

ABSTRACTS

OF THE
EIGHTH INTERNATIONAL CONGRESS OF BIORHEOLOGY
Yokohama, Japan, 3-8 August 1992

Submitted and selected with regard
to topics of clinical hemorheology
by Editor Y. Isogai
(President of the Congress)

(Received 22.4.1992 by Executive Editorial Office)

EFFECT OF HUMAN RECOMBINANT ERYTHROPOIETIN ON BLOOD RHEOLOGY IN RAT
N. Maeda and T. Shiga*. Dept. Physiol., Sch. Med., Ehime Univ., Onsen-
gun, Ehime, JAPAN and *Dept. Physiol., Sch. Med., Osaka Univ., Suita,
Osaka, JAPAN.

Rheological properties of blood upon intravenous administration of
human recombinant erythropoietin (rh-EPO; 180 or 1800 IU/kg) daily for 7
days to Sprague-Dawley rats (6 weeks-old, male) were investigated.

The following findings were resulted upon rh-EPO administration:
Rheologically, (1) drastic increase of blood viscosity (measured with a
cone-plate viscometer) was entirely dependent on the hematocrit (plasma
viscosity and composition of plasma proteins were not altered). (2) Red
cell deformability (a "deformation index", measured with a high shear
rheoscope) was not altered, in spite that the length of deformed red
cells were longer than that in control rats. (3) The velocity of
rouleau formation (measured with a low shear rheoscope) decreased.
Hematologically, (4) red cell (and reticulocyte) count and hematocrit
increased in a dose-dependent manner remarkably. MCV increased and MCHC
inversely decreased, without alteration of MCH. Furthermore, the
individual red cells became light and inhomogeneous in their specific
gravity (determined using phthalate esters). Metabolically, (5) ATP and
GTP concentrations in red cells (and the energy charge of adenylates)
slightly increased, but 2,3-DPG concentration was not altered.

Conclusively, rh-EPO-induced alterations in blood rheology in rat is
exclusively derived from the increment of young red cells (and/or
reticulocytes) in circulation. Hematocrit is an essential index of
rheological impairment for the use of rh-EPO in clinical medicine.

TESTS IMPLEMENTING BLOOD AND PLASMA VISCOSITY

M.R. Boisseau and M.F. Roudaut Hôpital Cardiologique & INSERM Unité 8, Bordeaux, FRANCE.

Fascinating developments have been performed in the field of blood viscosity since the early observations of Fahraeus in the 1950s, related to the thixotropic properties of whole blood. It is strongly established that plasma viscosity (PV) associates with driving pressure and vasomotricity of arterioles in order to fix the resistance and the hindrance of vessels. Besides red cell aggregates dramatically increase the viscosity in areas where flow output is very low, i.e. in venules. Therefore two methods have to be settled in a Haemorheological Laboratory: the checking of PV and the study of the Rheogram of whole blood viscosity (WBV). These techniques have to be compared with connected levels of haematocrit, fibrinogen, CR protein, albumin and gamma globulins. Two situations are to be analysed : open states, as in Haematology where increment of blood mass leads to acute events and covert states mainly in vascular diseases. Cardiovascular risk factors (HTA, tabagism, diabetes) induce a plasma volume contraction through a defect in microvessels permeability, and/or a chronic phase of inflammation. CR protein and cytokin measurements distinguish between the two mechanisms. Aging also play an important role when increasing fibrinogen. Finally PV is of value in epidemiology of vascular thrombosis. PV and the Rheogram of WBV also yield results of treatment aiming at external or internal (drugs-induced) haemodilution in such patients.

ERYTHROCYTE AGGREGATION : METHODOLOGY, STANDARDISATION AND CLINICAL RELEVANCE.

G.M. Potron, P.V. Nguyen, B.A. Pignon, M. Guenounou. Laboratoire central d'Hématologie, CHU Robert-Debré 51092 REIMS (FRANCE).

Beside classical erythrocyte sedimentation rate or whole blood viscosimetry, erythrocyte aggregation (E.A.) can nowadays be measured by specific apparatus (Myrenne - Germany ; Erythragrégomètre - France) which can give kinetics parameters and aggregation/deaggregation thresholds. Standardization should be done in order to allow epidemiological studies. Interindividual variations remain however large on aggregation and deaggregation thresholds. Plasmatic influence on E.A. is well known whereas erythrocyte parameters are more difficult to interpret, as shown by the analysis of in vitro models. Contrast media inhibit erythrocyte aggregation by erythrocyte membrane alteration. Abnormal E.A. are observed during inflammatory process and in elderly. On the contrary, activated polymorphonuclear supernatant do not modify the results whereas agents such as platelet activating factor disturbs erythrocyte membrane. The influence of fibrinolytic drugs on red blood cell - plasmatic proteins molecular equilibrium will also be presented. They appear to improve microcirculation and decrease thrombotic risk.

MOLECULAR RHEOLOGY OF BLOOD CELLS IN PRACTICE

M. Donner, S. Muller, C. Szczepaniak, J.F. Stoltz - U.284 INSERM - CO 10 Plateau de Brabois - 54511 Vandoeuvre-lès-Nancy cédex, France

Blood cells are complex media and their rheological properties partly control the interactions of cells with the surroundings. These properties are related to molecular motions occurring within the plane of the membrane. Therefore, the understanding of these motions could allow to approach the role of microrheological properties in cell functions.

The methods used for approaching membrane motions which result in "cell fluidity" are spectroscopic techniques : NMR, EPR, Fluorescence, Fluorescence polarization.

For the Hemorheology laboratory, fluorescence techniques have become currently used in preclinical studies because of their practicability and high sensitivity. These methods (steady-state, time-resolved, at single cell level) involve the use of fluorescent probes either embedded in lipidic compartments of cells or bound on surface proteins.

Nevertheless, using various fluorescent markers labelling different areas of cells previously require both the study of the behaviour of probes in isotropic media and the modelization of probe interactions with organized assemblies. Examples of specific probes which could provide useful tools in clinical Hemorheology are discussed.

LEUKOCYTE ADHESION AND LEUKOCYTE ADHESIONS RECEPTORS IN CLINICAL INVESTIGATION

J.L. Wautier, B. Boval, F. Liote, D. Weill. CHU Lariboisière, 2 rue A. Paré, 75010 Paris, FRANCE.

Leukocyte adhesion to vascular wall components is a crucial step in the genesis of vascular lesion in inflammation and atherosclerosis. The better knowledge of the molecular structures involved in the adhesion permit to establish relationships between adhesion molecules such as the CD11 CD 18 complex or the CD49d (VLA4) CD49e (VLA5) CD49f (VLA6) receptors have been shown to be involved in adhesion. The adhesion receptors can be easily determined using specific monoclonal antibodies and flow cytometry analysis. The adhesion of monocytes and polymorphonuclear can be measured by optical method or by determining the myeloperoxidase activity using a colorimetric technique. The adhesion can be measured on protein coated surfaces and on endothelial cell cultures. Since some endothelial cell adhesion are only expressed on cytokine activated endothelial cells we also measured the adhesion to activated endothelial cells which expressed Endothelial cell Adhesion molecule. Using this system we found that monocytes from diabetic patients and monocytes from patients with rheumatoid arthritis have an enhanced expression of leukocyte adhesion receptor and an increase in adhesion. These techniques could be also applied to evaluate the activity of drug on leukocyte vascular wall interactions.

Hemorheological measurements on the blood coagulation and their clinical significance

Y. Isogai and S. Ikemoto. Third Department of Internal Medicine, Jikei University School of Medicine, Minato-ku, Tokyo, JAPAN.

There have been reported many rheological measurements of blood (plasma) clot including the thromboelastography (TEG), viscoelastorecorder (VER) and damped oscillation type rheometer. In our laboratory, we have investigated changes in the viscoelasticity of blood clotting process obtained from the healthy controls and the patients with various disorders. As the indices of viscoelasticity of blood clot, we employed dynamic rigidity (elasticity) modulus, G' , and rigidity loss (viscosity) modulus, G'' . In hypercoagulable state the maximum value of G' , $G'm$, was increased. Especially, in diabetics with advanced microangiopathy, the prominent increase of $G'm$ was observed. The high values of $G'm$ was correlated with high plasma fibrinogen level, high plasma viscosity, high erythrocyte sedimentation rate (ESR), and low hematocrit level in the blood samples obtained from various diseases. The blood clot viscoelasticity also gives us information about the fibrinolytic system. The degree of decrease in G' after its maximum value was attained, indicates the fibrinolysis of the clot and the fibrin-network relaxation resulting from the dissipation of platelet-contractile energy.

NEW TRENDS IN ISOVOLUMEIC HEMODILUTION AS A THERAPY OF DEGENERATIVE VASCULAR DISEASES IN OPHTHALMOLOGY, IN ANGIOLOGY AND NEUROLOGY. H. Schmid-Schönbein and H. Goslinga^{*}. Department of Physiology RWTH Aachen, Aachen, Germany and ^{*}St. Lucas Hospital, Amsterdam, Netherlands.

Intentional exchange hemodilution, i.e. the exchange of RBC against plasma, artificial or natural colloidal solutions with the aim of lowering the hematocrit level (while maintaining blood volume, and thence filling pressure of the heart) is gaining rapid acceptance in clinical practice. Note that we are not assuming that generalized hyperviscosity were a prominent feature of perfusion defects in the retina, the peripheral and the cerebral microvasculature. Instead, we have always postulated that clinically occurring low flow state were deteriorated by the complex sequelae of rbc-aggregate formation, which curtail blood fluidity only if associated with normal or high, but not with therapeutically reduced hematocrit. Clinical success of this concept is now available from studies documenting concomitant rheological, cardiovascular and functional benefit. WOLF et al. (1) have correlated improved retinal microcirculation (dynamic videofluorescence angiography) to functional improvement. FRIEBE et al. (2) have compared marked increase painfree walking distance under therapy (Hct 0.33): primarily due to better compliance of hemodiluted patients to physical exercise programs. In stroke patients (3), the extremely variable tolerance of different patients to aggressive hemodilution was recognized by monitoring the cardiovascular performance (pulmonary wedge pressure): the procedure therefore no longer be paraphrased as "normovolemic" hemodilution, but rather as "custom tailored hemodilution" to warn the clinician against the hazards of hyper- and hypovolemia. <1> Wolf et al.: Klin. Mbl. Augenheilkunde 1990 <2> Friebe et al. Münchn.Med.Wschr.1992, <3> Goslinga et al. Stroke 1992 (in press).

TREATMENT OF BLOOD HYPERVISCOSITY AND IMPAIRED OXYGEN UPTAKE

H.R.D. Wolf and S. Witte

Dept. of Medicine, Diakonissenkrankenhaus, Akademisches Lehrkrankenhaus der Univ. Freiburg, D-7500 Karlsruhe FRG

Increasing oxygen uptake and CO₂ exhalation during constant moderate work exercise (50 - 80watt) over 15min proved to be a sensitive method to characterize impaired microcirculatory performance in individuals with elevated blood viscosity parameters, due to persistent contribution of anaerobic glycolysis during steady state exercise far below expected "anaerobic threshold" (1). Acute improvement of viscosity parameters by PGE₁ (alprostadil 40 µg i.v.), naftidrofurylhydrogenoxalat (400mg i.v.) or hemodilution with hydroxymethylamylum (6%, MW 40000, 500ml i.v.) reduced oxygen gradient between min.5 and min.15 during constant exercise significantly. Long term treatment (4 - 6 months) with EGB (tebonin®; 40mg tid p.o.) reduced the elevated blood viscosity parameters as well as oxygen gradient in 35 out of 42 patients. Non responders can be identified clinically and alternate treatment approaches to lower viscosity can be tested.

(1) H.R.D. Wolf and S. Witte, BIORHEOLOGY, 27;913-919;1990

MIXTURES OF A BLOOD SUBSTITUTE AND ERYTHROCYTES

L. C. Cerny and E. L. Cerny. Utica College of Syracuse University, Utica, NY 13502 U.S.A.

An acellular resuscitative fluid has been synthesized from a Tetronic Polyol and a hemoglobin stabilized with glutaric acid. This compound has been successfully used in exchange-transfusion experiments in rats. The present investigation explores the *in vitro* biophysical measurements in mixtures of the blood substitute and red blood cells. Included in this presentation are the following:

- 1) Oxygen transport using biotometry;
- 2) Hemorheology as a function of shear rate and concentration;
- 3) Erythrocyte sedimentation rates;
- 4) Hemolysis by malonamide induced kinetics.

The results indicate that this acellular compound has adequate oxygen transport properties as indicated by the P₅₀ value and Hill constant. It also is a hemodiluent with its non-Newtonian viscosity following Casson's equation. This compound tends to protect the erythrocyte membrane during hemolysis. Therefore it can be tentatively concluded that a Tetronic Polyol Hemoglobin Complex may be a viable candidate as a blood substitute.

MODIFICATIONS OF HEMOLYTIC PARAMETERS DURING A 20 KM RUNNING RACE WITH AND WITHOUT HYDRATATION.

F.Durand *, M. Delamaire *, F.Carre **, JF Dezicr ***, P.Rochcongar **, B.Genetet *
* C.R.T.S. - Rue P-J Gineste 35000 RENNES -France ** Explorations Fonctionnelles et Médecine du Sport (Prof Le Bars) *** Laboratoire de Biochimie B - CHU Pontchaillou -35033 RENNES -France.

Long-distance runners often have hematological disorders whose etiology is controversial. An attempt was made at explaining this phenomenon by studying the following parameters in 10 athletes running for 20 km with (H) and without (NH) hydration : Hematocrit (Ht), Hemoglobin (Hb), MCV, Haptoglobin (Hp), Hemorheological work-up including erythrocyte rigidity index measurement, blood and plasma viscosities, erythrocyte aggregation before (T1), immediately after (T2), three (T3) and 24 hours (T4) after exercise. At T1 in both groups, blood cell counts did not reflect any anemia, hemorheological parameters were normal, whereas haptoglobin was significantly decreased. Erythrocyte deformability and erythrocyte aggregation time were reduced in both groups at T2, and haptoglobin still more decreased. Only the NH group had hemoconcentration at that time (Ht), in parallel, blood viscosity was increased. In the H group, the effects of hydration probably compensated those of hemoconcentration. Recuperation was quicker in the H group. On the whole, after a 20 km race, we could evidence a fragility of erythrocytes, which was correlated to a sharp decrease in haptoglobin, reflecting hemolysis; these disorders were not as marked in the H group.

EFFECT OF VENOUS OCCLUSION (VO) ON HEMORHEOLOGICAL AND MICROCIRCULATORY PROPERTIES IN NORMAL SUBJECTS

T. Khodabandehlou, C. Le Dévéhat, N. Testu, Unité de Recherches d'Hémorhéologie clinique, Service de Diabétologie-Nutrition, Centre Hospitalier 58000 NEVERS, France.

The effect of VO (during 10 min. at 100 mmHg by a cuff), on red blood cell (RBC) aggregation, deformability and microcirculatory properties is evaluated in healthy subjects (5 men). RBC aggregation and deformability were evaluated by Sefam aggregometer and Hanss hemorheometer respectively. Blood samples for hemorheological measurements were obtained from a foot vein either before and at the end of a 10 minutes VO just before removing the cuff. Microcirculatory studies consisted of the transcutaneous oxygen pressure (TcPO₂) measurements at 44°C by an Oxymonitor SM 361 (Hellige, France) and the cutaneous blood flow (BF) at 44°C by a laser doppler fluxmeter (PF3 Perimed, Sweden). Both TcPO₂ and BF were recorded continuously before, during and after VO on the dorsum of the foot. Results can be summarized as follows : RBC aggregation and deformability remained unchanged at the end of VO when compared to before VO. BF and TcPO₂ during a 10 minutes VO were found to be decreased respectively by 67% and 36% of preocclusion values. After VO, the BF raised significantly by 42% of preocclusion flow whereas the TcPO₂ reached only to its preocclusion value. These data suggest the presence of a possible relationship between the TcPO₂ and BF decreases as a deleterious effect of VO in healthy subjects whilst the RBC rheologic properties remained unchanged.

STUDY ON PATHOGENETIC ROLE OF THE BLOOD VISCOSITY,
VISCO-ELASTICITY AND THIXOTROPY IN LOWER EXTREMITY
DEEP VENOUS THROMBOSIS

Yang Ching-wen, et al.

Department of Internal Medicine, Shanghai Ninth Hospital,
Shanghai Institute of Hematology
Shanghai Second Medical University, Shanghai

Hemorheology including blood viscosity, visco-elasticity and thixotropy, has been studied in 71 patients with deep thrombosis in lower extremity. All the cases were confirmed by phlebography. The results showed high blood viscosity, decreased red blood cells elasticity and increase value on every point of thixotropy-Hysteresis Loop in all. As compared with the control group, there is significant difference ($p < 0.01$). However, the area of the Hysteresis Loop and the energy consumption have no remarkable difference ($p > 0.05$) between the two groups. It reveals that the blood viscosity, red blood cell visco-elasticity and thixotropy take part in the formation of thrombosis.

HEMORHEOLOGICAL DISORDERS AND STAGES OF VENOUS INSUFFICIENCY

F. Zuccarelli (Department of Phlebology and Angiology,
Saint-Michel Hospital, Paris). A. CHABANEL - A. TACCOEN.

For about ten years numerous studies have confirmed *ex vivo* the hemorheological disturbances in venous insufficiency. These troubles can be well understood from theoretical data allowing to attribute a main role to blood rheology on the venous side within the circulation. Therefore *in vivo*, physiopathological signification of rheological abnormalities in venous insufficiency remain unclear and their delay of appearance are still misunderstood.

In order to bring more informations about these conditions, we conducted a rheological study in 57 patients suffering from venous insufficiency without any clinical and therapeutic confusing factors. Venous insufficiency was divided into 3 stages : stage 1 : only pain, heaviness or oedema ; stage 2 : no complicated main trunk varicosis ; stage 3 : varicosis with skin changes.

Fibrinogene levels and erythrocyte aggregation were assessed by laboratory examinations.

Results showed a significant difference between the three stages for fibrinogene and erythrocyte aggregation. The markest was the rheological deficit, the most severe was the stage.

This data suggest that erythrocyte aggregation has the same evolution than the venous disease and could play an important role in venous aggravation by increasing the viscous resistance.

EFFECT OF TROXERUTIN ON CLINICAL, FUNCTIONAL AND RHEOLOGICAL PARAMETERS IN VENOUS INSUFFICIENCY: A DOUBLE BLIND STUDY

A. Chabanel, F. Vin*, J. Ducros, J. Gruffaz, B. Hutinel, P. Nizard, P. Maillet*, A. Taccoen and M. Samama. Hôpitals Hôtel Dieu and Bon Secours*, Paris, France.

Several studies have suggested the presence of rheological abnormalities in venous insufficiency (VI). Troxerutin is used in VI treatment and seems to act by reducing capillary permeability and red cell aggregability (RCA). The aim of this study was to investigate those effects in a randomized, double blind, placebo-controlled study. Functional venous status was assessed by photo-plethysmography (PPG) and by a venous functional score including sensation of heaviness, pain and edema. RCA was measured with the SEFAM erythroaggregameter on blood drawn from the varicose vein (37°C, 40% hematocrit). After a 15 days placebo-treatment period, 34 patients received troxerutin (3.5 g) and 35 the placebo. Statistical evolution of the variables at 2 months in the 2 groups was compared with the *t*-test. The 2 groups were well matched with respect to baseline characteristics. Mean follow-up data showed improved clinical state ($P < 0.0003$), significantly increased PPG half-refilling time ($P < 0.05$) and decreased RCA ($P < 0.005$) in the troxerutin group when compared to the placebo group. Fibrinogen rose in the placebo group but remained stable in the treated group and the variation difference was significant ($P < 0.05$). Values and evolution of the rheological parameters were significantly related to the ankle perimeter, but there was no significant correlation between rheological parameters and clinical symptoms. This aspect of the study needs further clinical investigation. However, this work seems to confirm both actions of troxerutin: an effect on the venous tone as well as a rheological effect.

EVALUATION OF FUNCTIONAL CAPACITY OF RED BLOOD CELL AGGREGATION OF FIBRINOGEN DURING DIABETES MELLITUS

T. Khodabandehlou*, C. Le Dévéhat*, M. Ba**, M. Boynard***, M. Razavian*** ;*Unité de Recherches d'Hémorhéologie clinique, Service Diabétologie-Nutrition, Centre Hospitalier 58000 NEVERS, France. **INSERM U91, CNRS UA 607, Hôpital Henri Mondor, 94000 CRETEIL France, ***UFR Biomédicale, Université PARIS V, France.

The aim of the present work is to elucidate if fibrinogen undergoes any qualitative modification during diabetes which may affect its red blood cell (RBC) aggregative capacity. The study is performed on purified fibrinogen of 15 type 1 diabetics and 7 healthy subjects. The fibrinogen was purified and its aggregative effect at various concentration and in the presence of normal RBC was compared between diabetics and healthy subjects. Aggregation has been studied with a new ultrasonic interferometry method, which allows to measure the sedimentation rate of RBC aggregates. Results, expressed in terms of a mean size of sedimented aggregates, showed differences in RBC aggregation in the presence of only 7 diabetic fibrinogens when compared to the normals ; among them, 3 exhibited a significant more important aggregative effect and 4 had a significant less important aggregative effect. The 8 other diabetic fibrinogens showed similar aggregative capacity as the normals. Today, from our data, differences in aggregative effect of the studied fibrinogens can not be totally explained. Differences may probably be due to qualitative modifications and/or modifications in the structure of fibrinogen leading to functional abnormalities. Nevertheless, it seems from our findings that there may be different sorts of fibrinogen in diabetic patients which lead to different RBC aggregative effects.

STUDY OF BLOOD CELL DEFORMABILITY IN DIABETES MELLITUS

T. Yokose, H. Tanaka, J. Yamamoto, K. Kuchiba, T. Maeda, S. Ikemoto, Y. Isogai
3rd Dept. of Int. Med., Jikei Univ. School of Med., Minato-ku Tokyo JAPAN

It seems very important in diabetes mellitus and hemorheology to clarify the relationship between blood cell deformability and the development of diabetic microangiopathy. The purpose of the present paper is to determine the cell deformability related to diabetic microangiopathy. We used St. George's filtrometer (Carri-Med Ltd) which can measure erythrocyte and leukocyte deformability. We measured red cell suspension ($100 \times 10^4/\mu\text{l}$) and white cell suspension ($1000/\mu\text{l}$) with this device and studied the relationship between hemorheological abnormalities and diabetic microangiopathy. Venous blood was drawn and anticoagulated with EDTA from normal subjects and non insulin dependent diabetic patients. After each measurement, the computer gives immediately a result of the red cell transit time (RCTT). Red cell deformability was expressed as RCTT. Granulocytes suspension was measured by same apparatus. Fractionation of leukocytes subpopulation was carried out by layering anticoagulated whole blood on a two-step density gradient. White blood cell deformability was expressed as clogging rate (CR) which was calculated and printed out by the computer. A significant increase in red cell transit time was observed in the diabetic patients, especially in diabetics with nephropathy. The degrees of increase in RCTT was correlated with the duration of diabetes mellitus. The clogging rate (CR) in diabetic patients were significantly higher than those of normal subjects. We suggest that deformability of red and white blood cell in microcirculation are closely related to the tissue hypoxia and the pathogenesis in diabetic microangiopathy.

RED CELL RIGIDITY (Tk) — THE IMPORTANT FACTOR TO AFFECT THE
CAPILLARY BLOOD FLOW RATE IN RETINA OF DIABETES MELLITUS
— PRELIMINARY RESULTS

T. W. Chung*, A. G. Liu* and J. H. Yu. *Dept. of Biomedical Engineering,
Chung-Yuan Christian University, Chung-Li, 32023 Taiwan. Dept. of
Ophthalmology, Chung-Hsio Hospital, Taipei, Taiwan.

Diabetes mellitus (DM) is associated with impaired nutritive perfusion of the microvasculature. To investigate the issue, blood flow rates in a capillary of retina and the hemorheological factors are measured for DM group (n=8) and normal subjects (n=8). Both groups are age matched. Capillary blood flow rates (mm/sec) in retina are measured by Scanning Laser Ophthalmoscope (SLO). The results show that the blood flow rate in the capillary of retina for the DM group (3.05 ± 0.39 mm/sec) is significantly lower ($P < 0.005$) than that for the normal group (3.80 ± 0.40 mm/sec). The red cell rigidity (Tk) measured by viscometric method at $\dot{\gamma} = 225 \text{ sec}^{-1}$ and $\dot{\gamma} = 450 \text{ sec}^{-1}$ for the DM group ($\text{Tk} = 0.863 \pm 0.053$, and $0.840 \pm 0.050^*$, respectively) are also significantly higher ($P < 0.01$ and $P < 0.005$) than that for normal subjects ($\text{Tk} = 0.798 \pm 0.049$, and 0.759 ± 0.059 , respectively). However, other hemorheological factors such as hematocrit (Hct), mean corpuscular volume (MCV), plasma viscosity and whole blood viscosity in different shear rates, do not show statistical significance ($P > 0.05$) between the two groups. The preliminary results suggest that the higher value of red cell rigidity (Tk) may play a key factor to affect the blood flow rates of capillaries in retina of DM patients. *Note : n=7

PLATELET MEMBRANE FLUIDITY AND PLATELET METABOLIC PARAMETERS IN DIABETES MELLITUS

Caimi G., Serra A., Montana M., D'Asaro S., Catania A., Romano A., Sarno A. Istituto di Clinica Medica e Malattie Cardiovascolari, Università di Palermo, Italy.

The aim of this research was the evaluation of the platelet membrane fluidity, the membrane lipid composition and the Ca^{2+} cytosolic concentration in diabetics subdivided for type. In 12 type 1 diabetics (range 12-30 yrs), in 12 type 2 diabetics (range 44-67 yrs) and in 12 normal controls (range 22-44 yrs) we evaluated the platelet membrane fluidity (marking the platelets with TMA-DPH), the membrane cholesterol/phospholipid ratio (using the column chromatography), the membrane individual phospholipids (using the two-dimensional thin layer chromatography) and the Ca^{2+} cytosolic concentration (marking the platelets with Fura 2-AM). From the obtained results, it is evident that the platelet membrane fluidity differentiates normals from type 2 diabetics but not normals from type 1 diabetics. The membrane CL/PL ratio does not discriminate normals from diabetics of type 1 and 2. The membrane individual phospholipids do not distinguish normals from type 1 diabetics; in type 2 diabetics, instead, a slight difference regarding the phosphatidylserine is present. The Ca^{2+} platelet concentration does not differentiate normals from diabetics of type 1 and 2. In normals and in diabetics of type 1 and 2 no significant correlation is evident between platelet membrane fluidity and platelet metabolic parameters.

EFFECT OF THE PLASMINOGEN ACTIVATION ON ERYTHROCYTE AGGREGATION CHARACTERISTICS. APPLICATION TO DIABETICS

T. Khodabandehlou, C. Le Dévéhat, Unité de Recherches d'Hémorhéologie clinique, Service de Diabétologie-Nutrition, Centre Hospitalier 58000 NEVERS, France.

To better understand the problem of red blood cell (RBC) hyperaggregation tendency which occurs during diabetes, we have studied the effect of the plasminogen activation on RBC aggregation characteristics of diabetic blood suspensions. The plasminogen was activated in vitro by Streptokinase (SK). Patients, consisting of 14 type 1 diabetics and 12 type 2 diabetics with good and poor glycemic control, are compared with 11 healthy subjects. RBC aggregation measurements were performed with Sefam aggregometer. Results showed a significant decrease of RBC aggregation in healthy subjects as a result of the plasminogen activating by SK. In diabetic patients, the RBC aggregation changes, induced by the in vitro plasminogen activating, were found to be less important in regard to those obtained in healthy subjects. These findings would indicate that the plasminogen activating by SK leads to a less important fibrinogen degradation in diabetics than in healthy subjects. Furthermore, the fibrinogen degradation in diabetic patients seems to be associated with the type and the glycemic equilibrium of diabetes. These data might explain elevation tendency and thus the RBC hyperaggregation tendency in diabetics in relation with a decrease of fibrinolysis.

EFFECTS OF BUFLOMEDIL* ON CUTANEOUS BLOOD FLOW, VASOMOTION AND
TRANSCUTANEOUS OXYGEN PRESSURE IN DIABETIC PATIENTS IN AN OPEN STUDY

C. Le Dévéhat, T. Khodabandehlou, N. Testu, Unité de Recherches
d'Hémorhéologie clinique, Service de diabétologie-nutrition, Centre
Hospitalier 58000 NEVERS, FRANCE.

We investigated the acute effect of Buflomedil on cutaneous blood flow, vasomotion characteristics and also transcutaneous oxygen pressure (TcPO₂) in 10 diabetic patients. Cutaneous blood flow and vasomotion measurements, performed by a laser doppler fluxmeter (PF3 Perimed, Sweden), were carried out on the fingertip at rest and after a reactive hyperhemia. TcPO₂ was measured on the dorsum of the foot by an Oxymonitor SM 361 (Hellige, France) with arterialization at 44°C. The trial consisted of one intravenous perfusion of 400 mg of Buflomedil during 90 minutes. Patients showed before perfusion significant decreases of blood flow either at rest and after hyperhemia and also decreases in vasomotion frequency and amplitude in regard to control subjects. They had also decreases in TcPO₂ values in relation to the controls. In this study, Buflomedil is showed to improve the microcirculatory parameters :- an increase in cutaneous blood flow particularly after hyperhemia ; -increases in vasomotion frequency and amplitude ; - normalization of TcPO₂ values. Improvements in cutaneous blood flow and TcPO₂ may probably be secondary to those of vasomotion mechanism as a result of Buflomedil perfusion.

*Fonzylane, Laboratoire Lafon, France

HEMORHEOLOGICAL STUDIES ON THE EFFECTS OF HYPER-
GLYCEMIA ON MICROANGIOPATHY

F.Y.Zhuang, T.L.Wang, Z.S.Ma, Q.H.Hu And H.Zhao.* China-Japan
Friendship Hospital; Beijing Heart, Lung and Blood Vessel Medical
Center, Beijing, China.

Observation of diabetic retinopathy and measurements of hemorheological parameters in 27 diabetics showed that blood and plasma viscosity, plt aggregation increased more significantly in diabetics with retinopathy than without retinopathy, and hemorheological parameters were most deteriorated in preproliferative retinopathy of most active microangiopathy state. For further exploring the pathogenetic mechanism of microangiopathy, long term diabetic rabbits with normal plasma lipid and high blood sugar of 400 to 500 mg/dl were made. Results showed that in three months duration the abnormalities of hemorheology (blood and plasma viscosity, plt aggregation, RBC electrophoresis mobility) was obvious, where as microangiopathy was not obvious until nine months later of the models made. Transmission electron micrograph of glomerular of 9 months duration showed the thickening of the basement and the platelet adhesion on vessel. Conclusion: 1. Hemorheological disturbance could be caused by hyperglycemia and preceded microangiopathy in diabetes. 2. Hemorheological disturbance worse, the retinopathy (microangiopathy) was more serious.

MACRORHEOLOGICAL DETERMINANTS, RED CELL MEMBRANE DYNAMIC PROPERTIES AND ERYTHROCYTE Ca^{2+} CONTENT (TOTAL AND CYTOSOLIC) IN VAD SUBJECTS WITH AND WITHOUT NIDDM

Caimi G., Serra A., Lo Presti R., Grifò G., Francavilla G., Sarno A.
Istituto di Clinica Medica e Malattie Cardiovascolari, Università di Palermo, Italy.

In a group of subjects with vascular atherosclerotic disease (VAD) and in a group of VAD subjects with non-insulin-dependent diabetes mellitus (NIDDM), we evaluated the mean erythrocyte aggregation (employing the aggregometer MA1 of Myrenne), the whole-blood filtration (according to the Reid and Dormandy method), the erythrocyte membrane fluidity (marking intact red blood cells with pyrene), the red cell membrane protein lateral mobility (marking intact red blood cells with 3-PM), the total red cell Ca^{2+} content (using an atomic absorption spectrophotometer), and the cytosolic red cell Ca^{2+} content (employing Fura 2-AM). In both groups, compared to controls, it is evident that there is a significant difference in macro- and microrheological determinants. As regards the red cell Ca^{2+} content we observed that, in both groups, the total red cell Ca^{2+} content is not different from controls, while only in VAD subjects with NIDDM, compared to controls, a significant difference in cytosolic red cell Ca^{2+} content is evident. In normals and in VAD subjects with and without NIDDM, the mean erythrocyte aggregation and the whole-blood filtration are not related to the parameters reflecting the membrane dynamic properties and the red cell Ca^{2+} content.

ELEVATION OF BLOOD VISCOELASTICITY INCREASES THE INCIDENCE OF AGGRAVATION OF DIABETIC MICROANGIOPATHY

S. Ikemoto, H. Tanaka, J. Yamamoto, M. Akiyama, K. Kuchiba, T. Maeda, T. Yokose, and Y. Isogai. Third Department of Internal Medicine, Jikei University School of Medicine, Minato-ku, Tokyo 105 Japan

We observed the progression of diabetic microangiopathy after the cross-sectional determination of hemorheological factors and testified the pathological roles of these factors. The subjects were 34 diabetics (NIDDM). Hemorheological parameters, such as blood and plasma viscosity, blood viscoelasticity, red cell filterability, platelet aggregation, were determined between 1978 and 1988. The follow-up periods were from 3 years to 10 years. The definition on aggravation of microangiopathy was the progression from no or simple retinopathy to proliferative retinopathy, or the progression from no or simple proteinuria to massive proteinuria or renal failure. As results, the microangiopathy was aggravated in 12 patients, it was not changed in 18 patients, and the macroangiopathy was aggravated in 4 patients. When we compared the progression of microangiopathy between the group of normal blood-viscoelasticity and the group of elevated viscoelasticity, the incidence of aggravation was significantly higher in the group of elevated viscoelasticity. In conclusion, the elevation of blood viscoelasticity was considered to be a risk factor for the diabetic microangiopathy.

BLOOD HYPERFIBRINOGEN IN THE ELDERLY

Zou Qi-Jun, Sheng Feng, Liu Yan-Ying, Dept. of Internal Medicine
Shenzhen People's Hospital & Shenzhen Institute of Geriatrics
Shenzhen, China

Increased fibrinogen in blood is a risk factor for the developing thrombotic diseases. We report 404 elderly patients with a high level of fibrinogen. Among them were 43 cerebral thrombosis, 30 coronary heart diseases, 140 hypertensions, 85 acute and 50 old myocardial infarction and 16 simple obesity. The control was 40 healthy adults. Fibrinogen was measured by heat coagulation. The results were control 275 ± 37 mg%, cerebral thrombosis 420 ± 46 mg%, coronary disease 413 ± 92 mg%, hypertension 431 ± 53 mg%, acute myocardial infarction 526 ± 170 mg%. Snake venom antithrombotic enzyme (Svate) 0.5 U in 250 ml of 10% glucose was used to decrease the fibrinogen daily for 10 days. The level of fibrinogen in 17 cases was decrease from 534 ± 185 mg% to 267 ± 102 mg% ($P < 0.01$). We suggest that Svate is an effective drug to reduce the increased fibrinogen in the elderly.

EFFECT OF FIBRINOGEN AND HIGH DENSITY LIPOPROTEIN SUBFRACTION ON ERYTHROCYTE AGGREGATION IN NORMOTENSIVE HYPERCHOLESTEROLEMIC SUBJECTS

S.M. Razavian*, V. Atger°, M. Cambillau°, M. Del-Pino*, A. Simon*, N. Moatti°, J. Levenson* and PCV-METRA group. *Centre de Médecine Préventive Cardiovasculaire, INSERM U28, °Laboratoire de Biochimie, Hôpital Broussais, Paris, France.

Erythrocyte aggregation (EA) is known to be increased in cardiovascular disease including coronary heart disease (CHD). To investigate the role of lipoproteins on EA we studied in 57 normotensive hypercholesterolemic male (age 47 ± 8) EA by a laser reflectometry technique (EA, mean \pm SD, 19.7 ± 3.5). Total cholesterol (TC, 6.64 ± 0.84 mmol/l), HDL-Cholesterol (C-HDL, 1.38 ± 0.33 mmol/l), Triglycerides (TG, 1.33 ± 0.37 mmol/l) were measured by classical methods. C-HDL2 (0.44 ± 0.25 mmol/l) and C-HDL3 (0.94 ± 0.15 mmol/l) were evaluated by a direct electrophoretic method. Lipoparticle AI (LpAI, 0.52 ± 0.21 g/l) was assayed by immuno-electrophoresis. Fibrinogen (Fib, 3.48 ± 0.9 g/l) was also determined by a thrombin clotting method. In this population we found that EA was positively related to Fib ($r=0.72$, $p<0.001$) while among lipoproteins, EA was negatively correlated with C-HDL ($r=0.32$, $p<0.05$), C-HDL2 ($r=0.40$, $p<0.002$) and LpAI ($r=0.32$, $p<0.02$). No correlation appeared with TC, TG, C-LDL and C-HDL3. The significant negative correlation between EA and C-HDL2 and LpAI is confirmed by a multiple stepwise regression analysis ($p<0.001$) and suggests that they were independent of the fibrinogen effect. Thus, EA which occurs with macromolecules bridging the membranes of erythrocytes is strongly influenced by Fib. The negative relation between EA and HDL2, containing mainly LpAI, suggests a possible role of this lipoprotein subfraction in inhibition of red blood cell aggregation induced by Fib or others macromolecules.

STUDY OF HEMORHEOLOGY OF THE AGED HYPERTENSIVE & THE AMI PATIENTS

Zhang Jing, Li Lingzhi / Taiyuan Central Hospital

030009 Taiyuan, Shanxi Province, P.R.China

The article shows the determination, by the use of Type NXE-1 Cone-plate viscometer made in Cheng Du Instrument Factory, China, of 6 hemorheological indexes of 110 aged hypertensive and 19 AMI patients WHO are up to the WHO standard, and gives comparison with 82 healthy people over the age of 60. The results show that 6 hemorheological indexes of hypertensive group are generally higher than that of the healthy group ($P < 0.01$). The whole blood viscosity (η_b), reduction viscosity (η_r), ESR (mm/h), K value of the ESR equation (ESRK) of AMI group at different shear rates are all obviously higher than that of healthy group ($P < 0.01$), HCT (%) and aggregation indexes of red cell (VAT) has no difference with the healthy people ($P > 0.05$). The above results indicate that both aged hypertensive and AMI patients have higher blood viscosity. So we think it's very important for the hypertensive and AMI patients with hypercoagulation, hyperaggregation and hyperviscosity to cut down the blood viscosity. And the variation and severity of hemorheology of hypertensive and AMI patients are the important factor for prognoses. So a further study of its variable regulation can make great sense for cutting down of the death rate.

MECHANICAL MEMBRANE PROPERTIES OF RED BLOOD CELLS IN CHILDREN WITH END-STAGE RENAL DISEASE AND ITS ALTERATIONS AS A CONSEQUENCE OF ERYTHROPOIETIN THERAPY

W. Meier, D. Lerche and M. Paulitschke. Inst. Medical Physics and Biophysics, School of Medicine, Humboldt-University, Berlin, GERMANY

Erythropoietin is the major hormonal regulator of red blood cell (RBC) production. Besides a disturbed erythropoiesis, an impaired RBC deformability has been discussed as a determinant of renal anaemia. In contrast to a healthy reference group ($\mu = 4.01 \pm 0.71 \mu\text{N/m}$) we have found a significant higher value of the mean apparent elastic shear modulus μ in children with chronic renal failures undergoing haemodialysis ($\mu = 9.05 \pm 1.61 \mu\text{N/m}$) by means of a micropipette aspiration technique. Additionally, a statistically significant larger cell volume in uremic children could be detected than that in healthy donors ($91.7 \pm 6.9 \text{ fl}$ versus $86.5 \pm 5.4 \text{ fl}$ in control cells), whereas the mean RBC membrane area was unchanged. Under administration of recombinant human erythropoietin the deformability parameter μ was decreased and in the maintenance period (30th week of therapy with recombinant human erythropoietin, $\mu = 3.78 \pm 0.31 \mu\text{N/m}$) μ did not differ significantly from the reference group. In the course of therapy, the mean cell volume was found to increase further and consequently caused significant changes of the critical diameter. Considering this results and other evidences we would argue that the disturbed mechanical membrane properties in uremic RBC are the direct result of a disturbed erythropoiesis and not the result of direct effects of uremic toxins alone. We suggest that in the course of the erythropoietin therapy an increasing number of cells with normal elastic properties is produced and the cells with disturbed properties are diminished from the circulation.

THE CHANGES OF ERYTHROCYTE DEFORMABILITY IN CHRONIC RENAL FAILURE WITH AND WITHOUT HEMODIALYSIS TREATMENT

Hu Jinlin, Li Xianghong, Tian Niu, Jiang Gongwei, Zho Meihua Zheng Zhiyong, Li Leishi and Dong Wei. Microcirculation Research Center, Chinese Great Wall Hospital, Beijing 100853, China.

The erythrocyte deformability was compared between the healthy control group (n=17), patients group with chronic renal failure (CRF, n=17) and treated group with hemodialysis (n=28). The long axis of the erythrocyte diffraction ring in CRF group was significantly shorter than that in controls ($p < 0.001$), while the short axis was significantly wider ($p < 0.001$). The diffraction figure in CRF group looked like a olive. After a period of hemodislysis treatment, the diffraction figure became better ($P < 0.001$) and close to the normal shape. The results indicated that the number of sclerous erythrocyte was increased in CRF group, the deformability and membrane stability of erythrocyte in CRF were significantly lower than those in controls. These changes were possibly attributed to the condition of acidosis, water-electrolyte imbalance and the increase of endotoxin and SOD. The abnormality of the hemorheological properties of the erythrocyte will lead to and exacerbate the renal microcirculatory disturbance. Hemodislysis would improve the erythrocyte deformability effectively.

THE EXPERIMENTAL STUDY OF MYOCARDIAL ISCHEMIA AND REPERFUSION INJURY ON ERYTHROCYTE DEFORMABILITY AND ITS PROTECTION IN RABBITS

J.L.Hu, G.Y.Rong, F.Y.Liu and N.Tian
Microcirculation Research Center, Chinese Great Wall Hospital, Beijing 100853, China.

In the present study, forty New Zealand white rabbits (both sexes, 2.0 - 2.5kg) were used to study the changes of erythrocyte deformability when the circumflex coronary artery of the rabbits was occluded for a time period and then released to allow the reperfusion. We used the laser diffraction methods to study the erythrocyte deformability. The results were as follows: erythrocyte deformability were decreased more significantly in reperfusion group than in ischemia group, those results suggest that reperfusion could cause more injury to the erythrocyte deformability, and lead to the microcirculation disturbance and the further ischemia and cell death. Anisodamine and Anisodine could prevent the reduced erythrocyte deformability when they were given before reperfusion. The results suggested that it might have contributed to prevent the reperfusion injury to the erythrocyte deformability when the Anisodamine and Anisodine were given timely to the myocardial infarction patients. Clinical trials are needed to confirm their beneficial effects on patients.

THE RESEARCH ON RED CELL DEFORMABILITY (RCD) OF THE CASES WITH CORONARY HEART DISEASE (CHD)

Mao Xiuya, Xu Liqiang, Zhang Yali, et al.

The CVD Special Exam Dept. Luoyang No. 1 Hospital of TCM. Luoyang. Henan. P. R. of China

Zhang Yueli

Luoyang Municipal Public Health Bureau

The paper introduced TK value formula on stickness engineering put forward by Dintenfass. at temperature 25°C. to measure red cell deformability (RCD) TK value for the normal range of red cell hematocrit (Hct) and plasma viscosity among 92 CHD cases and 80 healthy persons (control group). so as to confirm the transformation of RCD in CHD cases. The results showed that TK value ($\bar{X} \pm S$) in CHD male group was 0.90 ± 0.04 . and that in the control group was 0.89 ± 0.04 . t test. 3.23. $P < 0.01$. TK value ($\bar{X} \pm S$) in the female group was 0.94 ± 0.03 . 0.91 ± 0.03 . respectively; t test 16.96. $P < 0.01$. there was a significant difference. Among the CHD groups. male $\bar{X} \pm S$ was 0.90 ± 0.04 and female. 0.94 ± 0.03 ; t test 6.14; $P < 0.01$. The males and females ($\bar{X} \pm S$) in the control groups were 0.89 ± 0.04 . 0.91 ± 0.03 . respectively; t test. 3.38; $P < 0.01$. The difference was so greatly significant. The result indicated that there were an increase of RBC rigidity and a decrease of RCD in the CHD cases. females are significantly higher than males. And also there was a remarkable significant difference.

It has been discussed in this paper that the decrease of RCD brings about the increase of blood viscosity at high shear rate. terminates in or makes worse microcirculatory disturbance. and directly gives rise to CHD incidence. There is a difference of RCD in sex. which comes from the difference of hematocrit in sex. Finally. the measured method applied in this paper has been discussed and we think that the combined method of TK value calculation and whole blood dilution (Hct and PV in the normal range) is more perfect. and convenient to be applied clinically.

A STUDY ON RED CELL AGGREGATION (RCA) IN THE CASES WITH CORONARY HEART DISEASE (CHD)

Mao Xiuya, Xu Liqing, Zhang Yali, et al.

The CVD Special Exam Dept. Luoyang First Hospital of TCM. Luoyang. Henan. P. R. of China.

In this paper. We have used the combine method of Plasma adjusting ESR (Dintenfass) and ESR's equation coefficient K (K value) (Prof Liang et al. Shanghai). and investigated erythrocyte aggregation (RCA) for 138 CHD cases. 104 CHD complicated with hypertension cases. and 139 healthy persons (control group). at the temperature of 25°C in order to confirm the correlation between CHD and RCA. and the correlation between the risk factors of CHD complicated with hypertension. and RCA.

The results showed that in the male CHD group ESR equation coefficient K value ($\bar{X} \pm S$) was 131.46 ± 78.28 . that in the control group 105.44 ± 63.81 ; t test 2.41; $P < 0.05$. the difference was significant. And females ($\bar{X} \pm S$) were 196.17 ± 64.36 . 148.27 ± 53.39 . respectively; t test 4.77. $p < 0.01$. the difference was of much significance. Males ($\bar{X} \pm S$) in the CHD complicated with hypertension group was 146.49 ± 87.20 . t test was 5.38. $P < 0.01$. as compared with the CHD group. and there was a great significance. Males ($\bar{X} \pm S$) in the CHD complicated with hypertension group was 146.49 ± 87.20 . t test was 5.38. $P < 0.01$ as compared with the CHD group. and there was a great significance in difference. And those ($\bar{X} \pm S$) in female groups were 206.51 ± 79.85 . t test 5.38 $P < 0.01$. as compared with the CHD group. The difference was remarkably significant. The results demonstrated that as the CHD group was compared with the control group. and the CHD group compared with the CHD complicated with hypertension group. male and female TK value were both higher. The difference had an obvious significance. It's proved that CHD cases' RCA went up. RCA in the Cases with CHD complicated with hypertension was significantly higher than that in CHD cases uncomplicated with hypertension.

The RCA incidence is dependent upon large molecular plasma substances. The increase of RCA can cause the increase of blood viscosity. As a risky factor of cardiovascular disease. rigid RBC aggregative masses can cause such pathological conditions as microvascular blockade. damaging all parts of cardiovascular system. and also can make blood pressure go up.

INFLUENCE OF PREOPERATIVE BLOOD VISCOSITY ON POSTOPERATIVE BLEEDING IN CARDIAC SURGERY

B. Liu, N. Hosseini, A. Belboul, G. Rådberg, D. Roberts, Z. Liang, O. Christensen and G. William-Olsson. Dept. of Thoracic and Cardiovascular Surgery, Sahlgrenska Hospital and Scandinavian Heart Center, Gothenburg University, Gothenburg, Sweden.

In order to find out if preoperative blood viscosity influences postoperative bleeding in cardiac surgery, 104 healthy blood donors and 62 patients undergoing heart operation with cardiopulmonary bypass (CPB) were studied. Blood cell count and blood viscosity was analyzed by a Sysmex F-800 cell counter and a computerized, multiple shear-rate, capillary viscometer at shear rates of 20, 30, 40, 60 and 80 S^{-1} respectively. Patients' samples were taken one to two days before the operation. Chest drainage from the end of operation to the removal of the drainage was defined as postoperative bleeding. Whole blood and plasma viscosities were both significantly higher in the patients when compared to the healthy donors. Postoperative bleeding in those (30.65%) whose whole blood viscosity was less than the mean value of blood donors was 774.53 ± 71.40 ml which was significantly lower when compared to 1191.98 ± 75.11 ml ($p = 0.0012$) in those (69.35%) whose whole blood viscosity was higher than the blood donors. Relative blood viscosity (whole blood viscosity per hematocrit per plasma viscosity) was positively correlated to postoperative bleeding at all the five shear rates. The r values at 20, 30, 40, 60 and 80 S^{-1} shear rates were 0.617, 0.59, 0.562, 0.535 and 0.495 respectively ($p = 0.0001$). Preoperative RBC count had a weak negative correlation to postoperative bleeding ($r = 0.286$, $P=0.023$) while the preoperative platelet count was not related to postoperative bleeding. The results of this study showed that significant increased viscosity values were related to postoperative bleeding in the cardiac surgical patients which raises the question of possible preoperative correction in the future in order to improve surgical morbidity and results.

HEMORHEOLOGICAL ABNORMALITIES IN ISOLATED OBESITY

C. Le Dévéhat, T. Khodabandehlou, M. Dougny, M. Vimeux. Unité de Recherches d'Hémorhéologie clinique, Service de Diabétologie-Nutrition, Centre Hospitalier ; 58000 NEVERS, FRANCE.

Excess weight as obesity is often evoked as a major risk for thrombosis and vascular diseases. The present study concerns the effect of obesity on microrheological properties of blood and without other concomitant pathology in 37 female obese patients with BMI of 36.2 ± 4.9 , mean age of 33.42 ± 12.7 years. They are non smoker and have a normal tolerance of glucose, cholesterol, triglycerides, Apo A1, Apo B, without arterial hypertension. These patients which present no vascular disease are compared with 32 normal females with a BMI of 22.4 ± 1.2 , mean age of 32.46 ± 0.9 years. Results showed a significant more important red cell aggregation in obese subjects in relation to the normals. In the same way, the red cell deformability is found to be significantly decreased. The red cell hyperaggregation is found to be accompanied by significant increases of plasma viscosity and fibrinogen level. By contrast, albumin level is significantly decreased. The red cell aggregation differences between the obese subjects and the normals could be explained in terms of fibrinogen and albumin level changes. It can be therefore concluded that the plasma protein metabolism and consequently the red cell aggregation can be altered as a result of an over-weight. These results might explain why obesity is considered as an important vascular risk factor.

LATE EFFECTS OF RADIATION ON HEMORHEOLOGICAL & MICROCIRCULATORY PARAMETERS

R.R. Punivani, M.M. Khan, N.G. Huilgol, School of Biomedical Engineering, Indian Institute of Technology Bombay, INDIA

Blood samples from 25 Head & Neck tumour cases of age group 35-70 years were analysed at three levels viz. before radiotherapy (group I), 5-7 days after radiotherapy (group II) and 10-12 months after the completion of radiotherapy (group III). Blood samples were analysed for Whole blood viscosity (WBV), Plasma viscosity (PV), Red cell aggregation (RCA), Hematocrit (Hct), Red cell rigidity (RG), ESR and Fibrinogen (FBR) & Cholesterol (CHL) levels in plasma in all the three groups. Group III data was compared with normals, group I and group II. Laser Doppler Perfusion Monitor PF 3 was used to study skin microcirculation over the tumour area in all the three groups and was compared. Student's 't' test was used for the statistical analysis of the data and 'p' values were calculated. The results show that radiation causes a change in the rheological parameters of systemic blood though it is administered locally. WBV, PV, RCA and FBR levels are raised in group I subjects which goes down following radiation in group II, but do not come down to normal range. It was interesting to observe that these values approach normal range in group III. Results of the skin perfusion studies show that in group I subjects skin perfusion is high, which is further raised in group II. This elevated perfusion comes down to the normal range in group III. It is concluded that fibrinogen is the main contributing factor in hemorheological as well as microcirculatory changes in the present study.

MODULATIVE EFFECTS OF CELL ADHESION PEPTIDE (RGDS) ON THE AGGREGATION OF STIMULATED PLATELETS FROM OPHTHALMIC PATIENTS

M. Babizhayev, Moscow Helmholtz Research Institute of Eye Diseases, Moscow, RUSSIA

Adhesion proteins are cofactors in the aggregation of human platelets, and can mediate the ADP-induced response of these cells. It was shown that the synthetic cell adhesion peptide, RGDS inhibits the aggregation of platelets from normal donors and ophthalmic patients with diabetic retinopathy, glaucoma and retinal vein occlusion. This effect increases in the relative order of activity retinal vein occlusion > glaucoma > diabetic retinopathy > control. Deaggregation due to the peptide appeared to be diminished in the order control (normal) > diabetic retinopathy > glaucoma > retinal vein occlusion after its addition at the maximum of aggregation curve. It is concluded that there are differences in the ability of RGD peptide to block the fibronectin adhesion receptor on ADP stimulated platelets from different clinical groups.

LEUCOCYTE FILTERABILITY AND PLASMA ELASTASE CONCENTRATION
IN OBSTETRICS AND GYNECOLOGY

M. Kaibara, Y. Marumoto and Y. Kaneko Department of Obstet.
and Gynecol., Ichihara Hospital, Teikyo University School of
Medicine, Ichihara City, Chiba, Japan

Leucocyte filterability and plasma neutrophil elastase concentration were measured in several obstetrical and gynecological conditions where thrombosis were frequently complicated. Leucocyte filterability was measured with St. George's filterometer using leucocyte-PBS suspension ($1,000 / \text{mm}^3$) and clogging rate (CR) and initial relative filtration rate were determined. Elastase concentration was measured immuno-enzymatically with GEL kit (E. Merk, Germany). CR's were significantly increased in late pregnancy, during delivery and in puerperal period compared to non-pregnant control. Patients with advanced cancer, with inflammatory pelvic disease and after gynecological operations showed also significantly increased CR values. Initial relative filtration rates were not significantly different in these conditions. Plasma elastase concentrations were significantly increased after delivery ($183.9 \pm 20.2 \mu\text{g} / \text{l}$), after surgery (177.0 ± 55.7) and in the end stage of gynecological cancer (197.0 ± 12.0) as compared to control (105.8 ± 35.6).

From these results it was suggested that the decrease of leucocyte filterability and increase of plasma elastase was closely related to thrombus formation.

EVALUATION OF MICROVASCULATURE STATUS AND BLOOD VISCOSITY FACTORS
IN HIGH RISK AND STROKE CASES

R.R. Punivani and Md. Asif Hussain

School of Biomedical Engineering, I.I.T., Bombay, INDIA

Aim of this study was to analyse the peripheral microvasculature status non-invasively in high risk i.e. hypertensive controls, normal controls and recent stroke cases having history of hypertension, using a combination of Laser Doppler Flowmetry and reactive hyperemia (RH). And also to evaluate significant blood viscosity factors which may be involved in altered microcirculation in high risk and recent stroke cases not more than 24 hours old. Blood viscosity factors were routinely evaluated in our laboratory. Reactive hyperemia (RH) was induced by arterial occlusion of 3.5 min. duration in the forearm of all the three groups. Basal skin perfusion (NP) before inducing RH, increased skin perfusion (MP) after inducing RH and recovery time (Trh) i.e. time taken for MP to return to NP, were almost same in hypertensive controls and stroke cases despite of significant difference in systemic blood pressure, but were less as compared to NP, MP & Trh values in normal controls. High fibrinogen concentration contributed to increased plasma viscosity in stroke cases while low red cell rigidity (RG) and hematocrit (Hct) values in these cases brought whole blood viscosity down to normal range, thereby reflecting RG and Hct to be the principal determinants of whole blood viscosity. Cholesterol level came down in stroke cases with simultaneous decrease in RG which means that cholesterol is an obvious contributor to red cell rigidification. In the follow up study of stroke cases on 4th day all blood viscosity factors were found elevated. Low NP, MP values and a reduced Trh in hypertensive controls and stroke cases irrespective of blood pressure difference and significant difference in blood viscosity factors lead us to conclude that with persistent hypertension there is a decrease in the effective vasomotor tone of arterioles which in turn is determined by the state of elastic tissues and smooth muscles.

STUDY OF HEMORHEOLOGY OF THE AGED HYPERTENSIVE & THE ACUTE CEREBRAL INFARCTION

Li Lingzhi, Zhang Jing, Zhao Yaoqin / Taiyuan Central Hospital
030009 Taiyuan, Shanxi Province, P.R.China

The article shows the determination, by the use of Type NXE-1 Cone-Plate viscometer made in Cheng Du Instrument Factory, China, of 6 hemorheological indexes of 110 aged hypertensive and 87 aged acute cerebral infarction (ACI) patients and gives comparison with 82 healthy people over the age of 60. The aged hypertensive patients are up to the WHO standard. ACI patients are those confirmed by CT scanning within one week of having the disease. The 6 hemorheological indexes are: whole blood viscosity (η_b), the viscosity (cp) at 6 different shear rates of 5.75/s, 9.60/s, 30.72/s, 38.40/s, 115/s, 230/s; reduction viscosity (η_r); ESR (mm/h) and HCT (%); K value of the ESR equation (ESRK) and aggregation indexes of red cell (VAT). The results show that 6 hemorheological indexes of hypertensive group are generally higher than that of the healthy group ($p < 0.01$). For ACI group, 5 out of the 6 indexes are obviously higher than that of healthy group ($p < 0.01$), HCT is also higher than that of healthy group, but not obvious ($p > 0.05$). Comparison of hypertensive and ACI indicates that η_b of ACI group at the shear rates of 5.75/s, 115/s is higher than that of hypertensive group ($p < 0.05$), ESRK of hypertensive group is quicker than that of ACI group ($p < 0.01$). The remainder terms of the two groups have no difference ($p > 0.05$). The results show that the abnormal increase of all the hemorheological terms of hypertensive and ACI is an indication of the patient's condition, e.g. the unusualness of the hemorheological terms is surely a dangerous signal for the ACI.

HEMORHEOLOGICAL CHANGE OF CEREBRAL THROMBOTIC PATIENTS TREATED WITH SVATE

Xue Jiaju¹, Zhang Jianbao², Fan Jiajun². ¹The centre of prevention and treatment of thrombotic disease, Xi'an hospital of General Company of China Railway Construction, Xi'an, 710032, P.R.China. ²Dept. of physics, The Fourth Military Medical Univ. Xi'an, 710032, P.R.China

During the past three years, 160 cases of cerebral thrombotic diseases have been treated with snake venom antithrombus enzyme (SVATE). After the treatment for one or two courses (28 or 56 days), we find that the whole blood viscosity, plasma viscosity, fibrinogen, cholesterol, triglyceride, β -lipoprotein and platelet counts have all, but prothrombin time, significantly decreased ($p < 0.01$). The results showed that the SVATE had effect of anticoagulation, thrombolysis, defibrinogen, decreased viscosity and platelet counts effectively. It should be considered to be a more satisfactory new drug for preventing and treating the cerebral thrombotic disease. SVATE is a preparation of enzyme which is extracted from the snake venom lived in Jiangsu and Zhejiang provinces, south east China. Its main components are thrombinoid, fibrinolysin and kallikrein. These enzymes belong to the serine proteinase, they are very specific to substrates and can only hydrolysis the arginine esterbond of the fibrinogen and kininogen. In vitro they can directly make the fibrin coagulate without blood coagulation factors as the thrombin does. In vivo they can continuously degrade fibrinogen and cause the fibrin clot unstable when formed, which is easily cleared out by the fibrinolytic system, thus forming a low-coagulating state. The kallikrein can make the kininogen release the kinin which can dilate the capillary, reduce the blood pressure and make the smooth muscle contract. The experiments showed that the snake venom of 1 mg/ml is equal to the streptokinase of 50 IU/ml or the trypsin of 10 μ g/dl for the fibrinolysis.

THE STUDY OF CORRELATION BETWEEN ERYTHROCYTE
SEDIMENTATION RATE(ESR), ESR EQUATION K VALUE AND PLASMA VISCOSITY
AMONG THE CASES WITH BLOOD-DEFICIENT CEREBRAL APOPLEXY.

Mao Xiuya, Xu Liqing, Zhang Yali, et al.

The Hemorheology Research Dept. Luoyang No. 1 Hospital of Traditional
Chinese Medicine, Henan China.

Lang Hongzhi

Neurology Teaching And Research Dept. Luoyang Medical College,
Henan, P. R. of China.

Zhang Yueli

Luoyang Municipal Public Health Bureau.

This paper represents the determination of ESR, ESR's equation k value and plasma viscosity among 560 cases with blood-deficient cerebral apoplexy, and researched on their correlation and effects of age on it. The results show that ESR and ESR's equation K value will be increased in the high plasma viscosity group. Through the statistic disposal, $P < 0.01$, difference is quite significant. It is obvious that ESR and ESR's K value increase with plasma viscosity, and illustrate very great positive correlation. Among the female (aged over 60) ESR group, $p > 0.05$, difference is not significant. So the age of the patients with cerebral apoplexy has some effect on the correlation between ESR and plasma viscosity. ESR will increase with the age and is not stable. And there is no correlation between ESR and plasma viscosity, and probably they have something to do with lower sex hormone level that makes Hct increase in this group. Afterward, ESR has been converted into ESR's equation K value, and excludes the effect from Hct on ESR. $P < 0.01$. Consequently, ESR, especially ESR, especially ESR equation K value is a stable, and reliable hemorheological index which indicates RCA and blood viscosity.

CLINICAL EVALUATION OF CEREBROVASCULAR DISEASE IN
DIABETES

S. Ikemoto, H. Tanaka, J. Yamamoto, K. Kuchiba, M. Akiyama,
T. Maeda, T. Yokose, Y. Isogai.

Third Department of Internal Medicine, Jikei University
School of Medicine, Minato-Ku, Tokyo 105 Japan

The purpose of this study is to investigate the pathophysiology of cerebrovascular disease (CVD) in diabetes mellitus both from the clinical characteristics of CVD in diabetics compared with those of non-diabetics and from the hemorheological abnormalities in diabetics with cerebrovascular disease. The subjects of this study were totally 128 patients with CVD and 78 of those were diabetics. In the diabetics with CVD, the incidence of cerebral infarction was significantly higher than that in the non-diabetics (81% vs 62%, $p < 0.05$). Concerning the localization of lesions of infarction, there was no significant difference between diabetics and non-diabetics. The significant increases of the concentration of both plasma fibrinogen and triglyceride were observed in the diabetic group, especially in the group with infarction. Hemorheological factors such as blood and plasma viscosity showed a tendency to increase in the diabetic group. The increase of plasma fibrinogen in diabetics was considered to be a risk factor for cerebral infarction, because fibrinogen is one of the main determinants of plasma and blood viscosity.

ORAL ADMINISTRATION OF ASPIRIN, TXA₂ SYNTHETASE INHIBITOR OR TXA₂ RECEPTOR ANTAGONIST DECREASES LEUKOCYTE ADHESIVENESS

C. Uotani, H. Asakura, H. Joka,ji, M. Saito, I. Kumabashiri, E. Morishita, M. Yamazaki and T. Matsuda, 3rd Dept. of Internal Medicine, Kanazawa Univ. School of Medicine, Kanazawa, JAPAN.

The adhesiveness of leukocyte to vascular endothelium has important roles in development of thrombosis. We measured leukocyte adhesiveness to glass surface by novel method using plastic tubes containing fine glass beads which have been used to assess the platelet adhesiveness, and examined the changes in adhesiveness following oral administration of mini-dose aspirin(40mg daily), TXA₂ synthetase inhibitor(TXSI; CV-4151 100mg daily) or TXA₂ receptor antagonist(TXRA; AA-2414 20mg daily) in healthy volunteers.

The following results were obtained. (1) The numbers of leukocytes which adhere to glass beads tended to increase with time and reached to a definite level after 60 min of incubation. (2) The proportion of leukocytes adhered to glass beads was high in the following order; monocytes, neutrophils, eosinophils and lymphocytes. (3) After the ingestion of aspirin, TXSI or TXRA, a statistically significant decrease in leukocyte adhesiveness was observed. (4) After the ingestion of aspirin and TXSI, a significant decrease in concentration of TXB₂ (metabolite of TXA₂) in serum was observed, while no changes were observed after the ingestion of TXRA.

From these results, it is concluded that TXA₂ plays an important role in adhesion of leukocytes.

SPONTANEOUS ACTIVATION OF GRANULOCYTES IN MYOCARDIAL AND CEREBRAL ISCHEMIA IN MAN.

G. W. Schmid-Schönbein, A. Grau, R. Chang, N. Chien, K.-M. Jan, K.-L. P. Sung, S. Chien, Institute for Biomedical Engineering, University of California in San Diego, La Jolla, California, and Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

An increasing body of evidence from experimental studies suggests that granulocytes and monocytes play a central role in the progressive organ dysfunction in different forms of ischemia. Manifestations of leukocyte mediated injury include entrapment of leukocytes in the microcirculation, occlusion of capillaries, oxygen free radical and enzymatically mediated injury of endothelial and parenchymal cells. In order to explore this situation in man, we investigated spontaneous granulocyte activation in venous blood samples of patients with myocardial and cerebral ischemic episodes early during hospitalization. Age matched individuals without symptoms served as controls. The blood samples were processed fresh and different forms of activation were explored. The results show evidence of spontaneous activation of the granulocytes in both groups of patients, although this was manifest only in form of pseudopod formation in the case of the heart patients and in form of adhesion in the stroke patients. The investigation brought to light several difficulties and limitations which accompany the investigation of venous blood sample in such patients. Venous blood from the arm contains only blood cells which can pass through the microcirculation and hence may not fully reflect the activation of leukocytes in ischemic regions of the circulation. Since fully activated leukocytes are trapped in the microcirculation, the leukocytes in the venous blood would exhibit a lower degree of activation compared with those accumulating in an ischemic region. Innovative techniques in addition to the investigation of venous blood samples are needed to further explore the degree of leukocyte activation in ischemia.

SHEAR-INDUCED PLATELET AGGREGATION AND ITS INHIBITION BY ANTIPLATELET AGENTS IN CEREBRAL ISCHEMIA

S. Uchiyama, M. Yamazaki, S. Maruyama and Y. Ikeda. Dept. of Neurology, Tokyo Women's Medical College and Dept. of Hematology, Keio Univ., Shinjuku-ku, Tokyo, JAPAN.

Recent evidence suggests that shear-induced platelet aggregation (SIPA) is an important mechanism of thrombosis in arterial bifurcations or stenotic lesions. We measured SIPA using a newly-developed equipment (Y. Ikeda et al: J Clin Invest 87:1234,1991) in 34 patients with cerebral infarction and 9 with transient ischemic attacks (TIA) as well as 25 controls. Platelet aggregation was induced by high shear stress at 108 dyne/cm², which is known to be dependent on von Willebrand factor, glycoprotein (GP) IIb/IIIa and GP Ib. The extent of SIPA using platelet rich plasma was expressed as the maximum change in light transmission. SIPA was increased in 25 patients with cerebral infarct over 8 days after onset (51±9%, p<0.05) and TIA patients (55±6%, p<0.01) but not in 9 patients with cerebral infarct within 7 days after onset (47±12%) in comparison with controls (46±10%). SIPA was reduced in 6 patients treated with ticlopidine (34±7%, p<0.01) but not in 10 patients treated with aspirin (50±8%). In in vitro studies, SIPA was completely inhibited by PGI₂ analogues (TEI-9090 and TEI-7165), PGE₁ and forskolin, all of which increase platelet cAMP by activating adenylate cyclase, but not by cyclooxygenase inhibitors, thromboxane synthetase or receptor inhibitors, lipoxygenase inhibitors or PAF inhibitors. The results suggest that measurement of SIPA is useful for evaluating abnormal rheology of platelets and drug therapy to inhibit SIPA may be potentially valuable for treating cerebral ischemia, a platelet-dependent disease state.

PLATELET-INDUCED GRANULOCYTE AGGREGATION AND PHARMACOLOGICAL AGENTS

T.Maeda, *G.B.Nash, T.Yokose and Y.Isogai. 3rd Dept. of Medicine, Jikei University School of Medicine, Tokyo, Japan, *Dept. of Haematology, The Medical School, University of Birmingham, Birmingham, U.K.

Responsiveness of granulocytes to stimuli has shown to be enhanced by the presence of platelets. Using an assay based on counting aggregates with Coulter Counter, we found that heparinised or citrated platelet rich plasma (PRP) directly caused aggregation depend on the platelet:granulocyte ratio, and increased rapidly over a period of 2-5 minutes of mixing, followed by a slower increase with time. Aggregation was calcium dependent, and increased by stimulation of platelets with ADP or collagen. A range of pharmacological agents were tested for ability to inhibit the process. The surfactant Pluronic slightly increased aggregation, and the stable prostacyclin analog Iloprost had no significant effect, if they were added to the PRP or granulocytes. However, if Iloprost was added to the whole blood immediately after withdrawal, then aggregation was inhibited at a dose of 10⁻¹⁰g/ml and above. Praxilene slightly reduced aggregation at 10⁻⁴M. Trental (10⁻³M) and Nitrendipine (10⁻⁵M) showed a similar reduction in aggregation, but this did not reach statistical significance. Platelet:granulocyte interaction could occur at sites of vascular damage, so that inhibition of this phenomenon might be beneficial in ischaemic or thrombotic condition.

RHEOLOGICAL MECHANISMS IN THE PATHOLOGY OF MALARIA

G.B. Nash. Department of Haematology, The Medical School, Birmingham, UK.

Marked changes occur in the rheological properties of red blood cells after invasion and maturation of malarial parasites. Loss of cellular deformability, adhesion to endothelium, and rosetting with non-parasitised cells are all induced by parasites of the species *Plasmodium falciparum*, which causes the most severe form of human malaria. The abnormalities are mainly limited to the second half of the intracellular life-cycle, when the parasitised cells disappear from the peripheral circulation and become sequestered in the microvasculature. Sequestration is thought to be the cause of the ischaemic, life-threatening complications of severe malaria. Adhesion to endothelium has been thought of as the major cause of sequestration, although recent attention has focused on the possibility that rosette formation could be an additional factor in promoting vascular occlusion. Changes in cellular deformability are thought to promote cell destruction in the spleen, and microvascular sequestration may be a parasite defence mechanism against splenic clearance. However, the relative circulatory influences of cytoadhesion, rosetting and cellular mechanical abnormalities remain to be fully evaluated. Comparisons of the properties of different strains and species of *Plasmodium* might clarify this question. Recent studies have used "wild" isolates of parasites from patients, to examine the correlation between adhesiveness or rosetting frequency and the clinical severity of disease. To date, these studies have not shown an unequivocal link between the properties of the different parasite isolates and the severity of disease, although rheological methods have rarely been used.

THE VALUE OF RHEOLOGICAL PARAMETERS IN ASSESSING VASCULAR DISEASE

A.M.Ehrly

Div. of Angiology, Dept. of Internal Med., J.W.Goethe-University Hospital, Frankfurt/Main, Germany

Haemorheological parameters have been found to be impaired in several types of vascular diseases. Some findings were not supported by other authors and the discussion about the value was often controversially. Most of the data have been described in patients with chronic arterial occlusive disease whereby the blood samples were obtained from the antecubital vein. Some hints suggest however that the flow properties of blood as measured in situ in an ischemic tissue would be different from the data as measured systemically. If we accept that there is a pathophysiological link between the symptoms of the disease and such in situ findings measurements of rheological parameters should make sense under following circumstances a) diagnosis, b) therapy control, c) prognosis d) prevention. A look on the literature demonstrates one dilemma very clearly: single case results do not allow to draw a statistical conclusion and statistical results do not necessarily hold for an individual patient. It seems possible to improve the value of haemorheological data (as measured ex vivo) for diagnostic purposes by so called exercise tests. With respect to drug studies it is necessary to verify rheological results with micro-circulatory methods like tissue oxygen pressure measurements.

EFFECT OF EXERCISE ON ERYTHROCYTE DEFORMABILITY IN ISCHEMIC HEART DISEASE

T. Fujino, H. Hoshii, T. Mori, T. Kuruma, R. Nagashima, T. Kaneko, J. Ejima, K. Makizumi, K. Ariyoshi, Y. Kaji, Y. Tsuda and S. Kanaya.

1st Dept. Int. Med., Kyushu Univ., Fukuoka, JAPAN.

Exercise test is useful method to assess cardiac function in ischemic heart disease (IHD). However, the mechanism of abnormal cardiac function induced by exercise has been rarely investigated in relationship with microcirculation. This study was done to clarify correlation between exercise and erythrocyte deformability in IHD. Subjects consisted of 23 patients with old myocardial infarction (mean age : 56 ys). Exercise test was performed stepwise from 25 W to 55 W on supine ergometer. In each stage of exercise test, erythrocyte filterability (RCF), electrocardiography (ECG), echocardiography (ECHO) and blood sampling were also performed. Mild exercise (25 W) induced worsening of RCF (73 %), decrease of ejection fraction (EF) by ECHO (62 %), ST depression of ECG (26 %) respectively. RCF was worsened in all the patients who showed the decrease of EF, while in 57 % of the patients accompanied with the increase of EF, RCF was improved. Blood norepinephrine during exercise was significantly higher in the patients showed worsening of RCF than in the patients who showed improvement of RCF. These data suggest that RCF is a sensitive indicator in exercise test and the change of RCF during exercise strongly correlates with catecholamine concentration.