a mobile carrier, but rather a dimerase. This enzyme, at least in the case of glucose and glycerol, aids in the formation of dimer solute molecules which causes a decrease in the number of free hydrophyllic groups. This allows the dimer to pass through the lipid layer more rapidly than the monomers. The dimer spontaneously disassociates into two monomers upon reaching the aqueous phase at the inner layer of the cell membrane.

According to Lieb and Stein (1971), transport in human red blood cells may proceed by internal transfer across a protein tetramer. Such a model could account for transport phenomena in other systems. Lieb and Stein (1972) suggested that transport occurs as a result of four sequences:

1. A sugar molecule binds to one of the subunits.

2. Following a conformational change, the sugar will be present within the internal cavity of the tetramer.

3. The sugar will distribute itself between the two inwardly facing binding sites according to energetic considerations.

4. A second conformational change occurs. If, while in the internal cavity, the substrate is bound to the binding site other than that on which it entered the cavity, then a transport event will have been achieved.

These studies have not only assisted in establishing the concept of facilitated diffusion involving a carrier mechanism, but also suggested new theories for glucose transport across membranes.

Hunter (1961), studying the effect of butanol on thiourea and glycerol penetration in human, rabbit, sheep and chicken erythrocytes, suggested that butanol increases the permeability in the case of simple diffusion but decreases the permeability in the case of facilitated diffusion. In each case in which it was assumed that simple diffusion occurred, n-butyl alcohol increased the thiourea permeability of the respective erythrocytes. The glycerol permeability was decreased by n-butyl alcohol in those instances where glycerol was postulated to cross the membrane by a carrier mechanism.

Subsequent studies using n-butyl alcohol and tannic acid (Hunter, George and Ospina, 1965; Ospina and Hunter, 1966), helped to distinguish between simple and facilitated diffusion systems. These data suggested that carrier

systems were found, among others, for urea in human erythrocytes. Hunter (1970), studying competitive inhibition in pigeons' erythrocytes, suggested that urea and thiourea shared the same carrier.

After research on saturation and competitive inhibition, it was found that the carrier for urea is shared with thiourea in the erythrocytes of rabbits and mice. The research suggested that thiourea entered by simple diffusion into human and rat red blood cells (Canielli et al., 1974).

Attempts to determine the half-saturation constant (ϕ) and maximum transport rate (K) for the glucose carrier were made by Widdas (1953, 1954) using sorbose-glucose competition, by LeFevre (1954) using competition between phlorentin and glucose, and by Miller (1965) using the light scattering method with glucose, mannose, and galactose.

The main purpose of this work is to determine the characteristics of the thiourea carrier in human erythrocytes taking into consideration the two parameters of transfer (half-saturation constant and maximum transport rate) and the energy of dissociation of the carrier complex. In the present experiment, the problem has been approached by observing the exit of thiourea, from cells previously equilibrated with thiourea, into a saline medium containing different concentrations of thiourea. The most important advantages of this method are the rapidity of the exit process and the fact that it can be easily measured.

MATERIALS AND METHODS

All of the experiments were carried out on human erythrocytes. Blood was obtained by venipuncture from a single individual for each series of experiments and dry heparin was used as an anticoagulant (cf. Hunter, Stringer and Weiss, 1940). The cells were washed three times with 1% NaCl, buffered to pH. 7.5 (6.05 gm Tris plus 3.45 ml concentrated HCl per liter), by centrifugation at low speeds for 3 to 5 minutes. After each centrifugation the supernatant and buffy layer were removed by aspiration.

Shrinking measurements for one volume of washed cells equilibrated with 10 volumes of 200 mM thiourea in 1% NaCl (pH. 7.5), were made by adding 0.25 ml of this cell suspension to 10 ml of 1% NaCl, with or without added penetrant in the chamber of a densimeter (Ørskov, 1935) and the rate of exit was measured (Sen and Widdas, 1962) at various temperatures (7, 12, 20, 25, 30, 35 and 40 C) with different concentrations of penetrant in the external solution (4.6, 9.5, 14.3, 18.7 and 23.1 mM).

The densimeter consists of a chamber in which the cell suspension is placed. A beam of light passes through this cell suspension and falls on a photocell which is connected to a D.C. amplifier and pen recorder. Temperature

is regulated by circulating water from a constant temperature bath through an outer jacket surrounding the chamber. The suspension is stirred to avoid settling of the cells. As the penetrant molecules leave the cells, the internal osmotic pressure is decreased, water leaves the cells and they shrink. A record of these volume changes is obtained with the pen recorder.

On the basis of a 70% hematocrit value following the washing of the cells and assuming that 50% of their volume is solvent, the internal concentration of thiourea following equilibration was calculated to be 188 mM. A small amount of thiourea outside of the cells, calculated to be 4.6 mM, was added to the salt solution with the cells. In the experiments, 0.25, 0.50, 0.75 and 1.0 ml of 200 mM penetrant were added to the external solution; thus, the maximum value of concentration outside the cell was 23.1 mM.

Tangents were drawn to the initial, steep portion of each shrinking curve. The times when these tangents intersected the base line drawn through the final equilibrium volume were measured and then plotted as a function of the external concentration of penetrant. The x-intercept of the straight line (calculated by the method of least squares) drawn through these points gives a value of $-\phi$, the concentration required for half-saturation of the carrier. These graphs were linear with external concentrations of 23.1 mM or less, although with higher concentrations outside the cells the effect was proportionately less.

Fick's First Law of Diffusion, (equation 1), gives the relationship between solute rate of transport across a cell membrane and the concentration gradient.

$$\frac{dS}{dt} = - K (C - D) \qquad (1)$$

K represents the permeability constant, C is the external solute concentration and D is the solute concentration inside the cell. The rate of penetration (dS/dt) is thus dependent upon the concentration gradient between the outside and inside faces of the membrane.

Widdas (1951, 1952, 1954) adapted equation (1) to fit those instances in which a carrier mechanism is involved in the transfer of a penetrant across the cell membrane. In instances of facilitated diffusion, he postulated that the transfer rate is proportional to the difference between the fraction of carriers combined with penetrant on the outside and inside faces of the membrane. This relationship is represented by the following equation:

$$\frac{dS}{dt} = K \left\{ \frac{C}{C + \phi} - \frac{S/V}{(S/V) + \phi} \right\} \qquad (2)$$

in which S = amount of penetrating solute in the cell, C = external medium solute concentration, K = permeability constant, V = volume of cell water, and ϕ = half saturation concentration of the carrier.

If the values of ϕ and of the concentrations are small, equation (2) can be integrated and one obtains the

expression:

$$t = \frac{(Si + \phi)}{\phi K} (C + \phi) \qquad (3)$$

in which Si = initial amount of penetrant inside the cells, C = concentration outside the cells, ϕ = half-saturation concentration of the carrier, K = maximum transport rate, and t = time (Sen and Widdas, 1962). This relation indicates that the times as measured in the manner described above should vary linearly with the outside concentration and the x-intercept of that line should give the value for $-\phi$ (Sen and Widdas, 1962).

Graphs were also made relating ϕ (mM) vs temperature, ln ϕ vs 1/T, K (isotones/min) vs temperature and ln K vs 1/T.

Miller (1965) has proposed a method for the determination of the half-saturation (K) and maximum transport rate (k) constants similar to that of Widdas but it does not require small values of ϕ and of the concentrations of penetrant.

Widdas' equation (2) is equivalent to Miller's following expression:

$$\frac{dx}{dt} = k \left(\frac{\bar{C}}{K + \bar{C}} - \frac{C}{K + C}\right) = \frac{kK (\bar{C} - C)}{(K + \bar{C}) (K + C)} \qquad (4)$$

where by k = maximum transport rate, $\bar{C}$ = external substrate concentration, K = affinity (half-saturation) constant, and C = internal substrate concentration.

Under conditions not requiring Widdas' approximations, (equation 3), one obtains the expression:

$$A\Delta t = \frac{\Delta t_o}{K} (\bar{C} + \Delta t_o) \qquad (5)$$

in which A = permeability factor, Δt_o = time interval, K = affinity (half-saturation) constant, and $\bar{C}$ = external substrate concentration where

$$A = \frac{E (E + \bar{C} - C_o)}{(E - C_o) (E + \bar{C})}$$

in which E = external osmolarity of non penetrating species, $\bar{C}$ = external substrate concentration, and C_o = internal substrate concentration. Thus by plotting $A\Delta t$ against $\bar{C}$ a straight line is obtained whose slope is $\Delta t_o/K$ and whose intercept is Δt_o.

Furthermore,

$$k = \frac{1}{\Delta t_o} (\frac{K + C_o}{E + C_o}) \qquad (6)$$

so that both k and K may be determined from such a plot.

RESULTS

Figure 1 shows a tracing of records of typical "exit" shrinking curves at 12 C and different outside concentrations of thiourea. The initial portion of these curves is linear which can readily be explained on the basis of a nearly complete saturation of the sites or carriers on the inside of the cell membrane and a low saturation of those on the outside. This low outside saturation is maintained because the external concentration is not sensibly changed by thiourea lost from the cells. As long as the inside of the membrane is nearly saturated, the process is proceeding at a constant and (at low outside concentrations) nearly maximal rate. When the inside thiourea concentration falls, so that saturation is reduced, the linear part of the record is not maintained and it curves toward the equilibrium volume.

In figure 2, the linear part of each exit record was reproduced on linear coordinates to cut the base line (representing the final equilibrium volume). The times were measured in minutes to the intersection of tangents drawn to initial, steep portion of typical shrinking curves with the horizontal base line drawn through the equilibrium volume. The times for the various experiments (Table I)

were plotted against the outside concentrations at different temperatures as shown in figures 3, 4, 5, 6, 7, 8 and 9.

From figure 2 it can be seen that, as the concentration of thiourea outside the cells is increased, the initial slope decreases; the time for the tangent to intersect the base line representing final equilibrium volume increases and the total deflection decreases slightly.

The Widdas' ϕ values were obtained from the graphs relating external concentration of thiourea and times. The K values were obtained from the expression $t = \frac{(Si + \phi)}{\phi K}$ $(C + \phi)$ solving for K for each value of C and averaging at each temperature. These data are summarized in table II.

The half-saturation constant (ϕ) was found to decrease from 7 - 20 C but increase from 20 - 40 C (Fig. 10). The maximum transport rate (K) did not change appreciably at low temperatures but increased markedly at higher temperatures (Fig. 12).

Since the half-saturation concentration is nearly analogous to the Michaelis-Menten constant of an enzyme reaction, a plot of the logarithm of this constant against the reciprocal of the absolute temperature was used to demonstrate the linear relationship. The result is shown in figure 11.

Such plots, first suggested by Van't Hoff, have been extensively used in enzyme studies (Dixon and Webb, 1958). The results plotted in this way are V-shaped with two linear

portions. From the slope of the continuous line obtained at higher temperatures (Fig. 11), the energy of dissociation of the carrier-penetrant complex was calculated to be approximately 8,500 cal/mol.

On the other hand, a similar plot of values for the maximum transport rate was not V-shaped but was linear (Fig. 13).

TABLE I

Average exit time in minutes of thiourea from human erythrocytes at different temperatures and concentrations.

TABLE I

Temperatures	Concentrations				
	4.6 mM	9.5 mM	14.3 mM	18.7 mM	23.1 mM
7 C	0.95	0.98	1.00	1.01	1.14
12 C	0.94	0.98	1.05	1.09	1.15
20 C	0.66	0.77	0.79	0.89	0.99
25 C	0.48	0.56	0.60	0.60	0.65
30 C	0.44	0.48	0.48	0.50	0.55
35 C	0.33	0.35	0.36	0.38	0.41
40 C	0.24	0.25	0.25	0.25	0.25

TABLE II

Values of half-saturation (ϕ) and maximum transport rate (K) for the thiourea carrier in human erythrocytes.

TABLE II

	Widdas'		Miller's	
Temp.	ϕ mM	K isotones/min	ϕ mM	K isotones/min
7 C	104.7	1.1	54.3	2.5
12 C	77.7	1.0	49.9	2.5
20 C	34.5	1.3	25.9	3.4
25 C	57.1	1.8	41.7	4.5
30 C	75.9	2.1	51.5	5.3
35 C	75.9	2.9	50.7	7.1
40 C	310.6	7.0	118.8	11.7

FIGURE 1. Shrinking of human erythrocytes at 12 C with different outside concentrations of thiourea.

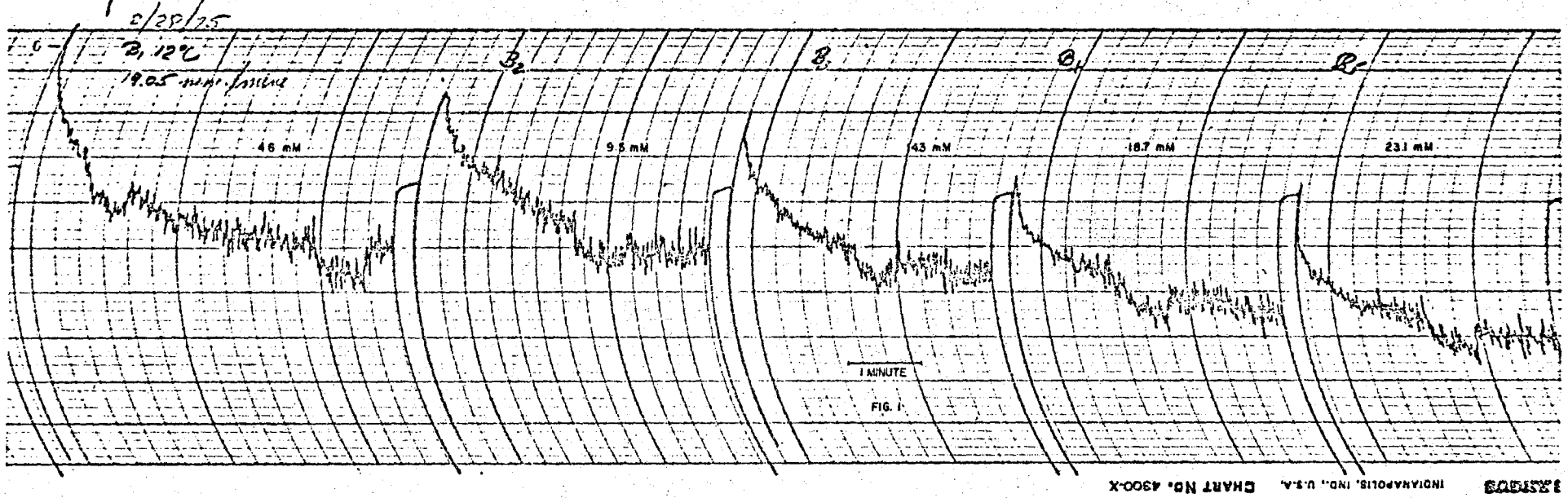
1 c/28/75
B, 12°C
19.05 mm. freezing
B2
B3
B4
B5
4.6 mM
9.5 mM
14.3 mM
18.7 mM
23.1 mM
1 MINUTE
FIG. 1
INDIANAPOLIS, IND., U.S.A. CHART NO. 430O-X

FIGURE 2. Initial portion of typical shrinking curves at 12 C with different outside concentrations of thiourea. Human erythrocytes were previously equilibrated with 200 mM thiourea in 1% NaCl. An aliquot of this cell suspension was added to 1% NaCl with increasing concentrations of thiourea. The linear part of each exit record was reproduced on linear coordinates to cut the base line (representing the final equilibrium volume). The times were measured in minutes to the intersection of tangents drawn to initial, steep portion of typical shrinking curves with horizontal line drawn through equilibrium. Average of 9 experiments.

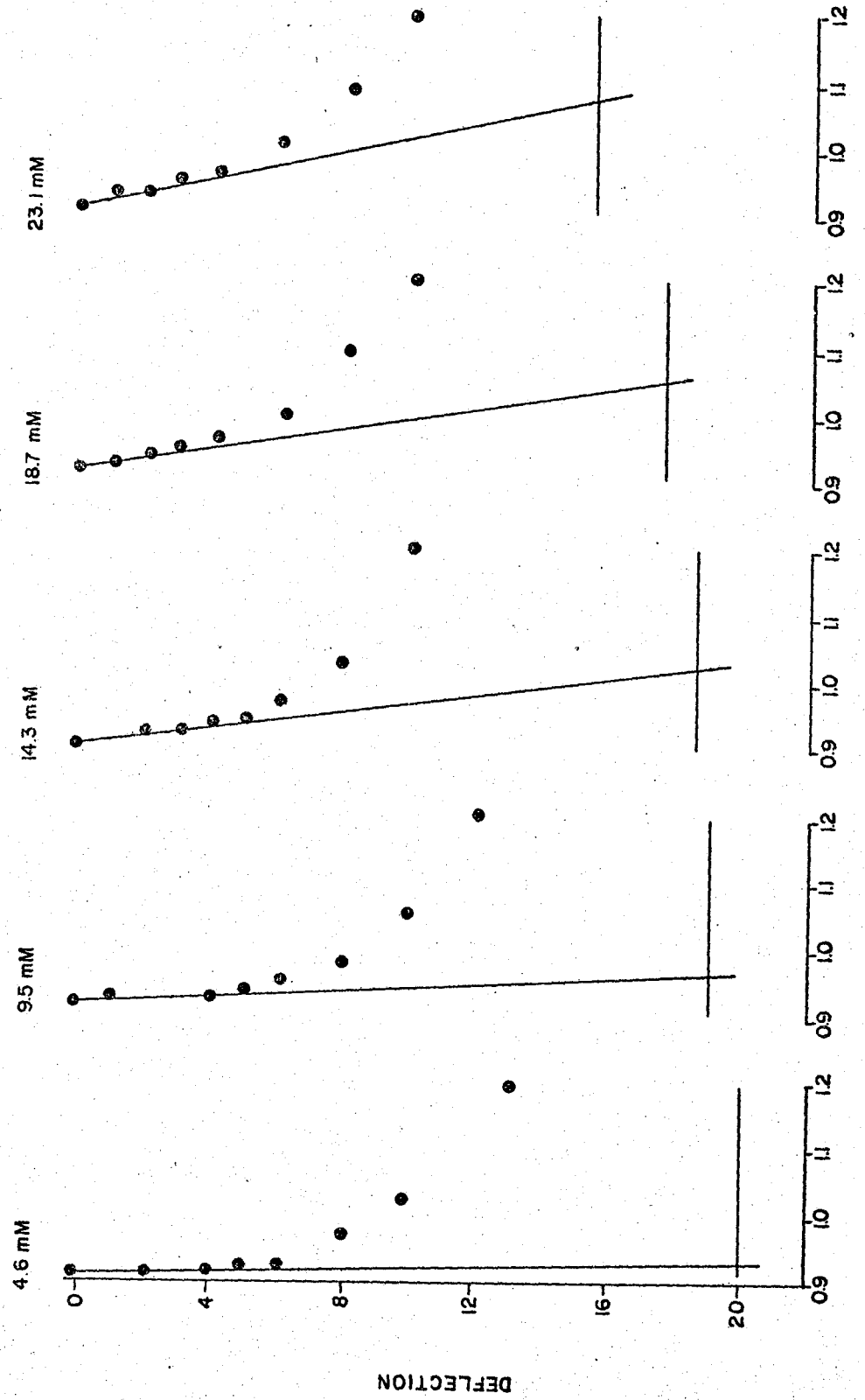
23.1 mM
18.7 mM
14.3 mM
9.5 mM
4.6 mM
0
4
8
12
16
20
DEFLECTION
0.9
1.0
1.1
1.2
TIME IN MINUTES

FIG. 2

FIGURE 3. Exit times obtained from typical shrinking curves at 7 C plotted against the concentrations of thiourea in the outside media.

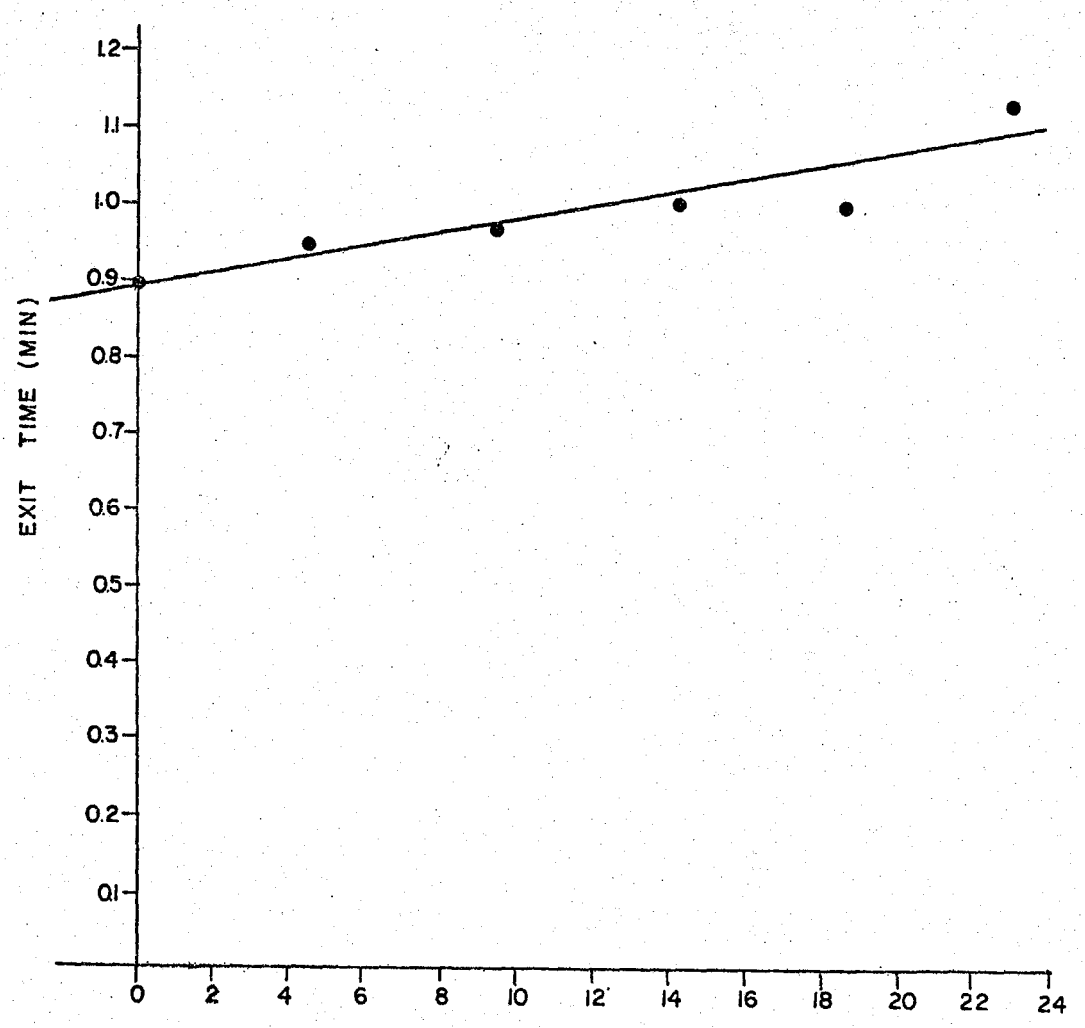
EXIT TIME (MIN)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
8
10
12
14
16
18
20
22
24
CONCN. OUTSIDE (mM)

FIG. 3

FIGURE 4. Exit times obtained from typical shrinking curves at 12 C plotted against the concentrations of thiourea in the outside media.

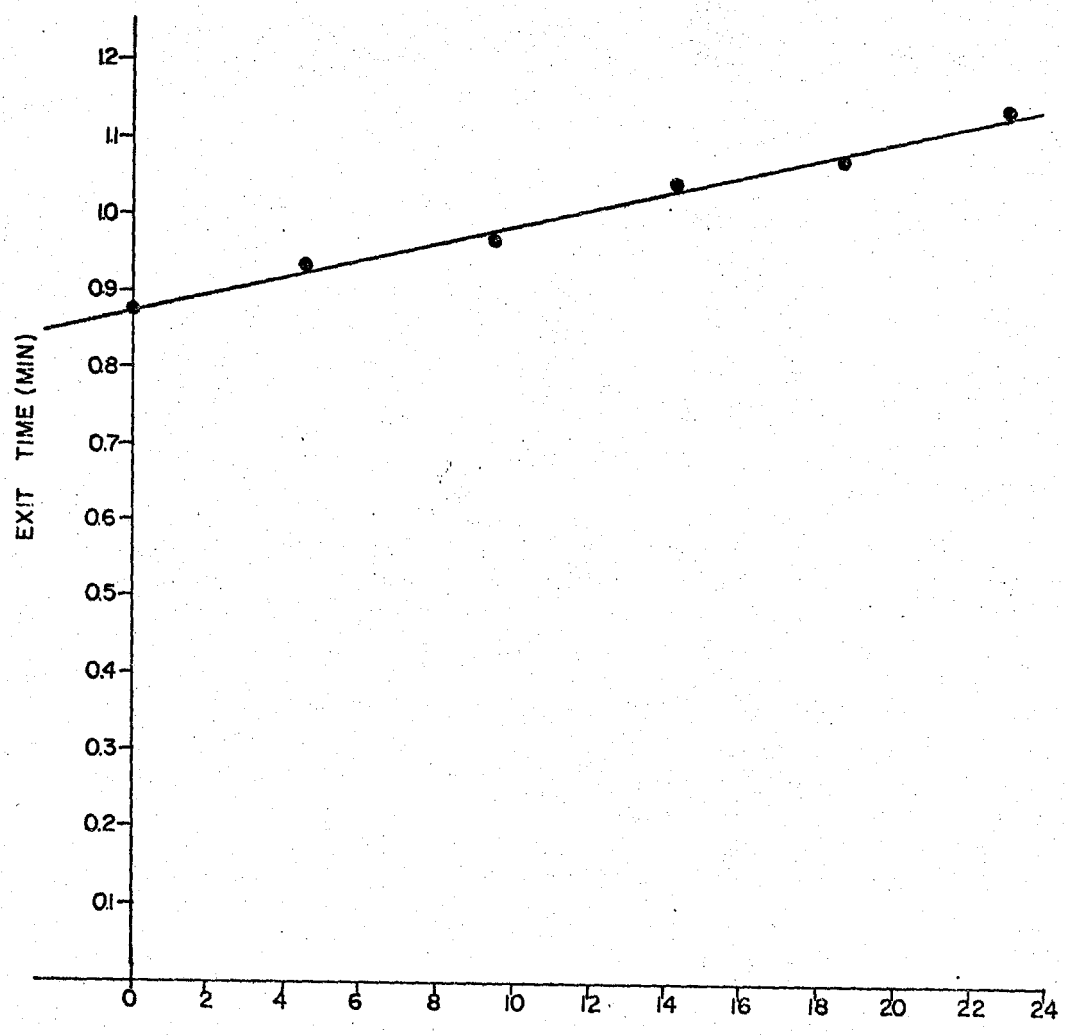
EXIT TIME (MIN)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
8
10
12
14
16
18
20
22
24
CONCN. OUTSIDE (mM)

FIG. 4

FIGURE 5. Exit times obtained from typical shrinking curves at 20 C plotted against the concentrations of thiourea in the outside media.

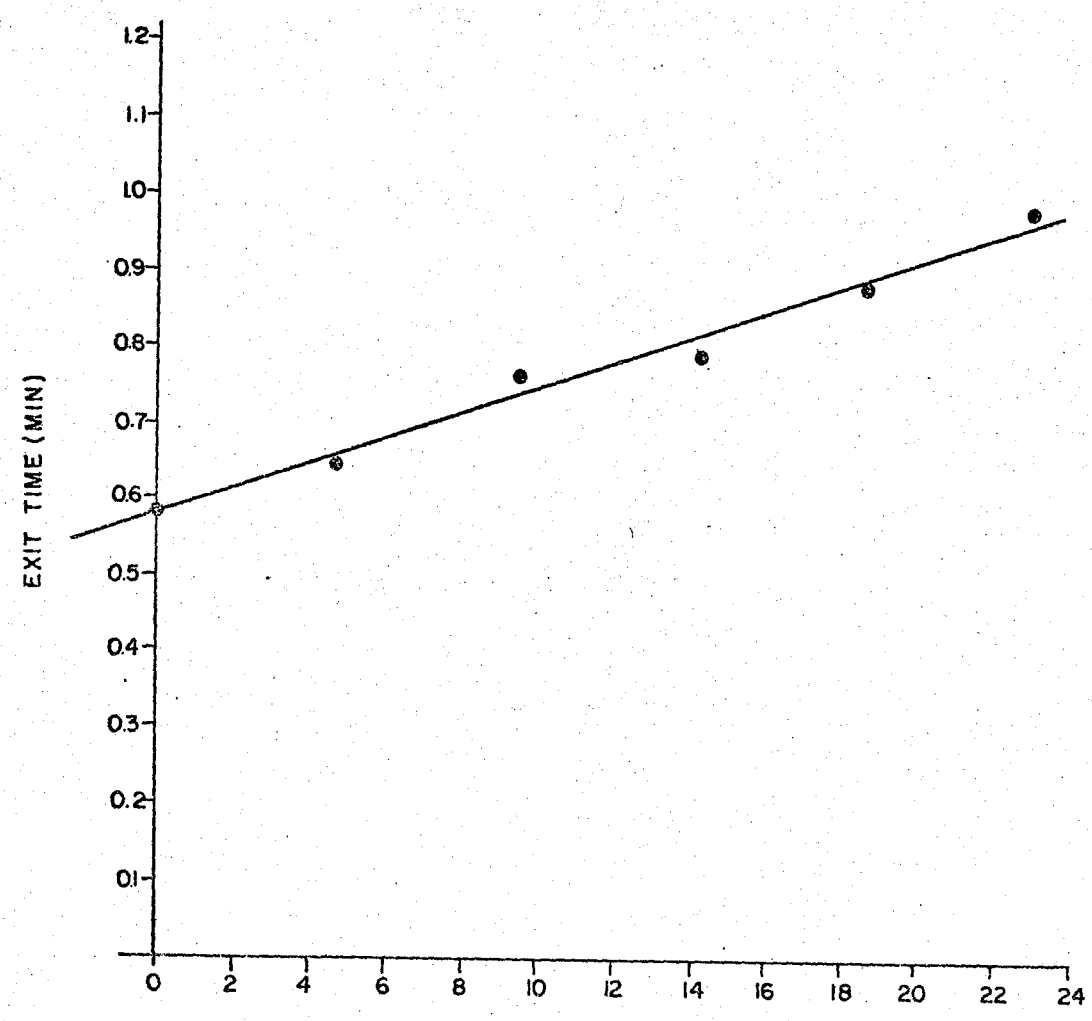
EXIT TIME (MIN)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
8
10
12
14
16
18
20
22
24
CONCN. OUTSIDE (mM)

FIG. 5

FIGURE 6. Exit times obtained from typical shrinking curves at 25 C plotted against the concentrations of thiourea in the outside media.

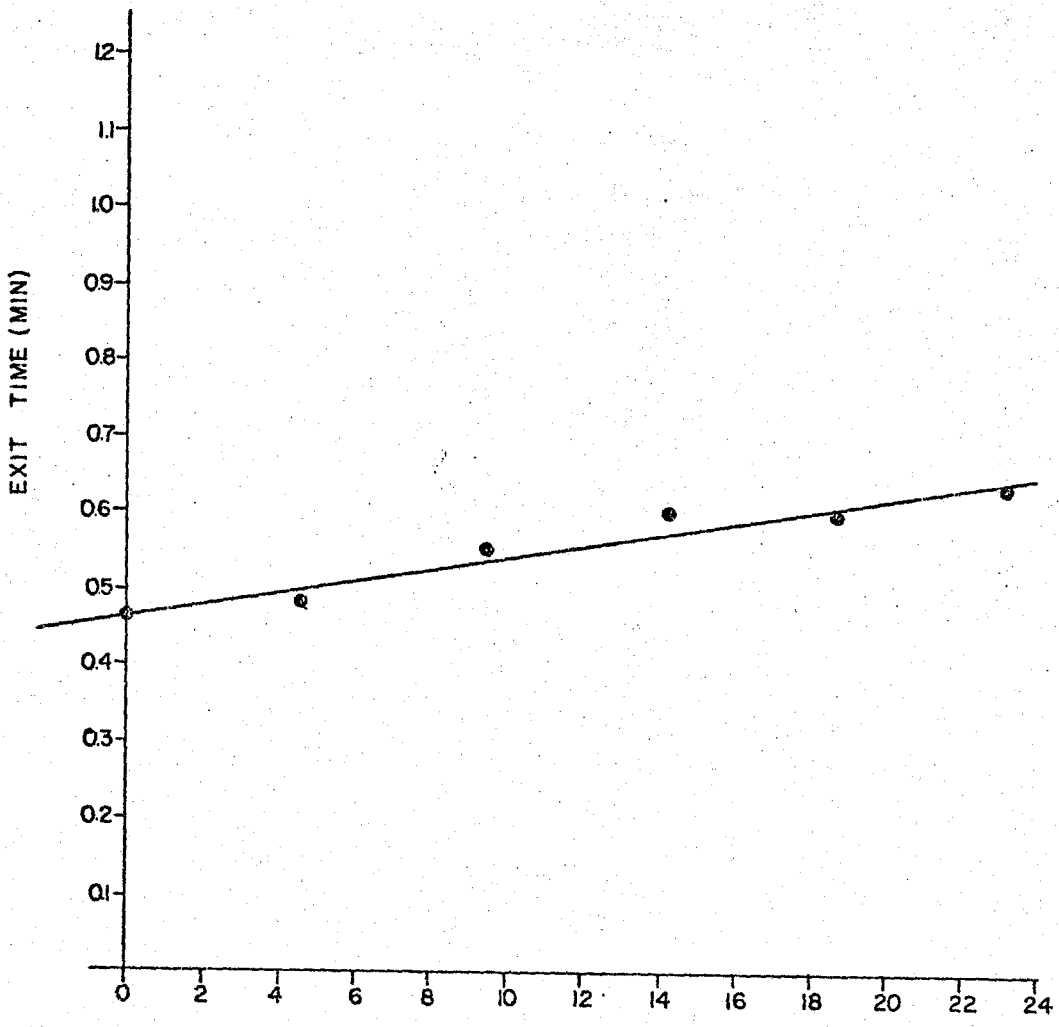
EXIT TIME (MIN)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
8
10
12
14
16
18
20
22
24
CONCN. OUTSIDE (mM)

FIG. 6

FIGURE 7. Exit times obtained from typical shrinking curves at 30 C plotted against the concentrations of thiourea in the outside media.

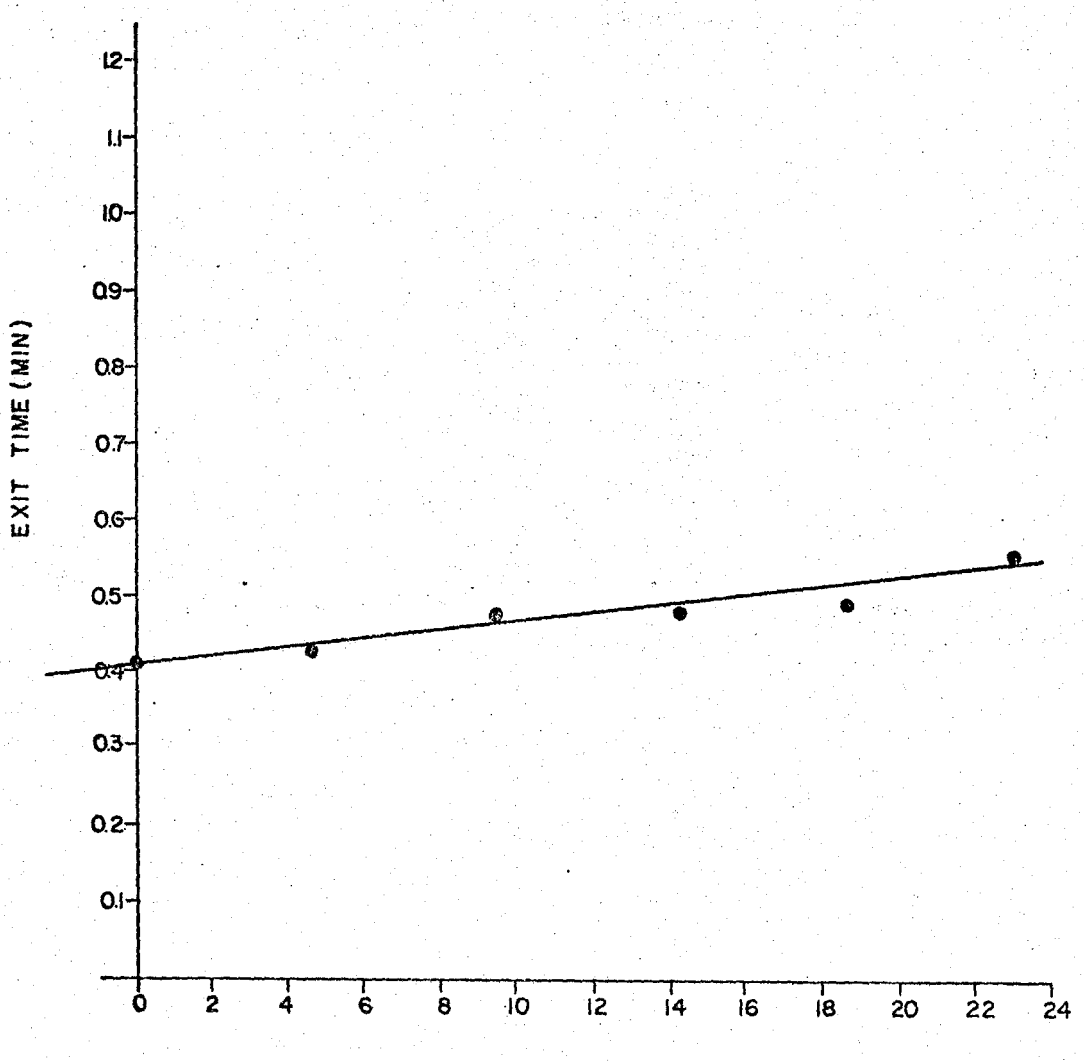
EXIT TIME (MIN)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
8
10
12
14
16
18
20
22
24
CONCN. OUTSIDE (m M)

FIG. 7

FIGURE 8. Exit times obtained from typical shrinking curves at 35 C plotted against the concentrations of thiourea in the outside media.

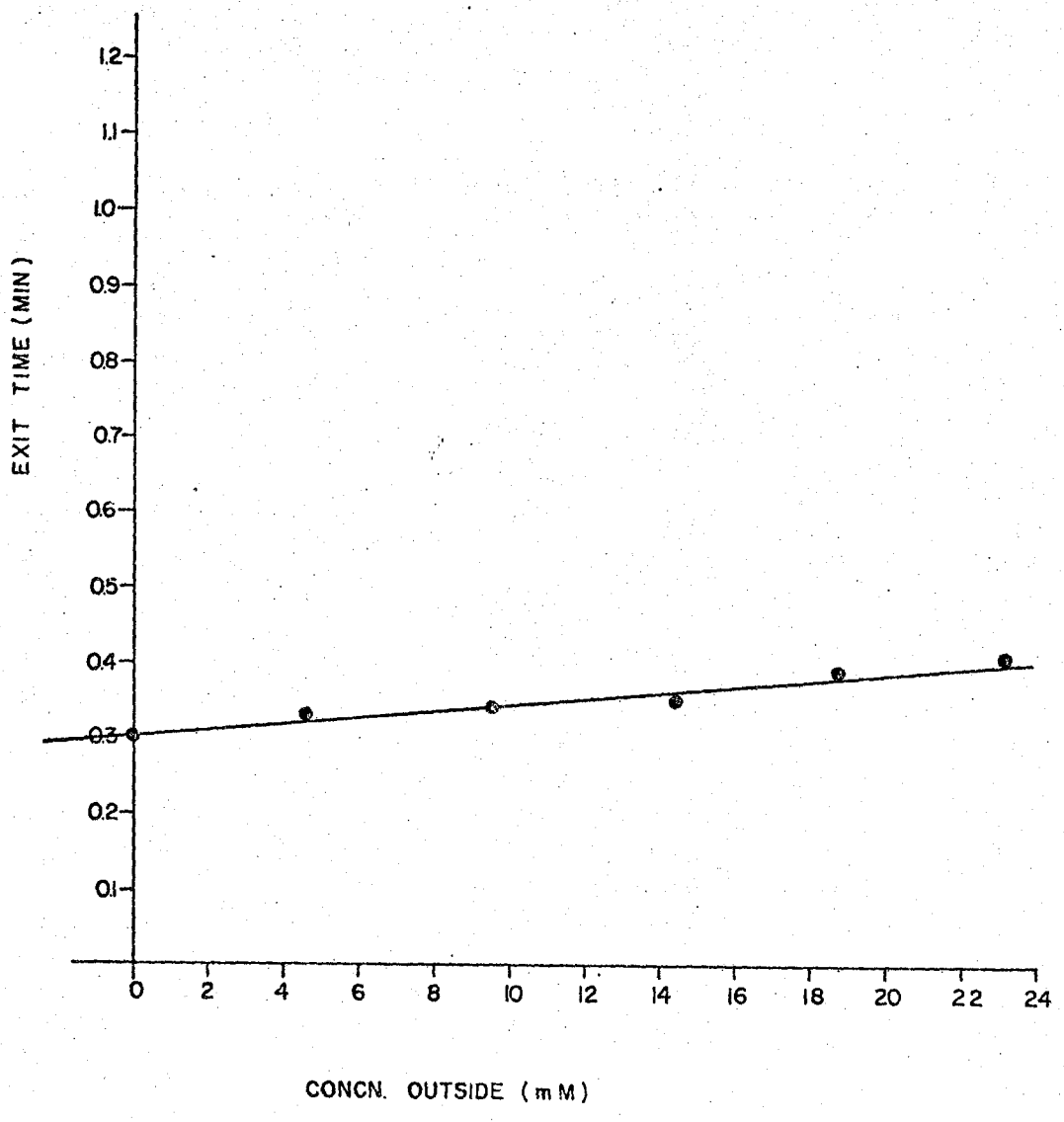
EXIT TIME (MIN)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
8
10
12
14
16
18
20
22
24
CONCN. OUTSIDE (m M)

FIG. 8

FIGURE 9. Exit times obtained from typical shrinking curves at 40 C plotted against the concentrations of thiourea in the outside media.

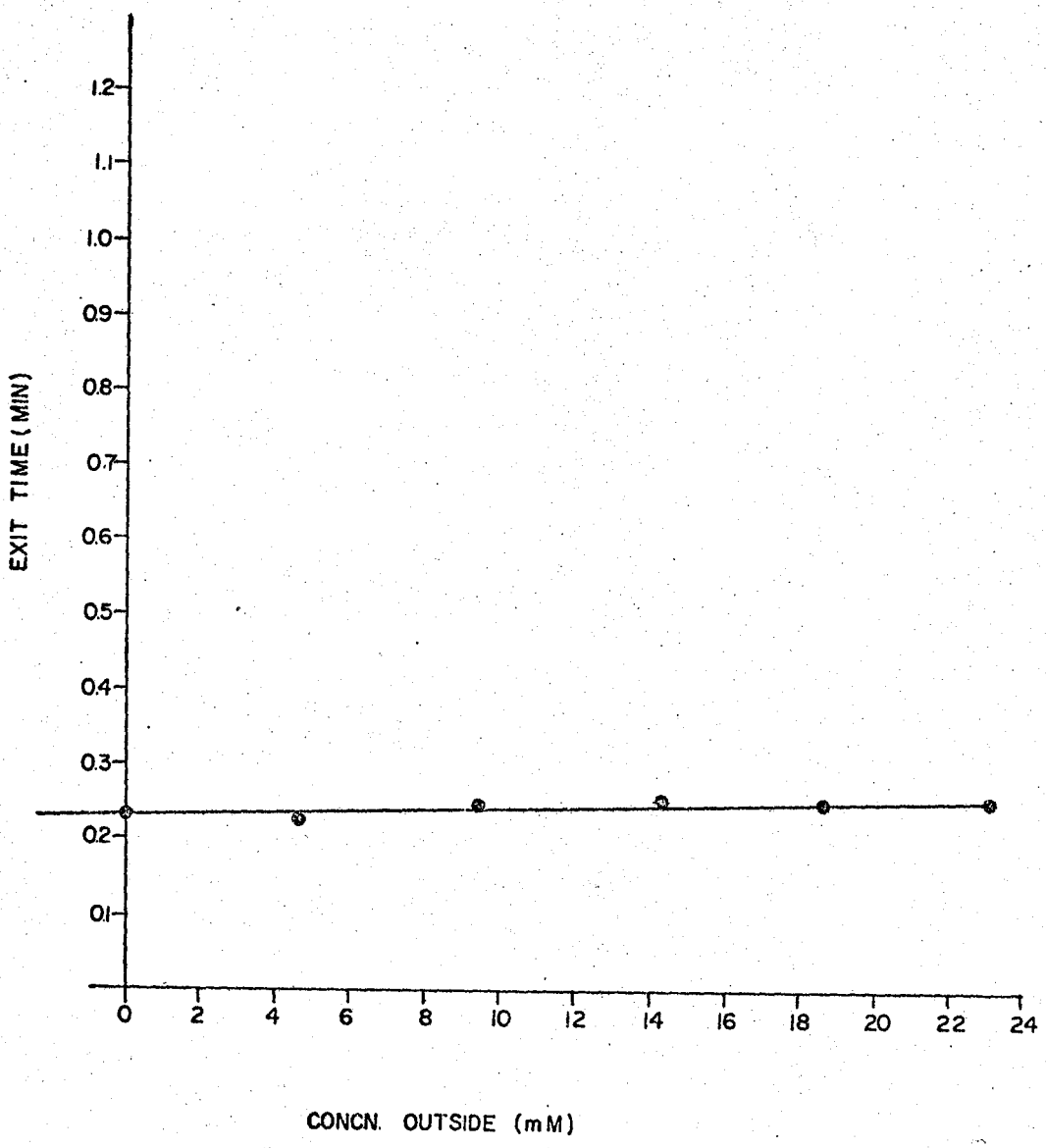
EXIT TIME (MIN)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
8
10
12
14
16
18
20
22
24
CONCN. OUTSIDE (mM)

FIG. 9

FIGURE 10. The effect of temperature on the half-saturation constant of the thiourea carrier in human erythrocytes.

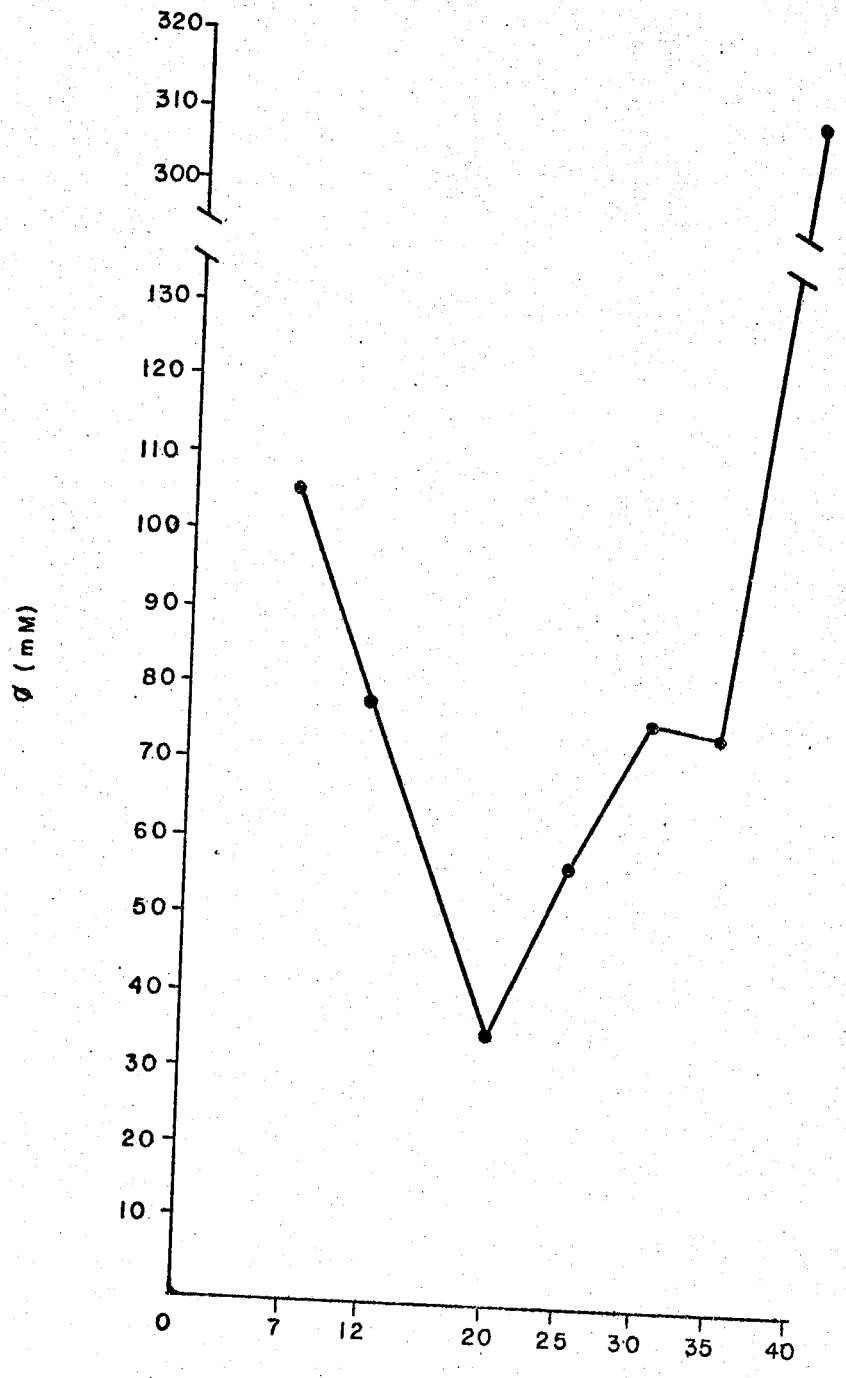
320
310
300
130
120
110
100
90
80
70
60
50
40
30
20
10
0
7
12
20
25
30
35
40
Ø (mM)
TEMPERATURE (°C)

FIG. 10

FIGURE 11. The Van't Hoff plot of data for the half-saturation constant. The slope of the continuous line indicates that an energy of 8,500 cal/mol is required for dissociation of the thiourea carrier complex.

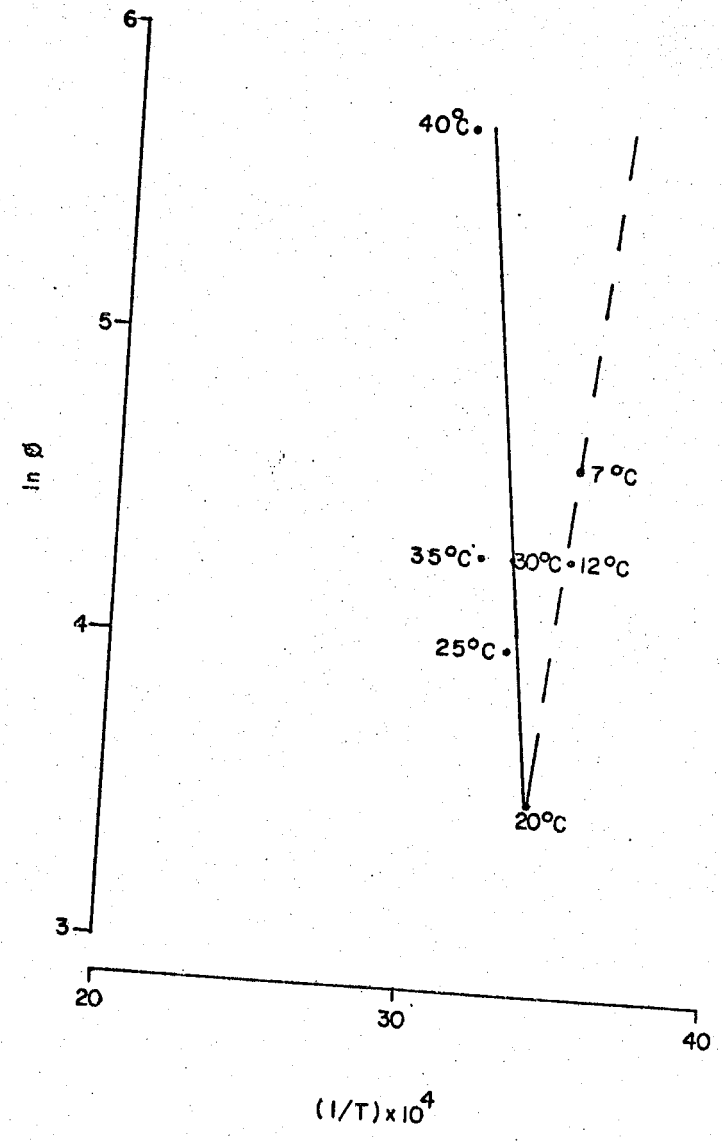

FIG. 11

FIGURE 12. The effect of temperature on the maximum transport rate of the thiourea carrier in human erythrocytes.

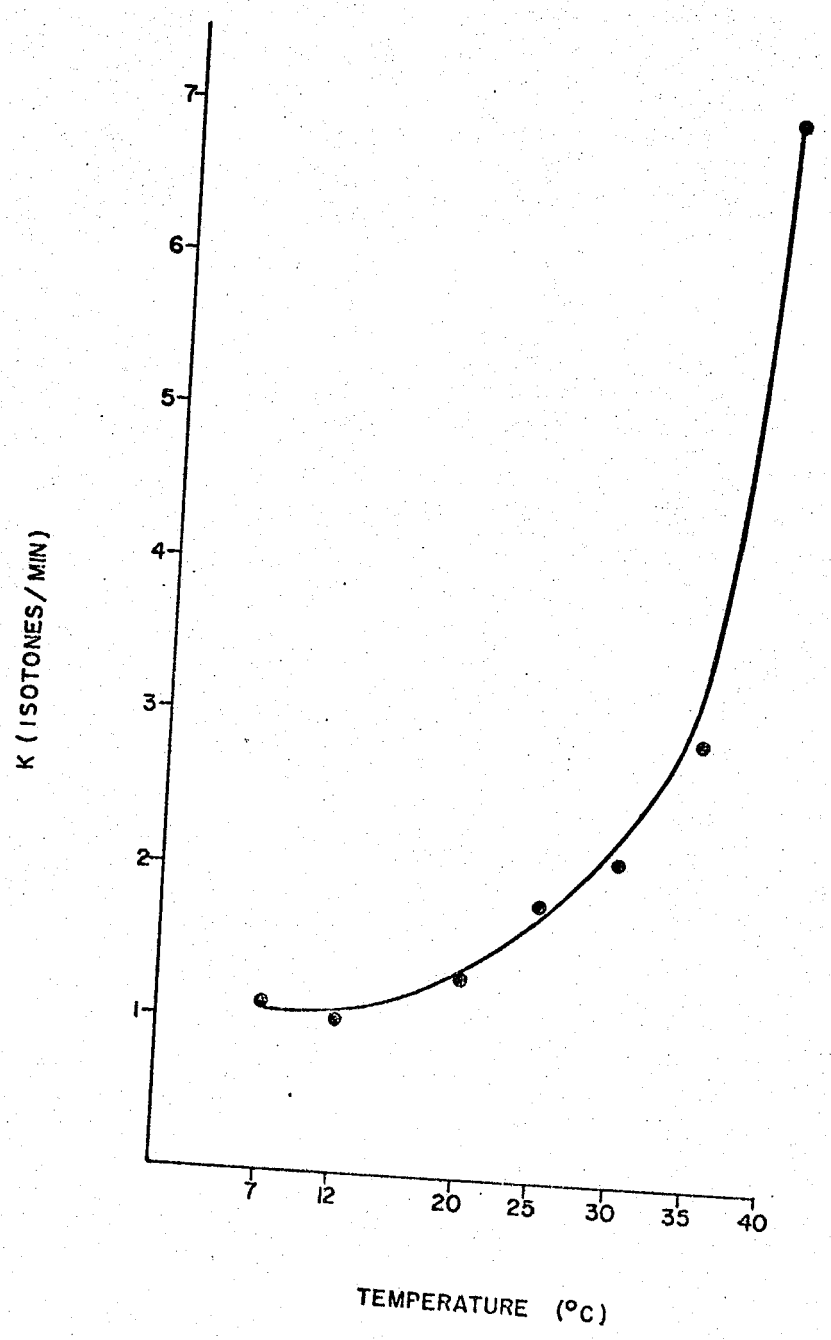

FIG. 12

FIGURE 13. The Van't Hoff plot of data for maximum transport rate.

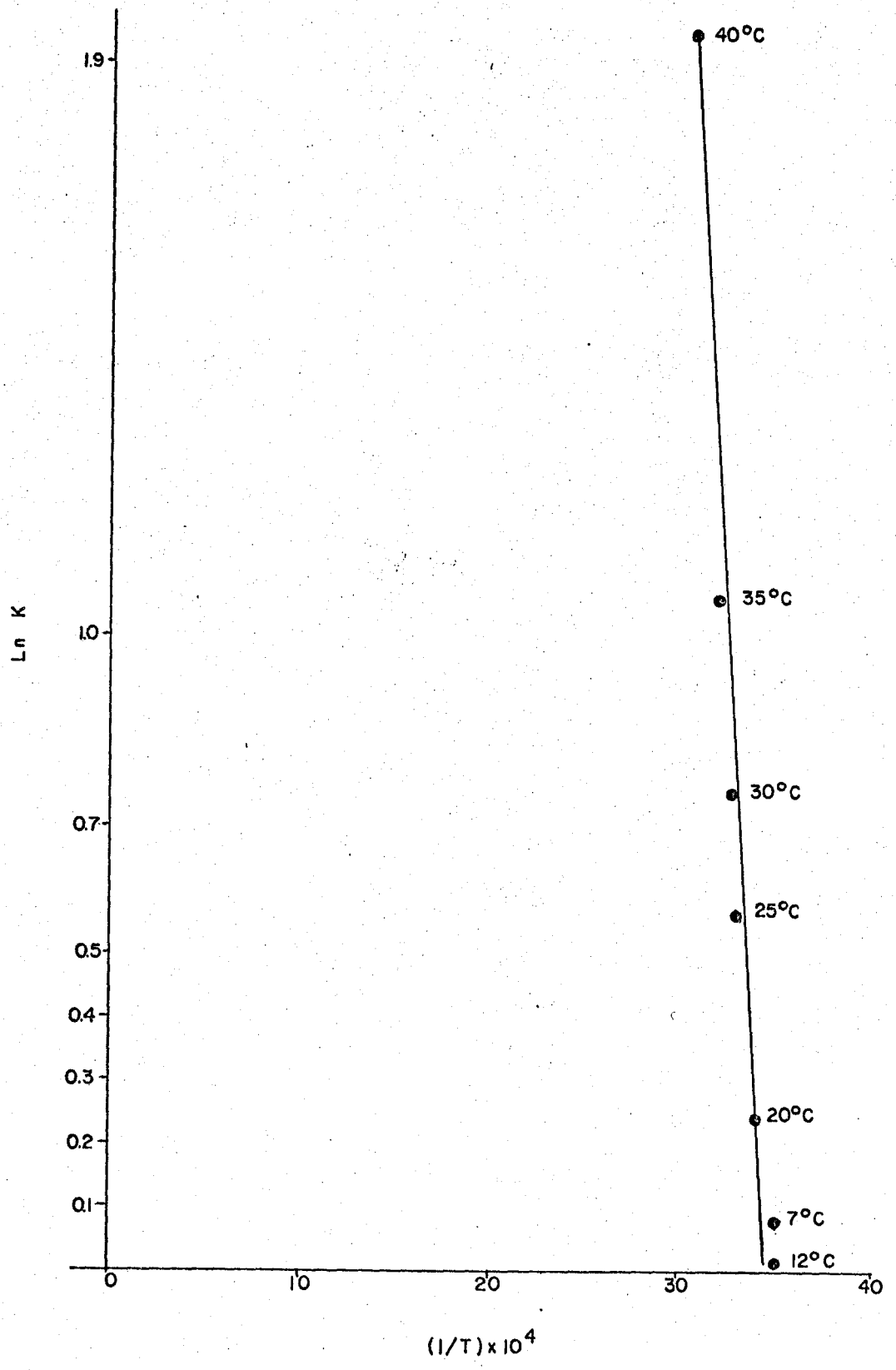
40°C
35°C
30°C
25°C
20°C
7°C
12°C
1.9
1.0
0.7
0.5
0.4
0.3
0.2
0.1
Ln K
0
10
20
30
40
(1/T) x 10^4

FIG. 13

DISCUSSION

Values of half-saturation constant (ϕ) and maximum transport rate (K) have been calculated by Sen and Widdas (1962) using data obtained with a photo-electric method and by Miller (1965) from data obtained using a light scattering technique. The method of Widdas requires that the concentrations of penetrant and the half-saturation constant are small. Miller's method does not require these conditions and therefore should be applicable to a wider range of experimental conditions. According to Miller (1965), his method should give higher values of half-saturation constants than Widdas' method. Miller (personal communication) subsequently agreed that the reverse should be true. In the present work, the values for the half-saturation constant obtained by Widdas' method were higher than those obtained by Miller's method.

Widdas' method assumes that concentrations and half-saturation constant are small. When half-saturation constant and concentrations are not small, the expression given in equation (3) can be obtained if one treats volume as a constant. Since the densimeter technique depends on volume changes, this introduces an error which, however, is not great if one considers only the initial, steep

portion of the shrinking curves.

Sen and Widdas (1962) measuring the rate of exit of glucose from human red cells by a photo-electric method obtained a value of 4 mM for the half-saturation constant at 37 C. The maximum transport rate as obtained by Widdas in his experiment at 37 C is approximately 0.8 isotones/min. Thus, it can be seen that the thiourea carrier for human erythrocytes has half-saturation and maximum transport rate values considerably larger than the human-glucose system.

The effect of temperature on the half-saturation constant and maximum transport rate of the glucose carrier for human erythrocytes was studied by Sen and Widdas (1962). They found that both of these parameters for this carrier increased as the temperature increased, but the change was not linear. In the present study, the half-saturation constant has been found to decrease at temperatures of 7 C to 20 C but to increase at temperatures of 20 C to 40 C (Fig. 10). The maximum transport rate did not change appreciably at low temperatures but increased markedly at higher temperatures (Fig. 12). It did not vary linearly which is in agreement with the data of Sen and Widdas.

In a Van't Hoff plot of data for the half-saturation constant, Sen and Widdas (1962) found a linear relationship and from the slope of the line they calculated the energy of dissociation of the carrier-penetrant complex to be approximately 10,000 cal/mol. The data obtained in

the present research when plotted in the same way show a V-shaped figure with two linear portions (Fig. 11). The dissociation energy for the carrier-penetrant complex calculated from the slope of the continuous line obtained at higher temperatures (Fig. 11) was 8,500 cal/mol.

The V-shaped figure with a break at 20 C (possible transition point) may suggest a conformation change in the carrier.

The present study demonstrates that the carrier for thiourea in human erythrocytes has different values for the half-saturation constant and maximum transport rate at different temperatures, as shown in table II.

LeFevre (1962) and Miller (1965) have shown that the maximum transport rate is the same for all sugars carried by a given transport system. LeFevre (1962) obtained a maximum transport rate of approximately 2 isotones/min at 37 C for six different sugars having a wide range of half-saturation constants. Similar results were obtained by Miller (1965) for three sugars tested. This might suggest that for the same penetrant carried by a transport system in different cells the characteristics of the carrier are not necessarily going to be the same. Because of that, it would be of interest to determine in further investigations whether or not the thiourea carrier in different red blood cells would have the same characteristics as those in human erythrocytes.

SUMMARY

1. A photo-electric method was used to measure the rate of exit of thiourea from human erythrocytes.

2. The half-saturation constant (ϕ) and the maximum transport rate (K) for the thiourea carrier were found at different temperatures using times measured on the exit curves. The half-saturation constant (ϕ) was found to increase at low and high temperatures with a break at 20 C. The maximum transport rate (K) did not change appreciably at low temperatures but increased markedly at higher temperatures.

3. This system had half-saturation values (ϕ) of the carrier considerably larger than the human-glucose system; consequently, higher concentrations of penetrant had to be used.

4. From the temperature dependence it was calculated that 8,500 cal/mol are required for the dissociation of the complex which thiourea is presumed to form with a component of the membrane.

LITERATURE CITED

Bowyer, F. and W. F. Widdas. 1956. The facilitative transfer of glucose and related compounds across the erythrocyte membrane. Disc. Faraday Soc. 21: 251-258.

Cainelli, Stephen R., Chui, Arthur, McClure, J. D. Jr. and Hunter, F. R. 1974. Facilitated diffusion in erythrocytes of mammals. Comp. Biochem. Physiol. 48A: 815-825.

Dixon, M. and Webb, E. C. 1958. Enzymes, pp. 8-34, 62-181. London: Longmans Green and Co.

Hunter, F. R. 1940. Heparin as an anticoagulant for permeability studies. Science 91: 579-580.

Hunter, F. R. 1961. The effect of n-butyl alcohol on the permeability of erythrocytes to non-electrolytes. J. Cell. Comp. Physiol. 58: 203-216.

Hunter, F. R., J. George and B. Ospina. 1965. Possible carriers in erythrocytes. J. Cell Comp. Physiol. 65: 299-312.

Hunter, F. R. 1970. Facilitated diffusion in human erythrocytes. Am. J. Physiol. 218: 1765-1772.

LeFevre, P. G. 1948. Evidence of active transfer of certain non-electrolytes across the human red cell membrane. J. Gen. Physiol. 31: 505-527.

LeFevre, P. G. and M. E. LeFevre. 1952. The mechanism of glucose transfer into and out of the human red cell. J. Gen. Physiol. 35: 891-906.

LeFevre, P. G. 1954. The evidence for active transport of monosaccharides across the red cell membrane. Symp. Soc. Exptl. Biol. 8: 118-135.

LeFevre, P. G. 1962. Rate and affinity in human red blood cell sugar transport. Am. J. Physiol. Vol. 203: 286-290.

Lieb, W. R. and W. D. Stein. 1971. New theory for glucose transport across membranes. Nature New Biology, Vol. 230: 108-109.

Lieb, W. R. and W. D. Stein. 1972. Carrier and non-carrier models for sugar transport in the human red blood cell. Biochim. Biophys. Acta. 265: 187-207.

Miller, D. M. 1965. Determination of transport constants for sugar movements in human erythrocytes. Biophysical Journal. 5: 407-415.

Miller, D. M. Personal Communication.

Ørskov, S. L. 1935. Eine methode zur fortlanfenden photographischen aufzeichnung von volumänderunger der roten blutkörperchen. Biochem. Z. 279: 250-261.

Ospina, B. and F. R. Hunter. 1966. Facilitated diffusion in mouse and rat erythrocytes. Nature. 211: 851.

Rosenberg, T. and W. Wilbrandt. 1955. The kinetics of membrane transport involving chemical reactions. Exptl. Cell Res. 9: 49-67.

Sen, A. K. and W. F. Widdas. 1962. Determination of temperature and pH. dependence of glucose transfer across the human erythrocytes membrane measured by glucose exit. J. Physiol. 160: 392-403.

Stein, W. D. and J. F. Danielli. 1956. Structure and function in red cell permeability. Disc. Faraday Soc. 21: 239-251.

Stein, W. D. 1961. Dimer formation and glucose transfer across the membrane of the red blood cell. Nature, Vol. 191, No. 4795: 1277-1280.

Widdas, W. F. 1951. Inability of diffusion to account for placental glucose transfer in the sheep. J. Physiol. 115: 36-72.

Widdas, W. F. 1952. Inability of diffusion to account for placental transfer in the sheep and consideration of the carrier kinetics of a possible carrier transfer. J. Physiol. 118: 23-39.

Widdas, W. F. 1953. Kinetics of glucose transfer across the human erythrocyte membrane. J. Physiol. 120: 23-24.

Widdas, W. F. 1954. Facilitated transfer of hexoses across the human erythrocyte membrane. J. Physiol. 125: 163-180.