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Measurement of RBC Velocities in the Rat Pial Arteries with an Image-Intensified High-Speed Video Camera System

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The mean centerline red blood cell (RBC) velocity of the rat pial artery was measured using an image-intensified high-speed (1000 frames/s) video camera system and RBCs labeled with fluorescein isothiocyanate (FITC). Some investigations measuring RBC velocity have been made in most organs, but the RBC velocity of the pial artery has not yet been measured with this system using FITC labeled RBC. After recording the emission of the FITC labeled RBC through a closed cranial window using this system, the authors analyzed the videotape. The movement of each individual RBC for several milliseconds over a distance of 50 μm could be pursued. The mean centerline RBC velocity in normal rats varied between 1.0 and 9.0 mm/s (most of the measurements we taken in vessels ranging between 20 and 80 μm in diameter). As the diameter of the pial artery becomes smaller, the blood flow rate ($\pi \times (\text{diameter}/2)^2 \times$ (mean centerline velocity/1.6)) tends to become smaller. During CO_2 inhalation, the pial artery diameter, mean centerline RBC velocity, and blood flow rate increased with statistical significance. Mean centerline RBC velocities in the cerebral microcirculation could not be measured directly with accuracy using the older methods (30 frames/s). However, this method is useful for investigation of the cerebral microcirculation and is considered to be applicable for studying the behavior of leukocytes or platelets, which will be examined in a subsequent study.

Key Words: microcirculation; fluorescence; FITC; velocity; CO_2; erythrocyte.

INTRODUCTION

Some investigations into mean centerline red blood cell (RBC) velocity have been reported in the main body organs, but measurement of the mean centerline RBC velocity is difficult because it is too fast to measure using a standard video system (30 frames/s) and it could not be measured accurately frame by frame. Due to these methodological difficulties, very few investigations of cerebral blood flow velocity have been reported (Gotoh *et al.*, 1982; Ma *et al.*, 1974; Rosenblum, 1969; Yamaguchi *et al.*, 1990). However, using an image-intensified high-speed video camera system (1000 frames/s) and RBCs labeled with fluorescein isothiocyanate (FITC), mean centerline RBC velocities could be measured accurately in the mesentery (Oshio *et al.*, 1991), the colon (Sekizuka *et al.*, 1991), the gastric mucosal surface (Sekizuka *et al.*, 1993), the liver, and

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the kidney (Tsukada *et al.*, 1997). In the present study, the mean centerline velocities of individual FITC-labeled RBC in the rat pial arteries were measured directly and accurately for the first time with an image-intensified high-speed video camera system (1000 frames/s) through a closed cranial window according to Morii *et al.* (1986). The relation between vessel diameter, mean centerline RBC velocity, and flow rate during CO_2 inhalation was investigated and compared with the normal state.

MATERIALS AND METHODS

Experiments were carried out with 20 male Wistar rats, weighing 300–320 g. The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and lidocaine (1%) was employed for local anesthesia. The femoral artery was cannulated to monitor the systemic arterial blood pressure and to sample arterial blood for blood gas analysis. The rats were tracheostomized, immobilized with tubocurarine chloride (1 mg/kg), and mechanically ventilated (SAR-830, CWE Inc., USA). Rectal temperature and temporal muscle temperature were monitored (MGA-III, Shibaura Electronics Co. Ltd., Japan) and maintained at 36–37.5° by a heating pad (Omron, E5C4) and a overhead lamp. The head of each rat was fixed in a stereotaxic frame (Narishige SR-6, Japan) and the left parietal bone was exposed by a longitudinal midline skin incision. After three polyethylene tubes (PE-50) were fixed on the skull with cyanoacrylate (Alon Alpha), a closed cranial window following a craniectomy (5 mm diameter) was made using a cover glass and quick self-curing acrylic resin (GC Unifast, Japan), as described by Morii *et al.* (1986). Artificial cerebrospinal fluid (CSF) consisted of Na^+ 147.8 mEq/L, K^+ 3.0 mEq/L, Mg^{2+} 2.3 mEq/L, Ca^{2+} 2.3 mEq/L, Cl^- 135.2 mEq/L, HCO_3^- 19.61 mEq/L, lactate$^-$ 1.67 mEq/L, phosphate 1.1 mM, and glucose 3.9 mM and was superfused at the speed of 0.1 mL/min.

Fluorescein isothiocyanate (FITC) was used for red blood cell (RBC) labeling (absorption peak 490 nm, emission peak 520 nm, Sigma) according to Sekizuka *et al.* (1993). 0.1 ml FITC-labeled RBC suspension (total RBCs × approximately 1/50) was injected for each rat. With an image-intensified high-speed video camera system (Kodak IMG 6000) to observe microcirculation on the surface of brain, fluorescent images of FITC-labeled RBCs were recorded under a fluorescent vital-microscope at the rate of 1000 frames/s. The recorded images were reproduced frame by frame at a slower speed, 1/33 of the original speed and the velocity of each FITC-labeled RBC and the diameter of each arteriole were analyzed with a digital analyzer (FOR-A, IV-560, Tokyo). The average of 20 RBC velocity measurements, taken along the centerline of the vessel, were used to define the mean centerline RBC velocity. The blood flow rate of a pial arteriole was calculated as the product of the cross-sectional area of the vessel ($\pi \times (\text{diameter}/2)^2$) and the mean velocity of cells plus plasma. For the relationship between the red cell centerline velocity (V_c) and the mean velocity of cells plus plasma (V_m), the ratio (V_c/V_m) = 1.6, is assumed (Baker and Wayland, 1974; Lipowsky and Zweifach, 1978). According to this formula, the blood flow rate in this study was calculated as the product of ($\pi \times (\text{diameter}/2)^2$) and (mean centerline velocity/1.6). Measurement of the vessel lumen diameter was possible because the FITC diffused to the parenchymal tissues and the vessels were labeled black after injection of the FITC-labeled RBCs.

In 10 rats the effect of CO_2 inhalation was examined by ventilating animals with 5% CO_2 in air. The mean centerline RBC velocity, pial artery diameter, and blood pressure were measured and $PaCO_2$, PaO_2, and pH were analyzed before and 3 min after the beginning of the inhalation.

RESULTS

FITC labeled RBCs were recognized (Fig. 1). When the RBC moved 50 μm for 10 ms (10 frames), the RBC velocity is calculated as follows: 50 μm/10 ms = 0.05 mm/0.01 s = 5 mm/s. A mean centerline RBC velocity in an artery was obtained by averaging the data from 20 centerline velocity measurements collected over several cardiac cycles. Depending on the extent of the

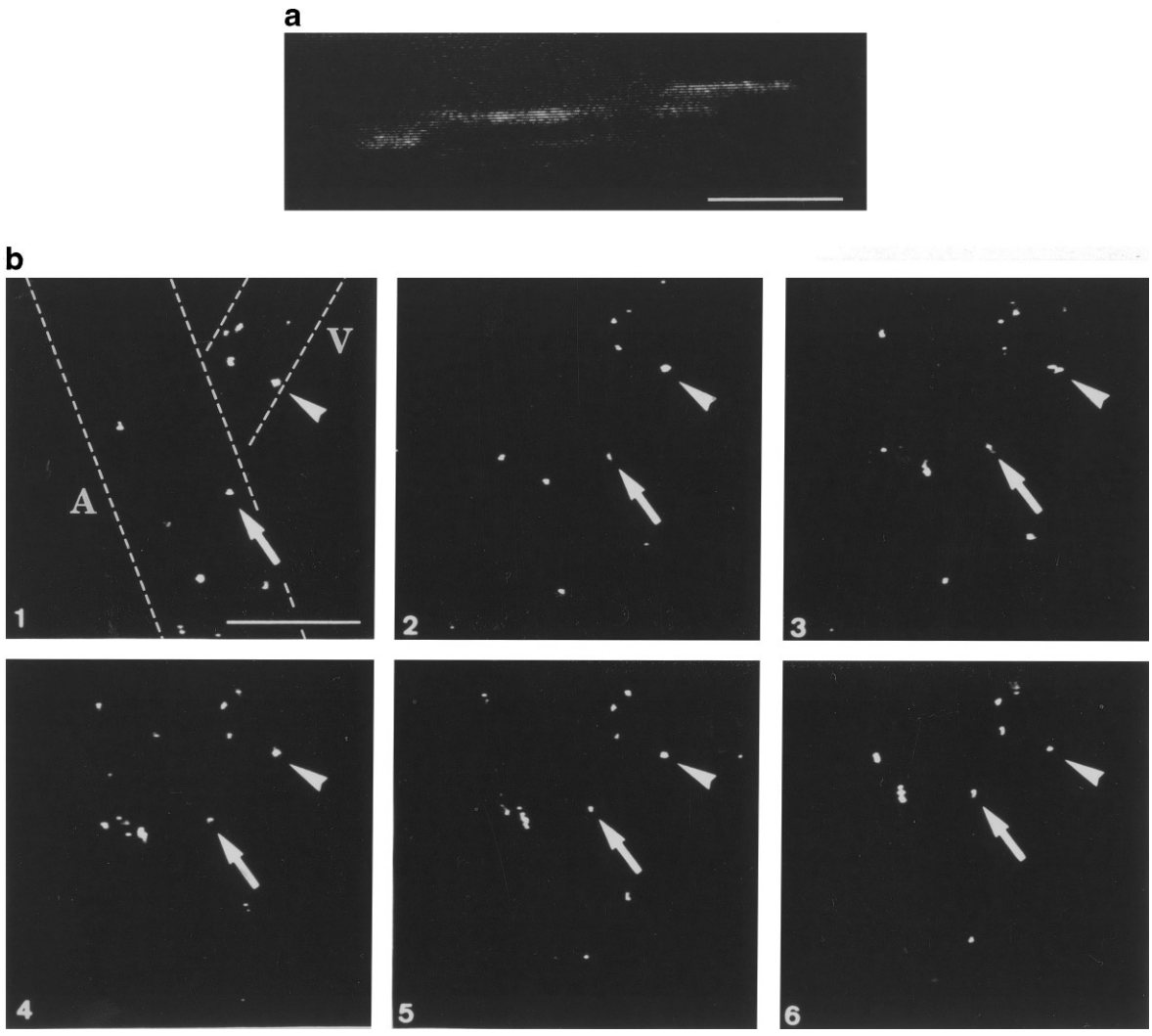

FIG. 1. (a) Fluorescent images of FITC labeled RBCs look like shooting stars with SIT camera at 30 frames/s. Movement of a RBC cannot be recognized with the usual speed video system. Scale bar, 50 μm. (b) Fluorescent images of FITC labeled RBCs taken with an image-intensified high-speed video camera system (1000 frames/s). In this figure it takes 1 ms between frames. The arrow shows an RBC in the arteriole and the arrowhead shows an RBC in the venule. It can be recognized that the RBCs in the arteriole move frame by frame and the RBCs in the venule hardly move over the six frames (5 ms). A; arteriole; V, venule. Vessel walls are marked with dotted lines. Scale bar, 50 μm.

pulsatility in the pial artery, the 10 values obtained by averaging the 20 sets of centerline velocity data were compared with the 10 values obtained by averaging 100 sets of centerline velocity data collected over more cardiac cycles, in the same videotape, recorded at the same point. There was no significant difference between the former (6.0 $\pm$ 2.1 mm/s, mean $\pm$ SD) and the latter (6.1 $\pm$ 2.2 mm/s), using a paired *t* test. Furthermore, there was a linear correlation between the 10 values obtained by averaging the 20 sets of centerline velocity data and the 10 values obtained by averaging 100 sets of centerline velocity data, with a regression coefficient of 0.99 and a intercept of 0.19 mm/s ($r^2 = 0.99$, $P = 0.0001$) (Fig. 2).

The relationship between the diameter and mean centerline RBC velocity of pial arteries in rats is shown in Fig. 3a. The mean centerline RBC velocity varied from 1.0 to 9.0 mm/s, with a wide range especially when the vessel diameter was less than 50 μm.

Figure 3b illustrates the relationship between the

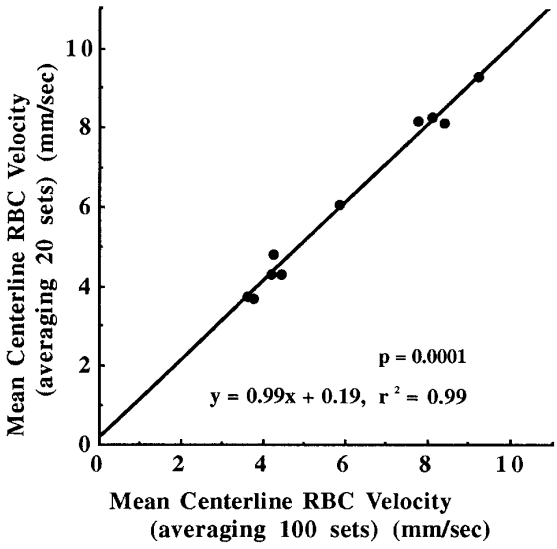

FIG. 2. Scatterplot illustration of the correlation analysis between the 10 values obtained by averaging the 20 sets of velocity data and the 10 values obtained by averaging 100 sets of velocity data.

diameter and blood flow rate of the pial arteries. As the diameter becomes smaller, the flow rate decreases.

During CO_2 inhalation, pial artery diameter, mean centerline RBC velocity, and blood flow rate increased significantly from 72.0 ± 11.4 (mean ± SE) μm to 76.8 ± 12.6, from 4.68 ± 0.55 mm/s to 5.76 ± 0.78, and from 12.3 ± 3.8 ($\times 10^3$) mm^3/s to 17.0 ± 5.3, respectively (Fig. 4). It seemed that the greater the pial artery diameter and the blood flow rate, the grater the effect of CO_2. Blood pressure, $PaCO_2$, PaO_2, and pH were summarized before and during inhalation of 5% CO_2 and the data are given in Table 1. $PaCO_2$ increased and pH decreased significantly compared with those before inhalation. Blood pressure and PaO_2 tended to increase but there was no significant difference between those before and those after CO_2 inhalation.

DISCUSSION

Measurement of cerebral blood flow or pial vessel diameter through the closed cranial window has proved useful for estimating various cerebral physiological and pathological conditions (Gotoh *et al.*, 1982; Morii *et al.*, 1986). However, few investigation of blood flow velocity in cerebral microcirculation have been reported because of the methodological difficulties. Rosenblum (1969) measured the velocity of erythrocytes in mice arterioles with high-speed microcinematography (500 frames/s). Because crowded red cells made the measurement obscure, the measurable velocity was limited to distances of less than 30 μm and in vessels up to 30 μm in diameter the RBC near

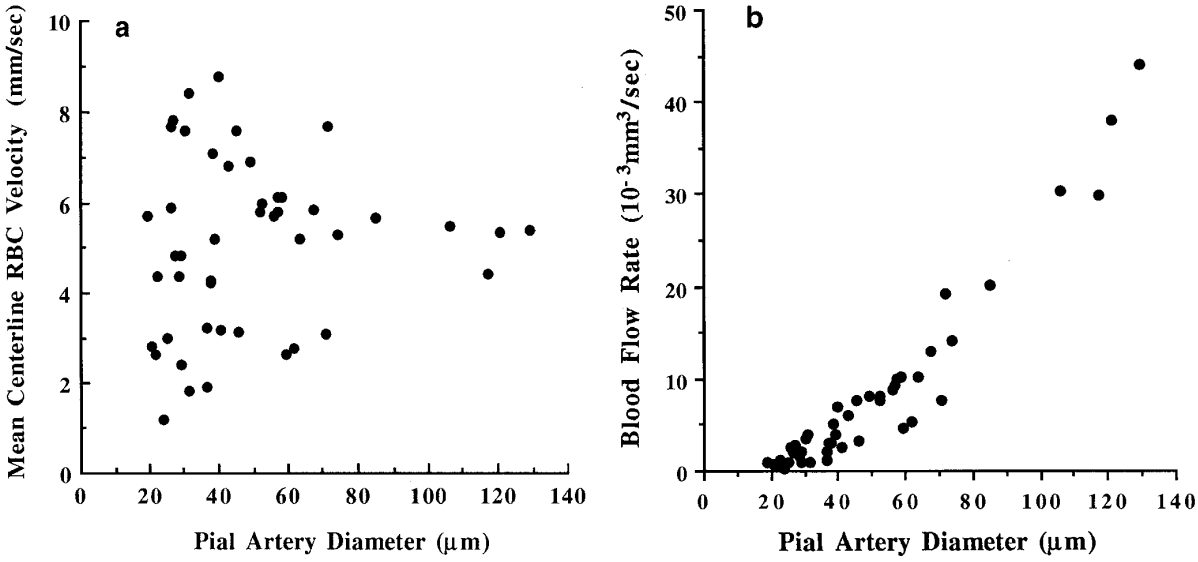

FIG. 3. Mean centerline RBC velocities (a) and blood flow rates (b) in pial arteries of normal rat plotted against arteriole diameter.

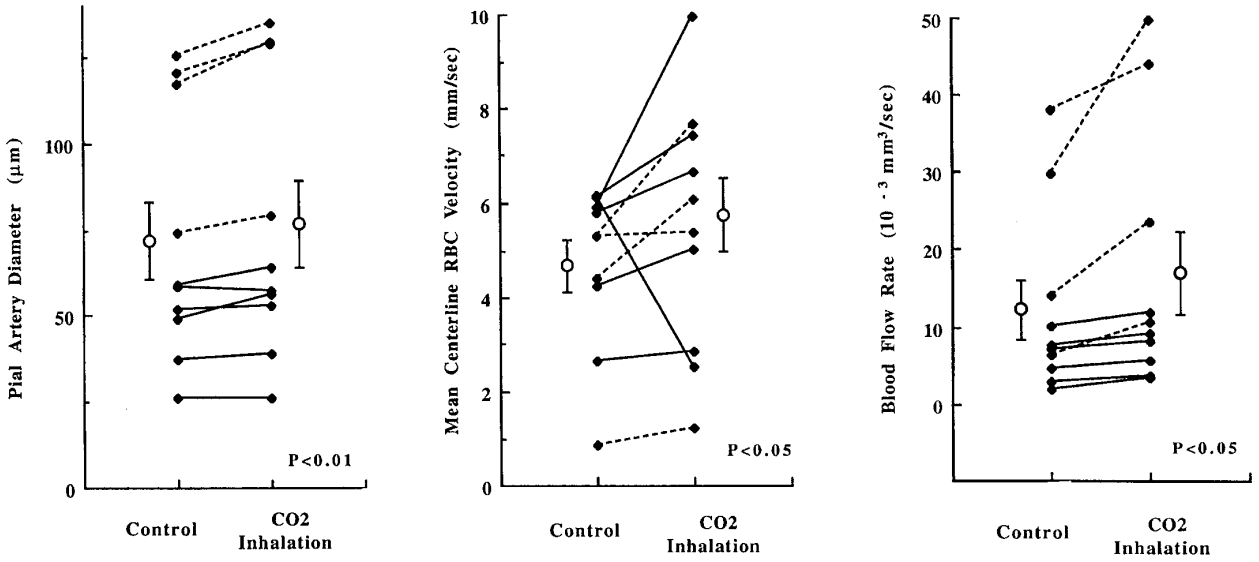

FIG. 4. Change in pial artery diameter, mean centerline BRC velocity, and blood flow rate before and during CO_2 inhalation. Dotted line shows that the artery diameter is grater than 70 μm. The 20 blood flow rate data points derived from the diameter and mean centerline RBC velocity data points are shown in this figure.

the vessel wall, where the hematocrit is reduced, may be visualized with sufficient clarity. However, the velocity near the vessel wall is less when compared with the mean centerline velocity. In our study we used only 0.1 ml FITC-labeled RBC suspension (total RBC $\times$ approximately 1/50) for one rat and each RBC was recognized clearly without any overlap. The mean value of arteriole velocity has been reported as 3.4 mm/s (Rosenblum, 1969) in agreement with our result. Ivanov *et al.* (1981) determined capillary blood flow velocity in rat brain by measuring the rate of motion of plasma-filled "gaps" in the continuous erythrocyte flow using a movie camera at the rate of 40 frames/s. The mean velocity of capillaries 2–5 μm in diameter was 0.79 mm/s. Arteriole velocity was not found in the study, but the value of the capillary velocity is lower following our data in arterioles.

TABLE 1
Physiological Parameters during 5% CO_2 Inhalation

	Control	CO_2 inhalation
Mean blood pressure (mm Hg)	111.9 ± 4.0	118.8 ± 5.3
PH	7.40 ± 0.03	7.30 ± 0.01*
$PaCO_2$ (mm Hg)	26.3 ± 1.3	39.4 ± 1.7**
PaO_2 (mm Hg)	94.7 ± 8.7	100.9 ± 6.9

Note. Values are means ± SE (n = 10).
*$P < 0.01$ versus control values before CO_2 inhalation.
**$P < 0.001$ versus control values before CO_2 inhalation.

Blood flow velocity in the cat was measured by the injection of a small amount of saline through the lingual artery visualized on a television monitor at 60 frames/s using both a width analyzer and a video densitometer (Gotoh *et al.*, 1982; Kobari *et al.*, 1984; 1987). Kobari described the relationship between the diameter and blood flow velocity of pial arteries in cats, which were between about 3 and 25 mm/s when the diameter was below 100 μm. These values were in good agreement with the results reported by Ma *et al.* (1974), who investigated the RBC velocity in pial arterioles (diameter below 50 μm) using two-slit transit time velocity, but were not able to be corroborated by our results. The difference in these results may be due, in part, to interspecies variation. Considering the small arterioles with a diameter of less than 100 μm in the Kobari's report, there did not seem to be any linear relationship between blood flow velocity and the diameter of the pial artery but the blood flow rate increased with an increase in the diameter. This tendency was in accordance with our result and the arteriole diameter plays a more important role in the cerebral blood flow than the arteriole velocity. Yamagu-

chi (1990) reported that using a dual window technique with FITC labeled RBCs and a silicon-intensified target tube (SIT) camera, the RBC velocity (diameter, 20–60 μm) was between 2 and 7.5 mm/s, which was in good agreement with our results. Therefore, a velocity of the small amount of saline may be faster than a RBC velocity. However, Ma *et al.* (1974) reported the faster RBC velocity in rats using two-slit technique than that we found in the present study. Recently, Hudetz (1996, 1997) measured RBC velocities in rat cerebral capillaries with the dual window digital cross-correlation technique and reported that 65% of all velocities fell in the range 0.5 to 1.8 mm/s. On the other hand, the RBC velocities of mesenteric arterioles were measured with the two-slit technique and the mean values were 7.04 mm/s (Gaehtgens, 1970) and 10.0 mm/s (Intaglietta, 1970). Compared with values of these capillaries or mesenteries, Ma's results seem to be faster. Using an image-intensified high-speed video camera system (1000 frames/s), mean centerline RBC velocities in arterioles have already been measured directly in the mesentery (Oshio *et al.*, 1991), the colon (Sekizuka *et al.*, 1991), the gastric mucosal surface (Sekizuka *et al.*, 1993), the liver, and the kidney (Tsukada *et al.*, 1997). Mean centerline RBC velocities in the pial arteriole in this study coincided with these previously reported values of other organs. The direct measurement of the RBC velocity with an image-intensified high-speed video camera system and the FITC-labeled RBC is the most accurate methodology at the present time.

Fluorescently labeled RBCs have been used as tracers to measure flow in microvessels and it is considered that the rheological behavior of the labeled RBCs is sufficiently close to that of native RBCs (Tangelder *et al.*, 1986). However, with this reported method, the accurate velocity of each RBC is measurable but some demerits are seen for calculating the blood flow rate. First, there are variations in RBC velocities even when considering RBCs at the same point in the same vessel. RBC velocity is low near the wall and high at the center of a vessel (Rosenblum, 1972; Tangelder *et al.*, 1987) and arterioles have a pulsatile blood flow which is synchronized with the arterial pulse cycle (Kobari *et al.*, 1984; Oshio *et al.*, 1991; Rosenblum, 1969). In this study the authors regarded the centerline velocity as representative, because it was shown that $V_c/V_m = 1.6$. One value of mean centerline RBC velocity was gained by averaging the 20 randomly measured sets of data collected over several cardiac cycles. The effect of the pulsative blood flow on the 20 averaged RBC velocity was minimum, because the RBC velocities gained by averaging the 20 sets of data were not significantly different from those gaind by averaging the 100 sets of data collected over more cardiac cycles. Second, it has been observed that after the irradiation of filtered light of 400–500 nm in wavelength, platelets began to adhere to the irradiated endothelium and subsequently the aggregates gradually began to grow (Sato and Ohshima, 1987). In this study these phenomena were not observed, because the irradiating time was 1–2 s. The velocity could not, however, be measured continuously.

It has been reported that the pial artery diameter increases during CO_2 inhalation (Auer, 1978; Kobari *et al.*, 1987; Morii *et al.*, 1986; Wei *et al.*, 1980). The pH value then decreases and mean arterial blood pressure tends to increase. In this study mean centerline RBC velocity and blood flow rate increased significantly and these results coincided with Kobari's reports. Morii reported that smaller arterioles dilated more than larger ones. These tendency was not derived in our analysis of the data in 10 arterioles but it appeared that the greater the artery diameter, the greater increase the blood flow rate during CO_2 inhalation. Naturally, this is in agreement with the fact that the smaller the artery diameter, the greater the number of the artery and that the total increase in blood flow rate is the same when considering smaller arteries and bigger arteries.

It is considered that measuring blood flow rate as described in the present study is one new method for estimating cerebral blood flow as described in other studies of the main body organs. The method in this study is considered to be applicable for studying the mean centerline RBC velocity and the behavior of leukocytes or platelets in the disease model, which will be examined in a subsequent study.

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