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Influence of rheological parameters on the velocity of erythrocytes passing nailfold capillaries in humans

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Abstract
1256 subjects (apparently healthy subjects and patients with cardiovascular diseases) were registered in a prospective study including demographical and clinical data, rheological parameters (hematocrit, plasma viscosity, erythrocyte aggregation, erythrocyte deformability) as well as the erythrocyte velocity in human nailfold capillaries under resting and postischemic conditions.
A multivariate regression analysis showed that under resting conditions there was no correlation between rheological parameters and erythrocyte velocity in capillaries. The blood flow regulation seemed to be so effective, that pathological changes of the blood fluidity showed no effect on the velocity of an erythrocyte passing the capillaries.
During vessel paralysis in the early phase of the postischemic hyperemia following a stasis of three minutes in the vasculature distal to a pressure cuff at the upper arm a very clear correlation between the plasma viscosity and the maximum postischemic erythrocyte velocity in ipsilateral cutaneous capillaries could be observed ($p<0.0001$) while none of the other rheological parameters seemed to play a role. In a subgroup of diabetic patients the erythrocyte aggregation (measured during stasis) also correlated with the erythrocyte velocity ($p=0.0175$) besides the plasma viscosity.
This shows that a correlation of rheological parameters with the capillary perfusion could only be found during vessel paralysis. In of diabetic patients besides the plasma viscosity also the erythrocyte aggregation correlated with the mean capillary erythrocyte velocity. Theses results are in agreement with the hypothesis from Barras that plasma viscosity determines the perfusion of microvessels. Under certain conditions e.g. diabetic disorder, also the erythrocyte aggregation plays a role.

Introduction

Besides arterial blood supply and tissue oxygen consumption [29], the tissue oxygen tension is governed by the quality of the microvascular perfusion, and there by the capacity to regulate the vascular dilation especially of arteriolar blood vessels [15, 37]. When the local tissue oxygen tension (pO_2) is too low for the actual O_2 need, metabolic stimuli locally induce the dilation of resistance blood vessels. In consequence, blood and O_2 – supply, respectively, by the nutritive capillaries will rise until tissue oxygen need is met again. Due to alterations of the arteriolar lumen (Vasomotion) not only the local intravascular blood pressure, blood velocity and the minute volume of blood flow change, but also the hematocrit and blood viscosity. Blood viscosity changes depend on hematocrit and the dependence was shown to be different when regarded either in blood vessels in vivo or in rigid glass tubes, which was already described by Whittaker and Winton in 1933 [39]. Since then several studies followed analysing the influence of viscosity or other rheological parameters on the blood flow in large or small blood vessels [5]. Sometimes contradictory results were reported said to be related to differing models and/or experimental conditions [5].
Multivariate regression analysis was used to find which of the rheological parameters assessed ex vivo might be correlated to capillary perfusion. As microcirculation characterizing parameter the velocity of erythrocytes in human cutaneous capillaries was assessed first under resting conditions (during physiological blood vessel regulation) and then following a 3 minutes stasis in the vasculature of the arm inducing a postischemic hyperemia coinciding with the elimination of blood vessel regulation.

Material and Methods

Design of the registry

The study was performed according to the ethical guidelines of the journal „*Clinical Hemorheology and Microcirculation*“ [3]. The registry is based on the anonymized rheological and microcirculatory data of 1256 probands/patients. Among these 171 probands were apparently healthy and aged between 45 an 67 years. There were 1085 patients aged between 41 and 79 years. Among them 531 patients suffered from coronary artery disease (CAD), 112 patients suffered from peripheral occlusive arterial disease (POAD), 309 patients suffered from essential hypertension and 133 patients from diabetes mellitus. Of the rheological parameters, the hematocrit (hct), the plasma viscosity (pv), the erythrocyte aggregation (SEA) and the erythrocyte deformability (SER) were assessed. Additionally, the erythrocyte velocity ($\mathbf{v}_{RBC}$) was measured in the

nailfold capillaries under resting conditions as well as during postischemic hyperemia applying intravital microscopy.

Intravital video capillary microscopy

The visualisation of erythrocytes in the nailfold capillaries was performed with a stereomicroscope (Zeiss AG, Germany) which was connected to a video system [21]. Due to the absorption of green light by hemoglobin, epi-illuminating light with a wavelength of 480 nm allowed erythrocyte detection. The erythrocytes in the capillaries appeared in dark shades of gray (Fig. 1) while the vessel wall itself was not visible.

Erythrocyte velocity measurements in the capillaries were performed by means of an image analysis system 'Cap-Image' (Zeintl Engineering Office, Heidelberg, Germany [24]). So-called plasma gaps are formed at sites without erythrocytes, and can be observed clearly so that the velocity of erythrocyte columns or single erythrocytes can be measured in a series of subsequent images. Figure 1 shows nailfold capillaries of the first row with the arrow indicating a plasma gap. After at least 10 measurements per minute in each capillary (in order to even out the vasomotion-dependent rhythmic fluctuations (6–10/s) of the erythrocyte velocity [35]) v_{RBC} was calculated as mean over time (mean time value).

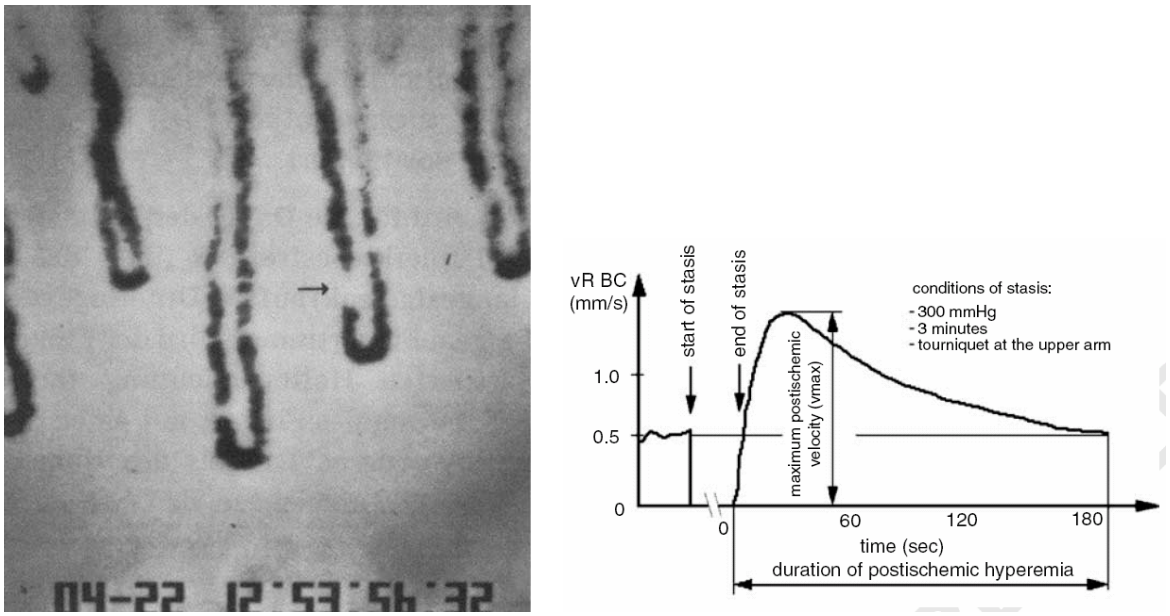

Figure 1: Left: Capillaries of the first row at the nailfold (the arrow marks a plasma gap, whose movement over time is used to calculate the erythrocyte velocity); Right: Recording of postischemic hyperemia (DpH)

With a normal capillary density (9/mm of the epidermal margin [20]), approximately 2–4 capillaries could be observed simultaneously at a magnification of 1:570. Owing to the marked dependence on the temperature of the cutaneous microcirculation, adaptation of the patient to room temperature with a skin temperature of at least 27.4°C was awaited before the measurements were started [19]. Details of the method and error analysis are described elsewhere [19].
To assess the postischemic hyperemia in nailfold capillaries an arrest of the blood flow was induced using a pressure cuff on the upper arm at 300 mmHg (Fig 2).

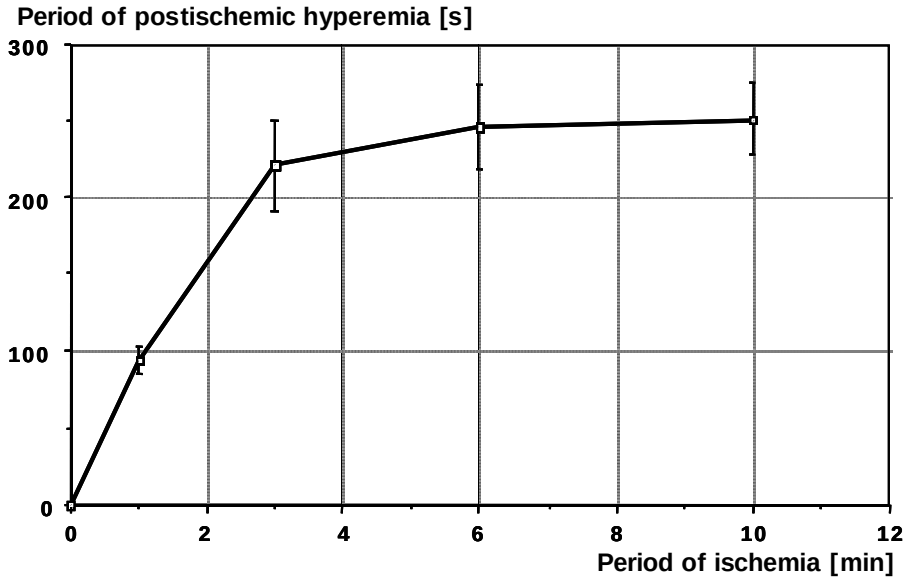

Figure 2: Period of reactive hyperemia in relation to the period of ischemia

The amplitude and the duration period of the postischemic hyperemia markedly increased with an increasing duration of the induced ischemia. After a 3 minute ischemia period 90% of the maximum effect was reached and longer periods of ischemia increased the effect only marginally [21]. Since longer periods of ischemia turned out to put serious inconveniencies upon the patients, the 3 minute arrest of blood flow was established to generate the vessel paralysis.

Hemorheological parameters

For the assessment of the hemorheological parameters (see table 1) the blood samples were drawn from the cubital vein of the sitting patients with the use of a cuff under standardized conditions [6]. The blood samples were stored in sealed polystyrole tube at room temperature. The rheological parameters were assessed within 2 hours after blood sampling [22].

Table 1: Parameter, methods of measurement and reference ranges [22]

parameter	symbol	method of measurement	reference range	
Plasma viscosity	PV	capillary tube plasma viscometer	1.14-1.34	mPas
Hematocrit	Hct	Impedance measurement	♂:39-52	%
			♀:34-50	%
RBC-Aggregation	SEA	RBC-Aggregometer	8-21	-
RBC-Rigidität	SER	RBC-Rigidometer	0.83-1.19	-

PV describes blood plasma viscosity and is proportionality constant between shear stress and shear rate. It was measured using a capillary viscometer [16].
Hct ist he cellular fraction in relation to the total blood volume. It was measured by means of an impedance measuring device [22].
SEA describes the reversible aggregation of erythrocytes leading to rouleaux formation under physiological conditions or to blood sludge under pathological conditions (e.g. due to elevation of a2-macroglubulin or immunoglobulins). SEA was assessed using an RBC-aggregometer (Myrenne, Roetgen, Germany) under the conditions of stasis [22]. Since SEA was shown to depend on Hct, the Hct was tuned to 45% before the assessment of SEA [17].
Erythrocytes can pass through blood vessels with diameters less than their own diameter due to RBC deformation. The deformability of RBCs was described by the time span which RBCs need to pass an artificial capillary of 4.2 µm and 45 µm length. Changes in electrical conductivity over the membrane due to the passage of RBCs through the capillary were used to measure the RBC passage time [34]. RBCs with decreased deformability are more rigid and need more time to pass the capillary. Passage times of 200 single erythrocytes were measured and the mean value displayed.

Results

1. Multivariate regression analysis under resting conditions

Table 2 shows mean values and standard deviations of rheological parameters as well as mean velocities of erythrocytes in nailfold capillaries and the results of the univariate and multivariate regression analysis.

Table 2: Rheological parameters and mean erythrocyte velocities $\mathbf{v}_{RBC}$ [mm/s] of 1256 probands/patients under resting conditions

Parameter	r-univariate analysis	p
$\mathbf{v}_{RBC}$=0.49±0.26 [mm/s]		
Hct=43.6±5.0 [%]	r (Hct – $\mathbf{v}_{RBC}$) = -0.002	p=0.414
PV=1.35±0.13 [mPas]	r (PV – $\mathbf{v}_{RBC}$) = -0.290	p=0.071
SEA=16.4±4.6 [-]	r (SEA –$\mathbf{v}_{RBC}$) = -0.020	p=0.619
SER=1.09±0.06 [-]	r (SER –$\mathbf{v}_{RBC}$) = -0.015	p=0.619

The correlation coefficient in the multivariate analysis of the rheological parameters and $\mathbf{v}_{RBC}$ amounted to r=0.367 (p=0.0025). A very weak tendency (p=0,071) indicated a relation between PV and $\mathbf{v}_{RBC}$ (Fig. 3).

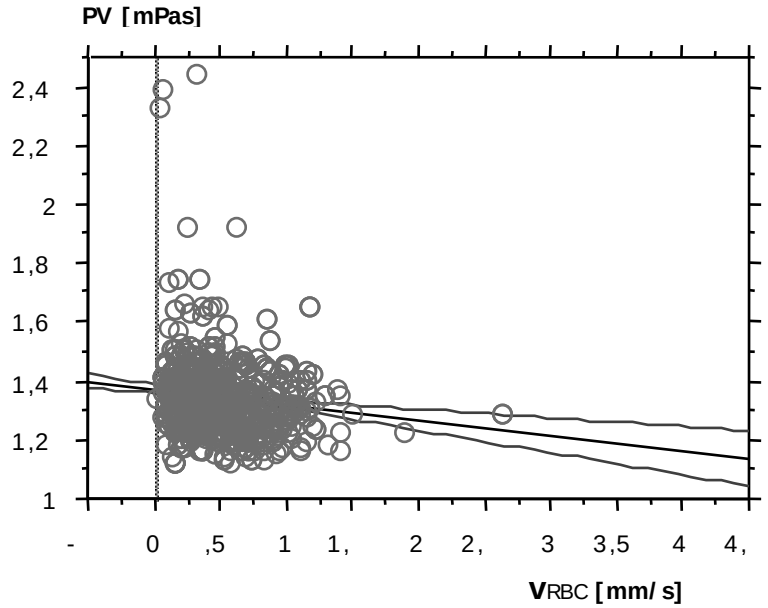

Figure 3: Correlation between plasma viscosity PV [mPas] and mean capillary erythrocyte velocity $\mathbf{v}_{RBC}$ [mm/s] under resting conditions

2. Multivariate regression analysis of results from postischemic hyperemia

Under these conditions a remarkable and statistically valid correlation between PV and $\mathbf{v}_{RBC}$ was revealed (Tab. 1).

Table 3: Rheological parameters and maximal capillary erythrocyte velocity $\mathbf{v}_{max}$ [mm/s] during postischemic hyperemia in 1256 probands/patients

Parameter	r-univariate analysis	p
$\mathbf{v}_{max}$=0.88±0.26 [mm/s]		
Hct=43.6±5.0 [%]	r (Hct – $\mathbf{v}_{max}$) = -0.004	p=0.1658
PV=1.35±0.13 [mPas]	r (PV – $\mathbf{v}_{max}$) = -0.790	p<0.0001
SEA=16.4±4.6 [-]	r (SEA – $\mathbf{v}_{max}$) = -0.006	p=0.1146
SER=1.09±0.06 [-]	r (SER – $\mathbf{v}_{max}$) = -0.006	p=0.1213

The correlation coefficient in the multivariate analysis of the rheological parameters and the maximal capillary erythrocyte velocity $\mathbf{v}_{max}$ [mm/s] during postischemic hyperemia amounted to r=0.484 (p=0.0001). In the univariate analysis a significant correlation (p<0.0001) between PV and $\mathbf{v}_{max}$ was revealed (Fig. 4).

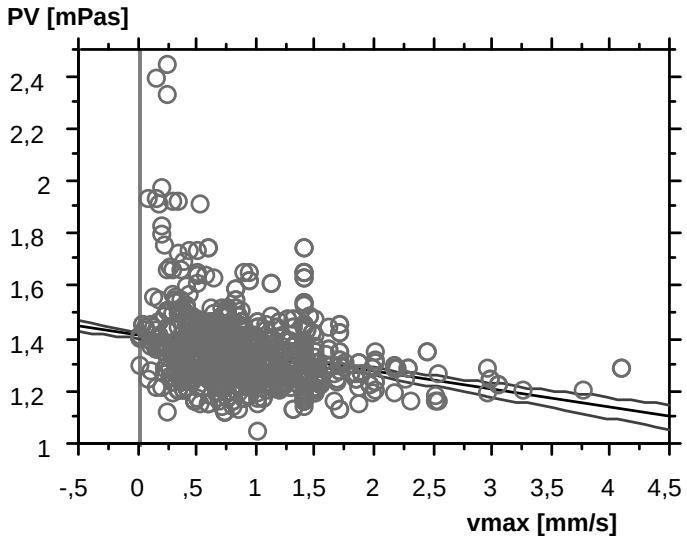

Figure 4: Correlation between plasma viscosity PV [mPas] and the maximal capillary erythrocyte velocity $\mathbf{v}_{max}$ in [mm/s] during postischemic hyperemia

3. Subgroup analysis of Diabetes mellitus patients

3.1 Multivariate regression analysis under resting conditions

Under resting conditions and a physiological blood vessel regulation the subgroup of patients with diabetes mellitus (n=133) did not show a statistical

correlation between rheological parameters and the erythrocyte velocity in nailfold capillaries (Tab. 4).

Table 4: Rheological parameters and mean capillary erythrocyte $\mathbf{v}_{RBC}$ [mm/s] in 133 patients with diabetes mellitus under resting conditions

Parameter	r-univariate analysis	p
$\mathbf{v}_{RBC}$ =0.42±0.26 [mm/s]		
Hct=44.2±5.9 [%]	r (Hct – $\mathbf{v}_{RBC}$) = -0.004	p=0.1658
PV=1.41±0.17 [mPas]	r (PV – $\mathbf{v}_{RBC}$) = -0.590	p=0.057
SEA=18.5±4.5 [-]	r (SEA – $\mathbf{v}_{RBC}$) = -0.226	p=0.2176
SER=1.13±0.07 [-]	r (SER – $\mathbf{v}_{RBC}$) = -0.011	p=0.133

The correlation coefficient in the multivariate analysis of the rheological parameters and the mean capillary erythrocyte velocity under resting conditions amounted to r=0.189 (p=0.192). A very weak tendency (p=0.057) indicated a relation between PV and $\mathbf{v}_{RBC}$ (Fig. 5).

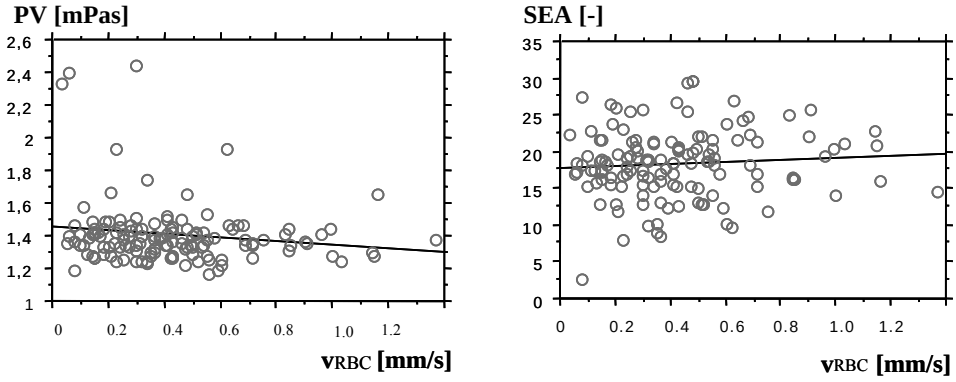

Figure 5: Correlation diagrams between plasma viscosity PV [mPas] and mean capillary erythrocyte velocity $\mathbf{v}_{RBC}$ [mm/s] (left diagram) and erythrocyte aggregation SEA [-] and mean capillary erythrocyte velocity $\mathbf{v}_{RBC}$ [mm/s] (right diagram) in patients with diabetes mellitus under resting conditions

3.2 Multivariate regression analysis during postischemic hyperemia

When the local blood vessel regulation was abolished almost completely in the early phase of postischemic hyperemia there were statistically valid correlations

between PV and the maximal erythrocyte velocity $\mathbf{v}_{max}$ and between SEA and $\mathbf{v}_{max}$ in the nailfold capillaries (Tab. 5).

Table 5: Rheological parameters and maximal capillary erythrocyte velocity $\mathbf{v}_{max}$ [mm/s] during postischemic hyperemia in 133 patients with diabetes mellitus

Parameter	r-univariate analysis	p
$\mathbf{v}_{max}$ =0.68±0.41 [mm/s]		
Hct=44.2±5.9 [%]	r (Hct – $\mathbf{v}_{max}$) = -0.004	p=0.1658
PV=1.41±0.17 [mPas]	r (PV – $\mathbf{v}_{max}$) = -0.590	p<0.0001
SEA=18.5±4.5 [-]	r (SEA – $\mathbf{v}_{max}$) = -0.226	p=0.0175
SER=1.13±0.07 [-]	r (SER – $\mathbf{v}_{max}$) = -0.026	p=0.1330

The correlation coefficient in the multivariate analysis of the rheological parameters and the maximal capillary erythrocyt velocity $\mathbf{v}_{max}$ during postischemic hyperemia amounted to r=0.41 (p<0.0001). The maximal erythrocyte velocity $\mathbf{v}_{max}$ was significantly correlated with the plasma viscosity PV (p<0.0001) and the erythrocyte aggregation SEA (p<0.0175, Fig. 6).

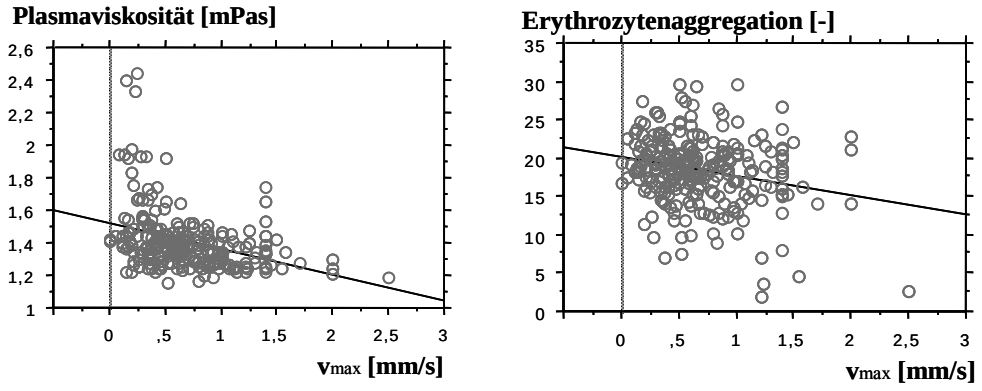

Figure 6: Correlation diagrams between plasma viscosity PV [mPas] and maximal erythrocyte velocity $\mathbf{v}_{max}$ [mm/s] (left diagram) and erythrocyte aggregation and maximal erythrocyte velocity $\mathbf{v}_{max}$ [mm/s] (right diagram) during postischemic hyperemia in 133 patients with diabetes mellitus

Discussion

There was practically no correlation between rheological parameters and the mean velocity of RBCs in cutaneous microvessels during intact blood flow regulation under resting conditions. Assuming that the Hagen-Poiseuille equation may describe the blood flow in vessels in a first order approximation [21], small changes of the vascular calibre then lead to changes of the blood flow to the 4^{th} order whereas viscosity changes influence the blood flow only linearly. It was shown that small arterioles can change their diameter up to 300% [2, 21]. This means that theoretically a 300% increase of the diameter of a small arteriole (ø 30 µm) results in an 1,296-fold increase of the blood flow in the supplied tissue. In contrast, an increase in viscosity of the same magnitude (300%) would theoretically result in a 3-fold decrease the blood flow only. This simple calculation may show that as long as the blood flow regulation is intact the blood viscosity or the rheological parameters, respectively, do not have a significant influence on the capillary perfusion.
However, even under resting conditions the relatively weak influence of rheological parameters can cause a deterioration of the microcirculation sometimes seen in patients with severe arteriosclerotic disease or in patients with endstage heart insufficiency. It could be that in patients with low cardiac output (low Δp in the Poiseuille equation) or with an inertia of vascular geometry (low $\Delta Ø$ due to e.g. calcinosis or a severe endothelial dysfunction with a loss of autoregulation of vascular geometry) an increase of blood viscosity (due to increased plasma viscosity or erythrocyte rigidifaction) might be a key event leading to a aggravation of a microcirculatory disorder [1, 10]. At the same time these conditions offer the chance for a therapy foccussed on the rheological parameters e.g plasma viscosity or erythrocyte rigidity [2, 7, 8, 13, 18, 26, 28].

Of the different available possibilities to outrule the blood vessel regulation we decided for the stasis induced silencing of vascular regulation. The pharmacological option is not appropriate to be applied in humans (due to adverse effects like severe hypotension with the risk of massive neurological and circulatory disorder).
In order to arrive at a correlation between the rheological parameters and the capillary perfusion an ischemia of the vasculature– using a blood pressure cuff - was performed. Carlson et al. could show that upstream vascular occlusion between 1 and 3 minutes enabled an increasing blood flow up to a maximum. The prolongation of the occlusion time over 3 minutes only led to an increase of duration of the reactive hyperemia [9]. Our own results demonstrated (Fig. 2) that a maximal blood vessel dilation and about 90% of the duration of the postischemic hyperemia were reached after a 3 minutes ischemia [21]. That is

why a 3 minutes stasis of arm vessels was chosen to arrest the blood vessel regulation in the downstream vasculature.

During vessel paralysis – as it occurs in the early phase of the postischemic hyperemia when the arteries are dilated and the blood flow regulation is turned off [14] – a correlation between PV and $\mathbf{v}_{RBC}$ in human cutaneous capillaries was demonstrated. This is thought to be an argument in favour of the hypothesis of Barras [4] that PV determines the perfusion of capillaries (capillary Hct is about 10 - 20%, so that blood viscosity in capillaries approaches PV [18, 23, 27]).

This should be true as long as the capillary diameter is big in relation to the RBC diameter [11, 38]. If the capillary diameter is smaller than the RBC diameter, the cells have to be deformed by shear forces during capillary passage. In this case the blood flow velocity should depend additionally on the RBC deformability [31, 32]. This view, which results from a fluid dynamical consideration and from experimental data [27], is often <u>not</u> met in patients: neighbouring capillaries with different diameters and fed from the same arteriole showed the same $\mathbf{v}_{RBC}$. Driessen could demonstrate that rigidified RBC slowed down the capillary blood flow (down to stasis) <u>only</u> if the blood pressure was lowered clearly below the normotensive state [12]. Since the mean capillary diameter in cutaneous capillaries is about 9 µm [20] it seems comprehensible, that in this analysis the deformability did not play a role regarding the capillary perfusion.

The problem is more complicated in case of a pathologically elevated RBC aggregation, as is known to be the case in diabetic patients due to elevated fibrinogen and α2-macroglobulin concentrations [33, 36]. Under these conditions a massive sludging can be found - firstly described by M.H. Knisely in conjunctival vessels [25]. This can occur even in small cutaneous capillaries. Under physiological conditions the shear rates in arterioles are high enough to disperse RBC aggregates so that the single RBCs can pass the capillaries in a single cell flow. But in diabetic patients the attracting forces holding RBCs together can be higher than the shear forces to disperse the aggregates so that small aggregates enter into the capillaries [30]. In these cases the aggregates pass the capillary very slowly but later a single cell perfusion in the same capillary can occur with significantly higher velocities. This might be the basis for the result that in the subgroup of diabetic patients not only the PV but also the SEA correlated with the mean capillary $\mathbf{v}_{RBC}$.

Conclusion

The study shows that a correlation of rheological parameters with the capillary perfusion could only be found during vessel paralysis. In the subgroup of diabetic patients beside of the PV also the SEA correlated with the capillary VRBC. These results confirm the hypothesis from Barras that PV determines the

perfusion of microvessels but shows as well that under certain conditions also the SEA plays a role.

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