A DISCUSSION ON ASPECTS OF CELL-SURFACE ADSORPTION PHENOMENA

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Some Sorption Phenomena at Mammalian Cell Surfaces

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Although the nature of the cell surface is of obvious importance in any discussion of sorption phenomena it is not proposed to consider this in detail here (see Weiss, 1962*b*). In the present discussion 'cell surface' means simply the first part of the cell with which the adsorbend comes into contact. Concepts of the surface region are not clear, partly because of the difficulty in deciding where environment ends and cell surface begins. Functionally the cellular microenvironment should be considered as part of the cell surface, which implies that in the case of free cells, adsorption may initially be to an ionic 'cloud' or to water. For quantitative work on surfaces it is often necessary to have some idea of surface area. It is, however, quite impossible to estimate the true surface area of living mammalian cells partly because of the presence of irregularities such as microcrenations, microvilli and possibly the endoplasmic reticulum, which may increase cell surface area by many orders of magnitude without being reflected in measurements of cell diameters or volumes. It will be pointed out that surface shape may affect some adsorption phenomena; this presents analytical difficulties in that the free edges of living cells are continuously moving and changing shape.

Cells carry a net negative charge at their surfaces due to ionogenic surface groups and to ion-induced dipoles. The approach of two cells in a fluid medium, and the

adsorption of one by the other may therefore be considered initially in terms of energies of attraction and repulsion between two charged particles. A useful discussion of the physical chemistry of cell adhesion is given by Pethica (1961). Two cells approaching each other in a 'physiological' environment can be regarded as being attracted by a potential energy of attraction V_A , due to dispersion forces where

$$V_A = \frac{-Aa}{12x} \quad (\text{Verwey \& Overbeek, 1948}),$$

A is a constant which depends on the nature of the cell surface; a is the radius of the approaching surfaces and x is the distance separating them. Other attractive energies at this range of separation are thought to be of minor importance.

As the two cells get closer together (approximately 20Å) they are separated by a potential energy (repulsion) barrier, V_R , mainly due to ionic double-layers, where

$$V_R = \frac{1}{2}Da\psi^2 \log_e (1 + e^{-Kx}),$$

D is the dielectric constant of the medium; a is the radius of curvature of the relevant parts of the cell surfaces which are separated by distance x ; $1/K$ is the thickness of the ionic cloud and ψ is the surface potential. Without attempting to fit real values to this equation it can be seen that the repulsion barrier which tends to prevent adsorption is reduced when the radius of curvature (a) of the approaching cells becomes less, and similarly with the surface potentials (ψ) and the depth of the ionic (Debye-Hückel) cloud ($1/K$).

Bangham & Pethica (1960) have made the suggestion that the approach of cells via pseudopodial projections of low radius of curvature may represent a method whereby cells may penetrate the repulsion barrier to make contact. The properties of living and dead cells may differ in respect to this.

Reducing the surface potentials on erythrocytes results in agglutination, and has been described by Coulter (1920) and others.

The potential ψ in a liquid medium surrounding a surface with a potential ψ_0 decreases exponentially with the distance. $1/K$, the Debye-Hückel mean double-layer thickness, is that distance at which

$$\psi = 1/e \cdot \psi_0$$

$$\frac{1}{K} = \sqrt{\frac{DkT}{8\pi n e_1^2 V^2}} \quad (\text{Glasstone, 1948})$$

where n = number of ions per ml.; e_1 = the electronic charge; V = valency of ions; D = dielectric constant of the medium and k = Boltzmann's constant. Therefore $1/K$, and hence the repulsion barrier to cell contact, can be reduced by increasing ionic strength and by introducing multivalent ions into the environment. The ionic double layer in relation to the erythrocyte has been discussed by Seaman & Heard (1960) and adsorption of viruses to a variety of surfaces has been affected in a manner predicted from this equation by altering these two variables (Valentine & Allison, 1959).

The systematic study of ionic effects is technically difficult with mammalian cells, since cells immersed in solutions of non-physiological osmolarity and chemical constitutions may not be considered normal in some respects. Wilkins, Ottewill & Bangham (1962) attempted to study flocculation in leucocytes in low concentrations

of sodium chloride, by maintaining the physiological tonicity with the non-electrolyte sorbitol. However, Pulvertaft & Weiss (to be published) showed that with some cells sorbitol may induce massive ingestion of fluid leading to gross vacuolation. Whether or not such cellular trauma inflicted during observations affects their validity is a difficult point to settle.

Another factor preventing cell contact in the 20 Å range may be solvation barriers around cells. There is no doubt that these zones can exist, but opinion is divided on their existence at the cell surface. If both ionic clouds and solvation barriers exist in this region, it might be expected that in environments of 'low' strength repulsion is primarily due to ionic clouds, and in environments of 'high' ionic strength (i.e. 'physiological' and higher) repulsion will be due mainly to solvation barriers.

In systems exhibiting contact phenomena, cells almost always seem capable of overcoming the various barriers before any evidence of biological specificity is seen. From this it is concluded that the initial contact between cells is non-specific, and that the physical bases for biological specificity in these circumstances are not to be found in considerations of forces operating over long and medium ranges.

After overcoming the obstacle of the repulsion barrier two cell surfaces can approach to less than 5 Å separation. At this range the most powerful attractive forces binding surfaces are primary chemical bonds having energies of the order of hundreds of kilocalories per mole. It should be noted that, *in vitro*, most mammalian cells will adhere strongly to the surfaces of pure gold, tantalum, platinum and polytetrafluorethylene (Weiss, 1962*a*) where primary chemical bonding appears unlikely. Other short range attractive phenomena include hydrogen-bonds and coulombic attractions.

Attempts have been made to 'explain' specific adsorption in terms of these various forces, relating them to chemical groups bearing particular spatial relationships one to another. When enzyme adsorption is considered, the work of Bangham & Dawson (1959) is of interest since it suggests that the positioning of certain enzymes at substrate surfaces, which is reflected in the magnitude of enzyme/substrate interaction, is dependent on the surface charge of the substrate among other factors. 'Fine' positioning of antibodies adsorbed on the cell surfaces may also be charge-dependent. Estimates of surface charge obtained from measurements of the electrophoretic mobility of various mammalian tissue cells show that this may vary appreciably with the growth rate of the cells (Heard, Seaman & Simon-Reuss, 1961; Eisenberg, Ben-Or & Doljanski, 1962). It seems conceivable that the final positioning of a closely approaching adsorbent to a cell surface could be brought about by internally regulated changes in surface charge. The specificity of much of the adsorption process in the case of tissue cells in a physiological environment is perhaps questionable, since *in vitro* tissue cells will adhere (adsorb) to a very wide range of non-toxic surfaces (Weiss, 1962*a*).

The separation of material from a cell surface may result from a reverse process to adsorption, in which case the term desorption may properly be used. On the other hand, surface material may be lost from cells, when the plane of separation is not the same as the plane of adhesion or adsorption between the adsorbent and cell surfaces. If cells growing on glass *in vitro* are detached from these surfaces, by shearing pressures transmitted through their fluid environment (Weiss, 1961*a*),

separation occurs by means of non-lethal surface rupture of the cells (Weiss, 1961*b*), and the cells leave species specific surface antigen behind on the glass (Weiss & Coombs, 1962). From these and other observations (Weiss, 1962*a*) it has been suggested that the active or passive separation of cells from cellular or non-cellular surfaces is accompanied by surface rupture.

In vivo, cells move actively through tissues by 'amoeboid' movement. Whatever propulsive mechanism is responsible for this type of movement, it is essential that some sort of adhesion or friction exists between the moving cell and the surfaces over which it moves. As cells are not infinitely extensible, amoeboid movement must be accompanied by making and breaking of cellular contacts with a surface. It has been suggested that breaking such a contact is not a 'desorption' of a cell (process) from a surface in the sense that it is a separation along the cell/surface interface, but that contacts are broken by rupture in the surface of the moving cell, or the surface over which it moves, or both. It can thus be visualized that active amoeboid movements of cells within tissues are accompanied by two-way exchange of surface materials, for which the term 'epipheryocytosis' has been proposed (Weiss, 1961*a, b*). It has also been suggested that this may result in exchanges of surface antigens, antibodies and other 'information' during round-cell infiltration for example.

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