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# Effect of Normal Human Erythrocytes on Blood Rheology in Microcirculation

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## Abstract

***Background***

Effects of RBCs on blood rheology were studied using a microchannel array flow analyzer (MC-FAN).

***Methods***

Fluidity of four types of samples prepared from the venous blood of healthy volunteers was examined in terms of passage time through the microchannel array of MC-FAN (model KH-3): (1) physiological saline-RBC suspensions and plasma-RBC suspensions, each adjusted to a predetermined hematocrit value; (2) suspensions of glutaraldehyde-treated hardened RBCs; (3) fibrinogen-RBC and albumin-RBC suspensions; and (4) dextran-RBC suspensions.

***Results***

Hematocrit positively correlated with passage time. Both plasma and fibrinogen prolonged passage time significantly. Hardened RBCs completely obstructed the microchannel. The passage time of dextran-RBC suspensions was prolonged in a dextran molecular weight- and concentration-dependent manner and was dependent on the passage time of the solution alone.

***Conclusions***

Blood rheology, as determined by MC-FAN, is affected not by RBC aggregation but hematocrit, RBC deformability, and the passage time of the solution.

Key Words: Erythrocytes; Microcirculation; Blood Rheology; Microchannel Flow Analyzer

## Introduction

Peripheral circulation directly contributes to skin and other systemic cell environments. Therefore, in diseases associated with peripheral circulatory failure, such as diabetes, collagen disease and arteriosclerosis, changes in color tone and a functional disorder of the skin first

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develop and serious conditions leading to ulceration often ensue. Identifying factors that affect blood fluidity in peripheral circulation is essential for preventing and treating these diseases[1]. However, no established method of hemodynamic assessment at the capillary level is available.

In 1994, Kikuchi et al developed a microchannel array flow analyzer (MC-FAN) for the evaluation of blood rheology in capillary-level microcirculation[2-5]. This device assesses blood rheology as the time required for 100 μL of whole blood to pass through the microchannel array, while simultaneously providing a view of the blood flow under microscope on the monitor. The microchannel array with 7 μm wide and 30 μm long grooves is a model of the capillary, used for the assessing of the enhanced state of platelet aggregation and decrease in white blood cell (WBC) adhesion and deformation capacity in the flow channel. The device is also drawing attention for its capability of enabling global assessment of circulating blood, including interactions between RBCs, platelets and WBCs, with the use of heparin-added venous blood withdrawn from subjects.

Blood rheology, a critical factor determining tissue blood flow levels, is influenced by the volume and function of RBCs, WBCs, platelets and plasma. RBCs in particular are, to a greater degree, influential in regard to rheology, occupying about 45% of the blood volume. RBCs affect peripheral circulation through (1) RBC volume; (2) RBC deformability; (3) fluidity of the plasma in which the RBCs lie; and (4) interactions between RBCs (RBC aggregability)[6-8]. While the mechanism of RBC aggregation is yet to be elucidated, the presence of macromolecules is deemed essential, and its extent depends on the molecular weight and concentration of macromolecules such as fibrinogen and immune globulin[6]. Albumin, another plasma protein, is of low molecular weight and does not induce RBC aggregation. Owing to complicated mechanisms such as this, conventional clinical laboratory tests alone have not been sufficient to demonstrate the kinetics of RBCs in various peripheral environments. In the present study, to clarify interrelationships between RBCs and hemodynamics at the capillary level, normal human RBCs were evaluated with a microcirculation assessment model MC-FAN and compared with previous findings. First, using RBC suspensions prepared with physiological saline and autologous plasma, the impact of hematocrit and plasma on passage time was assessed. Next, the state of passage of RBCs with decreased deformability through the channel array was observed using glutaraldehyde-treated hardened RBCs[6,7]. Albumin and fibrinogen, major plasma proteins, were used to assess the impact of plasma proteins on the passage time of RBC suspension. Lastly, to evaluate the degree of impact of RBC aggregability on passage time, the passage time of RBC suspensions prepared with macromolecular dextrans was measured, and the effects of dextran concentrations and molecular weights were assessed. Furthermore, the ratio of passage time of dextran-RBC suspension to that of dextran solution was calculated to estimate the influence of the fluidity of dextran solution on that of dextran-RBC suspension.

## Subjects and Methods

### *Subjects*

Healthy volunteers ranging in age from 26 to 35 years (4 men and 4 women) were recruited from Osaka City University campus. They had no history of chronic diseases and no medication history over the previous 1 year. Before the experimental work, they were informed of the objective and design of the study and consent was obtained thereafter. Venous blood samples

from healthy volunteers were taken via antecubital veins with anticoagulation by heparin sodium.

***Red blood cell suspensions adjusted to various hematocrit values***

RBCs obtained from healthy volunteers by centrifugation were washed with physiologic saline three times, and suspended in native plasma or physiologic saline adjusted to 9%, 27%, 45%, 55%, 63% hematocrit, and the fluidity of these RBC suspensions was determined according to Kikuchi's microchannel method.

***Kikuchi's microchannel method***

Kikuchi's microchannel method coupled with microchannel flow analyzer (MC-FAN: Hitachi Haramachi Electronics Co., Ibaragi, Japan) was used to determine the fluidity of RBC-suspensions and dextran solution, as described previously[2-5]. The time taken for 100 μL of each of the sample to flow through a microchannel, 7 μm in width and 30 μm in length, at a pressure of 20 cm $H_2O$ was measured. The passage time of the samples was calibrated with 100 μL of physiologic saline.

***Glutaraldehyde-treated red blood cells***

Fresh human RBCs obtained from healthy volunteers by centrifugation were washed in physiologic saline three times and were hardened by incubation in 1.6% glutaraldehyde solution (1.6% glutaraldehyde, 58.2 mM NaCl, 3.9 mM $K_2HPO_4$, and 0.7 mM $KH_2PO_4$) for 3 days. After incubation, hardened RBCs were washed in physiological saline five times, and suspended with physiological saline at 20% hematocrit[9]. These RBCs with hardening of the membrane have often been used because erythrocytes reduced their deformability significantly[6,7].

***Fibrinogen and albumin solution***

Fibrinogen and albumin from human plasma were purchased from WAKO-JYUNYAKU (Osaka, Japan). Fibrinogen was dissolved at 37℃ in physiological saline at a concentration of 1000 mg/dL, and albumin solution was prepared at a concentration of 5 g/dL. Separated RBCs were suspended at 45% hematocrit in fibrinogen solution or albumin solution, as well as physiological saline or native plasma RBC suspensions.

***Dextran solution***

Dextran 40 (Dex-40, M. W. 40000), dextran 70 (Dex-70, M. W. 73000), and dextran 500 (Dex-500, M. W. 496000) were purchased from Pharmacia (Stockholm, Sweden), and were dissolved in physiological saline at a concentration of 1 g/dL, 2 g/dL, or 4 g/dL. The transit time of each dextran solution was measured by MC-FAN. Separated RBCs were suspended at various hematocrit values in dextran solutions. Erythrocyte aggregation in dextran solution at rest was observed directly using the monitor of the microchannel flow analyzer (MC-FAN). The passage time of the RBC suspensions was measured by MC-FAN, and we investigated the influence of dextran concentration and molecular weight on the passage time of the RBC solutions. To examine whether the increased passage time was due to the increased plasma viscosity or due to the erythrocyte aggregation, we defined the ratio passage time of the RBC suspensions to dextran solution. Here, the concentration and molecular weight of dextran in the RBC suspensions were the same as those of the dextran solution.

***Statistical analysis***

Spearman's correlation coefficient analysis and simple regression were used to assess the

relations between hematocrit values and passage time. Other results were expressed as means±SD deviation. The means were compared by Student's *t*-test or Kruskal-Wallis test with Scheffe multiple comparison tests. A value of $p<0.05$ was considered to indicate statistical significance.

## Results

### *Influence of hematocrit on the transit time of RBC-suspensions*

Strong positive correlations were found between hematocrit values and passage time in physiologic saline-RBC suspensions ($\gamma^2=0.9137$, $p<0.0001$), and native plasma-RBC suspensions ($\gamma^2=0.8393$, $p<0.0001$). The regression lines using passage time as outcome variable (y) and hematocrit as predictor variable (x) were $y=0.363x+10.017$ and $y=0.4979x+17.438$ for physiologic saline-RBC suspensions and native plasma-RBC suspensions respectively (Fig. 1).

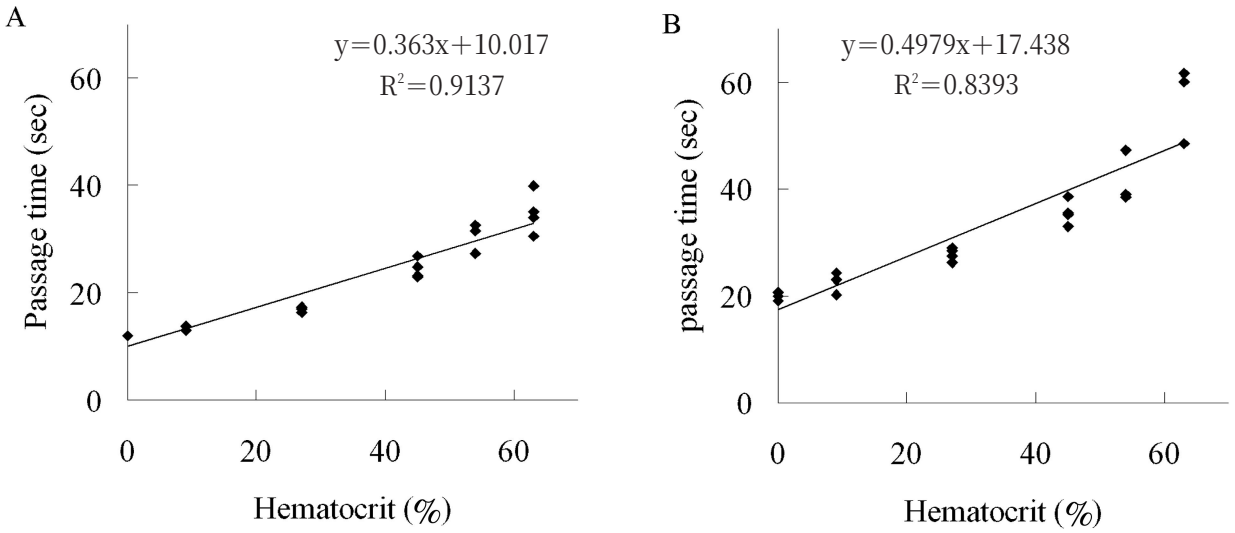


**Figure 1.** Relationship between RBC suspension passage time and hematocrit (Ht). A, Physiological saline (PSS)-RBC suspension. Passage time and Ht correlated positively ($\gamma^2=0.9137$, $p<0.0001$). When y=passage time and x=Ht, the regression line was $y=0.363x+10.017$. B, Plasma-RBC suspension. The passage time and Ht correlated positively ($\gamma^2=0.8393$, $p<0.0001$). When y=passage time and x=Ht, the regression line was $y=0.4979x+17.438$.

### *The passage time of RBC suspensions in native plasma, fibrinogen solution and albumin solution*

The mean passage times of native plasma-RBC suspensions were significantly longer than those of physiological saline-RBC suspensions (Fig. 2). Fibrinogen prolonged the passing time of RBC suspensions significantly compared to those of physiological saline ($p=0.0058$), but albumin did not ($p=0.6738$). There was no significant difference between the passage times of RBC-fibrinogen solution suspensions and those of RBC-plasma suspensions ($p=0.1281$) (Fig. 3). This result shows that the passage time is influenced by plasma, especially fibrinogen, which raises plasma viscosity and induces red blood cells to aggregate at low shear stress.

### *Glutaraldehyde-treated RBCs as a model remarkably decreasing deformability*

Glutaraldehyde-treated RBC suspensions could not pass through MC-FAN, because hardened RBCs became stuck in the microchannels (Fig. 4). This result indicates that RBC deformability is important to pass through narrow passages such as capillary and MC-FAN.

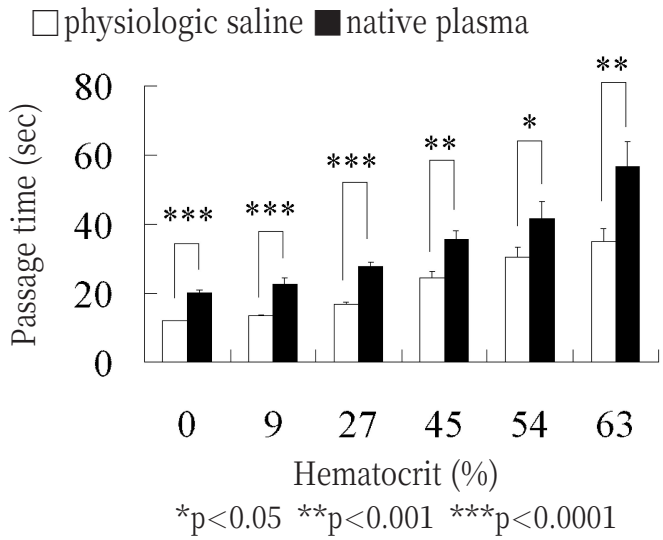


**Figure 2.** The passage time of plasma-RBC suspension was prolonged significantly compared with that of PSS-RBC suspension.

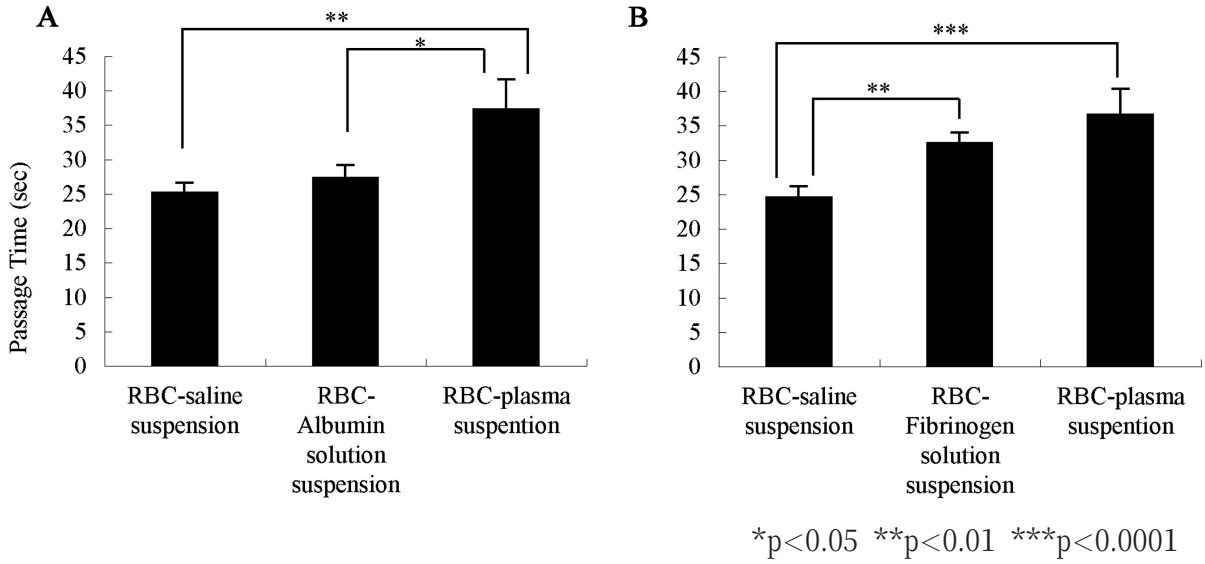


**Figure 3.** A, The passage time of PSS-RBC suspension and that of albumin-RBC suspension did not differ significantly (p=0.6738). The passage time of albumin-RBC suspension was significantly shorter (p=0.013) than that of plasma-RBC suspension. B, The passage time of fibrinogen-RBC suspension was significantly prolonged (p= 0.0058) compared with that of PSS-RBC suspension. The passage time of fibrinogen-RBC suspension and that of plasma-RBC suspension did not differ significantly (p=0.1281).

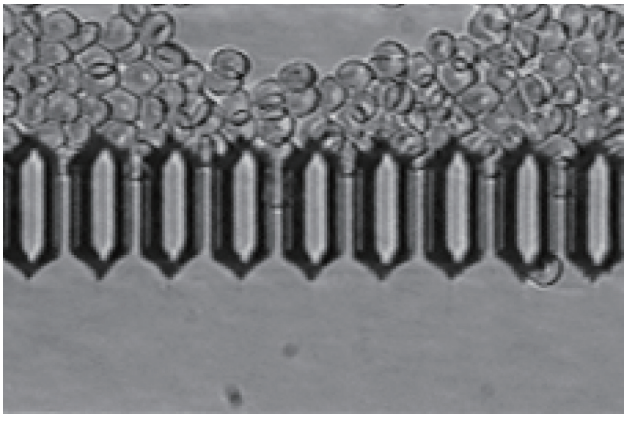

**Figure 4.** The suspension of glutaraldehyde-treated hardened RBC failed to pass through the microchannel array of MC-FAN. Because the channel was completely clogged, the transit time was undeterminable.

***The influence of dextran solutions on the transit time of RBC suspensions***

RBCs aggregation in dextran solution at rest was observed directly using the monitor of the microchannel flow analyzer (MC-FAN). RBCs aggregation were induced in suspensions with dextran of molecular weight=70000 and 5000000, but not 40000 (Fig. 5). Strong positive

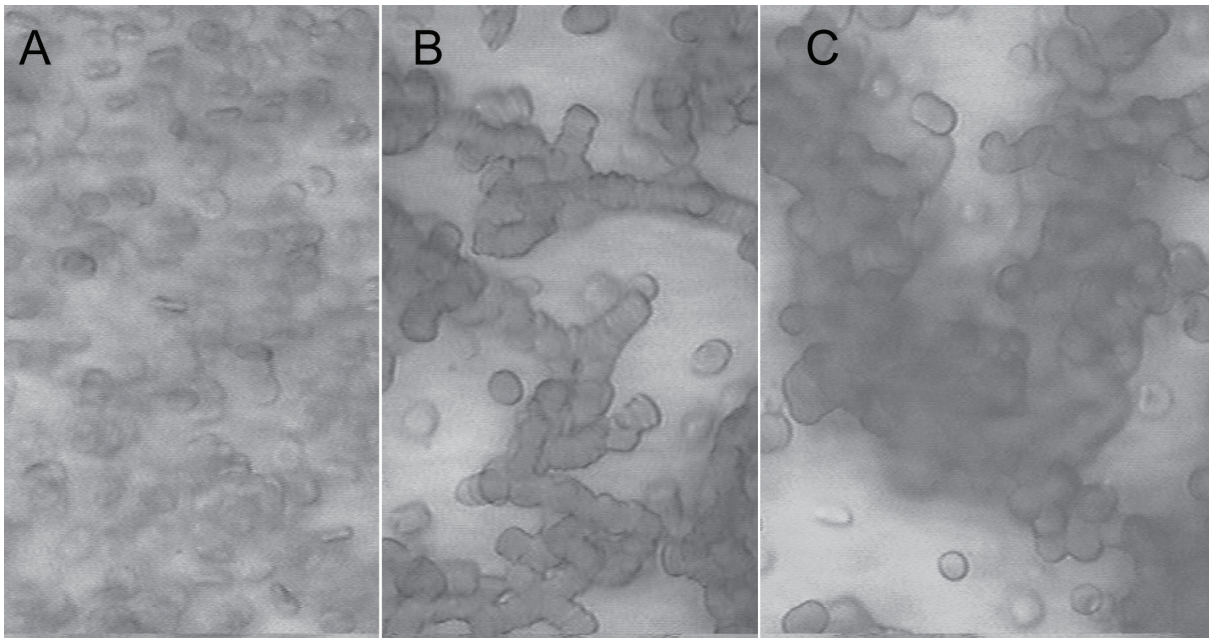


**Figure 5.** RBC state in the absence of flow in dextran solutions was observed on the MC-FAN monitor. A, Dextran (molecular weight [MW] 40000) solution. No RBC aggregation was noted. B, Dextran (MW 70000) solution. Coin columns were observed. C, Dextran (MW 5000000) solution. Coin columns larger than those in the dextran (MW 70000) solution were noted.

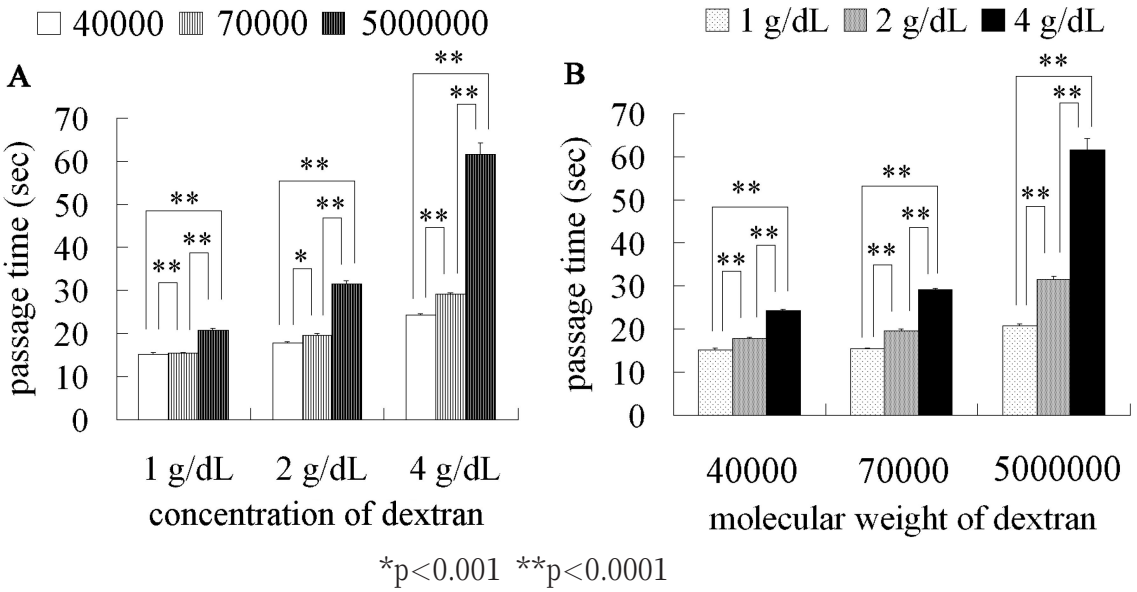


**Figure 6.** The passage time of dextran solutions. A, The passage time and dextran MWs. For solutions at equal dextran concentrations, the larger the MW, the greater the prolongation of passage time occurred with significance. B, The passage time and dextran concentrations. For solutions of dextran of the same MW, the higher the dextran concentrations, the greater the prolongation of passage time.

correlation was found between hematocrit and the passage time of RBC suspensions in dextran solution of molecular weight (40000, 70000, and 5000000), and of the concentration (1 g/dL, 2 g/dL, and 4 g/dL) (Data was not shown). The fluidity of dextran solution was detected by the concentration and molecular weight of dextran (Fig. 6). At the same hematocrit value, the transit time of dextran-RBC suspension was significantly longer than that of dextran-RBC suspension with lower molecular weight (Fig. 7), and than that with a lower concentration of dextran (Fig. 8). These results show that the passage time of dextran solution and of dextran RBC suspensions extended in samples with higher molecular weight of dextran or in more concentrated samples.

***The ratio of the passage time between dextran-RBC suspensions and dextran solution***

The ratio of the passage time of RBC-dextran suspension to that of dextran solution (suspension to solution ratio) was assessed. The suspension to solution ratio, as in the case of

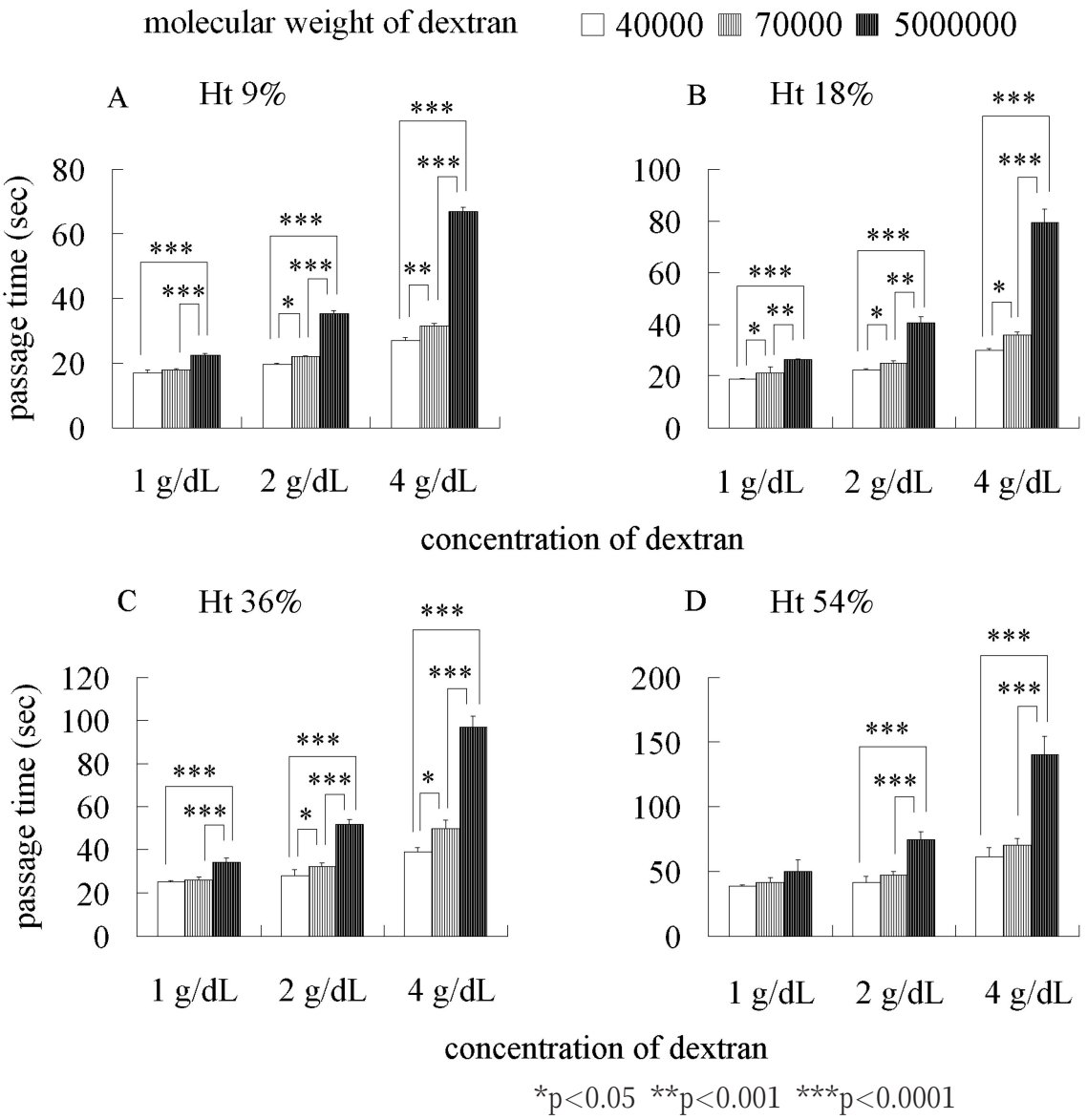


**Figure 7.** Relationship between dextran concentrations and dextran-RBC suspension passage time. Ht 9% (A), Ht 18% (B), Ht 36% (C), and Ht 54% (D). At a constant Ht, the higher the dextran concentration of the suspension, the greater the prolongation of passage time occurred with significance.

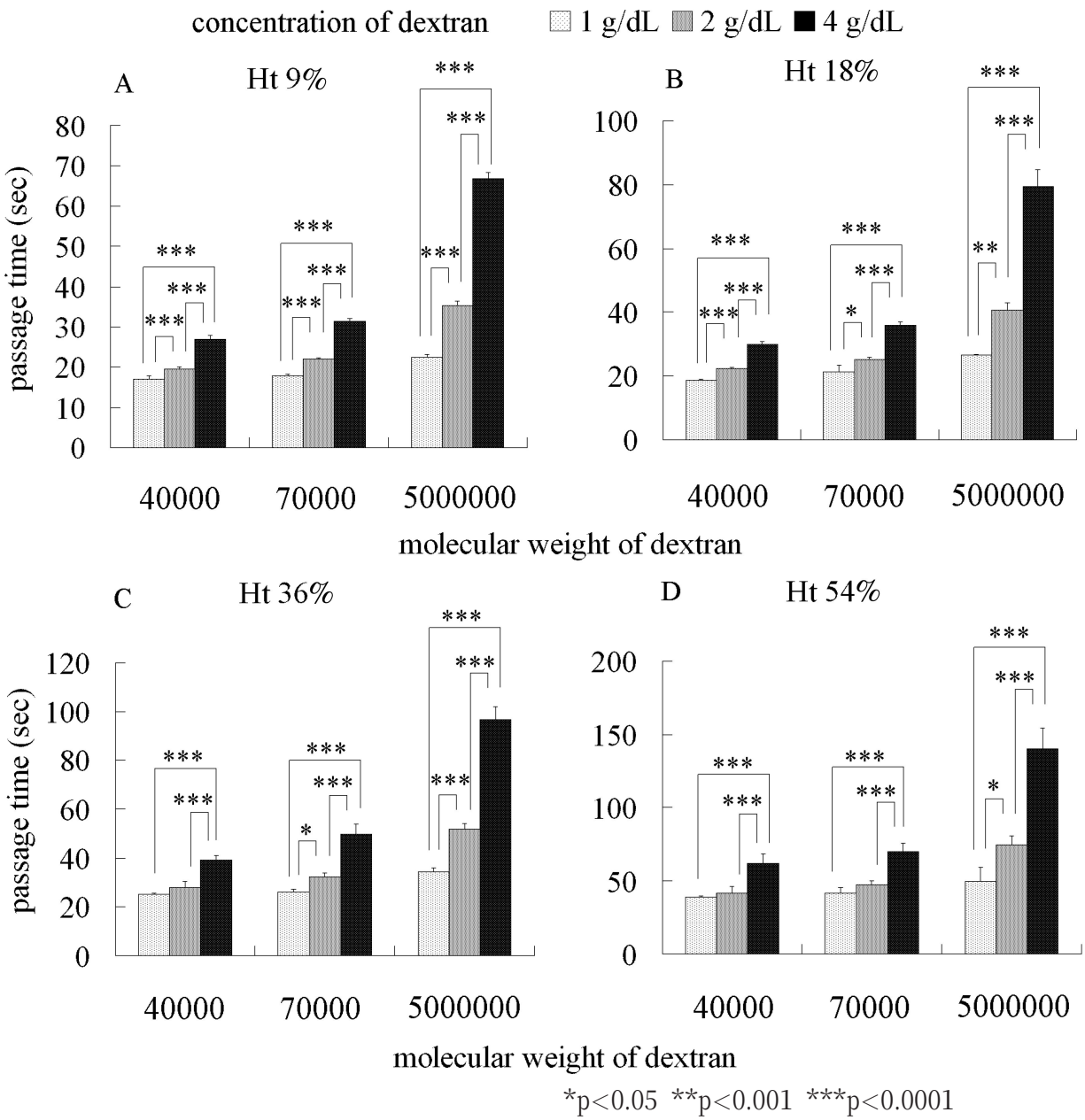


**Figure 8.** Relationship between dextran MWs and dextran-RBC suspension passage time. Ht 9% (A), Ht 18% (B), Ht 36% (C), and Ht 54% (D). At a constant Ht, the larger the MW of dextran in the suspension, the greater the prolongation of passage time occurred with significance.

RBC suspensions prepared with dextran or other solutions, exhibited a strong correlation with hematocrit (data not shown). However, at a constant hematocrit value, the suspension to solution ratio did not show any significant increase at higher dextran concentrations of the solutions (Fig. 9), or at larger molecular weights of dextran in the solutions (Fig. 10). This indicates that under a constant hematocrit value the transit time of dextran-RBC suspension is determined by the transit time of dextran solution alone. Between dextrans of a molecular weight of 40000 (which does not induce RBC aggregation) and dextrans of larger molecular weights of 70000 and 5000000 (which induce the aggregation), the suspension to solution ratio did not differ significantly (Figs. 9 and 10), suggesting that RBC aggregability would hardly

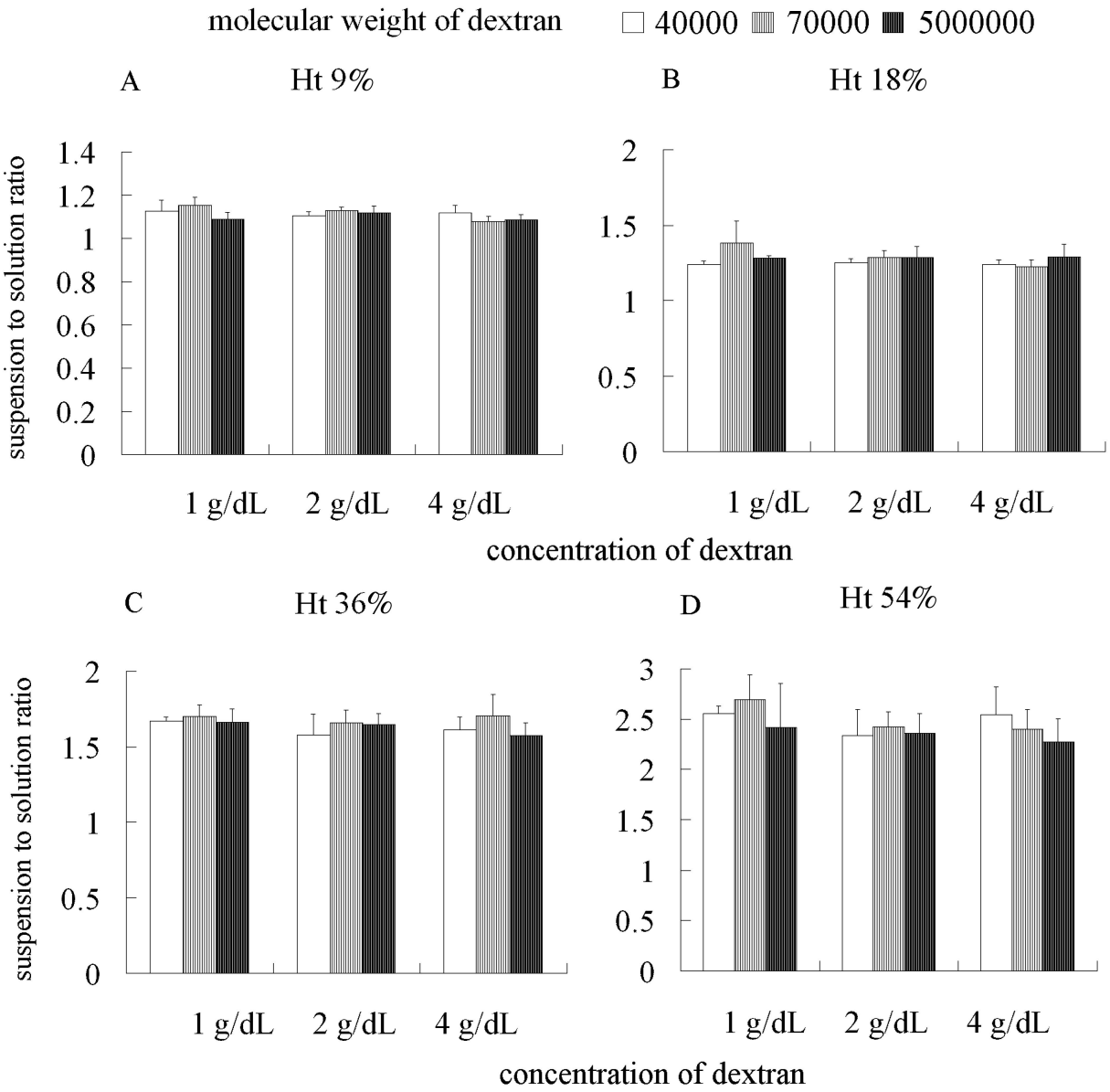


**Figure 9.** Relationship between dextran concentrations and the suspension to solution ratio. Under a constant Ht, Ht 9% (A), Ht 18% (B), Ht 36% (C), and Ht 54% (D). The suspension to solution ratio did not differ significantly at different concentrations of dextran in the suspension.

affect the passage time measured by MC-FAN.

## Discussion

The physicoproperty of blood in peripheral circulation *in vivo* is closely related to the transport and exchange of substances. Decreased peripheral circulation may result in multiorgan functional disorder followed by the development of various diseases, and affect the prognosis thereof[8]. Although a variety of methods to evaluate blood rheology were explored, no established simple methodology was available.

In 1994, Kikuchi et al developed a blood rheology analyzer (MC-FAN)[2-5]. This device has been used since then to assess the impact of drugs on blood fluidity and blood rheological states in various diseases[10-17]. MC-FAN also allows the effects of compound factors on hemodynamics to be

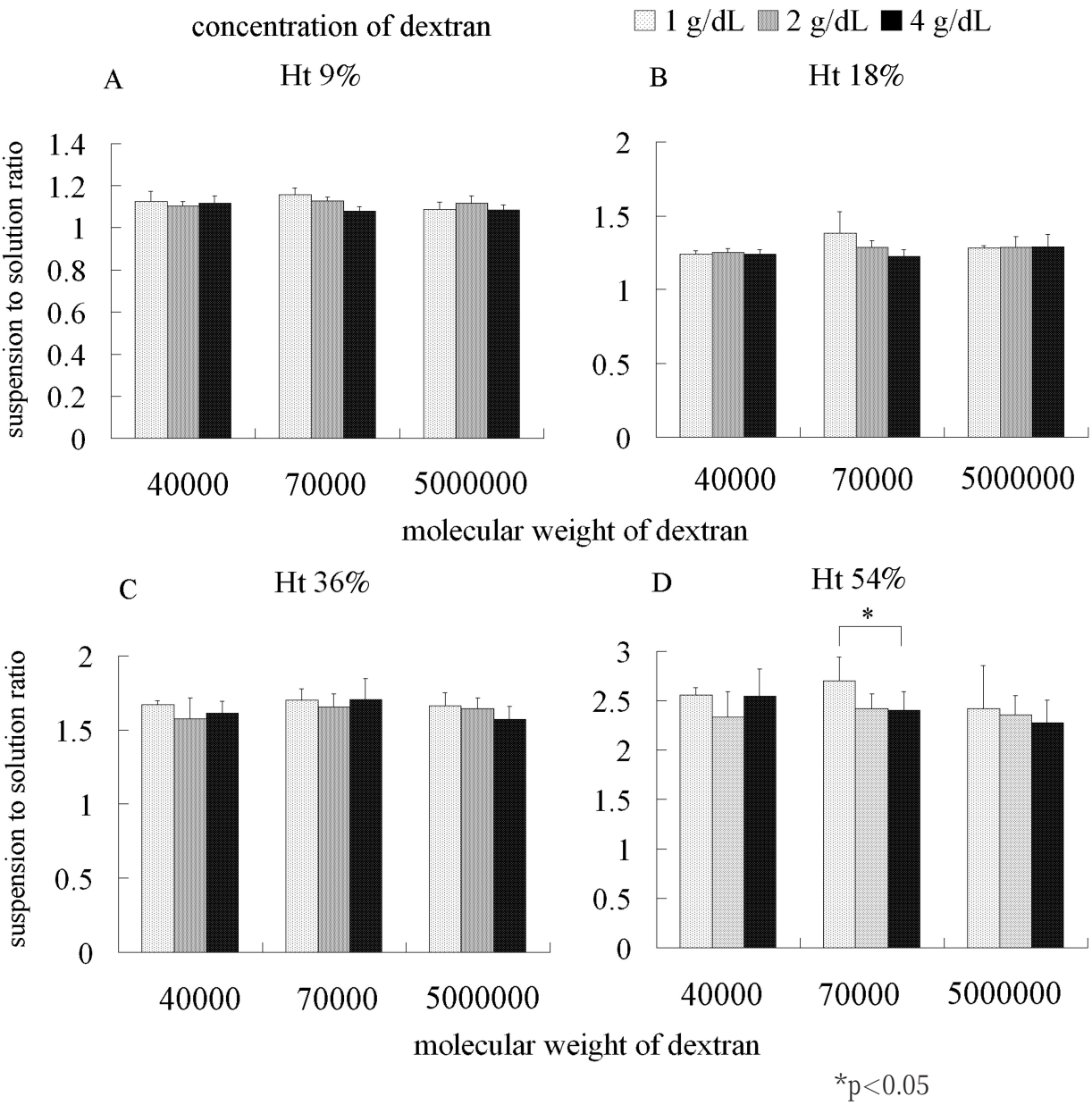


**Figure 10.** Relationship between dextran MWs and the suspension to solution ratio. Under a constant Ht, Ht 9% (A), Ht 18% (B), Ht 36% (C), and Ht 54% (D). The suspension to solution ratio did not differ significantly at different MWs of dextran in the suspension.

observed. Expectations are therefore high for its use in the assessment of food, traditional drugs, and so-called complementary and alternative medicine[1]. In the present study, we investigated the possible impact of RBCs, blood cells that occupy 45% of the blood volume having a major effect on hemodynamics, on the passage time as measured by MC-FAN. The following observations were made: (1) hematocrit and passage time were positively correlated; (2) hardened RBCs that lost deformability completely blocked the channel; (3) compared with physiological saline, plasma prolonged the passage time of RBC suspension significantly; the passage time was prolonged significantly by fibrinogen, but was unaltered by albumin; (4) the passage time of dextran-RBC suspension was prolonged in a dextran molecular weight- and concentration-dependent manner; and the ratio of dextran-RBC suspension passage time to

dextran solution passage time remained unaltered at different molecular weights and concentrations of dextran.

***(1) Impact of increased hematocrit***

Hematocrit increase is accompanied by elevated blood viscosity. According to Poiseuille's law, because peripheral vessel flow resistance is proportional to blood viscosity, hematocrit increase results in greater resistance, thus decreasing peripheral circulation[6]. A dermal microcirculation study *in vivo* showed decreased dermal blood flow due to hematocrit increase, and significant elevation in blood pressure in spontaneously hypertensive rats[18]. In another study with MC-FAN, hematocrit increase was associated with a prolongation of RBC suspension passage time as determined by MC-FAN.

***(2) Impact of RBC deformability***

Because the inner diameter of a capillary is smaller than the RBC diameter, RBCs become deformed while passing through a capillary. In blood flow, cell deformation in adjustment to the flow contributes to a reduction of flow resistance[8]. RBC deformability is thus critical to blood fluidity in microcirculation. In the clinical setting, conditions such as diabetes and hyperlipidemia are believed to cause decreased RBC deformability and thereby lower microcirculation, triggering functional disorders[13,19]. MC-FAN showed that hardened RBCs that had lost deformability failed to pass through the microchannel array and clogged it. Each groove of the channel is 7 μm wide while the mean RBC size is 8 μm. This indicates that the passage time of RBC suspension determined by MC-FAN is regulated by RBC deformability.

***(3) Impact of plasma component***

MC-FAN detected a significant prolongation in the passage time of RBC-plasma suspension compared with that of RBC-physiological saline suspension. This led to an evaluation of the transit time of RBC suspension prepared separately with each of the major plasma proteins, fibrinogen and albumin. A macromolecular protein fibrinogen prolonged the passage time of the suspension. High fibrinogen levels are a risk factor in the cardiovascular system[20,21]. Patients with hyperviscosity syndrome secondary to increased blood levels of immunoglobulin exhibited high plasma viscosity and a significantly prolonged arteriovenous passage time compared with that of healthy subjects, presenting with retinal venous dilatation, increased petechia, and intensive edema of the retina and optic nerve, due to high plasma viscosity[22]. The presence of excessive macromolecular proteins such as fibrinogen decreases microcirculation, triggering disorders.

***(4) Impact of RBC aggregation***

Macromolecular proteins such as fibrinogen and immune globulin trigger RBC aggregation as well as plasma viscosity increase[6,7]. It is assumed that RBC aggregation occurs due to imbalance between aggregating and segregating forces. The segregating forces include electrical resistance and cell membrane elasticity emerging in shear stress in the flow and among RBCs[8]. In the absence of flow, RBCs aggregate in the shape of a coin stack, so-called coin columns. This aggregation readily resolves in the presence of flow. In disordered conditions, marked RBC aggregation develops, decreasing fluidity, which leads to the formation of coin columns in microcirculation and further impediment of flow. This results in insufficient blood flow and thereby unsatisfactory substance transport into peripheral tissues[6-8]. In the study of RBC

aggregation, dextrans are commonly used since they are easier to handle than macromolecular proteins[6]. We thus evaluated the extent of the impact of RBC aggregation using dextrans.

RBC aggregation is not triggered by dextran with a molecular weight of 40000 while it is triggered by dextran with a molecular weight of 70000 or more in a molecular weight- and concentration-dependent fashion[6]. In the same fashion, the passage time of dextran-RBC suspension was prolonged. To determine whether the prolongation was due to a decrease in dextran solution fluidity or to RBC aggregation, the ratio of the passage time of each dextran-RBC suspension to the passage time of the solution of the matching dextran was computed; this suspension to solution ratio was regulated by hematocrit, but was unaffected by the molecular weight or concentration of dextran. The above observation indicates that the prolongation of dextran-RBC suspension passage time that accompanied the higher molecular weights and concentrations of dextran was due solely to the longer passage time required of dextran solution and was almost unaffected by RBC aggregation. This is supported by the fact that the 7 μm-wide grooves of MC-FAN are not spacious enough for RBCs to adhere to each other in a reversible manner to form coin columns.

***(5) Significance of MC-FAN use in the study of RBC kinetics in peripheral circulation***

Given the above, the transit time of RBC suspension as determined by MC-FAN was shown to be regulated by hematocrit, RBC deformability, and the passage time for the solution. RBC deformability can be assessed using RBC suspensions prepared at a constant hematocrit value and with a single type of solution. Various methods of RBC deformability assessment tried thus far require tools and technical skills specific to this assessment, and the procedures are often troublesome. The MC-FAN method is simple, requiring only the preparation of RBC suspensions with hematocrit adjustment. In a study with healthy volunteers with high cholesterol levels and those with normal cholesterol levels using phosphate buffered saline-RBC suspension at 10% hematocrit, passage time was prolonged significantly in the high cholesterol group[13]. This result demonstrates that the sensitivity of the assessment method is satisfactory.

MC-FAN measures the fluidity of whole blood and performs global assessment including the fluidity of blood cells, and that of plasma components separately, and their interactions. The device is therefore effective in the screening of peripheral circulation. In addition, determination of the passage time of RBC suspension and that of plasma may provide, together with the above data, details on intrinsic and extrinsic environmental factors that affect RBCs, a major blood component in peripheral circulation. Such information may be useful in the treatment evaluation and prevention of life style-related diseases and collagen disease, in which peripheral circulation plays an important role.

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