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RAPID TRANSPORT OF MACROMOLECULES IN POLYSACCHARIDE-SYSTEMS BY MEANS OF DISSIPATIVE STRUCTURES

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The observation that polymers have high apparent diffusion coefficients in concentrated solutions (1) led to the discovery that macroscopic finger-like structures can be formed at the interface between two polymer solutions and that these structures move in the gravitational field and transport high-molecular weight material (2). Subsequent work has shown that the formation of finger-like structures is a common phenomenon at boundaries established in concentrated multicomponent systems (3-5) but that the reverse, i.e. self-sharpening of the boundary also can occur (5). Work is now in progress to understand the mechanism of the structure formation. It has been shown by dialysis experiments that in systems where structures are formed the pre-requisites for density inversions exist (6,7). Two models have been proposed to describe the process which leads to the density inversion. In one model the diffusion of solutes over the boundary leads to an instability (8,9) and in the other an osmotic flow of solvent between two polymer compartments yields a reversal of density between them (5). The structured flow affords a transport mechanism in which by counter-current diffusion high-molecular weight material is transported faster than low-molecular weight material (6). This mode of transport as well as the formation of the regular structures may be of biological significance.

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THE ENDOTHELIAL CELL SURFACE

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Until the early 1970's, endothelium was regarded as an inert smooth-surfaced, non-thrombogenic semipermeable monolayer of cells that served primarily to separate blood from parenchyma. However, our interests in the structure and function of the endothelial cell surface did not arise from studies of known or presumed properties of endothelium but arose from efforts to understand the mechanisms by which certain oligopeptide hormones, notably bradykinin and angiotensin I, are eliminated during a single passage of blood through the pulmonary vascular bed. In the late 1960's we postulated that these hormones are metabolized by enzymes situated along the luminal surface of endothelium, and it was through efforts to prove our hypothesis that we began to appreciate the complex metabolic activities that occur at the surface of the endothelium and began to explore for structural correlates of these activites. Contrary to the common view of the time, we found that endothelial cells are neither inert nor smooth-surfaced. The cells possess many enzymes, enzyme inhibitors, transport structures and receptors that continuously interact with blood solutes and colloids. Similarly, the surface of endothelium is thrown up into projections approx. 500 nm in diameter and 500-3,000 nm long. The projections may branch or anastomose with other projections, and, like other areas of the cell membrane, possess their own caveolae. These surface structures obviously contribute to the enormous surface area of the vasculature. Whether caveolae, also known as pinocytotic vesicles, participate in pinocytosis is controversial. Nonetheless, these structures not only increase surface area, they possess enzymes; ATPase, ADPase, 5'-nucleotidase, angiotensin converting enzyme (ACE), carboxypeptidase N and carbonic anhydrase being among them. The structural complexity of endothelium is not fully appreciated by viewing the cells in cross-section. The cells have disposed near their nuclei the full complement of intracellular organelles required for protein and membrane synthesis and appear not only to synthesize their own surface enzymes such as ACE but to synthesize and export plasma proteins such as fibronectin. The presumed non-thrombogenic nature of endothelium is misleading in at least two respects. Normally, the cells are actively anti-thrombogenic, a property owing in part to their ability to synthesize (via enzymes of the endoplasmic reticulum) and secrete PGI_2, a process stimulated by circulating hormones, such as bradykinin, acting via receptors on the cell surface. On the other hand, damaged endothelium can become actively thrombogenic and, in turn, thrombolytic. The cells also contain the filamentous structures required for contraction and are capable of shape changes and movement. Until recently, means were not available for examining the true cell surface at the resolution of transmission electron

microscopy and many cell surface components could only be evaluated in terms of immunocytochemical reactions. We have now developed means of replicating the true cell surface a procedure that yields replicas of a quality to allow viewing of structures such as ACE (80Å in diameter) at the molecular level. The replication technique, however, has revealed yet another surface structure. The replicas show an en face view of the glycocalyx or endothelial fuzz, a layer postulated to occur on theoretical grounds but visible only after ruthenium red staining. The glycocalyx is not an amorphous cell-coating but is a regular, well-organized carpet work which enmeshes the surface enzymes and may allow molecular sieving. The glycocalyx becomes disrupted and disorganized by conditions such as exposure to antibodies to surface enzymes in the presence of complement or exposure to proteolytic enzymes, conditions which allow expression of otherwise latent receptors. Endothelial cells normally express Clq receptors. Given that the glycocalyx is believed to contain fibronectin, a substance for which Clq has high affinity, the glyocalyx may itself be the Clq receptor. Fc and C3b receptors are not expressed by normal endothelium but become active in virus-infected cells, cells reacted with antibodies to surface antigens and cells exposed to granulocyte lysates. Thus, while the role of the organized glycocalyx and the effects of its disarray on blood flow or permeability are not known, the glycocalyx may well play a central role in intravascular coagulation and complement activation in some forms of immune disease with attendant loss of transmural pressure gradients.

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Conformational Changes of Biological Macromolecules

E Katchalski-Katzir

(This Lecture was not available in time for press)

- Executive Editorial Office

Electro-Biorheology

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The deformation and flow of the matter are the main subject of Rheology. In most biological systems, the deformation and flow are closely associated with the electric polarization and current. When the fluid carries ions during flow, the streaming potential is produced. When the deformation is coupled with the dipolar rotation, the piezoelectric potential is produced(1).

Piezoelectricity can be observed where biopolymers are uniaxially orientated. The epidermis of fish consists of multilayers of oriented collagen fibers. Circular samples with a diameter of about 18 mm were cut of the dried skin of mackerel fish. The sinusoidal strain at 10 Hz was given at different directions. The angular dependence of the apparent elastic and piezoelectric constants were determined(2). The two maxima of the elastic constant were observed against the angle between the head to tail direction and the strain direction. The anisotropy for the piezoelectric constant d was approximately expressed by

$$d = (d_{14}/2)\cos(\theta_2 - \theta_1)\sin 2[\theta - (\theta_1 + \theta_2)/2],$$

where d_{14} is the shear piezoelectric constant for uniaxially oriented collagen fibers and θ_1 and θ_2 are the two directions of preferential orientation of collagen fibers.

The piezoelectric constant $d = d' - id''$ is a complex quantity depending on the measuring frequency and temperature. For a sample of fish bone at relative humidity of 93 percent, the value of d' decreases with increasing temperature from -150°C and reverses its sign at about -30°C. In order to model the inhomogeneous structures of bone consisting of piezoelectric collagen layers and nonpiezoelectric hydroxyapatite, the composite films of collagen and gelatin were prepared(3,4). A piezoelectric collagen layer was sandwiched between two nonpiezoelectric gelatin layers. The sign reversal of the piezoelectric constant against increasing temperature and water content was also observed for the composite films.

In static experiments, when static stress was applied to the sample, positive current was observed in the output circuit at low temperature but negative current was observed at high temperature. The piezoelectric polarization in the collagen layer is neutralized by macroscopic ionic currents in both collagen and gelatin layers, whoes relaxation time is denoted as τ. This current is positive and accumulated space charge at boundaries between collagen and gelation layers. The piezo-

electric polarization of collagen decays internally by the diffusion of counter ions surrounding dipoles, whoes relaxation time is denoted as τ_0. At low temperature and low hydration, $\tau_0 > \tau$ and the neutralizing ionic current gives the positive current. At high temperature and high hydration, $\tau_0 < \tau$. During the accumulation of space charge, the piezoelectric polarization rapidly disappears. Consequently the flow back of the space charge ions gives the negative current.

Unoriented gelation films do not show piezoelectric effect because the molecules and crystallites are randomly oriented. If a d.c. bias field is applied to the gelation film, the induced piezoelectric effect can be observed(5). When sinusoidal strain is given to the film, the capacitance and the conductance are changed, resulting the change of the polarization of the film. The value of apparent piezoelectric constant is proportional to the magnitude of the d.c. bias field.

The piezoelectric effect and its relaxational phenomena were investigated for oriented films of fibrin, cellulose derivatives and several kinds of synthetic polypeptides. The shear piezoelectric potential was detected for ethylene dichloride solution of poly-γ-methyl-L-glutamate(PMLG)(6). Alpha-helical molecules were oriented by applying a d.c. electric field. Ultrasonic shearing stress was then imposed to the solution. The apparent piezoelectric coefficient observed was 1×10^{-9} Vm^2/N and five orders of magnitude as small as that for the oriented solid PMLG.

Piezoelectric films wrapped around the femur of animal induced bone formation. The callus formed by electretized teflon film tended to disappear with the elapse of time because of the decay of polarization. The callus formed by piezoelectric film of PMLG continued to grow with time because the piezoelectric property did not decay(7).

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5 BIORHEOLOGY AND THE PRACTICE OF MEDICINE

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Biorheological disturbances in the human organism which can be looked at as a disease are applying for example to abnormalities of bronchial mucus or to other specific fluids produced by several organs - not to mention the many more or less solid constituents of the body with their very different rheological qualities. The most important role in this respect is played by the blood - vessel wall organ.

For diagnostic reasons the blood as one part of this organ is easy of access. The vessels, especially the microcirculation, often can be assessed only indirectly. Therefore measurement of fluidity of blood and its components considerably has been extended. Its unique results generally are not sufficient for a comprehensive diagnostic definition. The valence of disturbed fluidity of blood in the sense of a disease can only be judged if the clinical background is known. A laboratory screening of fluidity in this sense can only discover a risk, but it will not furnish a diagnosis. A diagnostic and likewise a therapeutic disposition therefore only will be possible, if, except from specifications of blood fluidity, there are enough facts or clinical evidence available about the situation of microcirculation.

The probability for a disease primarily appointed hemorheologically will be the highest, if microcirculation in its most sensitive reaction on disturbances of fluidity is not functioning correctly. Nearly always there are feed-back mechanisms included insofar as disturbances of fluidity are impairing the function of microcirculation. An impaired function of microcirculation,

for example in types of autonomous obliteration, conversely acts upon fluidity of blood, for example by increasing the hematocrit in the sense of autoprogression. The small vessel disease in diabetes mellitus is presenting similar findings. In addition consistent biochemical changes coincidently will be followed by increased aggregability and/or rigidity of red cells, enhancing the vitious circle. Increased friction of rigid red cells may destroy many platelets at the endothelial lining of the capillary, some kind of an ideal activation of clotting process, possibly with joining fibrin production.

Starting point for therapy of biorheological disorders is their aetiological and pathogenetical background. For the overwhelming disorders of the system blood - vessel wall the greatest part of therapy is directed to the underlying diseases. In addition the measure of hemodilution or sometimes reduction of fibrinogen level by snake venom has become important, though this is more or less a symptomatic treatment. Another therapy is plasmapheresis which sometimes for months may cause a regression of the underlying immunological process.

A further therapeutical action is to influence the behavior of red cells by different pharmacological substances. This may partly preserve platelets by reducing the rubbing effect of rigid red cells.

A special protection from platelet activation seems to be an efficient measure in many (small-) vessel diseases, though not always extent, mechanism and statistical significance of the drugs effect has been clarified.

Especially in small vessel diseases with trophical tissue damage physical as well as nursing measures are of great importance. They often can furnish an indispensable additional treatment for recovery of the supplying circulation and consequently revival of viable tissue.

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THE BIORHEOLOGY OF TUMOR CELLS

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Most fatalities caused directly by cancer are due not to growth of the primary tumor but to the appearance and development of secondary tumors, or metastases, at sites distant from the primary one. The majority of metastases result from malignant cells which successfully complete a sequence of invasion of the circulation, transportation via the blood stream to one or a series of microcirculatory beds, arrest in the capillary network, extravasation and growth into secondary tumors. Studies of experimental metastasis in which radiolabelled tumor cells are injected into the venous circulation of syngeneic mice and their location and fate followed typically show that within a few hours the majority of the injected cells are dead, and within 24 hours 95 to 99% of the tumor cells have perished. Hence, the circulatory system is a hostile environment for metastasizing cells. The damage inflicted upon circulating and arrested cells can be regarded as the most effective defence the body mounts against the spread of malignant disease. Little is known about the mechanisms important in this process, although it is clear that host natural immune defence systems cannot account for the extent and consistency of the tumor cell destruction observed. We are currently involved in a program aimed at examining the ability of metastatic cell lines to withstand shear stresses typical of the circulation, at determining the metastatic potential of subpopulations of tumor cells which have survived prolonged exposure to shear and at determining the ability of pressure drops typical of the microcirculation to deform tumor cells of known metastatic potential in a micropipette system.

In the shear survival experiments, B16 mouse melanoma lines are grown in tissue culture, suspended in medium and subjected to steady shear at 37^oC in a cone-plate viscometer equipped with either a 0.8^o or 1.57^o cone. At regular intervals samples are taken, the fluid volume replaced and cell counts and viabilities determined via vital dye exclusion and/or plating. Typically it is found that the rate at which viability is lost, $\dot{V}$, increases strongly with shear rate over the range in which stable flow occurs. For instance at 2,200 s^{-1} with the 0.8^o cone greater than 95% of the cells are dead after 5 hours of shear. Variation of the surface to volume ratio of the viscometer has little consistent effect on the results (at constant shear rate) suggesting that wall effects are not responsible for the lethal damage. If the shear rate is reduced mid-way through an experiment, $\dot{V}$ decreases to the value characteristic of continuous shearing at that rate, indicating that killing is not due to products released from damaged cells. $\dot{V}$ is

initially constant with time but the average decreases somewhat as an experiment progresses. Interestingly, if $\dot{V}$ is expressed as the number of cells losing viability per unit time, $\dot{V}$ is found to be independent of cell concentration, suggesting that cell-cell interactions are not responsible. Effects of plasma components, erythrocytes and other blood cellular elements on shear-induced loss of viability will be presented and models for the results will also be presented.

The deformabilities of two lines of B16 cells with differing metastatic potential, at the same stage in the cell cycle, have also been examined via micropipette aspiration experiments carried out under videomicroscope control, in collaboration with Dr. Evan Evans. The equilibrium tongue lengths aspirated into the micropipettes following successive pressure steps have been obtained from analysis of video tapes. Up to at least two pipette radii the lengths are linear in aspiration pressure, the slopes being greater the smaller the cell diameter. The initial results are that the tongue lengths are larger at a given pressure for the cell line with lower metastatic potential. That is, the more metastatic line appears to be less deformable on the average than the line exhibiting the lower propensity to form successful metastases. The other interesting observation made on these two lines is a difference in their ability to withstand an aspiration step from 0 to 2,000 dyne/cm^2 without blebbing off relatively large, membrane-bound vesicles, presumably filled with cytoplasm. The cells with the higher metastatic potential were again considerably more robust in this respect, 14% of the cells examined (n=14) showing blebbing in the highly metastatic line versus 80% (n=15) of the cells from the line exhibiting lower metastatic potential. To the extent that a rigid micropipette models a capillary, then, it would be expected that considerably more damage would be suffered by cells from the less metastatic cell line were they arrested in a microcirculatory bed across which pressure drops of this magnitude occurred. This result is not inconsistent with the ultimate fate of these cells _in vivo_.

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FIBRIN GELS
THEIR FORMATION AND RHEOLOGICAL PROPERTIES

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Th fibrinogen to fibrin conversion is an example of formation of biological polymers and the incorporation of them into gel structures, which display interesting viscoelastic properties.

The fibrinogen is a plasma protein, which before its gel formation must be activated. The activation proceeds through limited proteolysis of the molecule by which two peptides, fibrinopeptide A and fibrinopeptide B, are released from the N-terminal and of the molecule. The activated molecules form linear polymers in a half-staggered fashion in such a way that the activated molecules in the polymers cover each other by about half of their length. The polymerization reaction like the activation itself may be first order and resemble free radical polymerization. The polymers being formed will at the point of gelation crosslink into the network structure we call the gel.

As shown by Ferry and co-workers the properties of the gels depend on many factors, such as ionic strength, pH, protein, and enzyme concentrations. We have in liquid permeation studies shown that the porosity of the final fibrin gel is directly related to the clotting time of the fibrinogen-thrombin system and inversely to enzyme concentration. High enzyme concentration leads to formation of gels with small pores and low enzyme concentration to gels with large pores. Our results suggest that the events preceeding gel formation determine the final gel structure. At high enzyme concentration short polymers are formed which at the gel point crosslink into a fine network structure. At low enzyme concentrations longer polymers are formed, which create larger pores in the gel.

By means of particle permeation studies we have shown that the pores in fibrin gels are surprisingly uniform, which is a further indication of the regularity of these structures. Further support for this conclusion has been obtained by light microscopic and electron microscopic studies of the gels.

At a given enzyme concentration several parameters such as pH and ionic strength influences gel formation and porosity of fibrin gels. We have shown that dextran is present during gel formation, the porosity of the gels is drastically changed. This applies also to albumin.

We propose that the framework of the final gel structure is created already at the gel point. Subsequently formed polymers are accomodated into this framework structure leading to increase in strand width, but very little change in pore size. The gels are to be considered as micell structures in which the polymers grow out from initiation points in the solution phase, eventually forming contact in a regular fashion with other polymers and thus forming an infinite network structure. The higher the enzyme concentration, the thighter are these micells.

The viscoelastic properties of different fibrin gels and the roles of these gels in physiological and pathophysiological conditions will elaborated on.