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REPORT AND ABSTRACTS OF THE 4th NATIONAL SYMPOSIUM ON HEMORHEOLOGY, NOVEMBER 1987, KÜHLUNGSBORN, GERMAN DEM. REP.

The 4th National Symposium "Hemorheology" was organized by the Working Group "Theoretical, Experimental and Clinical Hemorheology" in conjunction with the Society for Physical and Mathematical Biology and the Society for Pathological and Clinical Physiology, both of the G.D.R..

The symposium was attended by more than 80 participants from technical, scientific and clinical fields, among them 15 foreign guests from Austria, Bulgaria, Berlin(West), CSSR, Great Britain, Hungary, Japan, Poland, Switzerland and U.S.S.R.. The opening lecture was given by Prof. P. Gaehtgens (Secretary General of the International Society of Biorheology) on "Rheological determinants of resistance to flow of blood through narrow tubes". In 52 papers, results were presented concerning 4 main topics: Theoretical Hemorheology, Experimental Hemorheology, Microcirculation and Clinical Hemorheology.

During the symposium a business meeting of the Working Group "Theoretical, Experimental and Clinical Hemorheology" for the Society for Physical and Mathematical Biology and the Society for Pathological and Clinical Physiology of our country was held. It was stated that successful work had been done since the last meeting in 1985 (cp. Biorheology, 24(1987)275-276). In future the cooperation with physicians and the standardization of hemorheological parameters measured should be strengthened. The 5th Symposium on basic and applied hemorheology will be organized in the spring of 1990.

Abstracts of the Symposium concerning non-clinical hemorheology are published on the following pages in this issue of BIORHEOLOGY. Abstracts dealing with clinical hemorheology are published in CLINICAL HEMORHEOLOGY, A Companion Journal of 'Biorheology'.

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METHODS AND RESULTS OF STUDIES OF VASCULAR PERMAEBILITY AND RED CELL SWELLING IN THE CAPILLARY SYSTEM AT HYPERTHERMIA AND LOW pH

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The increase in vascular permeability at low pH-values, supposed by us, was studied on the mesentery of rat as a function of temperature. The tissue exposed from the anaesthetized animal was placed in a thermostatized photometric cuvette, containing buffer solution. After iv. injection of 75 mg Evans' Blue (EB) per kg b.w. into the rat, the permeation ψ of EB from the tissue into the surrounding fluid could be measured down to 5 µg/l and was clearly discernible from any capillary haemorrhage (sensitivity 0.2 µl blood/l). In the temperature range from 37 to 45°, ψ increased from 3.2 to 20.7µg EB/min. At 41.4°C, ψ increased when the pH was lowered from 7.4 to 6.0. At 37°C, ψ did not exceed the initial value during 230 mins (pH 7.4) and 180 mins (pH 6.0). - Videodensitometric measurements revealed an increasing translucence of the venules due to intravasal swelling of erythrocytes, when the pH was reduced stepwise from 7.4 to 5.5. This effect was not always reproducible. - Using capillary viscosimetry, a significant raise in the apparent viscosity η of blood was seen at pH 6.8 and 37°C and at a shear stress between 1 - 6.5 Pa, due to the higher haematocrit (red cell swelling). After correction of haematocrit to its initial value, η remained only slightly increased. When, at the same pH, the temperature was elevated to 43°C, the pH-conditioned increase in η disappeared almost completely. The studies are being continued.

THE INFLUENCE OF ELECTROSTATIC AND STERIC ENERGY ON THE ROULEAU-FORMATION OF HUMAN RED BLOOD CELLS IN DEXTRAN SOLUTIONS

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The dextran (Dx) mediated aggregation of red blood cells (RBC) depends on the Dx concentration as well as the molecular weight M_w. In literature it is concluded from electrophoretic data that the so-called ζ -potential estimated rises with increasing Dx-concentration and M_w. In contrast to these conclusions we could show (studia biophysica 120(1987)113) that the electric potential in the presence of Dx is not significantly altered. Instead a Dx depletion layer is responsible for the high electrophoretic mobility of RBC in Dx solutions. Accordingly it can be concluded that the disaggregation of RBC could not be due to an enhanced electrostatic repulsion.

By means of the mean field theory Dx concentration profiles as well as the attractive energy in the case of interacting RBC have been calculated. It is shown that there is a balance between steric attractive energy and steric repulsive energy. The depth of the energy minima and their position are determined by the Dx concentration, M_w as well as the conformational state of glycocalyx. The Dx concentrations calculated necessary to disaggregate the RBC are in agreement with experimental data.

INVESTIGATION OF RHEOLOGICAL PARAMETERS OF WHOLE BLOOD OF HEALTHY DONORS BEFORE AND AFTER PLASMAPHERESIS

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Fresh citrated blood (1+9) of 59 healthy donors (29 male, 30 female, 19-26 years) was investigated immediately before and after plasmapheresis(2x500ml double plasmapheresis). The aggregation of red blood cells (RBC) was determined by means of a hydrodynamically controled light backscattering technique (aggregation index AI, dublet formation time t_2), a shearing parameter S (τ=400 mPa) for characterizing the deformability and/or orientability of RBC under shear and the plasma viscosity η .

Mean values and S.D. of rheol. parameters, T=25°C, hematocrit 0.45

	AI	t_2(s)	η(mPa s)	S
before	0.48±0.04	9.3±5.6	1.40±0.06	0.877±0.025
after	0.42±0.04	29.5±20.9	1.31±0.04	0.839±0.027

All mean values determined before plasmapheresis were significantly different to those after plasmapheresis. AI and as well as t_2 and η related to the RBC aggregation were highly correlated to each other (p=0.01). The correlations were significantly different before and after plasmapheresis.

INFLUENCE OF ACTIVATORS ON PLATELET ELECTROROTATION BEHAVIOUR

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The electrorotation behaviour of biological objects depends significantly on their electrical membrane and internal properties. The electrorotation spectrum of living cells shows two characteristic frequencies usually located in the kHz and in the MHz range of the applied external electrical field, respectively. Applying a single shell model it is possible to estimate membrane capacity, membrane resistance and internal conductivity. We hypothesizied that by means of electrorotation membrane conductivity changes of the platelet during the exocytotic release reaction can be followed. For electrorotation measurements a digital rotation-field-generator (RFG-5) was used. ^{14}(C)serotonin release and anti-field rotation of adrenalin-, arachidonic acid-, ADP-, thrombin-, collagen-, PAF-(Semi-synthetic-PAF-acether) and Ca^{2+}-ionophore A23 187-activated platelets were simultaneously measured and subsequently compared. In general, under our conditions of release efficiencies above 60% ^{14}(C)serotonin release correlated strongly to the decrease in anti-field rotation. Weak inducers with a release potency of less than 40% ^{14}(C)serotonin release did not show a correlation between the decrease of the anti-field rotation and ^{14}(C)serotonin release. The dramatic decrease of the anti-field rotation could only be explained within the framework of the above model assuming an increase of the membrane conductivity.

RHEOLOGICAL DETERMINANTS OF RESISTANCE TO FLOW OF BLOOD THROUGH NARROW TUBES

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Recent experimental studies of blood rheology in tube flow (Circ. Res. 59(1986)124-132; Am. J. Physiol. 253, in press) have shown that the effect on apparent viscosity of well known phenomena like red cell aggregation differs substantially from that seen in viscometric flow. In tube flow, the redistribution of red cells across the tube cross-section, which results from aggregation, core compaction, and sedimentation, represents the phenomenon dominating rheological resistance. This includes the assembly of aggregates from single red cells as well as the contraction of the aggregated network which leads to an expression of interspersed plasma, white cells and platelets. As a result, apparent viscosity falls with shear rate reduction to low values (1 sec^{-1}) in vertical tubes eventually leading to a disappearance of the Fahraeus-Lindqvist effect. In horizontal tube flow, non-axis-symmetric cell distribution caused by sedimentation dominates blood flow behaviour at low shear. As a consequence, apparent viscosity rises with shear rate reduction. In both situations, substantial time-dependent variations of viscosity occur. Predictions of hemodynamic aspects of blood flow in microvascular networks in vivo on the basis of these findings are additionally complicated by flow partition effects at vascular bifurcations. It is quite likely that substantial heterogeneity of flow at reduced perfusion pressures or increased aggregation tendency may result from these rheological phenomena.

THE RETENTION OF PLATELETS ON PROTEIN OR POLYMER COATED GLASS MEASURED BY MEANS OF COLUMN BEAD METHOD

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The platelet adhesiveness is considered to be an important parameter for evaluation of hemocompatibility of biomaterials. It was the aim of this study to demonstrate the applicability of the column bead method for the characterization of platelet retention onto protein and polymer coated glass. Glass beads coated with different plasma proteins or polymers were situated in plastic tubings. Anticoagulated whole blood was pumped through the columns and collected in fractions. The platelet number in influent and effluent blood was estimated using a Coulter Counter S Plus IV. The platelet retention was measured for glass coated with fibrinogen (FG), fibronectin (FN), gamma globulin, human serum albumin (HSA) and two types of polymers. The platelet retention was found to be 100% for FG, 45% for FN, 12% for gamma globulin and 6% for HSA. The polymers tested caused an increase of platelet retention in comparison with clean glass that indicates a low hemocompatibility of both polymers. In conclusion the column bead method was shown to be a useful in vitro method for evaluation of hemocompatibility of biomaterials in the presence or absence of preadsorbed plasma protein layers.

INFLUENCE OF THE CROSSLINKING OF SPECTRIN OF HUMAN ERYTHROCYTES ON RHEOLOGICAL PROPERTIES

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The membrane proteins of erythrocytes play an important role in maintaining the properties of the membrane. Diamide as a specific oxidant for the sulfhydryl groups crosslinks the membrane proteins and pertubs the membrane organization. The aim of the present paper was to characterize rheological properties such as deformability and aggregation behaviour of erythrocytes after diamide treatment (0.5 - 5 mM).

The oxidation of sulfhydryl groups was estimated over the reduction of intracellular glutathion, which was about 80% reduced after 5mM diamide. The apparent membrane elastic shear modulus μ was determined by the micropipette aspiration technique. In dependence on diamide concentration μ was increased from 300% to 600%. By light back scattering technique aggregation of erythrocytes (aggregation index AI and time constant α) was determined in solutions containing 4g/dl Dx70 and 1g/dl fibrinogen and was decreased for both with rising diamide concentration. There were qualitative differences of the concentration dependency of α between Dx70 and fibrinogen aggregation.

The changes obtained are discussed in connection with crosslinking of membrane proteins, especially changes in the cytoskeleton and in the surface structure of the erythrocyte membrane.

INFLUENCE OF IONIC STRENGTH ON RHEOLOGICAL PROPERTIES OF HUMAN RED BLOOD CELLS (RBC)

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The membrane elastic shear modulus (μ), surface area (S) and volume (V) of RBC were estimated with a micropipette aspiration technique. Lowering the salt concentration at constant pH=7.4 and osmolarity 300 mOsmol enhanced significantly the mean value of μ by 9.25% at 50mmol/l and 95% at 5.8mmol/l (normal ionic strength (162 mmol/l) μ=4.1$\pm$0.35μN/m). S and V were found not to be affected under low ionic strength conditions up to 50mmol/l salt concentration. Progressive RBC crenation was observed at 5.8 mmol/l. The aggregation behaviour of RBC after addition of 3g/dl Dx75 was investigated by means of light back scattering technique. The aggregation index (AI) and time (t_2) of formation of erythrocyte doublets were calculated using a nonlinear fit of the experimental curves. The mean value of the reflectometric AI for RBC at normal ionic strength conditions of 0.55$\pm$0.02 decreased continuously with reduction in salt concentration, reaching 50% of the initial value at 75mmol/l. At 50mmol/l the erythrocytes did not aggregate. Lowering the ionic strength increased drastically t_2: from 2s at 162mmol/l to 73s at 75mmmol/l. The changes of the rheological parameters studied are related to alterations in transmembrane potential and interaction force balance.

CHARACTERIZATION OF RBC DEFORMABILITY AFTER LONG-TIME STORAGE AT SUBZERO TEMPERATURES

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Deformability of RBC after long-time storage in frozen state at -28°C and -196°C were characterized by their filterabilities and compared with commonly used liquid stored cells. All samples were processed and stored routinuously under blood bank conditions.

Filterability measurements were performed by means of a Hemofiltrometer on gravity filtration principle with low starting pressure (290Pa), using cellulose filters and suspensions of washed cells (Hct=0.60). From 5 to 6 estimations a standardized relative filtration time (FI) is calculated.

In comparison with liquid stored (+4°C, 35 days, FI=+10%) cells, the storage at -28°C led to a more pronounced deformability loss after 2, 8 (FI=+40%) and 15 months (FI=70%). The storage at -196°C up to 9 years resulted in surprisingly small changes of cell deformability. Whereas after 2 months a slightly decreased filterability was measured, it was improved after 1, 4 and 9 years of storage as a consequence of decreased internal viscosity of the cells. Measured filterability changes are interpreted taking into account MCHC-changes during storage and equilibration by sample preparation. Substitution of -196°C-storage by -28°C should not be recommended from the viewpoint of cell deformability.

TUMOUR MICROCIRCULATION AT DIFFERENT BODY TEMPERATURES

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The film shows results of intravital microscopic examinations of the blood flow in mesenteric vessels in rats with hepatoma ascites.

Experimental and observational period is 120 minutes. A rise in temperature to 40°C leads to minor bleedings, a granular flow and few stases. A decrease in temperature to 25 °C always results in addition to a granular flow and aggregates in increasingly irreversible changes in the form of extended stases in the newly formed vessels. In the already existing vessels only insignificant changes of the blood flow occur.

CALCULATIONS OF STREAMING PROFILES IN SMALL VESSELS AND SOME SPECULATIONS ON THE RADIAL HEMATOCRIT PROFILE BASED ON IN VIVO LITERATURE DATA.

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As in vivo data concerning the radial hematocrit profile in small vessels are lacking, the aim of this paper was to apply a recently developed approach for velocity profile computation to literature data of in vivo velocity profiles. The theoretical profile works with an arbitrary radial hematocrit profile and takes the non-Newtonian flow properties viscosity of blood into account. Apparent blood viscosity in dependence on local hematocrit was computed by the Quemada equation.

Physiological pressure gradients and volume flows result in flattened velocity profiles. The plaque flow was more pronounced with higher assumed mean vessel hematocrit and increased axial migration of RBC. By changing the axial hematocrit distribution, the computed velocity profiles can be approached to experimentally determined in vivo profiles. A pronounced axial RBC flow is the prerequisite for a good fit.

NEGATIVE SPONTANEOUS MEMBRANE CURVATURE AND ITS SIGNIFICANCE UPON THE RESTING RED BLOOD CELL SHAPE.

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In general, biophysical hypotheses for RBC shape assume, beside bending and shear rigidity, a spontaneous membrane curvature due to membrane molecules of active bending or a bilayer couple effect. Based on recent knowledge of the steric and electrostatic structure of the RBC membrane, i.e., taking into account the electrostatic repulsion of charged phospholipids and spectrin at the interior membrane as well as the charge interaction inside the glycocalyx at the exterior side of the membrane, a quantitative model was developed (Lerche et al. Biorheol. 24(1987)23) estimating the free electrostatic energy changes due to bending.

Taking the lipid and electrostatic part of spontaneous curvature into account, th shear and bending energy was computed using the finite element programm of Zarda et al.(J.Biomech. 10(1977)211) for deflating a spherical cell up to a standard discocyte. It was shown that discocytes can be obtained for different combinations of the shear modulus, bending modulus and spontaneous curvature. Without spontaneous curvature, a bending rigidity is at least 10 times higher than the experimentally estimated value of about 2×10^{-19} J. The bending rigidity could be reduced by increasing the value of negative spontaneous curvature. For $C_o = -6$ µm standard discocytes can be computed for experimentally determined shear and bending rigidities.

VISCOELASTIC AND VISCOPLASTIC PROPERTIES OF THE ERYTHROCYTE MEMBRANE

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In dependence on the kind of mechanical loading the membrane of erythrocyte shows viscoelastic or viscoplastic properties, respectively. It behaves like a solid body, when only small forces act for a short time. The mean apparent elastic shear modulus of red cell membrane, measured on 643 cells of 37 healthy donors , was equal to (4.2 ± 0.7) μN/m.

The entry process of red cell membrane into a micropipette (inner diameter 1.6 μm) can be described by two characteristic phases. 80% of the entire deformation is realized in the first, fast phase with a characteristic time of $t = 0.2$ s. The following creeping phase has a characteristic time of few seconds.

A cycling loading of red cell membrane during few minutes led to a phenomenon of hysteresis. But a relatively large force during a long time causes a continuous deformation (flow). Under these conditions we could observe a fragmentation of the erythrocytes inside the capillary.

MICROFLUORIMETRIC ESTIMATE OF NATRIUM-FLUORESCEIN AND ALBUMIN PERMEABILITY CHANGE OF THE VENULAR VESSEL WALL CAUSED BY PEROXIDE OF HYDROGEN

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The active forms of oxygen, particularly their free radicals, play an important role in the genesis of permeability disturbances of blood vessel walls. By vital microfluorimetry the transport kinetics of Na-fluorescein and FITC-albumin across venular vessel walls of 30 rat mesenteries were investigated under control conditions and after application of 10^{-2}M peroxide of hydrogen. A new method of registration and analysis of the temporal changes of indicator concentrations in tissue near the vessel provides us with quantitative evidence on the tracer transport across microvessel wall. While the permeability coefficient for Na-fluorescein doesn't essentially change in the course of a 40 min control observation period, it linearly increases by about 25% per 10 min after H_2O_2-addition. A significant increase of venular permeability for FITC-albumin was registered during the first 10 min and in the interval between 40 and 50 min after H_2O_2-addition. It can be assumed that peroxide of hydrogen differently affects the diffusive and convective transport mechanisms across the venular vessel wall.

LOCAL HEMATOCRIT CHANGES. PRINCIPLES OF THIS HEMODYNAMIC PHENOMENON

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Changeable hematocrit in regional vascular beds is primarily accounted for by unequal distribution of red blood cells and plasma in the arterial branching sequence carrying blood to individual microvascular beds. This phenomenon was first substantiated in regional circulation and microcirculation in the 1950's when distribution of blood with unequal red cell concentration was found to be dependent upon blood velocity differences in the appropriate vascular branches arising from bifurcations. Therefore, ischemic vascular beds with reduced blood flow display a far lower hematocrit than those with arterial hyperemia, i.e., with enhanced blood flow. Recently, this phenomenon was discovered in the largest arteries of the body. Peculiar anatomical arrangement of the aortic arch seems to be responsible for the asymmetric distribution of red blood cells and plasma in both the forelimbs (hands in primates), as well as for higher hematocrit in the head, as compared with the caudally located parts of the body.

RED BLOOD CELL SEDIMENTATION IN BLOOD FLOW THROUGH INCLINED MICROVESSELS

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Red blood cell (RBC) falls through blood under the action of gravity. The rate of RBC sedimentation may be enhanced in settling vessels which are inclined to the direction of gravity (Boykott effect). To investigate steady blood flow in inclined microvessels, we flowed RBC suspension through a glass capillary at various angles of inclination. We visualized the flow by putting latex (approximately 1μm diameter) into the suspending medium. The behaviour of RBCs and latex flowing through the capillary was observed using a microscope-TV system. A two-phase flow approach was also made to analyze the pressure-flow relation and the growth of the RBC-free layer at the wall.

At low flow rates, the flow structure was influenced greatly by the inclination angle of the tube. Especially, the RBC-free layer appeared along the upper wall in the upward flow against gravity, but it disappeared in the downward flow for gravity. The RBC-free layer results from the natural convection at the downward facing wall of the tube. The pressure-flow relation was closely related to the growth of the RBC-free layer.

It is suggested that the gravity may affect the organ microcirculation at a low perfusion pressure.

IMPACT OF ERYTHROCYTES ON THE CAPILLARY WALL

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In microcirculation the capillary wall is considered as non-elastic and inflexible. This point of view is supported by numerous experimental and theoretic investigations. If the capillary wall is regarded as an elastic system, then capillary wall movements must occur and therefore it should be possible to observe them. Intravitalmicroscopic investigations in the mesentery of the "bloodless" rat were carried out to prove capillary wall movements. The model "bloodless" rat was obtained by hemodilution with an oxygen-transporting blood substitute. As one example the rectangular impact of erythrocytes in a capillary bifurcation was registered by means of highfrequency-microcinematography (2,000 pictures/sec.). Frame by frame evaluation during 1,000 ms demonstrates capillary wall movements up to 1 µm in the site of the impact. The intervals between the successive capillary wall amplitudes do not demonstrate any dependence on the heart frequency. The intervals between the successive capillary wall amplitudes and the velocity of the arriving erythrocytes demonstrate a contrary dependence on the investigation duration. In experimental investigations and theories of microcirculation it should be taken into consideration that microvessels are no inflexible tubes: capillaries have an elastic wall.

INFLUENCE OF FISH DIETS ON RHEOLOGICAL AND BIOPHYSICAL PROPERTIES OF HUMAN ERYTHROCYTHES

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People eating diets rich in fish take low risk to get a coronary disease. Recent investigations showed an increase of polyunsaturated fatty acids, especially of eicosapentaeic acid, in phospholipids of the cytoplasmatic membrane of human erythrocytes in dependence on fish diet. The aim of our paper was to study the deformability and artificially induced aggregation behavior of erythrocytes after 100 days of fish diet depending on number of meals per week.

The red cell deformability, characterized by a capillary passage time analyzer, was significantly increased in blood samples from people eating fish diet compared to the control group. The mean passage time of erythrocytes, measured with a 3.4 µm micropipette at a pressure difference P=300 Pa, was decreased by about 40% in persons eating fish five times per week. The artificial aggregation of washed erythrocytes in a suspension medium of an ionic strength of 5.6 mM and pH of 6.1 was changed in dependence on fish diet. The aggregation results showed an increase of the dead-time and a decrease of the aggregation extent. The results were discussed as an increase of the stability of the erythrocyte suspension and as an improvement of flow properties of erythrocytes.

RHEOLOGICAL PROPERTIES OF ERYTHROCYTES DURING ENDOTOXIN SHOCK

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Some rheological properties of erythrocytes during experimentally induced endotoxin shock in rabbits were investigated. Blood samples were drawn through cardiac puncture 2 hours after endotoxin administration. The electrophoretic mobility of red blood cells (RBC), determined by cell electrophoresis, was found to decrease to 30% with development of shock state. Using a modified Teitel technique it was shown that filterability of RBC under the same conditions was decreased between 14-70%. The RBC deformability, assessed with an electrooptical method, was also reduced between 30-50% after endotoxin administration. The changes in this parameter varied between the animals probably due to the different stage of shock development. These results give evidence that the rheological properties of RBC are impaired during endotoxin shock, probably due to alterations in membrane structure. Under the conditions of low flow state the reported changes in flow behaviour of RBC may evoke sludging in the microcirculation, as commonly observed in different shock state.

THE ROLE OF THE SHEAR AND TIME DEPENDENCY OF BLOOD FLOW PROPERTIES IN THE CARDIO-VASCULAR SYSTEM DYNAMICS

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The shear rate and time dependent flow properties of blood due to the structural breakup following an increase in shear rate or structural recovery after a decrease in shear rate influence the blood flow in the vascular system. In the present paper we investigate, on the basis of the proposed structural description of blood rheological behaviour, the importance of this influence using a residence time technique. The investigation reveals that, generally, the mean residence times are very short in the various parts of the vascular system. Consequently, the time needed for the structural changes is not sufficient to affect the flow. In addition the shear rates are very high, so that the shear viscosity ranges in the constant value region. If this is taken into account when estimating the importance of the blood flow properties, the shear rate and time dependency of blood can be ignored. The exception are local recirculating flows in branching vessels, which can increase the blood residence time, so that structural as well as viscosity changes could occur.

THE DENSITY AND THE ULTRASONIC VELOCITY OF BLOOD: THE CONTINUOUS MEASUREMENT OF THE CONCENTRATION OF THE RED BLOOD CELLS (RBC)

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Under the assumption that the distribution volume of the RBC is equal to the circulating blood volume, the concentration of the RBC may be used to monitor blood volume changes, which are due to shifts of the suspending fluid to and from the vascular compartment. Because of the differences of the acoustical properties of the plasma and of the RBC (density difference 60-70 kg/m^3; difference of the ultrasonic velocity 75-85 m/s), the measurement of the density (ϱ) as well as the measurement of the acoustic wave velocity (v) provides a means to determine RBC-concentrations. The ϱ of blood was determined by the mechanical oscillator technique and the v by the measurement of the acoustic wave travel time through the specimen. Both methods stand out for high precision. The resolution in the determination of Hct-changes may be as good as 0.01-0.02% and therefore permits to calculate fluid shifts from the concentration measurements upstream and downstream of a filtrating section of a circulation system. Filtration has been monitored in vitro with porcine blood making use of a dialysis system put at our disposal by Gambro, Sweden. In addition, adiabatic compressibilities ($\varkappa_{ad}$) of porince blood were calculated from the values of and v according to the relation $v = (1/\varrho\varkappa_{ad})^{0.5}$. The values of $\varkappa_{ad}$, and v were determined for different RBC-concentrations and for different temperatures (20.0 to 40.0°C).

THE EFFECT OF PENTOXIFYLLINE ON THE HAEMATOCRIT OF CONCENTRATED RED BLOOD CELL (RBC) SUSPENSIONS

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Published data concerning the influence of pentoxifylline (P) on the RBC volume are scarce and don't offer clear-cut evidence of effects of this kind. We have therefore reinvestigated that question using a microhaematocrit method with improved accuracy. To 50 vol of preserved blood (buffy coat removed) was added 1 vol either of Ralofekt® or of a 9 g/l solution of NaCl (ref. sol. A, isoosmotic to Ralofekt) or of a 6.94g/l solution of NaCl (ref. sol. B, NaCl concentration equal to that of Ralofekt). Subsequently the haematocrit (Hct) was measured. Addition of Ralofekt resulted in a Hct by a factor 1.0059 ± 0.0014 (n=10) greater than the Hct after addition of the isoosmotic ref. sol. A. Comparing the effects of Ralofekt and ref. sol. B no significant change in Hct was seen, the Hct ratio being 1.0005 ± 0.0009 (n=10). Apparently, P behaves osmotically inactive in concentrated RBC suspensions at the concentration used (1.4 µmol/ml RBC suspension). Neither a binding to the RBC membrane or to plasma proteins nor a colloidosmotic swelling caused by membrane damage account for this finding. Our results are consistent with the view that improved RBC filterability due to P is mainly effected via a membrane-directed action of the drug rather than a reduction of the internal viscosity of the RBC.

RADIAL SPREADING RHEOMETRY FOR DETERMINATION OF ERYTHROCYTE DEFORMABILITY AND PLASMA VISCOSITY

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Radial spreading rheometry was originally designed to measure the deformability of erythrocytes. A calibration time (CT) for radial spreading of buffer through a horizontally suspended filter paper is used to calibrate individual filter papers. The radial spreading time for washed erythrocytes is then expressed as the ratio RST/CT and adjusted to a standard packed cell volume of 0.50. The ratio RST/CT is independent of temperature within the range of 20-35°C, leucocyte count up to $5.0*10^9$/l and platelet count up to $76*10^9$/l. The results of the method are similar to those obtained with two membrane filtration methods and suspension viscometry using in vitro manipulated and sickle cell erythrocytes.

Recently the radial spreading technique was also evaluated for the measurement of plasma viscosity. After the measurement of CT for 50 µl buffer a "flushing dose" of 100 µl plasma was dispensed on the filter paper and, after completion of its radial spreading , the radial spreading time of a test dose of 25 µl plasma was measured and divided by CT. This quotient showed a linear correlation with plasma viscosity as determined by a Contraves rotational viscometer (r=0.98, n=58). Radial spreading rheometry is a simple and low-cost technique for the assessment of erythrocyte deformability and plasma viscosity.

ELASTIC STRETCHING OF ERYTHROCYTES AT SUBZERO TEMPERATURES

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During cryopreservation of cell suspensions, the cells remain in amorphous channels between the ice crystals. In the ranges of freezing and thawing the cell membrane is exposed to shear and bending stresses which arise directly or indirectly from the ice matrix. Maybe these mechanical forces induce cell lysis which is observable by low temperature light microscopy. It seems that mechanical membrane damage at subzero temperatures occurs beside chemical cell damage. From a theoretical point of view such a physical membrane damage is dependent on the membrane flexibility. In this report a cryomicroscopical method for measuring the elastic deformability of cell membranes was examined and first results on erythrocytes are given.

The cells were polarized in a high frequency electric field of 1 MHz. Increasing field strength up to 1.5 kV/cm^{-1} elongates the discocytes which is measured at decreasing temperatures. The cells are suspended in low ionic strength solutions without and with 2M glycerol for cryoprotection. The results show a rapid increase of the elastic modulus with decreasing subzero temperatures. No influence of the glycerol substitution was noticeable. At nearly -20°C the deformability of the erythrocytes is zero. These experiments explain that cells only survive if small shrinkage and small amounts of intracellular ice arise, because the cell deformation is minimized and the nearly brittle membrane can withstand.

CONVECTIVE THEORY OF MEMBRANE FILTRATION OF COLLOIDAL DISPERSIONS

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The mechanism ensuring continuous operation during membrane (crossflow) filtration of suspensions of particles (cells) with colloidal dimensions has remained elusive. The application of a "concentration polarization" model, explaining the ultrafiltration behaviour of macromolecules, is impeded due to the small diffusivity of colloidal particles. A theoretical model is developed taking into account only the convective mass-balance during crossflow filtration. In this way particles are postulated not to return to the bulk, but rather to flow along the surface of the membrane in a layer whose thickness increases in the direction of feed flow. The model relates the hydraulic resistance of the close-packed layer to the technological parameters (transmembrane flux and pressure, length of the filtration path, shear rate). The role of the rheological behaviour of the cake layer is emphasized.

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RED BLOOD CELL ADHESION TO GLASS AND POLYURETHANE - EFFECT OF CELL SHAPE

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By means of a streaming method it was shown that the adhesion of human red blood cells in solutions of low ionic strengths to glass and to polyurethane depends on the shape of the cells. When we increased the ionic strength from 6 to 10 mM we found a reduced adhesion. Additionally, we investigated the time-dependent shape change induced by the surface. An enhanced ratio of echinocytes (stage I) was observed within the remaining adherent cells after gravity or flow. A shape change in favour of more advanced stages of echinocytes was found to be responsible for a reduced adhesiveness. This led us to the hypothesis that echinocytes of stage I were more adhesive than the other shapes present.

Indomethacin and chlorpromazine were used to induce stable cell shapes on glass surfaces. Together with untreated cells we were able to produce samples covering the whole range of different cell shape from advanced stages of echinocytes to stomatocytes. As compared to echinocytes and stomatocytes of stage I and discocytes the adhesion of advanced echinocytic as well as stomatocytic shapes decreased by approximately a factor of two.

It was concluded that the local deformability of the cell membrane together with the total membrane area of closest contact were signifcant factors in determining the strength of red cell adhesion.