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Capillary Blood Cell Velocity in Full-Term Infants as Determined in Skin by Videophotometric Microscopy

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ABSTRACT. In order to study the neonatal microcirculation, the capillary hemodynamics in skin was investigated in 43 full-term infants 2–7 days after birth. The nailfold capillaries of the thumb were visualized by means of television microscopy and the capillary blood cell velocity (CBV) was videophotometrically quantified in 107 microvessels. The skin temperature, mean arterial blood pressure, and heel puncture hematocrit were measured simultaneously to evaluate any relation with the CBV. The mean CBV in all infants was 0.38 ± 0.21 mm/s, with a range of 0.04 to 1.2 mm/s in individual capillaries. There was no correlation between CBV and skin temperature (27–33° C), mean arterial blood pressure (44–68 mm Hg), or postnatal age. However, a significant correlation was found between the log CBV and the skin prick hematocrit ($r = -0.64$, $p < 0.001$). It is concluded that the mean CBV during the 1st wk of life is not significantly different from the capillary velocity reported in adults. Normal variations in skin temperature and mean arterial blood pressure, as well as age differences 2–7 days after birth, do not significantly influence the neonatal skin capillary blood flow. However, the hematocrit is of major importance for skin capillary perfusion in the newborn infant. (*Pediatr Res* 23: 585–588, 1988)

Abbreviations

CBV, capillary blood cell velocity
MAP, mean arterial blood pressure

The blood flow through the smallest branches of the vascular tree, *i.e.*, the arterioles, capillaries, and venules, is generally referred to as the microcirculation. Although it has been found that the blood circulation in the neonate differs in several respects from that of the adult, very little is known about the microcirculation in infancy. Thus, the microvascular significance of the previously reported high peripheral blood flow and low blood pressure in neonates (1, 2) is still obscure. Moreover, the microcirculatory role of the specific properties characterizing neonatal blood, *i.e.*, mainly a hematocrit that is higher and a plasma viscosity lower than in adult blood (3, 4), has not been elucidated *in vivo*. During the last decade microvascular hemodynamics have been studied in adult humans (5, 6). In most of these investigations skin has been the target tissue inasmuch as the cutaneous microvasculature can be fairly easily observed and studied under physiological conditions. In the newborn infant, microscopic appearance of the skin capillary structure has been described and the regular adult microvascular architecture was found to be poorly developed at birth (7–9). The typical horizontal loop capillaries, suitable for microscopic studies of capillary hemodynamics, have been observed only in the nailfolds (9).

The aim of our study was to study capillary hemodynamics in the full-term infant, using nailfold capillaries as a model system. The CBV was quantified by a videophotometric microscopy method. Simultaneous recordings of skin temperature, blood pressure, and hematocrit were performed to determine whether normal variations in these parameters affected the CBV.

MATERIALS AND METHODS

A total of 43 full-term infants of normal weight, all with a history of uncomplicated vaginal deliveries after normal pregnancies, was studied at 2–7 days of postnatal age (Table 1). Investigations were performed at room temperature (23 ± 0.5° C) with the infant cot-clothed and sleeping in the prone position 30–60 min after feeding. During microscopic studies the infant's eyes were closed, the breathing pattern was regular, and no gross movements were observed, i.e., behavioral state 1, according to Prechtl (10). The study was designed to minimize the influence of variations in total limb blood flow, which has been reported to change with gestational and postnatal age (2, 11), mode of delivery (11), temperature (12), and feeding (13). Parental consent was obtained before each investigation and the experimental protocol was approved by the local ethics committee.

Temperature measurements. In addition to the room temperature, the body temperature was measured in all experiments with an electronic axillary thermometer (Crafttemp, Crafon Medical AB, Lund, Sweden). A mean body temperature of 36.7 ± 0.2° C was found, which was accepted as normal for uncorrected axillary values. During microscopic studies, the local skin temperature was recorded with a temperature probe fixed with adhesive tape to the base of the left thumb (Fig. 1).

Blood pressure determinations. The MAP was oscillometrically measured in the left arm after microscopy (Omega 1400; Omega Optical, Inc., Brattleboro, VT). The cuff width was 50 mm and the MAP was calculated as the mean of two to three repeated measurements.

Hematocrit determinations. At the end of each experiment, the infant's unwarmed heel was punctured and two heparinized 75-mm microtubes were filled with blood. The hematocrit was calculated as the mean of the duplicate samples after centrifugation at 10,000 rpm for 10 min.

CBV measurements. The CBV was recorded and analyzed as described in detail by Fagrell *et al.* (5) (Fig. 1). The nailfold

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capillaries of the left thumb were visualized with a Zeiss light microscope connected to a TV camera (Ikegami CTC 2110, Ikegami Tsushinki Co., Ltd., Tokyo, Japan). Illumination was provided by a cold light source (Schott KL 1500, Schott Geaswerke, Wieshaden, West Germany) having a blue filter that increased the contrast between the erythrocytes and the surrounding tissue. A drop of paraffin oil was applied to the skin to make it transparent. The thumb rested on a miniature plate and, to minimize slight movements below the microscope, a small bracket was allowed to lightly touch the distal end of the nail. No other support or fixation was used. The image could be seen on a TV monitor (magnification ×200). All recordings were stored on videotape.

CBV was measured on the tape recordings, using a videophotometric analyzer and a cross-correlator (IPM, San Diego, CA). Two small optical "windows" were generated on the TV screen and placed along the axis of the arterial side of a suitable capillary loop. Different optical densities within the capillary, produced by the passage of erythrocytes, leucocytes, and plasma gaps, were quantified and converted to electronic signals from both these windows. The CBV was calculated from the inter-window distance, *i.e.*, the magnification, and from measurements of the inter-viewing transit time, *i.e.*, the delay between similar photometric signals. The CBV was computed by feeding the signals to the cross-correlator. The cross-correlator output in some recordings was continuous, whereas in others, due to a more compact erythrocyte flow with few plasma gaps, the CBV was computed at intervals.

The CBV in each capillary was calculated as the mean of at least 10 subsequent velocity determinations, in continuous readouts performed at 5-s intervals. All recordings used for CBV measurements had a duration of 1–5 min.

Inasmuch as recording time and capillary visualization varied, a different number of capillaries in each infant was studied. The CBV was measured in one capillary in 10 infants, two capillaries in 11, three in 13, and four in nine neonates, respectively. The plotted results are presented as the mean CBV in each infant, unless otherwise stated.

Table 1. *Infant data*

Postnatal age (days)	*n* (Male/female)	Gestational age (wk)	Birth wt (g)
2	9 (4/5)	40.1 ± 0.8	3510 ± 390
3	9 (2/7)	39.9 ± 0.6	3520 ± 360
4	9 (6/3)	40.0 ± 1.2	3700 ± 290
5	8 (4/4)	40.1 ± 0.8	3880 ± 330
7	8 (6/2)	39.8 ± 1.3	3790 ± 220
Total	43 (22/21)	40.0 ± 0.9	3670 ± 340

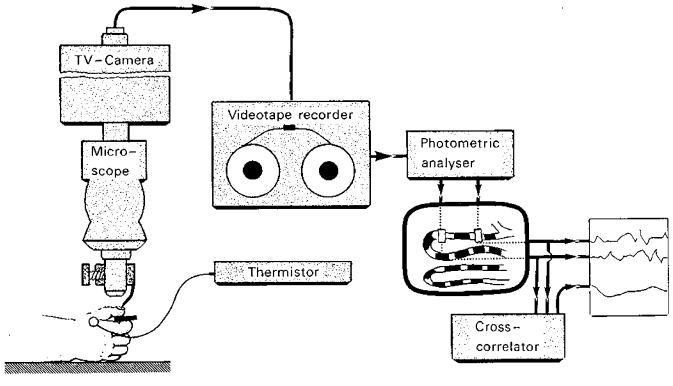

Fig. 1. Method description illustrating the procedure for skin capillary blood cell velocity determination.

Statistical analyses. In order to determine intra- and interindividual CBV variations, an analysis of variance was performed. To determine whether variations in skin temperature, MAP, hematocrit, or postnatal age affect the CBV, a multiple linear regression model was used and multiple-correlation coefficients were calculated. To determine nonlinear relations both the CBV and the log-transformed CBV values were included in the analysis. Unless otherwise indicated the results are presented as the mean ± 1 SD.

RESULTS

The mean CBV for all infants was 0.38 ± 0.21 mm/s with a range of 0.04 to 1.2 mm/s in individual capillaries. No sex difference was found; the mean velocity for boys was 0.42 and for girls 0.34 mm/s.

The CBV variations in time were small and occasionally a tendency toward periodic velocity fluctuations was seen (Fig. 2). The variance analysis showed that 69% of the estimated total variance was attributable to systematic differences between infants ($p < 0.001$). However, the spatial variation in the CBV between adjacent capillaries in the same individual was pronounced in some subjects (Table 2).

The local skin temperature varied between 27 and 33° C. It was stable in each infant during the recording and was not affected by postnatal age. The CBV was not influenced by normal variations in skin temperature (Fig. 3).

The average MAP was 56 ± 6 mm Hg and increased insignificantly from 52 at 2 days of age to 56 mm Hg at 7 days after birth. There was no significant regression or correlation between the CBV and the MAP (Fig. 4).

The mean skin prick hematocrit level was 60 ± 7% and ranged from 43 to 80%. In the three infants with hematocrit exceeding 70%, a venous sample was checked and found to be lower than this value, *i.e.*, below our limit for polycytemia in asymptomatic infants. A significant decrease in hematocrit was found from day 2 (mean = 64%) to day 7 (mean = 54%, $r = 0.45$, $p < 0.01$). A

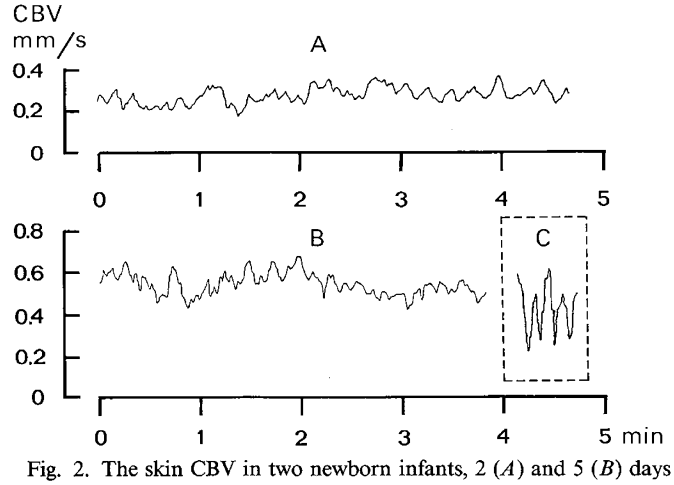

Fig. 2. The skin CBV in two newborn infants, 2 (*A*) and 5 (*B*) days old. *Inset* (*C*) is the adult CBV pattern (Fagrell B, unpublished data), illustrating marked periodic CBV changes.

Table 2. *Skin capillary blood cell velocity (mm/s) in four infants, illustrating the spatial velocity variation between adjacent capillaries*

Subject	Capillary a	b	c	d
1	0.85	0.92	0.84	
2	0.45	0.52	0.28	0.45
3	0.33	0.17	0.32	
4	0.13	0.13	0.12	0.09

significantly lower CBV was observed in the neonates with an increased hematocrit ($r = -0.50$, $p < 0.001$); after log-transformation of the CBV values the correlation increased ($r = -0.64$, $p < 0.001$), indicating a nonlinear relationship (Fig. 5). In the subject with the highest hematocrit (80%) the CBV was very slow and the capillary flow ceased completely at intervals of up to a duration of 40 s. In no other recording were such prolonged interruptions of flow observed; in a few other subjects momentary capillary flow standstills were seen.

The mean CBV increased from 0.22 to 0.41 mm/s in infants 2 and 7 days old, respectively, which, according to multiple regression analysis, could be attributed to the age-related drop in hematocrit (Fig. 6). However, when the 1-wk-old infants were excluded, a significant regression and correlation was found between the CBV and the postnatal age, irrespective of hematocrit changes ($r = 0.48$; $p < 0.01$).

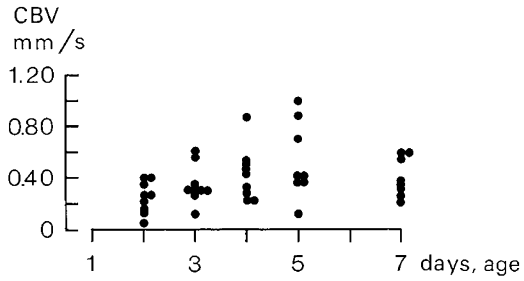

Fig. 6. The skin CBV *versus* the postnatal age. No significant correlation was found between the two parameters. The mean CBV values for each postnatal age group were 0.22 ± 0.11, 0.33 ± 0.14, 0.42 ± 0.21, 0.52 ± 0.30, and 0.41 ± 0.15 mm/s at 2, 3, 4, 5, and 7 days of age, respectively.

DISCUSSION

The accuracy of the cross-correlation technique for CBV measurements made with a dual photometric window analyzer has previously been documented (6). The method permits a noninvasive and continuous quantification of blood cell velocities in capillaries. Another method, laser Doppler flowmetry, has recently been used in microcirculatory studies of skin (14, 15). This technique facilitates only measurements of relative blood flow changes that are generated mainly in a group of skin microvessels larger than single capillaries (15). Consequently, the microscopic method used herein and laser Doppler flowmetry cannot replace, but rather complement each other.

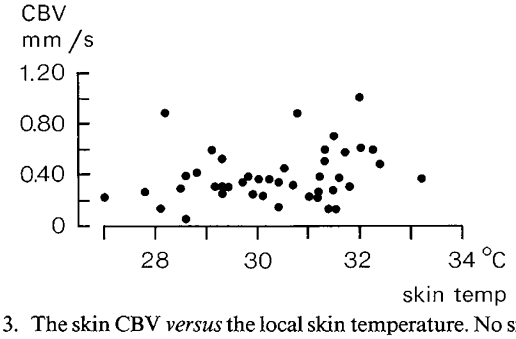

Fig. 3. The skin CBV *versus* the local skin temperature. No significant correlation was found between the two parameters.

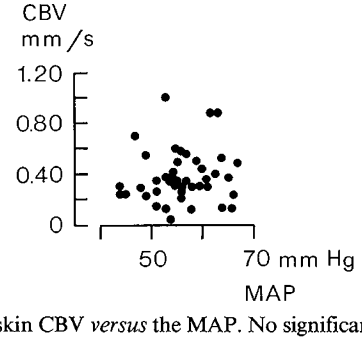

Fig. 4. The skin CBV *versus* the MAP. No significant correlation was noted between the two parameters.

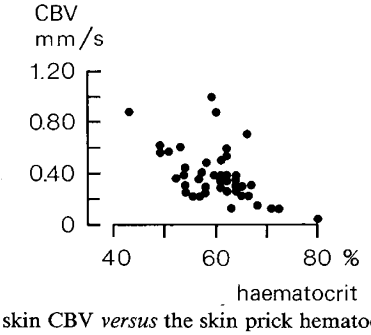

Fig. 5. The skin CBV *versus* the skin prick hematocrit. A significant correlation was found between the two parameters ($r = -0.50$, $p < 0.001$), and after log-transformation of the CBV ($r = -0.64$, $p < 0.001$).

The mean CBV recorded herein does not differ significantly from the nailfold CBV found in healthy adults (5, 6). This finding can be ascribed to an increase in the capillary cross-sectional area or density in infancy, because the total neonatal peripheral blood flow has been found to be about twice as high as in adults (1, 2). However, although it is difficult to quantify because of the limited and varying numbers of vessels observed, our recordings revealed poor skin capillary vascularization. This is in accordance with earlier morphological descriptions of the neonatal cutaneous microvasculature (7–9). Thus, the higher peripheral blood flow in newborn infants is more likely to bypass the superficial skin capillaries through deeper vascular beds, which suggests an increased flow resistance in neonatal skin capillaries.

Despite a similar mean CBV, the time-dependent velocity variation was found to be much less marked in newborn infants than in adults (5, 6, 15) as shown in Figure 2. Fluctuations in capillary velocity have been attributed to alterations in precapillary arteriolar diameter (16) and the rhythmic adult velocity changes are believed to reflect periodic contractions and relaxations of the supplying arteriole (17). The CBV pattern herein may be an expression of a low precapillary arteriolar tone, with only small active changes in vessel diameter. This assumption is supported by the findings of 1) a comparatively high limb blood flow, despite a low driving blood pressure, in newborn infants (1), and of 2) a CBV pattern in the adult similar to that in the neonate after the systemic administration of a vasodilating agent (18). Accordingly, the precapillary arteriolar or myogenic influence on the skin capillary perfusion appears to be less pronounced in the newborn infant, which means that other flow resistance factors, such as vessel geometry and the flow properties of blood, play a more significant role as CBV determinants.

In cutaneous tissues, unlike the more vital organs, the oxygen demand seems to have a minor influence on skin perfusion while the subject is at rest. Thus, the peripheral blood flow does not change in response to decreased (19) and increased (20) oxygen delivery, *i.e.*, the hematocrit, in newborn lambs and infants, respectively. Hence, our results suggest that the hematocrit plays a major role as a flow resistance factor in neonatal skin capillaries. Despite the counteracting effects of a low plasma viscosity (4) and a low red cell aggregation tendency (21), the hematocrit is the principal factor producing the hyperviscosity commonly found in neonatal blood (3, 4). The increase in capillary flow resistance may be due not only to the increase in the number of red cells but also to that in the larger size of the erythrocytes in the newborn, because the neonatal erythrocytes, although they

have been reported to be equally deformable (22), have less *in vitro* capillary filterability than adult red cells (3, 23). Furthermore, the absence of rhythmic CBV changes in neonates indicates a less effective precapillary hematocrit control in neonatal skin microcirculation; periodic arteriolar contractions have been shown to substantially reduce the downstream skin capillary hematocrit in adults (17). Thus, both specific rheological and myogenic factors in neonatal skin microcirculation may, to some degree, cause the slowing of the capillary flow associated with a high hematocrit level. The significance of this association is underlined by the prolonged interruptions of capillary flow seen in the infant with the highest hematocrit. The common finding of a hematocrit obtained by heel puncture exceeding the corresponding venous value during the 1st wk of life (24) may also be attributed to these microcirculatory factors in neonatal skin because such a hematocrit reflects the red cell volume fraction in a group of skin microvessels.

The adult skin microvascular architecture is characterized by a superficial layer of loop capillaries running perpendicular to the skin surface in the cutaneous papillas and of deeper lying, subpapillary, horizontal vascular plexuses formed by larger microvessels and arteriovenous anastomoses. This arrangement enables the major part of the skin circulation to be transferred via the arteriovenous shunts that regulate body temperature, whereas tissue nutrition is mainly provided by a minor part of the total cutaneous blood flow that passes through the superficial capillaries (25). Morphological studies of the cutaneous microvasculature in infancy have revealed a disorderly arrangement of the vascular network (7–9). Despite this immature morphology, rapid changes in the neonatal limb and total skin blood flows have been demonstrated after temperature alterations (12, 14), indicating a mature circulatory response. However, although wide variations in skin temperature were noted among the infants in our study, these were not associated with changes in the CBV. This finding suggests that the thermoregulation under normal conditions is not mediated via blood flow changes in the superficial skin capillaries, and, consequently, deductions of the normal skin capillary perfusion cannot be made from skin temperature measurements.

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