Deformability of Mammalian and Fish Erythrocytes:
Comparison of Mean Pore Transit Times
of Cells and Estimation of Cellular
Viscosity and Elasticity

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Abstract Red blood cells (RBCs) of various species of mammals and fish were subjected to a modified Nuclepore filtration test, which determines average time required for cells to pass through filter pores under constant suction. The values so far obtained, when plotted against the ratio of mean cell diameter to pore diameter, fall in a region given by a formula, which has been derived from a simple viscoelastic model for the deformation of a cell, for a small range of cellular viscosity ($1.0\text{--}2.0 \times 10^{-3} \text{ dyn}\cdot\text{s}/\text{cm}$ for mammals at 37°C , $3.0\text{--}5.0 \times 10^{-3} \text{ dyn}\cdot\text{s}/\text{cm}$ for fish at 15°C). Furthermore, estimates of cellular elasticity given from pore transit times at the cell-pore diameter ratio beyond 2.0 also fall in a similar range ($0.5\text{--}1.0 \text{ dyn}/\text{cm}$) for both the mammalian and fish cells. It is thus suggested that RBC filterability may not differ greatly, when compared at a comparative temperature, between different species studied so far, including mammals and fish, for a relatively large range of the extent to which cells are caused to deform. A question then arises about the shape and cytoplasm factors which have been accepted as the primary determinants of RBC deformability.

Key words: erythrocyte filterability, membrane viscoelasticity, nucleated erythrocyte.

Red blood cells (RBCs) show a remarkable ability to change shape when subjected to mechanical stresses. It is well documented that such deformability of cells plays a significant role in blood flow mechanics particularly in the microcirculation (WEED, 1970; CHIEN, 1987).

The high deformability of RBCs has been understood to be associated with their unique cellular structure. For example, the biconcave disk shape allows a

stressed cell to take a great variety of forms without its plasma membrane being stretched (FUNG, 1966), and the cytoplasm behaves merely as a hemoglobin solution against these shape changes. Such considerations have led to a consensus among researchers that RBC deformability is primarily regulated by the following three factors: (a) cell surface area to volume ratio, (b) cytoplasm viscosity, and (c) cell membrane viscoelasticity (WEED, 1970; LACELLE, 1972; MOHANDAS *et al.*, 1980; MEISELMAN, 1981; CHIEN, 1987).

This widely-accepted view based on mammalian studies implies a lower deformability of RBCs in lower vertebrates because of their thin oval shape and nucleated nature. In our previous studies, however, filterability of RBCs in some fish species was found to be similar to that of human RBCs (HUGHES *et al.*, 1982) with effects of temperature related to their ambient temperatures (HUGHES *et al.*, 1982; KIKUCHI *et al.*, 1982a, 1985b; HUGHES and KIKUCHI, 1988). Although there are many factors relating to the microcirculation and also other measures for RBC deformability, this finding has raised the question as to whether the suspected rheological impediment to the microcirculation does really exist in those animals, which have shown remarkable acclimatization and activities in various habitats which they have occupied.

The aim of the present study is to extend the comparison of RBC filterability to a wider range of species. Values of the average time determined for cells to pass through Nuclepore filter pores under constant suction have already been reported by the present author and coworkers as an index of RBC filterability for various species under various conditions. So far, however, no meaningful comparison could be made, since this transit time obviously depends on, in addition to cellular deformability, sizes of both cells and pores.

A scaling rule is given in the present study for differences in cell and pore diameters. A simple viscoelastic model applied to the deformation which a cell undergoes when it is drawn into a pore gives the deformation time, i.e. time required for a cell to enter into a pore (denoted as T_{in} in KIKUCHI *et al.*, 1980), as a function of the cellular viscosity, elasticity, and the ratio of cell diameter to pore diameter. Although the mean pore passage time includes the time for a cell that has entered into a pore to flow through and out from the pore, the formula has been applied as a first approximation of the mean pore passage time. This assumes that the exit time would be smaller than the deformation time and/or might be relatively constant for different size cells in given diameter pores because of the effect of plasma lubrication.

MATERIALS AND METHODS

(a) *Species.* The values of the mean pore passage time of RBCs taken from our previous studies are for human (KIKUCHI *et al.*, 1982b, 1983, 1988; KIKUCHI and KOYAMA, 1984), rabbit (KIKUCHI and KOYAMA, 1983), yellowtail (HUGHES *et al.*, 1982), carp (KIKUCHI *et al.*, 1982a), rainbow trout (HUGHES and KIKUCHI,

1984; KIKUCHI *et al.*, 1985a; HUGHES and KIKUCHI, 1988), Pacific salmon (KIKUCHI *et al.*, 1985b), and ray (HUGHES and KIKUCHI, 1988). Additional measurements were made in the present study for rat (Wistar, male, $n=10$), mouse (ddY, male, $n=10$), dog (mongrel, male, $n=3$), guinea pig (Hartley, male, $n=3$), sheep (male, $n=4$), and goat (male, $n=2$).

(b) *Blood sample preparations.* Blood of rats, mice, and guinea pigs was taken from the abdominal aorta after slight anesthesia with ether. Blood was obtained from conscious intact dogs and goats via venepuncture of an antecubital vein and jugular vein, respectively. Sheep blood was taken via chronic canula placed in a jugular vein from animals which were being used for other (lung lymph) studies. Each blood sample was anticoagulated with heparin solution (1,000 IU/ml) of 0.5 part to 9.5 part blood. RBC-plasma suspensions with hematocrit values of 10 to 20% were prepared from separated RBCs (5,000 rpm, 5 min; minimal buffy coat contamination) and plasma, and, after incubation for 15 to 30 min with gentle agitation in a water bath at 37°C, were subjected to a modified Nuclepore filtration test (KIKUCHI *et al.*, 1979, 1980, 1983) using 5 and 3 μm diameter pores. Mouse blood was used only for 3 μm pore filtration test because of a small available sample volume.

(c) *Filtration and related measurements.* A sample volume of 0.6 ml was used for each determination of the Nuclepore filtration rate. The test suspension was transferred to a 0.5 ml graduated cylinder connected to the inlet of a filter holder (both surrounded by a water jacket) and caused to flow through a Nuclepore filter membrane by applying 10 cmH₂O negative pressure to the outlet. The times for the sample meniscus to cross graduation marks (every 0.1 ml from 0.5 to 0 ml) were recorded with an electronic timer. The filter was repeatedly flushed with saline before every measurement to remove air bubbles trapped in the pores; this process is essential for making reproducible measurements. A fresh filter was used for each blood sample, the pore density of each being calibrated before use by determination of the saline passage time.

A small portion of each suspension was used for measurement of hematocrit (microhematocrit, 11,000 $\times g$, 5 min, in duplicate) and RBC count (auto cell counter). The mean pore passage time of RBCs was calculated from values of blood passage time, hematocrit, mean corpuscular volume using a previously-derived equation (KIKUCHI *et al.*, 1980, 1983; KIKUCHI and KOYAMA, 1984). These procedures for determination of RBC mean pore passage times are the same as those used in the previous studies except for use of more strongly heparinized whole blood samples at ambient temperatures and mainly 8 μm pore in the fish studies.

(d) *Analyses.* The mean pore passage time values were plotted against the ratio of mean cell diameter to pore diameter. The relations thus obtained have been subjected to an analysis by the following formula, whose derivation is given in the APPENDIX:

$$t_d = (\eta/c\Delta P)((r_c/r_p)^2 - (r_c/r_p))\sqrt{1 - (r_p/r_c)^2}, \quad (1)$$

where t_d is the deformation time required for a cell to enter into a pore, r_c the cell radius, r_p the pore radius, c the cell thickness, ΔP the pressure difference across the pore, and η the cellular viscosity; or the equation before approximated,

$$t_d = -(\eta/\mu) \ln(1 - (\mu/c\Delta P)((r_c/r_p)^2 - (r_c/r_p))\sqrt{1 - (r_p/r_c)^2}), \quad (2)$$

where μ is the elastic modulus used in the Kelvin-Voigt viscoelasticity model, to take into account the effect of the cellular elasticity on the deformation time when the cell-pore diameter ratio exceeds 1.8.

RESULTS

Sizes of RBCs in the species studied so far and in the present study are summarized in Table 1. The diameter values for the mammalian cells are taken from references (CANHAM and BURTON, 1968; CHIEN *et al.*, 1971; EVANS and FUNG, 1972; SCHALM *et al.*, 1975). The mean thickness of cells was calculated by dividing the mean corpuscular volume (MCV), which was our determination, by the cell radius squared. Since this value differs only slightly among the species, the average was taken for them for use in the above equations. Sizes of the fish cells are those determined in our previous studies. For the sake of comparison with mammalian cells, the geometrical mean of the major and minor axes of the ellipsoid $\sqrt{ab}$ and the mean cell thickness $4V/\pi(ab)$ were taken as the cell diameter and thickness, respectively. Because of such a presumption and limit as a first approximation, the average of the mean cell thickness values was also taken for the fish species in the present comparison.

The mean pore passage times of RBCs of the mammalian species are plotted against the ratio of cell diameter to pore diameter in Figs. 1 and 2 with different scales of X - and Y -axes. The values are for $5\text{ }\mu\text{m}$ pores unless otherwise indicated, and at 37°C . A similar comparison within the fish species is shown in Figs. 3 and 4, where, unless otherwise indicated, the values are for $8\text{ }\mu\text{m}$ pores and at 15°C . The solid lines shown in Figs. 1 and 3 are the deformation times calculated from the above Eq. (1) for cells to enter into 5 and $8\text{ }\mu\text{m}$ pores, respectively, using given values of the viscous modulus. The curves include the time for a cell with deformation completed, i.e. cell fully entered into a pore, to flow through and out from the pore, which is assumed to be constant for the different size cells and also to be equal to the transit time of the cell when no deformation is needed, i.e. at the diameter ratio 1 (0.45 ms for $5\text{ }\mu\text{m}$ pores in Figs. 1 and 2 from the value obtained for sheep cells). The corresponding value for $8\text{ }\mu\text{m}$ pores is assumed to be 0.3 ms (see DISCUSSION) in Figs. 3 and 4. The broken line in Fig. 1, which is given by the above Eq. (2), is to show a minor effect of the cellular elasticity on the deformation time in the range of a diameter ratio lower than 1.6 (the value of elastic modulus used is 0.7 dyn/cm). Meanwhile, the cellular elasticity becomes dominant in the

Table 1. Dimensions of erythrocytes.

Mammals	Diameter* d (μm)	MCV V (μm^3)	Mean thickness $4V/\pi d^2$ c (μm)
Goat	3.2	19.1 ± 0.9	2.4
Sheep	4.8**	38.1 ± 1.9	2.1
Mouse	6.1	54.6 ± 3.2	1.9
Rat	6.3	57.0 ± 2.3	1.8
Rabbit	6.7	58.9 ± 3.1	1.7
Dog	7.0	65.4 ± 7.3	1.7
Guinea pig	7.5	82.8 ± 2.6	1.9
Human	8.0***	97.3 ± 5.8	1.9 (mean 1.9)

Fish	Diam. $\times$ diam. $\times$ thickness (a (μm) $\times b$ (μm) $\times c$ (μm))	MCV V (μm^3)	Mean diam. $\sqrt{ab}$ (μm)	Mean thickness $4V/\pi ab$ c (μm)
Yellowtail	$11 \times 7 \times 3.3$	133 ± 12	8.8	2.2
Carp	$12 \times 9 \times 3.3$	186 ± 24	10.4	2.2
Rainbow trout	$13 \times 10 \times 3.3$	213 ± 8	11.4	2.1
Pacific salmon	$17 \times 12 \times 4.2$	498 ± 64	14.3	3.1
Ray	$25 \times 16 \times 4.2$	753 ± 111	20	2.4 (mean 2.4)

*From SCHALM *et al.* (1975) except for sheep and human. **From CHIEN *et al.* (1971). ***Mean of $8.1 \mu\text{m}$ by CANHAM and BURTON (1968) and $7.8 \mu\text{m}$ by EVANS and FUNG (1972).

Fig. 1. Mean pore passage times of RBCs of various mammals plotted against the ratio of cell diameter to pore diameter. The pore diameter is $5 \mu\text{m}$ and measuring temperature is 37°C . The solid lines are given by Eq. (1) in the text for given values of viscous modulus. The broken line includes elastic modulus 0.7 dyn/cm .

range of diameter ratio beyond 2.0. The solid curves in Figs. 2 and 4 are given by Eq. (2) for given values of elastic modulus, where viscous modulus is fixed to be $1.5 \times 10^{-3} \text{ dyn} \cdot \text{s/cm}$ in Fig. 2 and $4.0 \times 10^{-3} \text{ dyn} \cdot \text{s/cm}$ in Fig. 4.

While most of the passage times of RBCs appeared explicable by the present model as shown in Figs. 1 through 4, sheep and goat RBCs showed an anomalous resistance to passage through the $3 \mu\text{m}$ pores. Sheep RBCs could not pass through $3 \mu\text{m}$ filter pores under the present suction pressure. This result appeared particularly peculiar, as mouse, rat, and rabbit cells, which are larger than sheep cells, showed relatively small resistances (Fig. 5). A large fraction of goat cells also appeared to be unpassable through $3 \mu\text{m}$ pores. The dotted line in Fig. 5 indicates, from the present data, a possible relation of the resistance to $3 \mu\text{m}$ pore passage with cell diameter.

DISCUSSION

Blood filtration through polycarbonate sieves has widely been used as a simple

Fig. 2. Mean pore passage times of RBCs of various mammals plotted against the ratio of cell diameter to pore diameter (the ratio extends beyond 2.0). The pore diameter is $5\mu\text{m}$ unless otherwise indicated and measuring temperature is 37°C . The solid lines are given by Eq. (2) in the text for given values of elastic modulus and viscous modulus fixed at $1.5 \times 10^{-3} \text{ dyn}\cdot\text{s}/\text{cm}$.

means to study RBC deformability (USAMI *et al.*, 1975; REID *et al.*, 1976). However, it requires great care such as complete removal of air bubbles from filter pores and calibration of pore number every time to use the filtration as a measure (KIKUCHI *et al.*, 1979). Furthermore, it is necessary to eliminate the other factors interfering with the transit of single RBCs by sample preparations (STUART *et al.*, 1983; RITCHIE, 1985) or by analyses of changes in the filtration rate (KIKUCHI *et al.*, 1980, 1983; SKALAK *et al.*, 1983). Discrepancies still appear to exist between reports, probably because of different modifications taken (KIKUCHI *et al.*, 1988).

Despite many studies using human RBCs, few applications have been undertaken of the filtration or other methods to blood of other species particularly other than mammals. This appears to be not only due to interest of researchers but also due to technical difficulties. Differences between different species are not restricted to RBC morphology (Table 1), but include RBC vulnerability, relative size of

Fig. 3. Mean pore passage times of RBCs of various fish species plotted against the ratio of cell diameter to pore diameter. The cell diameter used here is the geometrical mean of the major and minor axes of the ellipsoid. The pore diameter and measuring temperature are $8\mu\text{m}$ and 15°C , respectively, unless otherwise indicated. The solid lines are given by Eq. (1) in the text for given values of viscous modulus.

WBCs to RBCs, RBC-RBC interaction, and coagulability. Therefore, any mis-modifications of the methods would result in a lack of consistency should they be applied for those different species.

Fig. 4. Mean pore passage times of RBCs of various fish species plotted against the ratio of cell diameter to pore diameter (the ratio extends beyond 2.0). The cell diameter used here is the geometrical mean of the major and minor axes of the ellipsoid. The pore diameter and measuring temperature are 8 μ m and 15°C, respectively, unless otherwise indicated. The solid lines are given by Eq. (2) in the text for given values of elastic modulus and viscous modulus fixed at 4.0×10^{-3} dyn·s/cm.

In the present study, an analysis has been undertaken to relate the deformation time for a cell to enter into a pore to physical properties of cell constituents. As already noted, the pore passage time includes the time required for a cell that has fully entered into a pore to flow through and out from the pore. A temporal assumption was made that this exit time would be relatively constant for different size cells in given diameter pores. This time was further taken to be equal to the

Fig. 5. RBC pore passage times in mammals determined for $3\mu\text{m}$ pores. The dotted line indicates a possible relation for the variation of pore passage time with cell diameter.

passage time determined for sheep RBCs (0.45 ms) for $5\mu\text{m}$ pores for which the cell-pore diameter ratio is nearly equal to 1. The corresponding value for $8\mu\text{m}$ pores was assumed to be 0.3 ms by an assumption that the time would be inversely

proportional to pore diameter (applied force which is proportional to cross-sectional area of the pore should be equal to frictional force which will probably be proportional to cell velocity multiplied by circumference). Despite the simplifications made for complicated force-deformation relationships actually involved in the entrance event and the above additional assumption, the present model appears to explain, as shown in Figs. 1 through 4, the relationship experimentally obtained between the pore passage time and the ratio of cell diameter to pore diameter.

Furthermore, estimates obtained for cellular viscosity agree well with those (10^{-4} – 10^{-2} dyn·s/cm) reported for cell membrane viscosity in much more sophisticated studies (EVANS and HOCHMUTH, 1976a, b; CHIEN *et al.*, 1978; HOCHMUTH *et al.*, 1980; MARKLE *et al.*, 1983).

Most of the difference indicated in the viscous modulus between the mammalian and fish RBCs (1.0 – 2.0×10^{-3} dyn·s/cm for the former at 37°C and 3.0 – 5.0×10^{-3} dyn·s/cm for the latter at 15°C) would be attributable to the difference in measuring temperature. HOCHMUTH *et al.* (1980) reported a four-fold increase in the surface viscosity at 15°C in comparison to 37°C for human RBCs. In accordance with this report, about a three-fold increase is obtained in the viscous modulus at 15°C compared to 37°C values for yellowtail RBCs (Fig. 3). Similar values of the elastic modulus shown for both the mammalian and fish cells, despite the difference in measuring temperature, would also be consistent with slight changes reported in the surface elasticity with temperature (HOCHMUTH *et al.*, 1980).

Although such a similarity among different species would be reasonable from the viewpoint of comparative physiology, the present suggestion appears contradictory to the consensus concerning the primary determinants of RBC deformability. The shape and cytoplasm factors, however, would be a supposition from the extreme cases of spherical and sickle cells, probably leaving a question as to whether the differences in cell morphology among species may result in significant differences in the cellular deformability. Several researchers have noted that relative importance of each determinant may vary depending on how RBCs are stressed or how RBC deformability is measured (MOHANDAS *et al.*, 1980).

The intuitive concept of deformability includes both elastic and viscous components, i.e. the extent or limit of deformation and the time or speed of deformation. These two components are commonly expressed by springs and dashpots in viscoelasticity models to characterize cellular deformations due to external forces (FUNG, 1981). The analyses made in the present study show that our "filterability" measure may be related mainly to the viscous term for small deformations, i.e. in the range of the cell-pore diameter ratio lower than 1.6, and mainly to the elastic term for large deformations, i.e. in the range of the cell-pore diameter ratio beyond 2.0.

Rather than between mammals and fish, differences in RBC deformability appear notable among mammals when tested using $3\text{ }\mu\text{m}$ pores. While much larger cells, even those of humans, could pass through $3\text{ }\mu\text{m}$ pores, sheep RBCs could not

pass through the pores under the present suction pressure. The fraction of goat cells having diameters larger than the pore diameter also appeared unpassable through $3\text{ }\mu\text{m}$ pores. Such a result would be consistent with viscometric and filtration studies by CHIEN *et al.* (1971) for sheep and goat, and with viscodiffractometric studies by SMITH *et al.* (1979) for goat, but contrary to filtration studies by ACQUAYE *et al.* (1987).

From a mechanical viewpoint, it appears possible that sheep and goat cells might show a greater resistance to passage through a $3\text{ }\mu\text{m}$ pore as do larger cells of the other mammals. Cells would become stiffer as their size becomes smaller. The change in stiffness with size is well documented in the shell theory, which gives a critical pressure to make a bulge in a curved shell as $E(h/R)^2$, where E , h , and R are Young's modulus, thickness, and curvature radius of the shell, respectively (LANDAU and LIFSHITS, 1986). An order-estimation using $P_{cr} \sim 10\text{ cmH}_2\text{O}$, $h \sim 0.01\text{ }\mu\text{m}$, and $R \sim 1\text{ }\mu\text{m}$ gives $E \sim 10^8\text{ dyn/cm}^2$, which falls in the range reported by RAND (1964) for Young's modulus of human RBC membrane. The filterability through the $3\text{ }\mu\text{m}$ pore would be critically dependent on the magnitude of pressure used, which might explain some of the above discrepancies. A higher deformability of larger cells has also been noted by CHIEN *et al.* (1971).

REINHART and CHIEN (1985) studied filterability of human RBCs exposed to various osmolalities using 3, 5, $8\text{ }\mu\text{m}$ Nuclepore filters and index of relative resistance β (SKALAK *et al.*, 1983) of RBC. They showed the importance of cell volume for $3\text{ }\mu\text{m}$ pore passage and cellular viscosity for $8\text{ }\mu\text{m}$ pore passage. The present study would confirm their conclusion by further clarifying the cell filterability in terms of the cellular viscosity and elasticity.

The values indicated for the elastic modulus ($0.5\text{--}1.0\text{ dyn/cm}$) are just intermediate between values reported for the area compressibility modulus (450 dyn/cm at 25°C) and those for the shear modulus ($0.004\text{--}0.0066\text{ dyn/cm}$ at 25°C) (MARKLE *et al.*, 1983). This result would probably characterize deformations which RBCs undergo when they are squeezed through pores with diameters smaller than a half of cell diameter.

In conclusion, a filterability test and analysis yielded similar and acceptable estimates for the cellular viscosity and elasticity in RBCs of various species, including mammals and fish.

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APPENDIX

Considerable efforts have been made to analyze as definitively as possible the viscoelastic and viscoplastic properties of the erythrocyte membrane (HOCHMUTH and MOHANDAS, 1972; EVANS and HOCHMUTH, 1976a,b; CHIEN *et al.*, 1978; HOCHMUTH *et al.*, 1980). However, the pore passage of an RBC involves so complicated a deformation of its membrane that such a rigorous approach appears out of reach. Let us take, therefore, a qualitative approach to the analysis of the deformation of an RBC entering into a pore.

A diagram for an RBC being drawn into a pore is given in Fig. 6(a). As indicated, the initial drawing force acting on the cell will be proportional to the cross-sectional area of the cell in the pore, and hence it may be given as

$$F = 2r_p c \Delta P, \quad (1)$$

where r_p is the pore radius, c is the cell thickness, and ΔP is the pressure difference across the filter. This drawing force is counterbalanced by the forces exerted by the edges of the pore on the cell (quasi-static condition). Since each counteracting force will be nearly directed to the center of the cell, the magnitude of its horizontal component f may be given as

$$f = (F/2)(\cos\theta/\sin\theta) = (F/2)(r_p/r_c)/\sqrt{1 - (r_p/r_c)^2}, \quad (2)$$

where θ is the angle between the direction from the edge to the center of the cell and the direction to the opposite edge, and r_c is the cell radius. The two opposite edges exert the two forces of the same magnitude directed toward each other in the horizontal plane, causing the cell to be squeezed. Let the decrease in cell diameter due to these two forces be denoted by u . Our assumptions are that the complicated cellular deformation may be represented by this single variable u and that the Kelvin-Voigt model of viscoelasticity (FUNG, 1981) may be applied to the relation between u and the sum of the two forces (Fig. 6(b));

$$\mu u + \eta \dot{u} = 2f, \quad (3)$$

where μ and η are the elastic and viscous modulus, respectively, and $\dot{u}$ is the time derivative of u . As a first approximation, we assume the right-hand side to be constant with time t , i.e. that the force acts on the cell in a step function manner with time, until it vanishes or becomes very small at the time point when the cell is fully drawn into the pore. The following relation then results:

$$u = (2f/\mu)(1 - \exp(-(\mu/\eta)t)). \quad (4)$$

If $(\mu/\eta)t$ is small in comparison to 1, this equation is further approximated by

$$u = (2f/\eta)t. \quad (5)$$

We apply this relation to the deformation time t_d required for the cell to be

Fig. 6. (a) Diagram showing the forces which act on a cell when it is drawn into a pore. (b) Diagram showing a viscoelastic model for the deformation which the cell undergoes to enter into the pore.

wholly drawn into the pore by giving $2(r_c - r_p)$ to u . By using Eqs. (1) and (2), this finally results in:

$$t_d = (2\eta/F)(r_c - r_p) \sqrt{1 - (r_p/r_c)^2} / (r_p/r_c) \\ = (\eta/c\Delta P)((r_c/r_p)^2 - (r_c/r_p)) \sqrt{1 - (r_p/r_c)^2}. \quad (6)$$

When Eq. (4) must be used, the following formula is derived straightforwardly:

$$t_d = -(\eta/\mu) \ln(1 - (\mu/c\Delta P)((r_c/r_p)^2 - (r_c/r_p)) \sqrt{1 - (r_p/r_c)^2}). \quad (7)$$

These final equations, Eqs. (6) and (7), are given in the text.