SHEAR STRESS AND VASCULAR WALL. EFFECTS OF BLOOD FLOW SHEARING IN NORMAL AND PATHOLOGICAL CONDITIONS

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INTRODUCTION

That the gravity forces and pulsations due to the beating of heart influence the human body and organs is known as a law of nature. As for the vessels, connections with physical forces appear particularly important: for the arteries there is a superficial influence of flowing blood which rubs against the wall, the *shear stress*. In addition of such a rubbing effect, the standing position extends gravity forces downwards in veins, this being particularly efficient in veins of the lower limbs. But there is also in vessels a radial pressure acting centrifugally on the wall.

Shear stress, related to laminar flow, is able to up and down-regulate functions of the wall, mainly acting on the endothelium layer. Beside the well known action on vasomotricity, numerous intracellular signalling pathways are sensitive to shear stress inducing activation of

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numerous genes. Such effects have recently been elucidated, but more is to be found in next future, as an open field of new research.

We will consider some basic studies related to the setting of shear stress in vessels. Then will be considered implications in vascular diseases, i.e. arterial, venous and disorders in microvessels.

THE FUNDAMENTALS OF SHEAR STRESS

Physical approach of flowing blood

According, traditionally, to Sir Isaac Newton (1), blood behaves in vessel, similar to tubes, as a streamlined or laminar flow, shearing apart adjacent fluid layers . *Shear stress* is a parameter which quantifies the magnitude of the force driving the flow process, and the *Shear rate* quantifies the speed of flow deformation. Looking at a section of liquid under shear stress, one can see that the driving force is acting on surfaces of fluid layers across the liquid : this is the shear stress τ (*tau*); it is given by the ratio of force to surface. The liquid's response is to exhibit a velocity gradient between fluid layers, the shear rate $\dot{\gamma}$ (*gamma punct*), which can be calculated by the ratio of velocity to layer thickness. Using these two parameters, the viscosity η (*eta*, dynamic viscosity) can be inferred from the ratio of shear stress to shear rate, quantifying the friction between, adjacent fluid layers. As for Sir Isaac Newton, he hypothesized that shear rate is proportional to shear stress, i.e. that viscosity was constant regardless to flow conditions, defining the Newtonian fluids. This is only true when blood is driven powerfully, i.e; for shear rates over 100 sec-1.

Jean Marie Léonard Poiseuille determined in 1844, in quite similar experiments, that volume flow rate (Q) depends on pressure gradient (driving pressure ΔP), the length of the vessel L, and the radius r at power 4, leading to the Hagen-Poiseuille equation (2):

$$Q = \ddot{A}P.\frac{\pi r^4}{8L\eta}$$

The distribution of velocity and shear stress across the tube is not uniform, for the velocity is very low and shear stress high for the layer close to the wall, as it is the opposite for central layers, where velocity is high and shear stress low.

From such an equation, shear stress τ is given by:

$$\tau = \ddot{A}P.\frac{r}{2L}$$

where r is the distance from the tube axis. And shear rate can be calculated from shear stress and viscosity;

$$\dot{\gamma}\left(\sec^{-1}\right) = \frac{\tau(mPa)}{\eta(mPa.\sec)}$$

From even more complex calculations one can demonstrate that the shear force reaches a maximum at the inner surface of the vessel wall permanently active provided blood is flowing.

The units used for measurements are: dynes per square centimeter or mPascal for shear stress and for shear rate the velocity gradient between layers (sec-1) is to be divided

by the distance between them (m), leading to units as reciprocal seconds (sec-1). Another tool is the Reynolds number, a non dimensional value, set for a given vessel, from mean diameter, mean red cell velocity and being special expression of the laminar aspects of the flow

Of course there are great differences between glass tubes and vessels, due to elastic and muscular components of their wall. Therefore the flow is *pulsatile* throughout the arterial tree exhibiting *oscillatory* waves and cycles : periodic deformation (ε = 1 to 3 Hz). Sometimes *turbulences* are present, increasing or decreasing the shear stress. Also is acting, mainly in veins, the hydrostatic pressure, square to the wall providing effect on endothelium and sub-cellular matrix as well. (3)

Of great importance are *stagnant zones* at flow separation around branching, in sudden expansions and in vein valvulae: in such areas, secondary flow of low velocity are set up in the shape of a vortex, allowing circulating cells, mainly leukocytes, to attach because the shear stress is very low (1 to 6 dynes/cm2). Such a physical phenomenon is important in order to understand how cells enter the wall, as usually the blood flow push them away. The way cells enter arterial or vein wall is the basic pathogenic factor of atherosclerosis and varicosities.

Shear stress as an activator of endothelium (TABLE 1)

The continuous tangential mechanical strain on lining cells is an actual and one of the main activator of endothelial cells. Within the range of "high values" i.e. 10 to 50 dynes/cm^2 "sensors "are activated, inducing biological actions, particularly on the very large bed of arterioles and the arterial side of microcirculation. Inversely a negative action occurs in zones where shear stress is low or abruptly decreased. The shearing is normally low in microvenulae (venous side of microcirculation), stagnant zones (see above), or when outflow abruptly decreases, when driving pressure collapses (heart failure, shock, etc…) or in vein stasis. The endothelium provide a pattern of physiological actions, which are different when shear stress is high of low, but very productive in the two states. Globally one can tell that high shear related actions are "protective", for example inhibiting expression of adhesion molecules and producing nitric oxide NO, as low shear induce the inverse effects.

In all situations where shear is low, and also usually driving pressure, blood has no longer a Newtonian profile and red cells aggregates contribute to the lack of rubbing at the wall: this is the mechanism of *rheological* action and disorders.

Mechanisms of shear stress effects

From basic functions to genes up regulation

Recent studies using transport of gold particles, have shown that *permeability* increased when low shear down regulates mRNA and protein level of occludin, the protein of tight junctions of endothelial cells, and up

regulated mRNA and protein of VEGF (4). Another work showed that low shear induced permeability is bound to be dependent on the presence of adherent leucocytes and NO, a condition of low shearing (5). Reversely high shear stress over 10 dynes/cm^2, reduces *apoptosis*, decreasing Fas-receptor mRNA activator of apoptosis and inducing mRNA of inhibitor Bcj-xl (6). Shearing is so a condition of life for the endothelium…! As for *shape maintenance* and *cytoskeleton*, high shear, mostly pulsatile, induces stress fibers in endothelial cells of arterioles, made of actin, myosin and alpha-actinin (7)(8)(9). Reversely in veins endothelial cells look polygonal as shear stress is lower, and actin si to be found at the periphery (9). Such phenomenon is due to influence of shearing on SAPK/JUNK pathway, the AP-1/TRE (10). Finally, from those examples and many others, it clearly appears that shearing is able to implement its action through gene up regulation.

Gene up regulation and related functions of vascular endothelium cells

The *mechano-transduction* represents mechanisms by which shear stress hand over its action through a physical force up to the promoters of genes inside the nucleus, and which is characterized by different steps: transduction on receptors, transduction of signals involving physiological cell functions, and variable cell responses including gene activation.

As for *sensors* some of them are structural: the *caveoli*, small membrane invaginations, housing microdomains, as preformed *eNOS isoforms* immediately available for NO production and extra cellular signal-regulated kinases for de novo synthesis (11) (Figure 1). The main constituent, caveolin 1, is shear sensitive, i.e. more concentrated where shearing is the highest (12)(13)(14). The *ion channels* are important sensors: K^+ channels, which open at low shear stress by "convex curving", Ca^{2+} channels an Cl^- channels, associated with G proteins. *Integrins* $\alpha v\beta 1$ and $\beta 1$ may be considered as sensors, for shear stress exerts a force upon the links to sub-endothelium, dragging up the integrins, anchored on fibronectin and vitronectin; that, in return, induces intra-cellular signalling pathways, mainly *tyrosine kinases receptors* and subsequently JNK (15). Also cytoskeleton is involved in such mechanisms.

It finally appears that an important step in signalling is the monitoring of *kinase inducing phosphorylation cascades* (16)(17). The most important is *stress activated protein kinases SAPK/JNK* where JNK dimers, in a final event, translocate into the nucleus. A second important pathway is *mitogen activated protein kinase* cascade MAPK/ERK where Erk dimers translocate to the nucleus (Figure 1). Such products reach the SSRE (*shear stress response element*), a 12 nucleotides sequence which specifically binds mechanic induced products.

Once expressed, some adhesion molecules can behave as shear stress receptors, when linked to MEK1/2, Ras, Raf, then c-fos. In the presence

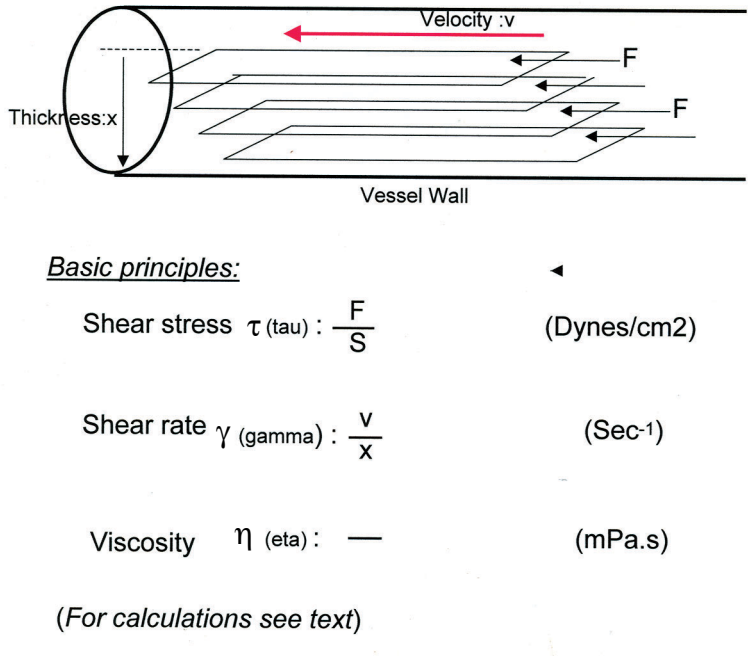

Figure 1 — Blood flow profile under shear stress

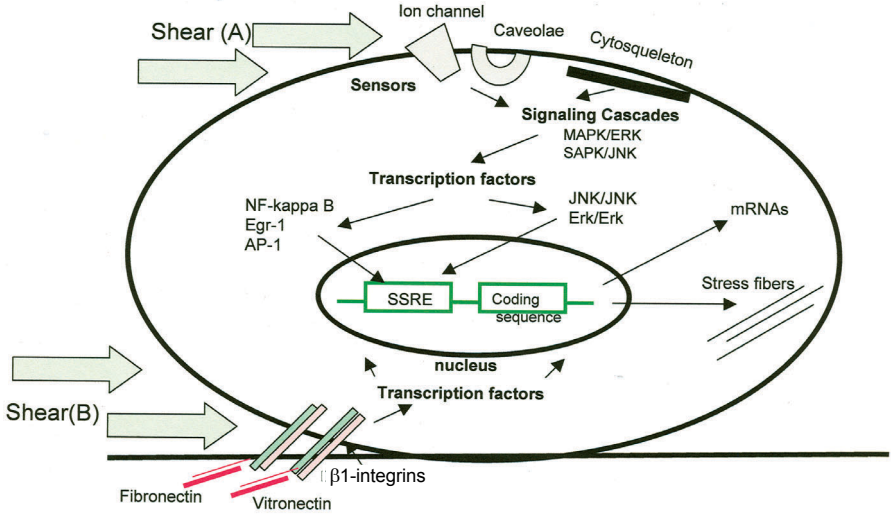

Figure 2 — Different ways of mechanotransduction: either (A) through membrane sensors, or (B) from sub endothelium components

TABLE 1
Mean values of hemodynamic parameters in human blood vessels

Vessel	Diameter (mm)	Number	Local velocity (cm/sec)	Shear Stress (dynes/cm2)	Shear rate (sec-1)	Reynold's number
Aorta	20 to 30	1	60	***5-10***	100-150	4500
Arteries	1 to 3	600	20 to 50	***20-30***	700	400
Arteriolae	0.5 to 0.1	40.10^6	0.5	***40 to 60***	1000	2.3
Capillaries	0.05 to 0.01	$1200.\ 10^6$	0.05	***60***	800	0.05
Postcapillary venulae	0.01 to 1	$8O.\ 10^6$	0.1 to 0.04	***1 to 5****	0.01 to 0.02	0.01
Veins	3 to 6	1000	5	***6 to 10****	100 to 200	400
Vena Cava	13 to 15	1	10 to 15	***10***	50	400

**depending on blood flowing or not*
All data are mean values from several publications and *Clinical hemorheology*, S Chien et al Edrs, Martinus Nijhoff Publisher 1967 and *Microcirculation and Hemorheology*, A Larcan and JF Stoltz Edrs, Masson Publisher 1970.

of adherent white cells, oxygen free derivatives are produced and can be involved in shearing activation, through Rac activation, as one part of SAPK/JNK and NADPH oxidase on another. Integrins act through the pathway SAPK/JNK and I Kappa B kinases (18)(19).

Finally numerous systems are starting from the luminal and the abluminal surfaces of endothelial cells. They form complexes, capable of "cross talks", giving flexible signals (in intensity and duration), depending on the kind of shear stress.

Molecules expression

Examples of products of endothelium, related to shear stress is listed on Table II. Endothelial cells discriminate between normal-laminar and abnormal-turbulent flow. High shears (10 to 50 dynes/cm^2) seem to be protective. Low shear (2 to 10 dynes/cm^2) induce adhesion and inflammatory proteins. Turbulent, oscillatory and cyclic strain are experienced by the cells as inducing inflammation products. Founded upon current large genes investigations, more than 10 000 genes seem to be under the influence of shear stress. Some effects concern basic functions.

Shear, for example, determines *vasomotricity*. When blood volume and pressure increase, preformed eNOS in caveoli produces NO within milliseconds by oxidation of L-arginine in L-citrulline (11). Laminar high shearing also activate COX-2 production of prostanoïd prostacyclin PGI2. This is particularly efficient in arterioles which are numerous (40.10^6 TABLE 1). A balanced situation exists between endothelin ET-1 and other vasoconstrictive factors (oxygen free radi-

TABLE 2
Examples of circulating products due to Shear Stress
(within 10 to 50 dynes/cm²)

1- Substances up regulated by laminar flow			
Name	***Product and effect***	***Level of mARNs****	***Time***
e-NOS	NO	Increased	Seconds
COX-2	Prostacyclin	Increased	Minutes
t-Pa	Fbrinolysis	Increased	> 1 hour
TGFb1	Cytokine	Increased	> 1 hour
Caveolin, actin	Stress fibers	Increased	Within 6 hours

2- Biphasic regulation (lower then relative higher shear stress than usual)			
thrombomodulin	Thrombin receptor	Increased then decreased	Disappears after 6 hours
Endothelin-1	Vasoconstriction	*Ibidem*	Low production then disappears over 0.6Pa
PDGF A & B	Cell growth factors	*ibidem*	Disappears after 1 hour

3- Substances down regulated by laminar flow			
VCAM-1	Leukocyte adhesion	decreased	Within 6 hours
ICAM-1	*ibidem*	*ibidem*	*ibidem*
PAI-1	Inhibitor of fibrinolysis	*ibidem*	*ibidem*

4- Very high shear stress regulation (occurring "picks")			
VCAM-1	Leukocyte adhesion	Increased (transient)	Within 1 hour
MCP-1	Monocyte adhesion	*ibidem*	*ibidem*
PDGF A & B	Cell growth factor	*ibidem*	*ibidem*

mRNAs checking
More than 11000 genes are presently known being regulated by shear stress.

cals and peroxinitrites) and NO-PGI2. Continuous *NO production* is also necessary for platelet de-activation, so when there is a drop in shear stress, NO production is stopped leading to vasoconstriction and more activated platelets in blood stream (21). This is also important in ischemia, below arterial stenosis, during vein stasis and skin related lesions. When shearing forces are applied 24 hours at a high value (1Pa) Weibel Palade bodies are extruded and the *Von Willebrand Factor* expressed (22).

Another important function of high laminar flow is *down regulation and distribution on the membrane of adhesion molecules*, such as VCAM, ICAM and MCP-1. So beside the fact that high shearing impairs rolling, there is no corresponding ligand at the endothelium surface. This phenotype is reversed when flow becomes low or turbulent. Some "peaks", however, generating focal high shear stress, can induce transient expression of ICAM and VCAM (20).

At the end shear stress appears to commit a lot of functions, i.e. numerous genes, which, to say shortly, look like two opposite systems, but carefully balanced.

PATHOLOGY OF VESSELS IN RELATION TO SHEAR STRESS CHANGES

Arterial tree

Atherosclerosis could be considered as a geometrically focal disease (23). Plaques develop in regions where flow is low and cells able to adhere in stagnant and recirculation zones: outer edges of bifurcations, coronary ostiums and every place where deformation and dilation, even small, could appear. It is now known that cells are *entering such zone,* where flow is low and receptors gathering in clusters: monocytes, mast cells, lymphocytes causing the wall remodelling, through biochemical processes including TGF β1, metalloproteinases MMPs. Polymorphonuclears usually stick to the wall providing MMP 9. Platelets, as NO production is low in such areas, are also activated. At the end sub endothelial thickening correlates with low shearing areas: carotid sinus, abdominal aorta, vertebro-basilar junctions… The *coronary artery* exhibits a high propensity to plaque formation and, as its radius is small shearing forces become high at the upstream surface of the plaque allowing platelets to be thrown up on it, entering it and bringing about growth factor as PDGF. Or they give way to aggregates flowing away (24). This "SIPA" (shear-induced platelet aggregation) is platelet Glycoprotein-1-factor Willebrand dependent, but little is known of its exact role in vivo. *LDL entry* is managed by a sustained activation of SREBP-1 (*sterol regulatory element binding protein*) by disturbed flow, translocating the transcription factor domain into the nucleus leading to mRNA encoding for LDL receptor and HMG CoA synthase (25). As soon as a plaque deforms stagnant zones at the wall are established behind them, where circulating cells can margin and inflammation develop. Vasospasm and ischemia occur. Inside the plaques

LDL lipoproteins are oxidized by monocytes and accumulate, as well as tissue factor, highly thrombogenic.

Hypertension is characterized by an "endothelial dysfunction", which is investigated by checking endothelial dependent increase in arterial diameter by an ultrasound method following "increased reactive hyperaemia shear stress". This latter test fails in hypertensive and diabetic patients. As NO production is related more to the fall than to the elevation of shears (here pressure is increased), the disorder is likely to be due to excess of vasoactive products coming out of smooth muscle cells (26). Another explanation could be a defect in vasodilatation response to bradykinin and angiotensin II hyper production (27). Finally flow-mediated dysfunction in hypertensive subjects is more related to metabolic disorders than to abnormalities in shear stress, especially in diabetics and hypercholesterolemics.

Angioplasty is followed by remodelling and restenosis induced by flow-reduced and disturbed shear stress. In experiments managing changes in shearing conditions, mRNA of TGFβ1 and integrins have been found, able to act inside the wall (28). The actual effect of immunomodulators used on stents, clearly shows the role of cells, as lymphocytes, which have been attracted inside the wall.

Vein diseases and shear stress

In human being ankle pressure, owing to the upright position, is permanently high. Therefore two hydrodynamic processes are implemented in veins: a centrifugal intravascular pressure aiming at distension of veins and downward accumulation of red cells mainly in venulae (rheological disorders with huge red cell aggregates). This is partly counteracted by the arteriovenous reflex, the muscular pumps and the valvulae. But the situation is permanently bad…! The shear stress at the wall, in standing position, is low, around 6 to 20 dynes/cm^2, and decreases below such values in blood stasis after a short time, and this becomes permanent in chronic venous insufficiency (29). Obviously all conditions for adhesion and migration of circulating cells are very frequently present, leading to the *white cell trapping* theory of vein wall deterioration. Indeed, along to the ageing of people, thickening of vein wall develops leading to varicosities, so due to processes quite similar to atherosclerosis (30). The trigger mechanisms are *hypoxia* and presence of *areas of low shear stress* along the vein wall.

Microcirculation disturbances due to shear stress changes

Robin Fahraeus showed in 1931 that blood moves differently in micro vessels (31). Due to the decrease in diameter of micro vessels compared with the immediate upstream arterioles, the flow regimen changes, turning into a plasma layer flow (the Fahraeus phenomenom). On the *arterial side* of microcirculation, the blood is divided in two parts: a central line of red cells moving rapidly (high velocity) and a peripheral one

deprived of cells (empty plasma layer). The haematocrit is very low and so viscosity, condition which facilitates red cells entry into small vessels. As driving pressure remains high, shear stress is high (>40 dynes/cm^2), as it will remain also in capillaries (Table I). On the *venous side* of microcirculation a dramatic change occurs. The last capillaries pour out blood into venulae of wider diameter (feeding vessels) and where the output is very low. Therefore shear stress and shear rate reach the lowest values in vascular tree (1 to 5 dynes/cm^2) and red cells accumulate in aggregates; here also viscosity is high. This special situation of post capillary venulae allow this large amount of small veins, lined with active and potent endothelial cells, to set up major functions, mainly migration of leukocytes, haemostasis and inflammatory products.

Such areas of microvenulae are sensitive to changes of shearing conditions, starting from trivial conditions so simple as the standing up…In *pathological conditions of arteries*, heart failure, arterial thrombosis, ischemia and ischemia-reperfusion, the driving pressure becomes negative and occlusion of microvessels appears in many fields. At the level of skin the "density" (capillaroscopy) of capillaries diminishes; vasomotricity disturbances, platelet activation, excess of leukocyte adhesion and inflammation develop, mainly due to NO defect and oxygen reactive products. Shunts are opening, increasing permeability (oedema). During *venous diseases* such events can occur in skin, participating in *ulcer* generation. Hypoxia and low shear stress are the determining processes for an accumulation of white cells, which is the main fact (*white cell trapping*) (29)(31). Recently it has been shown that migration cells, as mast cells, bearing TGF β1 to fibroblasts and subsequent activation of MMPs, should be considered as the biochemical link between hemodynamic disturbance in leg veins and biochemical disorders involved in leg ulcer formation (32)(33).

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