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Comparative Study of the Thiourea Carrier in Erythrocytes

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Summary. A densimeter technique was used to measure the rate of exit of thiourea from erythrocytes of various species of mammals. The cells were first equilibrated with a 200 mM thiourea solution in 1% NaCl. An aliquot of these cells was added to 1% NaCl containing 4.6–23.1 mM thiourea. Facilitated diffusion was demonstrated in each case. Using exit times or initial rates, calculations of half-saturation constants (ϕ) in mM and maximum transport rates (K) in isotones per min were made by three different methods. The following values were obtained: human – ϕ = 60, 42, 35; K = 1.2, 2.9, 0.9; rabbit – ϕ = 46, 33, 32; K = 0.8, 2.1, 0.8; mouse – ϕ = 46,40, 30; K = 3.4, 8.5, 3.2; rat – ϕ = 65, 42, 23; K = 6.1, 15.3, 3.7; ox – ϕ = 107, 63, 88; K = 0.6, 1.4, 0.4; sheep – ϕ = 56, 38, 56; K = 0.9, 2.2, 0.6; and pig – ϕ = 110, 64, 49; K = 1.6, 3.6, 1.1.

Jacobs and Parpart (1937) reported that butanol accelerated the hemolysis in glycerol of erythrocytes of some species but decreased the rate of hemolysis with other species. With hemolysis in thiourea, however, butanol always accelerated. In a study of the effect of butanol on the permeability of erythrocytes of several species to a number of different penetrants, it was suggested that butanol increases the permeability in the case of simple diffusion but decreases the permeability in the case of facilitated diffusion (Hunter, 1961; Hunter, George & Ospina, 1965). Evidence in support of this suggestion has been presented for a number of cases (Hunter, 1970*a, b,* Cainelli, Chui, McClure & Hunter, 1974). Studies of saturation and of competitive inhibition led to the conclusion that thiourea shares the carrier for urea in erythrocytes of rabbit and mouse but enters by simple diffusion into erythrocytes of man and rat (Cainelli *et al.*, 1974). These observations raised the possibility of rather fine distinctions in "recognition sites" of the urea carrier in erythrocytes of different species.

In connection with another study, the rate of exit of thiourea from red cells of ox and sheep was measured to illustrate certain aspects of the kinetics of simple diffusion. The data, however, indicated that the kinetics were those of facilitated, rather than of simple, diffusion (Hunter, Fayad & Mayorga, 1976). This observation suggested that the question of thiourea permeability of erythrocytes of various species should be reinvestigated.

Materials and Methods

Human and sheep (*Ovis aries*) bloods were obtained by venipuncture, rat (*Rattus norvegicus*) and mouse (*Mus musculus*) bloods by decapitation and rabbit (*Oryctolagus cuniculus*) blood by cardiac puncture. Heparin was used with these bloods. Ox (*Bos taurus*) and pig (*Sus scrofa*) bloods were obtained from a slaughter house and citrate or heparin was used as an anti-coagulant. As a minimum, three series of measurements were made on blood from at least three different individuals. All bloods were washed 3 times with 1% NaCl (pH 7.5−Tris). One volume of washed cells was equilibrated with 10 volumes of 200 mM thiourea in 1% NaCl (pH 7.5). An aliquot (0.25 ml) of this cell suspension was added to 10 ml of 1% NaCl, with or without added thiourea, in the chamber of a densimeter and the rate of exit was measured (*cf.* Sen & Widdas, 1962). Most of the data were obtained using a densimeter in which the photocell was connected to a sensitive galvanometer. Readings were taken at fixed time intervals and shrinking curves were plotted from these observations. Measurements were made at room temperature (20–21 °C). Other data were obtained using a densimeter with a DC-amplifier and a pen recorder (*cf.* Mawe, 1956). With this apparatus, temperature was maintained at 20 °C by a constant-temperature water bath.

The internal concentration of thiourea following equilibration was calculated to be 188 mM on the basis of a 70% hematocrit following the washing of the cells and assuming that 50% of the volume of the cells was solvent. A small amount of thiourea outside of the cells was added to the salt solution with the cells. This was calculated to be 4.6 mM. Exit measurements were made with different external concentrations of thiourea. A tangent was drawn to the initial, steep portion of each shrinking curve. The time when this tangent intersected a horizontal line drawn through the equilibrium volume was measured [Δt− Eq. (2)]. These times were then plotted against the external concentration of penetrant. The x-intercept of the straight line drawn through these points gives a value of $-\phi$, the concentration required for half-saturation of the carrier [Eq.(2)]. This type of graph will be referred to as "plot 1". These graphs were linear with external concentrations of 23.1 mM or less. With higher concentrations outside the cells the effect was proportionately less. The slopes of these exit curves were measured graphically and were expressed as dV/dt. Numerically, $dS/dt=(1+C)\ dV/dt$ as indicated in the derivation of Eq.(3). The reciprocal of the slope *vs.* outside concentration gave "plot 2" [Eq.(3)]. The intercepts in "plots 1 and 2" were calculated by the method of least squares.

Sen and Widdas (1962) and Widdas (*personal communication*) have shown the following relationships.

The basic equation is:

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

S=amount of substance inside the cells; t=time; K=a constant; C=external concentration; V=volume of cell water; and ϕ=concentration required to half-saturate the carrier. Concentrations and ϕ are expressed in isotonic units.

If C and ϕ are small with reference to 1 isotone, the following can be obtained:

$$\Delta t=\frac{(S_i+\phi)}{\phi K}\quad(C+\phi). \tag{2}$$

Δt=time to reach equilibrium if exit had continued at initial rate; S_i=initial amount of substance in the cells.

Since $V=\frac{1+S}{1+C}$, by differentiation one obtains the expression:

$$\frac{dV}{dt}=\frac{1}{1+C}\frac{dS}{dt}$$

and

$$\frac{dV}{dt}=-\frac{K\phi}{(1+C)(C+\phi)}$$

or

$$1\Big/\left(\frac{dV}{dt}\right)=-\frac{(1+C)}{K\phi}(C+\phi). \tag{3}$$

If C is not too large, a plot of the reciprocal of the initial slope (dV/dt) against the outside concentration should give a straight line. If C is larger, dS/dt should be used. The x-intercept$=-\phi$ and the y-intercept$=1/K$.

For simple diffusion $dS/dt=K(C-S/V)$. (4)

A plot of initial rate against difference in concentration on the two sides of the membrane should give a straight line passing through the origin.

Miller (1965*a*) considered the experimental situation in which concentrations and values for the half-saturation constant of the carrier are not small in comparison with unity. Under these conditions, experimental times should be multiplied by a factor, A.

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

in which isotonic units are used and E=external concentration of nonpenetrating species; C=external penetrant concentration; C_o=internal penetrant concentration at $t=0$.

A plot of $A\Delta t$ against external concentration should give a straight line with a slope equal to $\Delta t/\phi$, the y-intercept equal to Δt and the x-intercept equal to ϕ. In addition

$$K=\frac{1}{\Delta t}\left(\frac{\phi+C_o}{E-C_o}\right). \tag{5}$$

Values of K and ϕ were obtained using the present data.

Miller also considered a number of other experimental situations and methods of analyzing the data (1965*b*, 1968*a*, 1968*b*, 1971). One of these (1968*b*) results in a graph similar to our "plot 2".

To simplify experimental procedures and calculations, both 1% NaCl and 300 mM thiourea were considered to be isotonic with all of the bloods. Isotonic units were used in all calculations but the graphs are presented with conventional units.

Results

Typical exit curves are shown in Fig. 1. The initial tangents were drawn by eye. The horinzontal lines were drawn through the equilibrium volume in each case. It can be seen that as the outside concentration of thiourea is increased, the initial slope decreases, the time for the tangent to intercept the horizontal line increases, and there is a slight decrease in total deflection. In Figs. 2 and 3 are included "plot 1" (top row) and "plot 2" (bottom row). The vertical columns are data for human, mouse and rat bloods (Fig. 2) and rabbit, pig and ox bloods (Fig. 3). With each type of blood, the two graphs clearly indicate that thiourea leaves the erythrocytes of all six species by facilitated diffusion. The data on the sheep-thiourea system were included with other data. The "plot 1" and "plot 2" each gave a value of ϕ of approximately 56 mM (Hunter *et al.*, 1976). Qualitative studies of competitive inhibition with urea demonstrated that urea and thiourea share the same carrier. The values of ϕ obtained by "plot 1" are larger than those obtained by "plot 2". In no case does a plot of the difference in concentration of penetrant on the two sides of the membrane against dS/dt

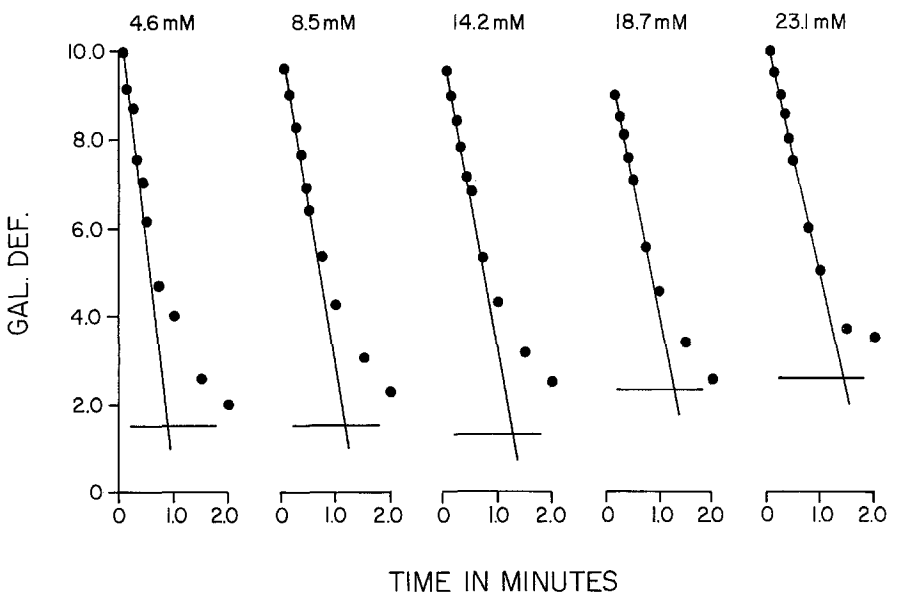

Fig. 1. Initial portion of typical shrinking curves. Sheep erythrocytes previously equilibrated with 188 mM thiourea in 1% NaCl added to 1% NaCl with increasing concentrations of thiourea as indicated at the top of each curve. Ordinates: galvanometer readings, arbitrary units. Abscissae: time in min. Times are measured to intersection of tangent drawn to initial, steep portion of curve with horizontal line drawn through equilibrium volume

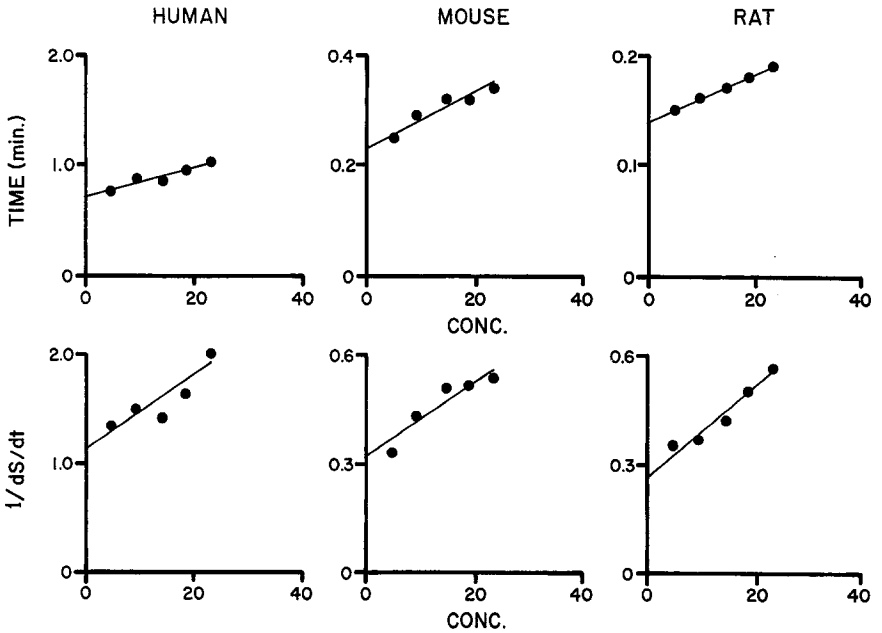

Fig. 2. Data demonstrating facilitated diffusion of thiourea into erythrocytes of human, mouse, and rat. Upper 3 graphs—Ordinates: time in min for initial tangent to intersect horizontal line drawn through equilibrium volume. Abscissae: concentration in mM of penetrant outside the cells. Bottom 3 graphs—Ordinates: reciprocal of initial velocity in isotonic units per min. Abscissae: concentration in mM of penetrant outside the cells

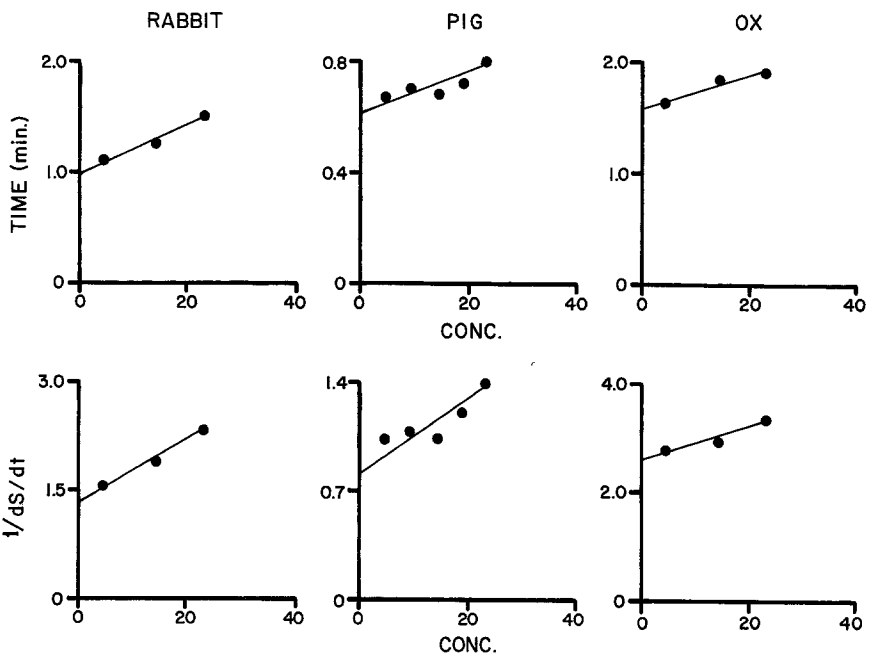

Fig. 3. Similar to Fig. 2. Data for rabbit-, pig- and ox-thiourea systems. All units same as in Fig. 2

Table 1. Values of half-saturation constant (ϕ) in mM and of maximum transfer rates (K) in isotones/min for the thiourea carrier in erythrocytes of various species of mammals

Species	ϕmM			K isotones/min		
	Plot 1	Plot 2	Miller	Eq. (2)	Plot 2	Miller
Human	60	35	42	1.2	0.9	2.9
Rabbit	46	32	33	0.8	0.8	2.1
Mouse	46	30	40	3.4	3.2	8.5
Rat	65	23	42	6.1	3.7	15.3
Ox	107	88	63	0.6	0.4	1.4
Sheep	56	56	38	0.9	0.6	2.2
Pig	110	49	64	1.6	1.1	3.6

give a straight line passing through the origin which would be true if only simple diffusion were involved. A summary of the values of ϕ and K, the maximum transport rate, is presented in Table 1. Three different values were determined. One was obtained from "plot 1" and Eq. 2, a second from "plot 2" and the third was calculated using Miller's method.

Discussion

During the last few years a number of authors have pointed out that the original mobile-carrier model cannot adequately be used to interpret all of the data obtained from different kinds of experiments. Several modifications of the original model, as well as new models, have been suggested. Mawe and Hempling (1965), for example, presented evidence which suggests that the loaded carrier crosses the membrane faster than the unloaded carrier. Baker and Widdas (1973) studied competitive inhibition and demonstrated an asymmetry in the hexose transfer mechanism. Edwards (1974) suggested that diffusion of the penetrant in the bulk phase may be rate-limiting.

Alternate models have been proposed by a number of different workers (e.g., Lieb & Stein, 1970; Naftalin, 1970; Karlish, Lieb, Ram & Stein, 1972; Ginsberg & Ram, 1975; Ginsberg & Stein, 1975). Miller (1969) and Regen and Tarpley (1974) have discussed various models, and LeFevre (1975) has reviewed the field.

Until there is general agreement on a model which is more satisfactory than the original mobile-carrier model, we shall continue to analyze

our data in accord with the latter. Our data can subsequently be reevaluated if a new model becomes generally accepted.

It has been shown that carriers for different penetrants have different maximum transfer rates (K) and different values for half-saturation (ϕ). Other data also suggested that carriers for the same penetrant but in different cells do not have the same characteristics (Hunter, 1976*a*). For this reason it seemed of interest to make a comparative study of a carrier for a given substance in erythrocytes of several different species. From Table 1 it can be seen that thiourea carriers in red cells of different species have a range of maximum transfer rates of almost one order of magnitude. The values for half-saturation show smaller differences. If one assumes that carriers are proteins, one might predict that the differences in the values of ϕ and K between species would be comparable to differences in K_M and V_{Max} for a given enzyme in different species. Barman (1969) contains many examples of comparable variations in these two parameters for different enzymes. In the case of a carrier, differences in maximum transport rates may depend not only on differences in the chemical nature of the carrier itself but also on other chemical differences in the membrane.

Jacobs, Glassman and Parpart (1935) reported rather small values of Q_{10} for hemolysis of rat, man and rabbit erythrocytes in thiourea. Data are not included for hemolysis in thiourea of the other species included in the present report. Low values of Q_{10} are a characteristic of facilitated diffusion while higher values suggest simple diffusion.

Kaplan, Hays and Hays (1974) suggested that urea enters erythrocytes of Agnatha, Chondrichthyes, Osteichthyes and Aves by simple diffusion but enters red cells of Amphibia, Reptilia and Mammalia by facilitated diffusion. These generalizations are supported by various workers, for example, Rabinowitz and Gunther (1973) for elasmobranch red cells, Hunter (1976*b*) for 4 species of trout, Macey and Farmer (1970) and Hunter (1970*a*, 1976*a*) for several species of mammals. Other data, however, suggest that facilitated diffusion is involved in the penetration of urea into red cells of some birds (Hunter, 1970*b*). Another suggestion of Kaplan *et al.* (1974) that urea may share the sugar-carrier is not supported by published data (e.g., Bowyer, 1954; Bowyer & Widdas, 1956; Cainelli *et al.*, 1974).

The significance of the effect of butanol on thiourea permeability is not clear. In all other instances of facilitated diffusion that we have studied, butanol decreases the permeability. With erythrocytes of some species it does have this effect on the permeability to thiourea (e.g.,

Table 2. Relationship of animals studied

Order:	Primates-man
	Lagomorpha-rabbit
	Rodentia
Family:	Muridae-mouse, rat
	Artiodactyla
Family:	Bovidae-ox, sheep
	Suidae-pig

Ospina & Hunter, 1966). With erythrocytes of other species it is difficult to demonstrate inhibition of thiourea penetration. In some cases, low concentrations of butanol will inhibit slightly, but in other cases it has been impossible, within experimental error, to demonstrate any effect. With higher concentrations of butanol an apparent increase in permeability to thiourea has been observed with erythrocytes of several different species. This means that failure to demonstrate a decrease in permeability in the presence of butanol may not in every case demonstrate simple diffusion. It should be remembered that thiourea is not very soluble in water, which may account for the difficulty in demonstrating saturation and competitive inhibition in some cases. While we have been unable to demonstrate the presence of a carrier for thiourea in certain instances using other techniques, the present technique indicates that facilitated diffusion is involved with the movement of thiourea across the membrane of human, mouse, rat, rabbit, sheep, pig and ox erythrocytes.

Looking at the data from a zoological point of view may suggest some relationships, but they are not completely consistent. Four different orders of mammals are represented by the 7 species (Table 2). Pig, ox and sheep are in one order. Two suborders are represented with the latter two animals in the same family. The ϕ-values for ox and pig are similar but the value for sheep is quite different. On the other hand, the K-values for ox and sheep are more similar than that for pig. Rat and mouse, representing a different order from the other animals, are both in the same family. The ϕ-values calculated by the Miller method are quite similar. The K-values are both large in comparison with the other data. A representative of each of the 4 orders, mouse, human, rabbit and sheep have similar ϕ-values but the K-values vary widely.

Widdas (1955) studied the transport of glucose and sorbose by red cells of fetal animals of various species. He found a similarity in affinities for a given penetrant but differences in maximum transfer rates. LeFevre (1962) and Miller (1965*a*), using human erythrocytes with different

sugars, found different values for half-saturation but similar values for maximum transport rates. The present values of ϕ obtained by the Miller calculation vary from 33 to 64 mM with 4 of the 7 in the 38–42 range. In every case the value is smaller than that obtained from "plot 1". Slightly greater variability in this parameter is obtained from the Sen and Widdas calculation. The values for maximum transfer rate obtained using the Miller calculation differ by more than an order of magnitude and are all considerably higher than those obtained from Sen and Widdas calculations. The latter values vary less than the former but still they are more variable than the ϕ-values. These data suggest that in different species there is less variation in half-saturation values than in maximum transport rates for a given carrier.

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