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ARTICLE

Urea transport in human red blood cells: Donor variation compared to chloride, glucose, and water transport

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We determined the permeability (P, cm/s) of unmodified human red blood cells (HRBC) to urea (P_u), chloride (P_{Cl}), glucose (P_{glu}), and water diffusion (P_d) under conditions of self-exchange (SE) with the continuous flow tube method at pH 7.2, 25°C. Among 24 donors, P_u at 1 mM varied >100%. Two of the donors were also tested in 1983. Their P_u had decreased by 77 and 90%. High age in males and Kidd genotype Jk(a+,b+), but not blood types AB0, appear related to low P_u. For one of the two donors, P_{Cl} (150 mM, 38°C, pH 7.2), P_{glu} (1 mM, 38°C, pH 7.2), and P_d (55.5 M, 25°C, pH 7.2) were determined then and now and showed no significant changes with age. The results from six more donors show donor P_{Cl}, P_{glu}, and P_d in the range of ≈1%. P_{Cl} and P_{glu} are vital for the metabolism of cells and tissues, and we see but little donor variation, and so far, no phenotypes without glucose (GLUT1) and anion (AE1) transporters in HRBC. Phenotypes with no urea transporter (UT-B) or no water transporters (aquaporin, AQP1) are registered and are compatible with life. Our results are in line with the concept that the solutes do not share pathways in common. The great donor variation in P_u must be considered in comparative transport physiological studies.

Introduction

About 30 yr ago, Fröhlich et al. (1991) demonstrated that the permeability of human red blood cells (RBCs) to urea and thiourea is related to the Kidd blood type. RBCs that lack Kidd blood group antigens, Jk(a−,b−), had a permeability to the two solutes as low as in lipid bilayer membranes (Vreeman, 1966; Galucci et al., 1971), while the permeability to ethylene glycol, chloride, and water was normal as in Jk(a−,b+) and Jk(a+,b−) cells with a facilitated urea transport. Fröhlich et al. (1991) concluded that their study strongly supports that urea transport function is separated from the transport of the other three solutes. The present study extends the work by Fröhlich et al. (1991) regarding the number of donors, their genotypes and age, and the transport function of the later named urea transporter UT-B, as well as transport of chloride by the anion exchanger AE1, glucose by the glucose transporter GLUT1, and water by the water transporting channel aquaporin AQP1 under physiological conditions. We exchanged measurements of ethylene glycol with the more physiologically relevant solute glucose.

Material and methods

Media

All media (in mM) were prepared from reagent grade chemicals (Merck). Medium A: 150 KCl, 2 KH_2PO_4, pH 7.2; medium B: medium A with 1 urea or 1 d-glucose, pH 7.2; medium C: 50 phloretin in ethanol.

Inhibitor

Phloretin (Sigma-Aldrich Chemie, GmbH) was dissolved in ethanol to obtain a 50 mM stock solution that was added to the medium to obtain a final concentration of 1 mM.

Preparation of RBCs

Blood was either freshly drawn obtained with consent from healthy persons, or discharged, not outdated blood packs from the Blood Bank, Copenhagen University Hospital. All blood samples were handled according to the Danish regulatory requirements. The bank blood was stored in a SAGM medium (salt, adenine, glucose, and mannitol) with CPD (citrate, phosphate, and dextrose) as anticoagulant (for details, see e.g., D'Amici et al., 2012). The blood was washed once in medium A or medium B containing the urea or d-glucose concentration concerned. The buffy coat was removed in the freshly drawn samples, and the cell suspension was next titrated to pH 7.2 at 25°C or 38°C. The cells were subsequently washed at least thrice in the appropriate medium A or B.

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Cell water content and radioactivity

Cell water content in RBC was determined by drying ≈300 mg packed RBCs at 105°C for 24 h. The packing was carried out in slender 0.8-ml nylon tubes that were centrifuged for 15 min at ≈40,000 *g* (Sorvall RC-5 centrifuge; DuPont Instruments-Sorvall Biomedical Division). A 2% correction was carried out for extracellularly trapped water. The cell solids determination was done to ensure the relationship that 1 g of cell solids equals 4.4 × 10^4 cm^2 membrane area (for details, see e.g., Brahm, 1982).

The radioactivity of the cells and the supernatant was determined by liquid β-scintillation spectrometry after precipitation with 7% (vol/vol) perchloric acid. The radioactivity in cell-free filtrates and centrifuged samples from the efflux and influx experiments was measured without preceding precipitation.

Cells for efflux experiments

After the last wash, the cells were resuspended to a hematocrit of ≈60%, and radioactive isotope (^{14}C-urea, ^{14}C-glucose, $^{36}Cl^-$, or ^3H_2O (Perkin-Elmer) was added to obtain an activity of ≈18 kBq (0.5 μCurie) per ml cell suspension. Finally, the cells were centrifuged (see above) in slender 0.8-ml nylon tubes for efflux experiments with the Millipore-Swinnex filtering method or 8 ml tubes for efflux experiments with the continuous flow tube method (Dalmark and Wieth, 1972; Brahm, 1977).

Flux experiments

All experiments were carried out under conditions of self-exchange (SE), where the net flux of the solute is zero. The distribution ratio, $r = C_{solute,i}/C_{solute,o}$ of the uncharged urea, glucose, and water is ≈1, while the ratio of chloride is ≈0.7 due to the intracellular content of negatively charged hemoglobin. Depending on the velocity of solute tracer efflux, we used either the continuous flow tube method or the Millipore-Swinnex filtering method.

Continuous flow tube method

Efflux of ^{14}C-urea, ^{14}C-glucose, $^{36}Cl^-$, and ^3H_2O show half times in the millisecond range. The continuous flow tube technique is suitable for use in these experiments (for details see Brahm, 1977, 1983a, 1989). In short, 0.7–1 ml of RBCs with the labeled compound that is stored in 1 ml Manteaux syringes is continuously injected with a preset constant velocity into a mixing chamber and mixed with ≈375 ml of efflux medium that is also injected with a preset constant velocity. The mixing initiates an efflux of radioactive tracer from the cells to the medium, a process that continues during the flow of the dilute suspension (hematocrit [hct] < 0.3%) along a tube originating in the mixing chamber. The flow of the suspension along the tube is constant, 200–400 cm/s, depending on the presetting during a run, and thus a certain distance from the mixing chamber equals a certain time after initialization of the tracer efflux. Ordinarily, at six predetermined variable distances, ≥12 mm, the tube wall is replaced by Nucleopore filters with a pore diameter of 0.4–0.8 μm, which withhold the cells and allow collection of small-volume cell-free filtrates with increasing radioactivity.

Millipore-Swinnex filtering method

Efflux of acute phloretin-inhibited ^{14}C-urea is >10–100 times slower than non-inhibited urea efflux and was determined by means of the Millipore-Swinnex filtering technique (for details, see Dalmark and Wieth, 1972). In short, ≈300 mg packed (hct ≈ 98%), labeled cells are injected into a temperature-regulated chamber with 30 ml well-stirred medium. Subsequently, a series of cell-free filtrates is collected through Millipore-Swinnex filtering units at times that are registered with a pedal-activated timer.

Calculations

With both methods, the tracer efflux is well described as a closed two-compartment model with finite volumes:

$$C^*(t) = C^*(o) \times e^{-k^{*'}t}, \quad (1)$$

where $C^*(t)$ and $C^*(o)$ are the radioactive solute concentration at time t and 0, respectively, and $k^{*'}$ (s^{-1}) is the rate coefficient (the fraction of tracer removed per time unit).

The determined rate coefficient ($k^{*'}$) relates to k^*, the rate coefficient for the unidirectional efflux by:

$$k^* = k^{*'} \times \frac{V_o}{V_i + V_o}(s^{-1}), \quad (2)$$

where V_i and V_o are, respectively, the intracellular water (donor) volume and the extracellular (recipient) volume. It follows that $k^{*'} \rightarrow k^*$ for $V_o >> V_i$ is the condition in tracer efflux experiments with a hematocrit <1%. k^* is used to calculate the tracer permeability P^* (cm/s). Under the assumption that the rate coefficient and permeability of the tracer (k^*, P^*) and non-tracer (k, P), respectively, are indistinguishable, we have:

$$P = k \times \frac{V_i}{A} = \frac{\ln 2}{T_{1/2}} \times \frac{V_i}{A}(cm/s), \quad (3)$$

where A (cm^2) is the cell membrane area available for the solute transport and $T_{½}$ is the half-time of the efflux process. A is related to the cell solid contents (corrected for any additional solute content >1 mM) as 1 g of cell solids equals 3.1 × 10^{10} "normal" cells with a total area of 4.4 × 10^4 cm^2 (see e.g., Brahm, 1982).

Results

Fig. 1 shows ^{14}C-urea efflux under conditions of SE at 1 mM urea in two donors in Table 1, respectively, with a slow and a fast urea transport in their RBCs. The linearity of the efflux curves in the semilogarithmic plot indicates that the efflux can be considered as a monoexponential process.

Table 1 summarizes the urea permeability in RBCs from 24 donors as calculated from efflux experiments carried out by means of the continuous flow tube technique and the Millipore-Swinnex filtering method described in the Materials and methods. The continuous flow tube technique was used to determine the noninhibited ^{14}C-urea efflux, while the Millipore-Swinnex method was used to determine the much slower phloretin-inhibited tracer efflux. The table includes the

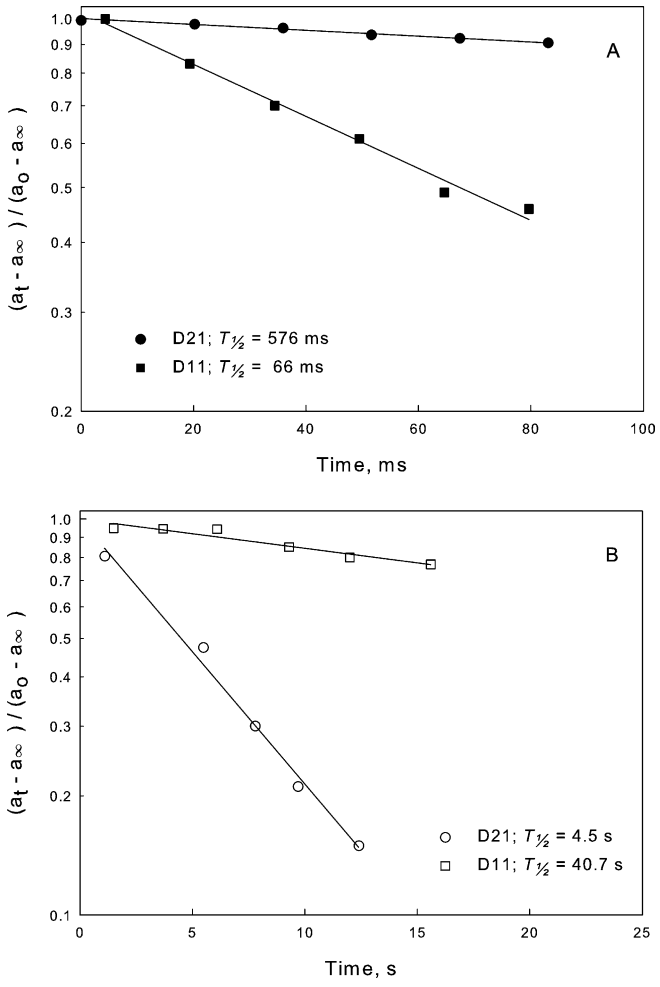

Figure 1. **Unidirectional ^{14}C-labeled urea efflux under SE conditions at 1 mM in human RBCs from two donors.** The ordinates express the fraction of tracer that remains in the cells at a given time, and the abscissa is time. Note the different time scaling of the abscissa. D21 represents a slow urea transport and D11 represents a fast urea transport in the present collection of donors in Table 1. **(A and B)** The experiments were carried out at 25°C, pH 7.2, by means of the continuous flow tube technique with control cells (A) and the Millipore-Swinnex filtering method with acute phloretin-exposed cells (B). The linearity of the efflux curves in the semilogarithmic plot indicates that the efflux is a monoexponential efflux process. The negative slope of the efflux curves equals the rate coefficient k (s^{-1}) and $T_{½}$ (ms or s) = ln2/k. The rate coefficient is used to calculate the permeability coefficient according to Eq. 3. **(A)** No inhibition. D21: P_u = 0.57 (SD ± 0.02, n = 3) × 10^{-4} cm/s. D11: P_u = 4.79 (SD ± 0.42, n = 4) × 10^{-4} cm/s. **(B)** Acute phloretin inhibition. D21: $P_{u,i}$ = 0.60 (SD ± 0.09, n = 3) × 10^{-6} cm/s. D11: $P_{u,i}$ = 0.76 (SD ± 0.24, n = 4) × 10^{-6} cm/s.

available information about the Kidd antigen, blood type, sex, and age of the donors.

With two of the donors, we have the option to compare the urea permeability obtained now with the urea permeability determined 40 yr earlier by Brahm (1983b) as shown in Table 2.

For comparison with other well-established transport systems in human RBCs, we have a collection of determinations of the permeability to chloride, glucose, and water in RBCs from another group of donors. Figs. 2, 3, and 4 illustrate the unidirectional efflux rates of the three solutes, and Table 3 summarizes the permeability coefficients of the RBCs in the seven donors. All experiments with chloride and glucose were performed at 38°C, pH 7.2, and those with water at 25°C, pH 7.2. The efflux curves of chloride (Fig. 2), glucose (Fig. 3), and water (Fig. 4) were obtained with blood from the same donor (D50).

Table 3 summarizes the data on chloride, glucose, and water permeability in RBCs from seven donors. One donor (JBR) is represented with data from 2018 and 35–40 yr before.

Discussion

The applied methods

The combination of the continuous flow tube method and the Millipore-Swinnex method offers a time resolution from a few milliseconds to minutes and hours. The two methods overlap as determined in a temperature study of chloride self-exchange in human RBCs (Brahm, 1977). In the present study, the continuous flow tube method was suitable for measuring the rapid diffusion of water with half-times ≈13 ms, the much slower transport of urea with half-times ≈580 ms, and the in between half times for chloride and glucose as illustrated in Figs. 1 A, 2, 3, and 4. Determination of inhibited urea transport requires a much lesser time resolution, and the well-established Millipore-Swinnex filtering method is here the choice (Fig. 1 B). We note the essential point that all curves show linearity in a semilogarithmic plot indicating that the radioactive tracer efflux can be described in terms of unidirectional efflux that follows first order kinetics with a monoexponentially decay. The negative value of the slope of the efflux curve, k (s^{-1}), is related to the half-time, $T_{½}$ (s), by $k = ln2/T_{½}$. Neither k nor $T_{½}$ can be used to compare transport processes in different cells unless the cells have the same intracellular water volume as illustrated by Eq. 3. Therefore, the permeability, P, is the parameter used for comparison. Since transport of chloride, glucose, and urea show a concentration dependence (Gunn et al., 1973; Gasbjerg and Brahm, 1991; Brahm, 1983a, 1983b), we used fixed concentrations of chloride (150 mM), and urea and glucose (1 mM) in the present comparative study.

The donor variation of urea permeability

Table 1 summarizes the RBC urea permeability determined in 24 donors. The Jk phenotypes, the ABO, the rhesus blood types, and the ages and sex of the donors are also included with a few exceptions. The range of urea permeability values is over 10-fold and thus larger than previously reported (Brahm, 1983b). It is well-established that the phenotype Jk(a–,b–) has no urea transporter as first reported by Fröhlich et al. (1991). We have not discovered the phenotype Jk(a–,b–) in our collection of donors, and only the three phenotypes, Jk(a+,b–), Jk(a–,b+), and Jk(a+,b+) are equally represented in Table 1 in a number of, respectively, 6, 5, and 6 (plus 7 ND). The obvious question is whether the magnitude of the urea permeability is randomly distributed or related to one or more factors. Cautiously, taking the limited number of 24 donors and the lack of unambiguous relationship between the permeability and one of the registered parameters in the table, we note that there appears to be a predominance of Jk(a+,b+) among the donors in the lower third of the table, with $P_u \leq 3.23 \times 10^{-4}$ cm/s. The UT-B protein in

Table 1. **Urea self-exchange permeability in human RBCs from 24 donors**

Donor	P_u	SD	n	Phloretin	$P_{u,i}$	SD	n	Inhibition	Jk (a,b)	Blood type ABO, Rh±	Sex	Donor age
	cm/s × 10^4			mM	cm/s × 10^6			%				Years
D57	6.14	±0.49	4		ND				ND	ND	M	55
D53	6.08	±0.47	4	1.00	1.71	±0.71	4	99.8	–,+	A,–	F	75
D52	5.65	±0.20	4	1.00	4.05	±0.88	3	99.3	+,+	0,–	F	77
D59	5.44	±0.07	2		ND				ND	ND	F	36
D01	5.42	±0.86	4		ND				ND	ND	NA	NA
PBN[a]	5.17	±0.02	3						+,–	0,+	M	28
D11	4.79	±0.42	4	1.25	0.76	±0.24	4	99.8	–,+	A,+	NA	NA
D05	4.68	±0.22	4	0.50	3.39	±1.17	4	99.3	–,+	A,+	F	55
D04	4.66	±0.57	4		ND				+,–	0,+	M	52
D03	4.59	±0.53	4		ND				ND	A,+	F	52
D09	4.43	±0.66	4	1.00	1.45	±0.28	4	99.7	+,–	0,+	M	26
D07	4.24	±0.31	4	1.00	1.29	±0.05	2	99.7	ND	A,+	M	48
D25	4.22	±0.45	4	1.00	3.39	±0.19	4	99.2	+,–	0,–	NA	NA
D08	4.15	±0.26	4	1.00	0.86	±0.30	4	99.8	–,+	A,+	M	57
D06	3.94	±0.36	4	1.00	1.73	±0.35	4	99.6	ND	B,+	M	26
D10	3.87	±0.49	4	1.25	0.94	±0.18	4	99.8	–,+	0,–	NA	NA
D02	3.78	±0.33	8	0.75	1.23	±0.72	13	99.7	+,–	0,+	M	40
D12	3.23	±0.64	4	1.25	1.10	±0.15	4	99.7	+,+	A,+	F	51
JBR[a]	2.67	±0.07	4					99.4	+,+	A,+	M	38
D17	0.87	±0.03	4	1.25	3.96	±1.20	3	95.4	+,+	0,+	M	67
D20	0.62	±0.02	3	1.00	2.32	-	1	96.3	ND	A,+	F	23
JBR	0.62	±0.15	4	1.00	1.02	-	1	98.4	+,+	A,+	M	75
D51	0.59	±0.05	4	1.25	0.65	±0.32	2	98.9	+,+	B,+	M	67
D13	0.58	±0.03	4	1.25	0.66	±0.24	4	98.9	+,+	A,+	M	31
D21	0.57	±0.02	3	1.00	0.60	±0.09	3	98.9	+,–	0,+	M	40
PBN	0.53	±0.08	3	1.00	1.47	±0.60	2	97.2	+,–	0,+	M	65

The self-exchange permeability coefficients were determined at 1 mM, pH 7.2, and 25°C. The inhibition of the urea transporter was determined by means of acute exposure of the cells to phloretin. NA, not available; ND, not determined.
[a]From Brahm (1983b).

human RBCs also carries the ABO antigens (Lucien et al., 2002), but there seems to be no correlation of the urea permeability to the ABO blood types.

Table 2. **Urea self-exchange permeability in RBCs from two donors determined in 1983 and 2022**

Donor	P_u in 1983[a]	P_u in 2022	$P_{u,2021}/P_{u,1983}$
	cm/s × 10^4		
JBR	2.67 (SD ± 0.05, *n* = 4)	0.62 (SD ± 0.15, *n* = 4)	0.23
PBN	5.17 (SD ± 0.03, *n* = 2)	0.53 (SD ± 0.08, *n* = 3)	0.10

The urea permeability was determined at 1 mM at 25°C, pH 7.2. Standard deviations (SD) are listed in brackets.
[a]The values are from Brahm (1983b). The standard deviations in 1983 are calculated from values in our databank.

A third parameter we have been attentive about is the time of storage of the donor blood. The blood from the collection of donors has been stored for different times, but the storage time does not correlate with the variation in urea permeability.

The urea permeability and donor age

Quite extraordinarily, the urea permeability of two of the donors in Table 1, PBN and JBR, was also determined under the same experimental conditions in our laboratory 40 yr ago. Surprisingly, the urea permeability has decreased by 90% and 77% of the values in 1983 (Table 2). Unfortunately, it has not been possible to get hold of other donors in Table 1 in Brahm (1983b). In 1983, the urea permeability of donor PBN was about twice that of JBR (Table 2). Apparently, the age-dependent decrease of the two donors approaches a common residual permeability of 0.5–0.6 ×

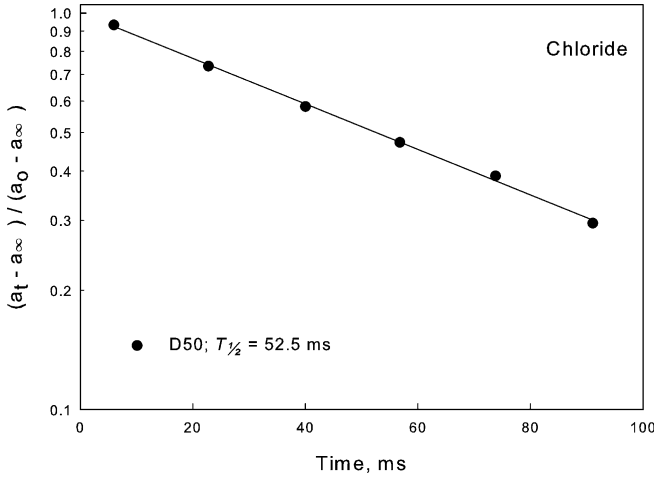

Figure 2. **Unidirectional $^{36}Cl^-$ efflux under SE conditions (C_o = 150 mM KCl) at 38°C, pH 7.2, from donor D50.** The monoexponential rate coefficient is used to calculate the permeability, P_{Cl} according to Eq. 3. P_{Cl} = 5.05 (SD ± 0.15, n = 3) × 10^{-4} cm/s.

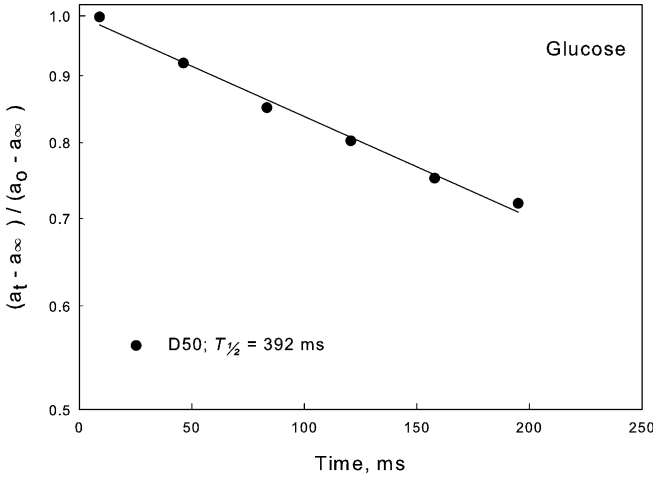

Figure 3. **Unidirectional ^{14}C-glucose efflux under SE conditions of 1 mM glucose at 38°C, pH 7.2, from donor D50.** The monoexponential rate coefficient is used to calculate the permeability, P_{glu} according to Eq. 3. P_{glu} = 7.69 (SD ± 0.50, n = 8) × 10^{-5} cm/s.

10^{-4} cm/s. We note that the residual permeability is about two orders higher than in lipid bilayer membranes and unmodified RBCs with no UT-B built-in (Vreeman, 1966; Galucci et al., 1971; Fröhlich et al., 1991; Brahm and Wieth, 1977; Brahm, 2013). Further, acute exposure to phloretin greatly reduces the residual permeability by 97% or more, indicating that UT-B still is the primary urea transport pathway in RBCs.

It is notably that all males above 65 yr exclusively are accumulated in the lower part of the table with Pu ≤ 0.87 × 10^{-4} cm/s. Inspired by the results of Table 2, we tested two female donors in their 70s. Unexpectedly, we found that their urea permeability was in top three of Table 1. Our working hypothesis is, therefore, that the age-dependent decrease of urea permeability is a phenomenon that refers only to male donors.

Inhibition of urea permeability

Most of the donor RBCs of Table 1 were used in another study in our laboratory to develop a method for influx measurements (Leifelt et al., 2023). In addition to determination of the Kidd antigen and AB0 blood type, we also determined the inhibition of urea permeability by means of acute exposure to phloretin. Phloretin is a very efficient non-specific and non-covalent inhibitor of many facilitated transport processes. Incubation leads to >99.4% inhibition, while acute exposure may result in somewhat less inhibition. The purpose of the inhibition in the present study was to show that whatever be the magnitude of the uninhibited urea permeability, it comprises a phloretin-sensitive permeability and a very low phloretin-resistant residual permeability, which is close to the permeability of lipid bilayer membranes and RBCs with no urea transporters. The high inhibition with phloretin in our studies opposes the model advanced by Yang and Verkman (2002) and supported by others (Li et al., 2012; Yu et al., 2019), in which as much as 2% of urea permeation in RBCs should be through the lipid phase of the membrane.

Comparison of permeability to urea, glucose, chloride, and water

As a follow-up on the results of the inter- and intra-urea permeability variation among our donors, we also looked at other transport processes that have been intensely investigated through the years. Table 3 summarizes an extract of data from our database on RBC permeability to chloride, glucose, and water in seven donors who have regularly contributed considerably through the years to transport studies in our laboratory by donating cells. The data from donor JBR spans several decades, while the data from the other six donors is obtained over much shorter time intervals. About donor JBR, the decrease in urea permeability in Table 2 is followed neither by a decrease in RBC permeability to glucose (7.81 × 10^{-5} cm/s), chloride (5.02 × 10^{-4} cm/s), nor diffusion of water (2.49 × 10^{-3} cm/s). The results with the other six donors demonstrate that the range of

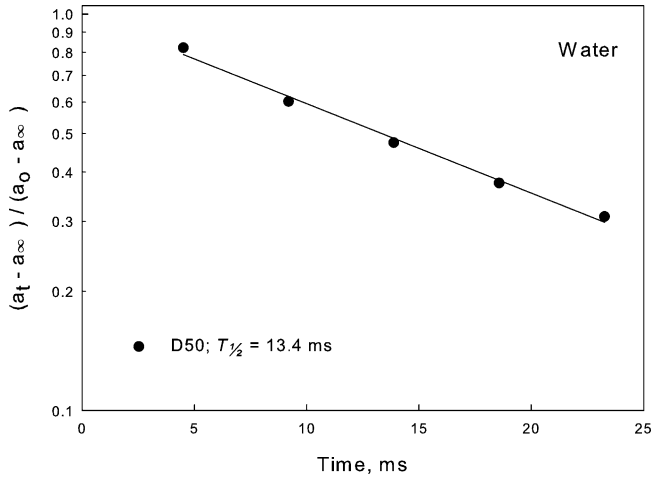

Figure 4. **Unidirectional ^3H_2O diffusional efflux at 25°C, pH 7.2, from donor D50.** The monoexponential rate coefficient is used to calculate the permeability, P_d according to Eq. 3. P_d = 2.38 (SD ± 0.09, n = 3) × 10^{-3} cm/s.

Table 3. **Permeability to chloride, glucose, and water in RBCs from seven donors**

Donor	P_{Cl}	SD	n	P_{glu}	SD	n	P_d	SD	n
	cm/s × 10^4			cm/s × 10^5			cm/s × 10^3		
JBR[a]	5.02	±0.42	6	7.81	±0.43	9	2.49	±0.23	8
D58	5.03	±0.01	3	8.75	±0.26	4	2.29	±0.19	3
D50	5.05	±0.15	3	7.69	±0.50	8	2.38	±0.09	3
D55	5.10	±0.41	5	ND			2.70	±0.75	4
JBR	5.11[b]	±0.09[b]	10[b]	7.66[c]	±0.42[c]	4[c]	2.4[d]	±0.2[d]	18[d]
D56	5.12	±0.20	8	ND			2.70	±0.65	4
D54	5.14	±0.10	2	ND			2.52	±0.25	4
D57	5.23[a]	±0.34	2	8.26	±0.13	3	2.43	±0.16	6

Chloride and glucose permeability at 150 and 1 mM, respectively, was determined at 38°C, pH 7.2–7.4. Diffusional water permeability at 25°C, pH 7.2. All experiments were carried out by means of the continuous flow tube method.
[a]Determined in 2018.
[b]Calculated from legend to Fig. 5 in Brahm (1977).
[c]From the databank of Brahm (1983c).
[d]From Brahm (1982).

membrane permeability to the three solutes has a very narrow donor variation. Our present results and another study from our laboratory (Jensen and Brahm, 1987; Brahm et al, 2023) at physiological temperatures and concentrations contribute and expand the conclusions advanced by Fröhlich et al. (1991) that the urea transport function is separated from other solute transport functions.

An obvious question related to the functional results in Tables 1, 2, and 3 is whether the >10-fold variation of P_u is due to a variation of the number of transporters. A review and a quantitative analysis of human RBC proteome (Burton and Bruce, 2011; Bryk and Wiśniewski, 2017) do not indicate a similar variation in the number of transporters. The number was determined in two earlier studies (Masouredis et al., 1980; Mannuzzu et al., 1993) to be 14,000 copies per cell. The comprehensive proteomics analysis by Bryk and Wiśniewski (2017) showed several differences from earlier determinations, including that UT-B should instead be 26,000 copies per cell with a SD of ± 8.2. Hence, there appears to be no obvious relationship between the morphological properties and the functional age and donor variation of the UT-B. The other relevant transporters in the present study, the anion exchanger AE1, glucose transporter GLUT1, and the water transporting channel APQ1 show a similar small variation in the proteomic analysis study.

If the number of urea transporters is constant as indicated by the cited studies, the present study indicates that the function of the transporters shows a >10-fold multifactorial variation. To our knowledge, such great interindividual or age-dependent variation has not been observed in other transport systems in RBCs besides the ones in the present study.

The physiological implication of a low facilitated UT-B mediated urea transport due to a donor and/or age variations is not clear. The lack of the transporter in Jk(a–,b–) in RBCs apparently reduces the maximum concentrating capacity of urine in the kidneys by about 20% (Sands et al., 1992). Whether a similar donor variation and age dependence may also apply to the UT-B transporter in the descending vasa recta in the kidneys remains to be determined. Most likely, a low UT-B capacity in RBCs also implies an overproduction of urine, though not as much as in Jk(a–,b–) persons. The great variation of the urea transporting capacity in RBCs as shown in the present and other studies from our laboratory (Brahm and Wieth, 1977; Brahm, 2013) has in common with APQ1 that the presence of the transporter in the RBC membrane is not essential for survival of the individual. The difference is that if the transporter is present, the capacity of the UT-B varies greatly, while the capacity of AQP1 appears to be the same (Table 3). About GLUT1 and AE1, we see little donor or age variations indicating that the transport functions are essential to human life. AE1 is essential in the vital transport of CO_2 in the form of bicarbonate from tissues to the lungs, and GLUT1 ensures that intracellular glucose becomes an important increase of the glucose transport capacity of the blood to the tissues.

Another noticeable outcome of the present study is to consider, when studying urea transport characteristics in human RBCs, that not taking the individual donor variations into account may blur the conclusions.

Data availability

The data are available from the corresponding author upon reasonable request.

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Author contributions: J. Leifelt and J. Brahm performed experiments, analyzed results, and assembled figures. M.H. Dziegiel contributed to design the experimental strategy, discussed the results, and revised the manuscript. J. Leifelt and J. Brahm designed the project, planned the experiments, and wrote the

manuscript. The three authors approve the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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