

SUMMARIES OF COMMUNICATIONS

INTERNATIONAL CONFERENCE ON CLINICAL ASPECTS OF THE
HEMORHEOLOGY OF DIABETES

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PENTOXIPHYLLINE AS AN ALTERNATIVE TO LOW MOLECULAR WEIGHT DEXTRAN IN PATIENTS
AFFECTED WITH PERIPHERAL VASCULOPATHY AT III, IV LERICHER-FONTAINE DEGREES
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The aim of our research has been to assess the efficacy of infusional and oral therapy with pentoxiphylline as an alternative to low molecular weight dextran in peripheral vasculopathy at III, IV LericHER-Fontaine degrees. 51 patients with degenerative and 45 patients with diabetic arteriopathy all at III-IV degrees have been examined. We have followed two patterns of treatment, labelled 2 Am and 2B by our School (the methods will be explained in detail in the article). Variations in considered parameters (clinical, instrumental, hemorheological) have been statistically analyzed. The results show: 1) Pharmacological dose-dependent effects on clinical and hemorheological parameters; 2) both patterns 2Am and 2B are efficient in withdrawal from infusional therapy; 3) absent modification of haematochemical tolerance parameters, of ECG and humeral arterial pressure. Results show that pentoxiphylline is a valid alternative to low molecular weight dextran.

ABNORMALITIES IN HEMORHEOLOGY AND NAILFOLD MICROCIRCULATION OF DIABETES
PATIENTS WITHOUT RETINOPATHY.

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200 Diabetes patients without retinopathy were studied by means of hemorheology and nailfold microcirculation. 123 age- and sex-matched healthy persons were used as controls. The results showed that the patients, though without retinopathy, had notable changes in their hemorheology and nailfold microcirculation. Compared with the control, they had higher whole blood and plasma viscosity, higher erythrocyte sedimentation rate, lower erythrocyte electrophoretic mobility and higher LDL, TCH and TC ($p < 0.005-0.001$). Disorderly arranged or seriously deformed capillary loops, clustered erythrocytes and slow blood flow were commonly seen. The results reveal that abnormalities in hemorheology and nailfold microcirculation present earlier in diabetes than does retinopathy. Hyperlipidemia and red cells with reduced deformability due to the metabolic impediments and increased aggregation due to lower surface charge now could be the major causes for the higher blood and plasma viscosity which might lead to abnormalities in microcirculation and further subsequent angiopathy. Thus, we conclude that abnormalities in hemorheology are promotion factors in the development of microangiopathy complications in diabetes.

THE ROLE OF ALTERED BLOOD RHEOLOGY IN THE PATHOGENESIS OF DIABETIC AND OTHER GLOMERULOPATHIES

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Raised blood viscosity may impair glomerular function by causing a proportionately greater increase in resistance to blood flow in the narrower post-glomerular vessels. This sequence of events would result in raised intraglomerular pressure and therefore to glomerular hyperfiltration, a change that is commonly found in the early phase of diabetic nephropathy. Preliminary findings of a serial study have shown significant differences in rheological indices in diabetic patients compared to healthy control subjects. Blood rheology was also found to be significantly different in diabetics with overt renal disease compared to diabetics with normal renal function or microalbuminuria (≥ 300 mg/dl). These findings suggest that while changes in blood rheology may be involved in the pathogenesis of early diabetic nephropathy the impairment in renal blood flow may become more manifest in the later phase of the disease. The results of this study will be discussed in conjunction with the findings of an earlier investigation carried out in this department that showed abnormal blood rheology in patients with IgA nephropathy (Clin Nephrol 29; 288-293 (1988)).

INFLUENCE OF HEMORHEOLOGICAL CHANGES IN DIABETES ON SYMPTOMS OF MYOCARDIAL ISCHEMIA

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The present study was designed to evaluate the possible hemorheological effect on symptoms of ischemic heart disease (IHD) by an improved therapy for poorly controlled type II diabetes. 23 diabetics with angiographically proven IHD who exhibited serum glucose >300 mg% and HbA_{1c} $>9\%$ at admission were regularly treated with diet and sufficient doses of Clibenclamid, 5 of them needed insulin s.c. additionally. Blood samples were obtained and an ergometer test was performed within 1-3 days of hospitalization and 3 weeks thereafter. Rheological measurements included plasma viscosity, extent and rate of RBC aggregation (Hct 45%) and RBC filtrability (Hct 10%). In the case of angina 1.2 mg Glyceroltrinitrate (GTN) was given. Within 3 weeks in all diabetics the serum glucose could be reduced below 200 mg%. The improved metabolic control led to significant ($p < 0.05$ or better) increase in RBC filtrability (+12%), decrease of plasma viscosity (-8%), fibrinogen level (-14%) and RBC aggregation constant (-32%). This was accompanied by a reduction in the number of angina episodes (-28%) and the number of GTN capsules needed (-22%). The maximum achieved ergometer load also improved significantly (+25%). These results indicate that the improvement of flow properties of blood in diabetes has a beneficial effect on the clinical symptoms of IHD. These rheological impairments thus most likely contribute to circulatory disturbances in IHD.

ELEVATED VISCOELASTICITY OF BLOOD IN DIABETIC MICROANGIOPATHY

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Abstract not available.

MICROCIRCULATION IN DIABETIC MICROANGIOPATHY

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It is generally accepted that hemorheological and microcirculatory changes may play important roles in the pathoetiology of diabetic microangiopathy. In this study a normal and two groups of diabetics (A,B) were examined for microcirculatory changes of venules on the human bulbar conjunctiva and hemorheological changes in vein blood before and after 75g-OGTT. Normal (N) and diabetics without retinopathy (A) and those with retinopathy (B) were examined. Microcirculatory changes were examined using an Intravital Video Microscopic System and Distance Meter developed by author. Viscosity of whole blood and plasma was measured with a Low Shear 30 (Contraves). Coagulo-thrombolytic factors were also measured. In the microcirculation, internal diameters, flow velocities of blood and flow volumes in the venules of the human bulbar conjunctiva showed significant increase in normal and diabetics without retinopathy, but not in diabetics with retinopathy. Also the viscosity of whole blood in diabetics without retinopathy was significantly higher than that in normals, but the viscosity of whole blood in diabetics with retinopathy was significantly lower than that in diabetics without retinopathy before glucose administration. After glucose administration, whole blood and plasma viscosity at high shear rate (94.5/sec) did not change significantly while fibrinogen, -macroglobulin, -antitrypsin increased and antithrombin III decreased significantly.

STUDY OF CERTAIN HORMONAL AND VASCULAR PARAMETERS IN IMPOTENT DIABETICS. A THERAPEUTIC TRIAL BY IMPROVING HEMORHEOLOGIC ASPECTS.

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Fifty-five adult diabetic males were studied, 35 with organic impotence and 20 with normal potency, 20 out of the impotents were NIDD and 15 were IDD. Ten out of the potent group were NIDD and 10 were IDD. Mean duration of impotence was 15.6 ± 11.8 months. Mean serum prolactin in both groups was within normal 16.06 ± 16.5 ng/ml in potent group and 13.62 ± 11.9 ng/ml in impotents. No statistical difference between both groups ($P > 0.05$). Mean serum testosterone was also normal in potents and impotents 435 ± 207 and 468 ± 202 ng/ml respectively, with no statistical difference between both groups ($P > 0.05$). Doppler examination for estimation of Penile-Brachial-Index (PBI) revealed no abnormal penile arterial flow in the potent group ($PBI > 0.75$). Thirteen of 35 impotents 37% were having penile vascular insufficiency ($PBI < 0.75$). This was detected in 47% of IDD and 30% of the NIDD impotents. Estimation of plasma volume, blood viscosity, red cell filtration rate and serum lipids in patients with vascular insufficiency is now under investigation. The use of Pentoxifylline (Trental-Hoechst) 1.2 gm/day and hypolipidemic agents (whenever indicated) are in trial to improve the hemorheologic properties. This is hoped will improve penile erection in such patients.

PENTOXIFYLLINE ACTION ON BLOOD VISCOSITY OF DIABETIC PATIENTS WITH RETINOPATHY

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From our experience, the administration of pentoxifylline (1200 mg daily), during a three month-period produces a decrease in blood viscosity, measured at constant temperature and hematocrit. This viscosity lowering effect results from a decrease of internal viscosity of red cells as shown by a significant reduction in Dintenfass's TK index. The value of studying blood viscosity under constant hematocrit and constant temperature to assess indirectly erythrocytic deformability, is confirmed through the correlation obtained in Dintenfass's TK. Pentoxifylline's capacity to reduce the blood viscosity through a diminution in erythrocytic rigidity makes us think that blood flow improves particularly at the capillary level. Such a pentoxifylline effect should be especially useful to prevent and/or improve the long term prognosis of a diabetic patient with retinopathy.

VARIATIONS OF BLOOD RHEOLOGY IN DIABETES.

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Following a survey of the rheological characteristics of blood from 68 diabetic patients, nine were selected for long term study and treatment with nicofuranose. Initially, these patients had a mean fibrinogen level of 4.95mg/ml (min. 4.2mg/ml), eight had a low erythrocyte flexibility relative to that expected for their fibrinogen level, and one was normal. Treatment with nicofuranose, two 250mg tablets three times a day, significantly ($P < 0.1\%$) reduced the level in six patients by an average of 1.16mg/ml, over the 9yrs of the trial. In two patients there was no significant change, and one patient dropped out. Three patients maintained a flexibility, as measured by the centrifuge technique, in the range for normal subjects, but differed in that their flexibility didn't vary with plasma fibrinogen ($P > 10\%$). One patient had a high flexibility, $13.8 \pm 2.5\%$ min (normal range 4.5-8%/min), which varied, as with normals, directly with the fibrinogen level. The remaining four patients were intermediate between these extremes. Estimates of KT were made from measurements of blood and plasma viscosities at high shear rates using a Coulter capillary viscometer. There is normally a linear increase of KT with decrease of erythrocyte flexibility, when the latter is below 8%/min. At the higher flexibilities observed in some diabetic patients the values of KT tend to stay constant. This is due to the presence of rouleaux. The invariance of flexibility with fibrinogen level in some patients, provides a means by which the formation of rouleaux at high fibrinogen levels can be reduced. There is some evidence that this is due to a factor in their plasma.

RHEOLOGY OF BLOOD IN DIABETES MELLITUS BEFORE TREATMENT

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There is a good deal of heterogeneity in results from hemorheological studies in diabetes mellitus. The variety of responses may depend on ABO blood groups and ethnic variations. Plasma viscosity has been found to be raised in diabetes. It is likely that red cell rigidity in diabetes is raised in the presence of microcirculatory complications. This study was conducted on 42 cases of maturity onset diabetes mellitus. The diagnosis was based on symptoms, signs, hyperglycemia (fasting and post prandial). These cases were free from any secondary complications of diabetes. The study was done before treatment was started. The parameters studied include whole blood viscosity at different rates of shear, plasma viscosity and red cell filterability. Whole blood viscosity is raised more significantly at higher rates of shear than at the lower rates of shear. Plasma viscosity is significantly higher. Surprisingly red cell rigidity is lower than in normal controls of comparable age and sex.

ERYTHROCYTE MEMBRANE FLUIDITY IN DIABETICS

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Erythrocyte membrane fluidity is an essential component of red cell deformability that has been studied in diabetes by several authors. For evaluation of this microrheological parameter a suitable technique is fluorescence spectroscopy. Our study included two groups of diabetics. In the first group (11 diabetics of type 1, 10 diabetics of type 2) the ghosts were marked with the 1,6-diphenyl-1,3,5-hexatriene (DPH) and the microviscosity (η) was calculated from the polarization degree (p). In the second group (7 diabetics of type 1, 17 diabetics of type 2) the membrane fluidity was evaluated on washed intact red cells marked with the fluorescent label 10-(1-pyrene)-decanoic acid, considering the dimer to monomer fluorescent intensity ratio (lex/lm).

Examining the first group, it is evident that:

- the degree of polarization as well as membrane microviscosity do not differentiate normals from diabetics;
- no relationship is evident between fluorescent parameters and whole-blood filterability; a slight significant correlation is evident, only in diabetics, between η and mean erythrocyte aggregation;
- no relationship is present between η and fasting blood glucose level and between η and diabetes duration.

Examining the second group, it is evident that:

- the lex/lm ratio does not discriminate normals from diabetics but shows that the ratio is lower in diabetics of type 2 than of type 1 diabetics;
- no relationship is present between lex/lm , whole-blood filterability and mean erythrocyte aggregation;
- no relationship is evident between lex/lm and fasting blood glucose level while a slight negative relationship is evident between lex/lm and diabetes duration.

From those results, the different behavior of the membrane fluidity evaluated on ghosts and on intact cells is evident. The relationship between lex/lm ratio and diabetes duration needs further investigation.

DIABETES, HISTORY AND SURVEY IN CHINA

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Diabetes was recognized by the ancient Chinese more than 2000 thousand years ago, the "sweetness" of diabetic urine and some treatment for diabetes was mentioned in some ancient traditional Chinese medical books. A large scale diabetes survey in the People's Republic of China showed that the total standard prevalent rate is 0.67% which is lower than in some western countries. The prevalence increased with age, reaching its peak at 60 yrs, both sexes were almost equally affected with obesity a predisposing factor in onset of diabetes. It is likely that higher diabetic prevalences are associated with better living conditions, less physical activity, aging and general living habits, lower prevalences were found in rural areas, heavy physical workers, young students and preschoolers.

ELEVATED PLASMA VISCOSITY IN DIABETICS WITH STROKE

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Diabetes mellitus is associated with impaired nutritive perfusion of the microvasculature and is considered to be a major risk factor for stroke. The pathogenesis of thrombotic stroke as well as the microvascular complications resulting from diabetes remain unclear although a common underlying factor may be abnormal rheological properties of blood. Published results from several sources have shown whole blood viscosity and plasma viscosity to be increased and erythrocyte and leukocyte deformability to be decreased in diabetics. Similar results have been reported for stroke patients. To date we have studied the hemorheological profiles of 216 individuals including 68 acute stroke patients with a subgroup of 14 patients with diabetes, 38 patients with transient cerebrovascular ischemic attack (TIA) (9 with diabetes), 50 individuals at high risk for stroke (10 with diabetes) and 60 healthy age-matched controls. Variables studied included hematocrit, whole blood viscosity, plasma viscosity, fibrinogen levels and albumin-globulin ratios. Plasma viscosity was significantly elevated in stroke patients with diabetes in comparison to non-diabetic stroke patients (1.96 vs. 1.82, $p < 0.02$). Interestingly, no intragroup differences in plasma viscosity are apparent in the TIA group of patients, whereas there exists a highly significant difference in the at-risk group, (1.93 vs. 1.80, $p < 0.01$). There appears to be no correlation between the increased intragroup differences in plasma viscosity and the fibrinogen levels, or albumin-globulin ratios. Intergroup differences in several rheological variables suggest that abnormal hemorheological properties are not an epiphenomenon in acute stroke. Abnormal plasma viscosity in diabetics may play an important role in their predisposition toward stroke.

COMPLEX HAEMORHEOLOGY OF DIABETES: INTERFACES WITH GENETICS AND IMMUNOLOGY.
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The fields of genetics, immunology and haemorheology are combined in the set of diseases described as "diabetes". The original division of diabetes into "juvenile-onset" and "maturity-onset", although differing in the clinical symptoms and rheological characteristics, become blurred as intermediate cases come to the fore. Thus, a variety of rheological factors, such as rigidity of red cells, aggregation of red cells, plasma viscosity, tendency to thrombus formation, etc. can be noted. The functions linking rheological factors and specific biochemical factors may differ greatly in groups of patients with different ABO blood groups or different ethnic background. While elevated blood glucose levels are characteristic of the group of diseases described as diabetes, they could be different in patients with different blood groups or of different ethnic background. A parallel elevation of plasma viscosity perhaps serves as a defense factor: by decreasing rigidity of red cells, while glucose might increase stability of the red cell membrane; also, an increase of plasma viscosity perhaps affects the presence, progress and direction of the chemical reactions in the cell membrane. Different complications in diabetes might be related to a variety of different factors and functions in haemorheology. The onset of a variety of complications might be related to the different inherent thresholds for particular rheological factors or functions in specific individuals.

WHOLE BLOOD VISCOSITY IN THE NEWBORN HUMAN AND ITS CLINICAL SIGNIFICANCE

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The whole blood viscosity and hematocrit was measured in 1,830 singleton live births with gestational ages ranging from 28 to 42 weeks. Hyperviscosity occurred in 6.6% of neo-nates. Using polycythemia to define and identify hyperviscosity does not give a true indication of hyperviscosity since only 34% of the hyperviscous infants were also polycythemic. A double blind long term follow up of 21 control and 24 hyperviscous infants showed a statistically significant difference in learning ability and motor co-ordination skills when tested at the age of 5 years.

CORRECTION OF BLOOD HYPERVISCOSITY IN DIABETIC MICROANGIOPATHY BY HAEMATOCRIT REDUCTION (THERAPEUTIC IMPLICATIONS)

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More than half of the diabetics with retinopathy have elevated erythrocyte rigidity, that makes blood flow deficient especially in the microcirculation. It seems that at some place in the circulatory system, probably at the capillary level, a viscoreceptor detects the erythrocyte high rigidity and develops a mechanism to produce a decrease of viscosity by hemodilution thus improving blood flow. The detection of the erythrocyte high rigidity and development of the compensating mechanism do not seem to function in all the diabetics with retinopathy. About one in five of these patients does not correct the hyperviscosity caused by a decrease of the red cell flexibility. In general, there are diabetics with bad metabolic control of their illness over a long period of time. Nowadays there are drugs that improve the erythrocyte rigidity and the blood viscosity. That is why it is important to include a study of these parameters when following these as it may improve the prognosis.

EFFECTS OF EICOSAPENTAENOIC ACID (EPA) ON PROTEINURIA OF STREPTOZOTOCIN-INDUCED DIABETES MELLITUS IN RATS

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Wistar rats (6wk old) were administered with streptozotocin (45mg/kg) intravenously through tail veins. After 10 days rats were divided into two groups. One group was fed semisynthetic lipid-free diet plus 8% (w/w) lard and 2% safflower oil for 4 weeks (SAF group, n=12). The other group was fed in the same way, except that safflower oil was replaced by 90% pure EPA ethyl ester (EPA group, n=13). Rats were fed 20g of diet each day. Twenty-four hour urine samples were collected just before starting the diets and during the dietary experiment at 7 day-intervals. The comparison of urinary protein excretion between the two groups is shown in the table below.

Table. Comparison of urinary protein (mg/day) between SAF and EPA groups.

Duration of Diet (week)	Before	1	2	3	4	
SAF Group	27±7	50±15	65±7	92±15	83±19	
EPA Group	27±10	38±12	49±15	62±25	71±20	p<0.01

Because EPA-rich diet reduced proteinuria of diabetic rats compared to linoleic acid-rich diet, EPA-rich diet may retard the appearance of diabetic nephropathy in diabetics.

PRECISE DIETARY FAT COMPOSITION DETERMINES DEVELOPMENT OF INSULIN RESISTANCE IN RATS

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Short-term feeding of rats on diets high in vegetable fat leads to markedly impaired insulin action in the liver and a range of peripheral tissues; effects completely prevented by replacing 20% of the vegetable fat with fish oil (Storlien et al, Science 237:885, 1987). The present study was aimed at determining whether the protective effects of fish oils related to some property of ω -3 fatty acids per se, and by what mechanism they act. Seven groups of adult, male, Wistar rats were fed equicalorically for 4 weeks on either a chow (high carbohydrate) or one of 6 high fat diets (59% of calories as fat). These high fat diets were varied along number and/or saturation. Insulin action was determined at \sim 140mU/L insulin levels using the euglycemic clamp with tracer 2-deoxyglucose/glucose. The unsaturated ω -6 (linoleic from safflower oil) and ω -9 (olive oil) diets led to whole-body insulin resistance (clamp glucose infusion rate (GIR mg/kg.min) of 8.9 ± 0.9 and 9.7 ± 0.4 respectively compared to chow controls 16.1 ± 1.0 , $p < 0.01$). This was associated with significant impairments in insulin action in both liver and peripheral tissues. Replacement of 11% of the linoleic with 20:5/22:6 ω -3 fatty acids from fish oil prevented the impaired insulin action (GIR 15.0 ± 1.3); however similar replacement with linseed oil 18:3 linoleic ω -3 had no beneficial effects (GIR 9.9 ± 0.5). The saturated fat diet led to even more profound insulin resistance (GIR 6.2 ± 0.9) but with this diet replacement with 11% linolenic completely prevented the insulin resistance (GIR 16.0 ± 1.5). The occurrence of insulin resistance in skeletal muscle of high-fat fed group of rats appeared to relate inversely to accumulation of long-chain polyunsaturated fatty acids in the phospholipid component of the fatty acids in muscle. We conclude that whether insulin resistance occurs as a result of increasing the fat content of the diet will depend greatly on the precise fatty acid composition of that diet and thus presumably the phospholipid composition and in turn the fluidity of the cell membrane in skeletal muscle, the major organ of insulin-stimulated glucose disposal.

OPTICAL CHARACTERIZATION OF ERYTHROCYTES SEDIMENTATION AND AGGREGATION IN DIABETES

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Hemorheological changes, such as increase in blood viscosity and decrease in erythrocyte deformability in diabetes are well established. These changes may affect the sedimentation and aggregation properties of erythrocytes. In the present study an attempt has been made to visualize these phenomena within blood samples by the scattering of He-Ne laser light. The erythrocyte sedimentation profiles are determined by measurement of laser transmitted intensity at various points in the blood column along its height and width. For aggregation studies, samples of low hematocrits are used and the changes at the center of the blood column determined by analysis of the optical fluctuations occurring in the transmitted signal. The results show that diabetes affects these parameters significantly compared to that of normal subjects. These results are in agreement with the rheological parameters measured by conventional techniques.