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26 February 1964

Urea: Apparent Carrier-Mediated Transport by Facilitated Diffusion in Dogfish Erythrocytes

Abstract. *The exposure of erythrocytes from the elasmobranch,* Squalus acanthias, *to solutions isosmotic with plasma (1M) but containing urea or hydroxyurea as the sole solute does not produce hemolysis. Exposure of these cells to 1M methylurea, thiourea and acetamide does produce hemolysis. Low concentrations of urea, which are associated with hemolysis, protect dogfish red cells against hemolysis by methylurea and thiourea. Dogfish red cells exposed to mediums containing high concentrations of urea, or no urea, reach 95 percent of their equilibrium concentration in less than 5 minutes.*

The presence of high concentrations of urea in the plasma (and presumably the tissues) of elasmobranchs is well known (*1*). This compound accounts for approximately one third of the total tonicity of plasma. Previous work has shown that the elasmobranch red cell is permeable to urea (*2*). By implication, it has been assumed that penetration occurs by means of simple passive diffusion. If this were true, then these erythrocytes, like mammalian erythrocytes, should be rapidly hemolyzed when exposed to solutions isosmotic with dogfish plasma, but containing urea as the sole solute. The experiments reported here demonstrate that there is no hemolysis of dogfish erythrocytes in isosmotic salt-free solutions of urea or hydroxyurea. Further, the behavior of dogfish erythrocytes when exposed to compounds chemically similar to urea suggests a carrier-mediated transport system for urea, similar to that described by a number of workers including LeFevre (*3*) and Wilbrandt (*4*) for glucose in primate erythrocytes.

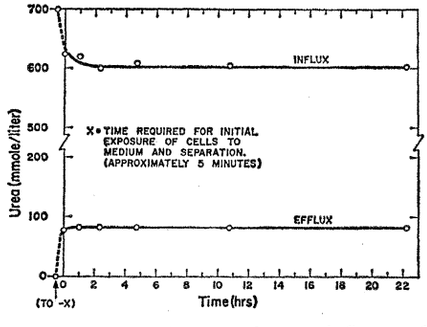

Fig. 1. Measurements of urea influx and efflux in dogfish red cells.

Erythrocytes from the spiny dogfish, *Squalus acanthias*, were used throughout this study. Blood was obtained from a caudal vessel in a syringe containing heparin. The red cells were separated from the plasma and white cells by means of centrifugation at 10°C in a refrigerated centrifuge. The erythrocytes were then washed once or twice and the red cells isolated by means of in elasmobranch Ringer solution (*5*) careful aspiration of the supernatant solution. Hemolysis was studied by adding approximately 0.1 ml of erythrocytes to 2 ml of the solution being tested. The red cell mixture was permitted to stand at room temperature for approximately 5 minutes; the mixture was then centrifuged for 2 minutes. The color of the supernatant was taken as an indicator of the degree of hemolysis (Table 1).

Solutions of the following substances (1.0 and 1.3*M*) were tested for their ability to produce hemolysis: urea (NH_2CONH_2), methylurea ($NH_2CONHCH_3$), thiourea (NH_2CSNH_2), acetamide (CH_3CONH_2), and hydroxyurea ($NH_2CONHOH$). A modified "urea osmotic fragility" test was performed by exposing dogfish erythrocytes to a graded series of urea concentrations varying from 0.1 to 1.0*M*.

Net urea influx was measured by suspending dogfish erythrocytes in a modified isosmotic dogfish Ringer solution containing twice the concentrations of urea normally found in dogfish erythrocytes (700 mmole/liter) (*5*) and determining the rate of decrease of urea concentration in the Ringer's solution. Net efflux of urea was measured by suspending dogfish erythrocytes in a modified isosmotic Ringer's solution containing no urea (*5*) and determining the rate of increase of urea concentration in the Ringer's solution. In these studies, the red cell hematocrit of the suspension was approximately 10 percent. Urea concentrations in the suspending media were determined in duplicate by the technique of Conway (*6*), an ammonia blank being used.

The results obtained, which were the same for three different animals, are shown in Table 1. The exposure of erythrocytes to 1*M* urea as the only solute produces no hemolysis. When the concentration of urea in the suspending media is reduced to 0.3*M* or less, hemolysis occurs in less than 5 minutes. There is slow hemolysis at intermediate urea concentrations. The behavior of hydroxyurea is similar to urea in that it produces no hemolysis at concentrations of 1.0*M* and above and causes rapid hemolysis at 0.3*M* concentrations or less. The three other urea-like compounds studied, when present as the sole solute in contradistinction to urea, produce rapid hemolysis although the degree of hemolysis produced by thiourea is somewhat less than that produced by methylurea and acetamide.

The addition of 0.3*M* urea to the suspending media protects the red cells

Table 1. Effect of urea and related compounds on the dogfish erythrocyte.

Solutes and concentrations used	Degree of hemolysis*
1.0 *M* urea	0
0.3 *M* urea	4
1.0 *M* hydroxyurea	0
0.3 *M* hydroxyurea	4
1.0 and 1.3 *M* methylurea	4
1.0 *M* methylurea + 0.3 *M* urea	4
1.0 and 1.3 *M* thiourea	3
1.0 *M* thiourea + 0.3 *M* urea	0
1.0 and 1.3 *M* acetamide	4
1.0 *M* acetamide + 0.3 *M* urea	4
1.0 *M* methylurea + 0.32 *M* hydroxyurea	0
1.0 *M* thiourea + 0.32 *M* hydroxyurea	0
1.0 *M* acetamide + 0.3 *M* hydroxyurea	4

* 0, No detectable pink color in the supernatant; 1, barely detectable pink color; 2, moderate pink to red color; 3, red color; 4, deep red color in the supernatant and no red cells in the sediment.

from hemolysis by 1.0*M* methylurea and thiourea but does not affect the hemolysis produced by acetamide. Similarly, 0.3*M* hydroxyurea protects red cells from hemolysis in 1.0*M* methylurea and thiourea but not from hemolysis by acetamide.

Figure 1 shows typical results (selected from four separate studies) of the flux measurements made with the solutions containing high concentrations of urea and no urea. It is evident that by the time the first sample of supernatant solution is obtained for measurement (generally after 5 minutes) equilibration of urea between cells and medium is approximately 95 percent complete. Thus there is apparently rapid bidirectional exchange of urea across the red cell membrane. By the end of 60 minutes net influx and efflux are essentially zero.

The term "facilitated diffusion" has been applied to the process by which glucose penetrates the plasma membrane of primate erythrocytes. The evidence for this mode of transport includes (i) the failure of primate erythrocytes to hemolyze in isotonic glucose (*7*); (ii) the apparent specificity of the transport process so that a given sugar may readily penetrate the red cell (*d*-xylose and *l*-arabinose) while other structurally similar compounds (*l*-xylose and *d*-arabinose) are excluded (*8*); and (iii) the competition between various substrates for the carrier so that low concentrations of glucose inhibit the penetration of other sugars like xylose and arabinose (*9*).

These features are clearly similar to the results obtained for the transport of urea into elasmobranch red cells. The failure of these cells to hemolyze in isosmotic urea; the hemolysis of these cells by methylurea and thiourea; and the inhibition of this hemolysis by small concentrations of urea indicate a similar mechanism. Our data also suggest that hydroxyurea shares the same carrier mechanism, whereas acetamide does not.

It should be emphasized that although our results are consistent with facilitated diffusion of urea, we have not rigorously established this as the mode of transport. The techniques we employed were chiefly qualitative. For the rigorous demonstration of facilitated diffusion, quantitative studies of the kinetics of urea transport; accurate measurement of changes in red cell volume; flux measurements of labeled urea; exploration of carrier inhibitors; and analysis of concentration gradients under a variety of circumstances will be required.

In view of the important role of urea in the economy of the elasmobranch, it is not entirely surprising that the individual cells of this group of fish possess unusual mechanisms for dealing with the compound. This demonstration of transport by facilitated diffusion may serve as an important model for studying the nature of urea distribution in other systems.

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27 January 1964

Strontium and Calcium Reabsorption in Renal Tubules of the Newt, Triturus pyrrhogaster

Abstract. *Reabsorption of calcium in the proximal tubule cannulated in vivo exceeded reabsorption of strontium. The diuretics Diamox and Salyrgan depressed the reabsorption of calcium but not strontium.*

During intestinal absorption and renal excretion in man and animals, if both calcium and strontium are present, calcium is preferentially absorbed (*1*). The phenomenon of preferential reabsorption of calcium is useful for evaluating the accumulation of Sr^{90} in man. Much less work has been done on reabsorption during renal excretion than during intestinal absorption because of the technical difficulty of performing experiments on a kidney in vivo. Although the importance of the renal tubules in the preferential absorption of calcium has not yet been demonstrated, it has been a subject of speculation (*2*).

Calcium reabsorption is thought to occur mainly in the proximal tubules of the kidney (*3*). We have measured in vivo the rates of reabsorption of Sr^{85} and of Ca^{45} in the proximal tubule of the newt, *Triturus pyrrhogaster*. The large size of the nephrons in the newt make this animal very suitable for such an investigation. A technique similar to ours was used previously for measuring reabsorption in single nephrons of *Necturus* (*4*).

A newt (weighing 4 to 5 g) was immobilized by destruction of the cerebrospinal system and the kidney was exposed under a microscope. A polyethylene tube (50 to 100 μ in diameter) containing a 0.6 percent NaCl solution between two air bubbles was inserted into a proximal tubule via the Bowman's capsule. Another tube, containing Ringer solution, was inserted into the corresponding distal tubule from the opposite side, and the Ringer solution was injected into the tubule.

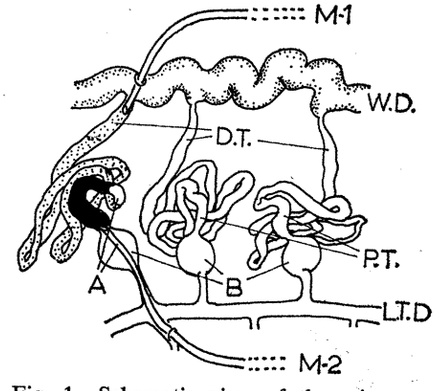

Fig. 1. Schematic view of the microperfusion method used for measuring the rates of reabsorption of Ca^{45} and Sr^{85}. *WD*, Wolffian duct; *M*, microinjector; *B*, Bowman's capsule; *PT*, proximal tubule; *A*, air bubble; the dark area represents NaCl solution containing either Ca^{45} or Sr^{85}; *LTD*, longitudinal duct.