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Problems in Blood-Tissue Reactions to Polymeric Materials

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SUMMARY

Intervention during circulatory insufficiency invariably produces certain basic changes in the blood itself and, as a consequence, in the functional aspects of blood-tissue exchange. Several categories of disturbances can be recognized: 1) Changes in the fluidity of the blood as a consequence of activation of blood coagulation mechanisms; 2) alterations in the physical properties of the red blood cells leading to abnormally high shear forces at the blood stream-vessel wall interface and to hemolysis; 3) inability of artificial systems to maintain integrity of vascular endothelium, and the blood capillary barrier in particular; 4) red blood cell aggregation and changes in the rheological properties of the blood.

The use of anticoagulants to avoid blood clotting leads to a loss in blood capillary integrity and suggests that it is necessary first to establish the factor in the blood clotting mechanism associated with normal permeability of the vessel wall. Macromolecules such as albumin are necessary for normal red blood cell structure. Osmotic and metabolic considerations should be carefully examined. It is suggested that mucoproteins may be useful in lining vascular protheses and in maintaining the smooth, nonwettable characteristics of vascular endothelium.

Of the many bodily functions, the never-ending circulation of blood through a closed system of arteries and veins lends itself especially well to physical analysis and to the application of engineering techniques. The design and behavior of the basic components of the cardiovascular apparatus, the heart, the elastic blood vessels, and the blood itself, have been intensively studied to the point where it has become possible to set up meaningful mathematical and physical analogs [1]. Natural structures have been replaced with man-made counterparts, and mechanical assist devices have been used to support the circulation of the blood during periods of emergency and surgery.

For the most part, intervention during circulatory failure has taken the form of assistance for the mechanical pumping of the heart, replacement of segments of large blood vessels and heart valves, duplicating the dialyzing function of the kidneys, and, in cases such as hemorrhagic shock, replacement of the blood with synthetic materials. Our own work has been directed for the most part at the transport and exchange functions of the circulation at the level of the microscopic vessels. Once the blood is distributed to the different organ systems of the body, the circulatory tree forms successively smaller subdivisions until thin-walled capillaries, some 8-10 μ wide and about 1 μ thick, provide access for the exchange of materials with the cells which make up the tissues proper. There is no doubt that many of the unanswered questions which confront the engineer in dealing with the circulation have their roots in disturbances at this level of organization.

All of these procedures are beset with certain basic problems in common (in terms of basic structure) involving the blood proper as well as the blood vessels.

1) Perhaps the most striking property of the blood is its tendency to undergo clotting when removed from the body. In the main, this stems from the exposure of the blood to abnormal surfaces and the disturbance of the set of checks and counterchecks that keep the potential conversion of fibrinogen to fibrin under control in the bloodstream.

2) A second set of problems arises from the difficulty of maintaining the viability of blood cells, in particular the red blood cells and the blood platelets. As is the case for other cells, the outer or plasma membrane of the red cell regulates the ionic content of the cell interior through active metabolic processes and thereby influences its physical properties [3]. Factors which interfere with cell metabolism lead among other things to alterations in the surface of the cell. As a result, the altered red cells

become much more susceptible to shear stresses at the vessel wall or at foreign surfaces. In addition they tend to form blood cell aggregates, which interfere with capillary perfusion and thereby with tissue nourishment, in the circulation.

3) The inner wall of the blood vessels consists of flattened endothelial cells whose state of health or viability is essential for maintaining optimal boundary conditions with the bloodstream [4]. In the capillary network proper, the mosaic of endothelial cells represents the barrier across which exchange occurs between the blood and tissue compartments. Here, the functional integrity of the endothelial cell is a major determinant of exchange.

4) The normal capillary wall permits the free passage of water and small molecular substances but it is relatively impermeable to the plasma proteins [5]. The capacity of this barrier to maintain its selective properties depends upon chemical factors present in the blood, and when they are absent in artificial perfusion solutions the capillary wall can be compromised to the extent that even blood cells penetrate into the tissues.

5) The end result of such abnormalities is an inadequate perfusion of the tissues, cell hypoxia, and extensive cellular destruction. In particular, the distribution of blood through the capillary network can be seriously impaired by the presence of red cell aggregates (so-called sludge), microemboli (platelet masses), increased leakiness of venules, and by changes in the rheological properties of the blood which in turn make the blood cells more susceptible to shear stresses [6].

It is the purpose of this report to discuss relevant aspects of each of these problems as a means of focusing attention on specific areas of fruitful research.

BLOOD FLUIDITY

Basic to most of the problems encountered in attempts to intervene in circulatory dynamics is the difficulty of maintaining the fluidity of the blood. The fluid portion of the blood is a complex mixture of salts and macromolecules which range in size from molecular weights of several thousand to several million. The cellular elements, which constitute some 40-45% of the total volume of the blood, are highly susceptible to changes in their environment. Unlike other fluids for which physical properties such as viscosity can be readily determined, the apparent viscosity or flow properties of the blood can change with time and with

varying conditions. The fact that the blood must flow through capillary channels, where the red cells are for the most part larger than the capillaries, has made it difficult to analyze pressure-flow relations in this all-important segment of the vascular apparatus.

During the continuous circuit of the blood through the vascular system, the propensity to clot is minimized by several factors including the motion of the bloodstream, the smooth surface of the endothelial lining, and the action of a fibrinolytic mechanicism – the plasmin enzyme system [7]. Materials which activate the clotting reaction can arise from different cellular elements (the leucocytes, the platelets, the red blood cells) and even from the endothelial cells lining the blood vessels themselves [8]. It is believed that under normal circumstances there is a balance between the small amounts of these chemical activators which are continuously generated and the capacity of the opposing plasmin system to destroy the fibrin. Outside of the body, however, even when blood is handled carefully it tends to clot unless chemical antagonists, so-called anticoagulants, are introduced. A neglected consideration is the fact that although anticoagulants maintain the fluidity of the blood, they in turn introduce other undesirable complications. It has long been known that patients who have a blood-clotting defect (hemophiliacs) or individuals subjected to whole body x-irradiation, who have a resulting deficiency in blood platelets and leucocytes, are prone to bleed through the minute venous capillaries and are continuously losing protein from the blood into the tissues [9].

There is good evidence that the normal impermeability of the vessel wall to proteins is in some way related to the blood coagulation system [10]. Thus, when an anticoagulant such as heparin is administered to animals in which the circulation is being observed through the microscope, it can be seen that the capillaries and small venules begin to lose protein and even red blood cells through defects in the vessel wall. This abnormality can be corrected by injecting substances that neutralize the anticoagulant. In other conditions, where capillary bleeding exists because of a platelet deficiency, the infusion of platelets or platelet extracts restores the normal permeability characteristics of the blood vessel wall. It is not clear whether the platelet factor acts directly on the vessel wall or operates through the repair of a blood clotting defect.

The terminal vascular bed is in a precarious state in animals in which blood clotting has been surpressed. When tissue blood flow is increased, the accompanying increase in transmural pressure in the small vessels leads to the rapid loss of plasma from the venous capillaries to the point where many of the vessels become impacted with solid masses of red cells.

Molecular fibrin is first laid down in monomer form and will with time tend to form chains of dimers, trimers, etc. The polymerization of fibrin will be considerably less in a rapidly flowing stream. The interface between the moving blood and the wall where the blood velocity is slowest is the point where fibrin formation is most likely to occur. Both the platelets and red cells contain chemical agents that accelerate the conversion of fibrin from its precursor fibrinogen, and these are the very cells that are subjected to abnormal stresses when handled extracorporeally [2].

Liproprotein-containing fragments of red blood cell membranes serve as a physical nidus for the attachment of fibrin molecules and in this way seed the formation of a thrombotic mass in the blood vessel [11]. In a similar context, damaged platelets form aggregates and serve to initiate thrombus formation [12]. As a consequence, a vicious cycle is set into motion which markedly distorts the rheological properties of the blood itself and soon disrupts flow through small blood vessels.

RED BLOOD CELL DETERIORATION

Attempts to support the cardiovascular system are hampered by the unremitting deterioration of the red blood cells themselves, as manifest by the appearance of free hemoglobin in the plasma. The red cell observed in the living circulation behaves like a fluid droplet surrounded by a flexible membrane. In vivo the erythrocyte assumes bizarre shapes under the small pressure gradients (5 to 8 cm H_2O) that drive the blood through the capillary vessels [13]. Under steady-state conditions or in a uniform pressure field the red cell quickly resumes its biconcave shape.

Despite the fact that the blood contains up to 45% of red cells which range in diameter from 8 to 10 μ, it behaves like a Newtonian fluid in the larger blood vessels [6]. The properties of the red blood cell are remarkable in that blood to which red cells have been added until they make up 85-90% of its volume still exhibits the properties of a fluid [14]. Cell-cell surface interactions must therefore be minimal. In the capillary bed proper, where the red cells circulate in single file, conditions are maximal for red cell-endothelial wall interaction. A thin layer of plasma is interposed between the red cell and the vessel wall proper and possibly serves as a lubricant [15]. It can be appreciated that changes in the physical properties of the red cell or in its surface will markedly influence the resistance to flow through the capillaries.

There are limits to which the red cell can be stretched and distorted

even within the bloodstream. This feature is illustrated during osmotic swelling of cells in hypotonic media where the biconcave shape becomes converted to a sphere, but where any additional swelling leads to hemolysis [16]. A similar situation develops when the cell is mechanically deformed with a glass microneedle. In the vascular system proper, part of a cell is frequently pinched or caught in a defect in the wall while the remainder is pulled out and deformed by the force of the blood stream. In cases where the blood flow is suddenly stopped by compressing the vessel with a needle within several minutes after the cell had become trapped, the deformed portion of the cell immediately resumes its original shape [4]. After being subjected to deformation for some 4-5 min, however, the cell remains permanently distorted and behaves as if it were a gelated structure.

Evidently the transfer of red blood cells from their normal plasma milieu to protein-free crystalloidal media will by itself alter the physical properties of the blood cells. It is interesting to note that the addition of small amounts of serum albumin to physiological salt solutions preserves the red blood cells for longer periods of time [17]. The amounts of albumin which are needed are much too small to operate by virtue of a purely osmotic mechanism.

Similar changes in the physical state of the red cell can be induced by mechanical compression and even by repeated washing in saline. Red blood cells that have been extruded through the vessel wall into the tissue proper from the bloodstream likewise show evidence of gelation after several minutes. It is probable that the extent to which the integrity of the red cell has been compromised contributes substantially to the difficulties encountered in attempts to provide mechanical support for the circulatory system. In most instances the presence or absence of hemolysis has been used as the indicator of the normal state of the blood cells. However, even microscopic inspection will not distinguish between normal and altered red blood cells. Differential centrifugation might reveal the percentage of different cells as in the case of young vs old blood cells.

Altered red cells can contribute to pathology in different ways. Most importantly, they undergo lysis more readily when subjected to shear stress during blood flow. The almost fluidlike character of the red cell under normal circumstances makes it possible for cells 8-10 μ in diameter to flow through capillaries as narrow as 4-5 μ. As the cell becomes less deformable, the flow properties or so-called viscosity of the blood will be shifted accordingly, and it becomes increasingly difficult for the blood cells to enter into the small capillary branches [18]. Changes in the cell surface will lead to cell aggregates and increase the shear stress at the wall of the vessels.

Red cell destruction liberates cell membrane fragments which initiate fibrin formation and form a nidus for microthrombi.

RED CELL AGGREGATION

There is considerable evidence that the red blood cells flow freely in the circulation as separate entities even when they are closely packed as in the large blood vessels. The cells may be attracted to one another and form stacks or rouleaux although such formations are temporary [10]. Current evidence indicates that the red blood cell surface is coated with a muco-protein, rich in the polysaccharide sialic acid [20]. The white blood cells or leucocytes are additionally coated with sugars which impart certain chemical or immunological properties to the cells. Red blood cells normally flow smoothly over one another or over the surface of the blood vessel lining. When carbon particles or other visible colloidal materials are injected into the bloodstream, they do not adhere to the surface of the red cell.

The polysaccharide component of the red cell surface is presumably synthesized by the cell cytoplasm. Mammalian cells in general seem to be enclosed in a thick husk which contains a glycoprotein [21]. Cells which normally are nonadhesive stick to one another and to other surfaces in the presence of divalent cations. Basic cationic polypeptides also have the property of inducing surface adhesiveness [22]. Protein-polysaccharide complexes are frequently encountered in living systems, especially at interfaces. Their precise role in such systems remains to be established. In the case of the red blood cell, factors which interfere with the rate of degradation and replacement of substances such as sialic acid can affect the cell surface and lead to abnormal cell-cell interactions.

Direct examination of the circulation in different disease states and in various traumatic conditions reveals that the red cells frequently flow in clumps as discrete aggregates [23]. Various explanations have been advanced to account for this phenomenon with the concensus favoring either some change in the surface of the cell directly or the adsorption of a material to the cell surface. Red blood cells show an especially strong tendency to form aggregates in artificially maintained circulations and in situations where synthetic agents have been used to expand the blood volume [24].

Under conditions where there is an increased tendency for the blood to undergo coagulation in the intact circulation, microthrombii that

contain red blood cells are formed. Initially such thrombii are white, that is, they are made up of platelets and white blood cells enmeshed in an amorphous precipitate. Early workers believed the mass to be made exclusively of fibrin. However it has been shown that similar aggregates can develop in the presence of anticoagulants. Red blood cells become entrapped only secondarily in such masses. In other situations, the surface of the red cells appear to have been changed and the cells aggregate or clump readily under conditions of slowed flow. Among the many conditions which give rise to such red cell aggregates are the injection of macromolecules such as dextran or PVP. The dextran effect appears to be related to the molecular weight of the polymer; dextrans with higher molecular weights have a greater propensity to induce aggregation [24]. In a similar context, the injection of dextran actually increases the blood flow in abnormal states where cell aggregation is suspect as an important factor contributing to the deficient tissue blood flow. Information is needed concerning the mechanism by which such polymers act to affect cell aggregation.

ENDOTHELIAL LINING

The key interphase in the circulatory system is the intimal or lining surface of the blood vessels. Here again some uncertainty exists as to the nature of the lining material. Evidence has been presented that the surface is a fibrin film [25], presumably not in fibril form since the characteristic cross-linking of fibrin is not seen in electron micrographs; other workers believe the endocapillary lining has a polysaccharide component [26].

If the proper surface is not maintained at the boundary layer between the vessel wall and the blood, the resulting shear forces may damage the endothelial cell proper. Under normal conditions the thin layer of mucoprotein or polysaccharide on the endothelial surface will serve as a buffer and dampen out the shear forces developed by the moving blood. The statement is made that the lumen surface of the vessel is nonwetable with respect to the blood, thereby minimizing any direct interaction between the two. The precise physical attributes of the vessel lining remain to be defined. Most of the materials used in prostheses to replace lumenal surfaces have been designed to be chemically inert. Nonetheless they permit the deposition of fibrin and other plasma proteins and in fact are not ideal interfaces for the blood stream.

BLOOD-TISSUE FLUID EXCHANGE

Although the microvasculature which separates the blood and tissue proper is freely permeable to water, the volume of blood in circulation is maintained at a constant level. The actual exchange of water is achieved by diffusion along a concentration gradient and by hydronamic or bulk flow as a consequence of a shifting of pressure forces generated by the actual driving pressure of the blood and the colloid osmotic pressure (c.o.p.) of the plasma proteins. The hydraulic pressure is modified by the resistance encountered in the small blood vessels, and it has been found to be slightly higher than the plasma c.o.p. on the arterial side of the tissue network and slightly below that of plasma c.o.p. on the effluent or venous side of the tissue. In this way a balance is believed to be maintained between the tendency of the hydraulic pressure to filter fluid out and the inward movement of water from the tissues because of the higher plasma c.o.p. [27].

In most in vitro systems involving artificial assist devices, little attention is paid to the maintenance of proper osmotic pressure relationships. For example, many artificial blood substitutes may satisfy osmotic requirements on the basis of molecular calculations but because of their inability to maintain the permeability characteristics of the capillary wall, they allow the macromolecules to escape and thereby lead to fluid loss into the tissues.

Normally, the capillary may retain molecules above a molecular weight of about 10,000. When isolated structures are perfused for physiologic studies, the blood is replaced by an artificial or synthetic mixture. A common organ for such studies is the hind limb of an animal. With artificial mixtures, the leg begins to swell despite the presence of macromolecules in the proper concentration to ensure isotonicity. When about 10% by volume of plasma is added to such perfusion mixture, the swelling, or edema, is halted and fluid can even be withdrawn back from the tissues into the perfusate [27]. Here again biological macromolecules have the capacity to affect the properties of the capillary membrane proper so as to maintain an impermeability to such molecules.

Organ transplantation has been hampered by the inability to maintain suitable donor tissues for sufficiently long periods in an organ bank. Much of the work concerned with the preservation of organs for transplant procedures has been directed toward mechanically pumping a given volume of flow, the maintenance of high oxygen tensions, and the use of subnormal temperatures to reduce the metabolic demands of the exteriorized tissues. Not enough attention has been given to the duplication of key

biological properties of blood that are essential for the integrity of the small blood vessel wall relative to its function in the exchange of materials between the blood and tissue compartments. As has been pointed out, the fluidity of the blood is maintained by anti-coagulant chemicals, a feature which, by itself, will compromise the integrity of the capillary wall. It would seem worthwhile to devise techniques for handling blood without anticoagulants, or better yet to determine what factors in the coagulation process are involved in "waterproofing" the small blood vessel wall.

A lead in this direction is seen in experiments where blood platelets or platelet extracts have been added to perfusion media and greatly reduce the edema in such organs. The kidney has been particularly difficult to maintain in vitro; excessively high pressures are needed to perfuse its vascular bed, the organ swells, and after 24 or 48 hr large areas can no longer be sustained and the tissue deteriorates. The simple addition of blood platelets has enabled workers in Boston to keep such organs viable for at least 6-7 days on an artificial pump. It would represent an achievement of major significance if the properties of the macromolecules in blood platelets responsible for this permeability effect could be defined and attempts were made to duplicate this property with synthetic macromolecules. The tendency for the walls of blood capillaries or venules to become leaky is associated with a separation of the individual endothelial cells which line the vessels [28]. Presumably, the restoration of a more normal state would be achieved by causing the cells to adhere more strongly to one another.

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