

Hypothesis: The epidermal permeability barrier is a porous medium

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The stratum corneum is a complex biological material characterized by very low permeability to water and most other molecules. This material may be thought of as a ‘porous medium’ composed of impermeable and permeable regions. Intercellular lipid membranes in the stratum corneum are postulated to exist in a mixture of two phases: solid (i.e. impermeable) and liquid crystalline (permeable). The corneocyte envelope is classified as impermeable. Diffusion mechanisms of solutes within, across and between the intercellular lamellae are discussed. This model represents a refinement of previous theories about the physical structures responsible for the low observed permeability of the stratum corneum. Key words: Stratum corneum; percolation.

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Our purpose here is not to review the extensive literature on the organization of mammalian stratum corneum and diffusion within it, but rather to propose that the stratum corneum is a “porous medium”, and that passive diffusion within and across it may be considered to be “percolation”.

POROUS MEDIA AND PERCOLATION

The study of this field has general application in real life (e.g., oil and gas extraction) and the theory is quite complex (1). A porous medium may be described as consisting of two phases, a solid or impermeable space and a permeable or pore space. In the simplest case, flow of material within the medium can only occur within the pore space. Common examples are cement, sand, and gel filtration columns, and porous media may also exist on vastly different scales (oil fields, semi-conductors).

If the interface is ignored, any point in the medium is in either the pore space or impermeable space. Connected points within the pore space form a *porosity*, and a porosity which connects two surfaces of a finite porous medium is said to be *percolating*. Thus, flow between these two surfaces can only occur through percolating pathways. If the pore space is occupied by a stationary material (i.e., one that does not flow), then the movement of a solute within the pore space can be described by diffusion, and the flux of solute across the medium will be strongly influenced by the architecture of percolating pathways.

We note at the outset that the existence of *aqueous pores* (continuous pathways containing water) within the stratum corneum has been debated. We believe such pathways do *not* exist under physiological or even most pathological conditions, but whatever the reality, our use of “pore space” is to be distinguished from such aqueous channels. As will be

discussed below, our concept of a percolating pathway across the stratum corneum with respect to, say, water, is that of interconnected regions of fluid intercellular lipid membrane and permeable associated interbilayer space, as proposed by Forslind (2).

BIOLOGICAL MEMBRANES AS POROUS MEDIA: 1972 AND ALL THAT

Singer and Nicolson (3) proposed the “fluid mosaic” model for the physical organization of biological membranes. Although much modified over the intervening time (e.g., (4)), the basic idea is that membranes resemble liquid crystals in which individual molecules are restrained in one dimension, but free to diffuse in the other two. Such diffusion may be lateral (mean squared displacement per unit time) or rotational (characterized by frequency of revolution). Other motion on longer length and time scales (say, undulation) may be of importance in cellular events such as fusion, endocytosis, and intracellular transport (5), but are ignored in the present discussion. The vast majority of biological membranes have been thought to approximate an “ideal” mixture, in which all molecular species (including membrane proteins) diffuse within the plane of the membrane at rates determined by their own physical properties. Lipid composition may be an important determinant of such diffusion: for example; contrast the more “fluid” mitochondrial membranes and the more viscous myelin. This problem is part of the larger question of “lipid diversity” among biological membranes (see for example, 6).

In model membranes (dispersions of membrane type lipids in aqueous media), modes of physical organization (“phases”) other than the liquid crystalline bilayer can be demonstrated. These vary from bilayers which are not liquid crystalline (“gel”, “crystalline”) in which there is severe restriction of lateral and rotational diffusion, to “non-bilayer” phases which may be important in membrane events such as fusion (7). Under certain conditions (of say, temperature) transitions occur between phases, during which there will be a “phase coexistence” region. In the case of bilayers, such a phenomenon may result in the coexistence of “fluid” (i.e., liquid crystalline) and “gel” (more solid) in an unknown architecture. Mouritsen and his colleagues (8) have simulated the existence of solid and liquid regions occurring within a model phospholipid membrane in the phase coexistence region near the main transition temperature. Such solid regions offer obstructions to diffusion within and across the membrane.

DIFFUSION IN THE PLANE OF THE MEMBRANE

For amphipathic molecules such as membrane phospholipids, diffusion in the plane of the membrane has been shown to depend on the proportion of solid phase lipids, and a

“percolation threshold” (i.e., the proportion of solid phase lipid required to prevent continuous diffusion) has been observed (9). In a phospholipid/cholesterol system, adding cholesterol (up to ~20 mol%) affected the phase architecture and percolation threshold (10). Obstacles to lateral diffusion need not be “solid” lipids of course, and might consist of arrays of membrane proteins (e.g., desmosomes, tight junctions). As proposed by Forslind (2) for the SC intercellular membranes, obstructions would indeed be presented by solid phase lipids, and there is evidence for such solid domains in these membranes that is, perhaps, more convincing than for any other mammalian membrane yet described (e.g., 11,12).

We point out that some drugs commonly applied to the skin (corticosteroids, retinoids) have an amphipathic quality, and that a considerable portion of their diffusion path may be within the plane of SC intercellular membranes, and therefore susceptible to obstruction.

TRANSBILAYER DIFFUSION

In the absence of convincing evidence that there are continuities of membranes across the SC (which would imply that aqueous channels might exist), diffusion *across* intercellular bilayers must be a significant part of all passive diffusion. There is evidence that such transbilayer diffusion of water (to choose an obvious example) is determined by the physical state of the component lipids, and in particular that gel-state membranes are much less permeable than those that are fluid (13). Thus, for water diffusing across an array of stacked bilayers (e.g., SC intercellular membranes or multilamellar liposomes) having co-existing fluid and solid regions, the membrane component of the diffusion path could be imagined to be a porous medium, with the “solid” lipids being impermeable space, and the fluid lipids being the “pore space”.

An argument may be made that the co-existence of gel and fluid lipid bilayer phases should result in increased permeability due to structural defects in the membrane. However, Carruthers and Melchior (14) found that this was not always so. In particular, the addition of cholesterol to a binary mixture of a sphingomyelin and dioleoyl-phosphatidylcholine (a phosphoglycerolipid) eliminated any abrupt increases in water permeability near the melting temperature of sphingomyelin. Thus the permeability characteristics (including the existence of defects) of complex membranes containing cholesterol should not be assumed, even when phases co-exist, and cholesterol may in fact act to “seal” phase boundaries (15).

For amphipathic molecules (e.g., membrane lipids) transbilayer diffusion (“flip-flop”) occurs in fluid lipids on a time scale very much slower than water, and is dependent to some degree on the characteristics of individual molecules. Such a phenomenon has been described for ceramides (16), in addition to other more typical membrane lipids.

INTERBILAYER DIFFUSION

In model membranes, aqueous interspaces between concentric lipid bilayers are thought to allow free lateral diffusion of water, although water associated with lipid headgroups is less mobile (17). Such spaces, typically on the order of 3 nm for

phosphoglycerolipids with or without cholesterol, are probably maintained by short range repulsive forces associated in part with the polar headgroups (see for example, the discussion by Israelachvili (18)). In the case of SC intercellular membranes, such headgroups are extremely small, the largest being cholesterol sulphate (present in very small molar quantities in normal mammalian stratum corneum). For the three most common classes of lipids found in SC intercellular spaces (ceramides, cholesterol, and free fatty acid), it is not clear what repulsive forces may exist between adjacent membranes. If the fatty acid carboxyl group is protonated at pH < 6.0 (reported to exist in SC intercellular spaces), then apposition close enough for hydrogen bonding of lipids in neighbouring lamellae may occur (perhaps via a small number of water layers), with the resulting exclusion of freely diffusing interbilayer water. We therefore suggest that unimpeded diffusion of water laterally between SC intercellular lipid sheets should not be assumed, and such diffusion may in fact be very restricted. Finally, the other properties of SC interspaces themselves are not well known; e.g., water content, hydrogen ion concentration, presence of protein, etc. Obstructions other than hydrogen-bonding of apposed lipid headgroups may occur.

Thus, the passive diffusion of molecules through the SC interspaces must be quite complex, and composed (at a minimum) of diffusion in the plane of the membrane, transbilayer diffusion, and interlayer diffusion.

STRATUM CORNEUM VIEWED AS A POROUS MEDIUM

A great deal of work has been done to define the nature of the mammalian epidermal permeability barrier. The original formulation of the problem by Lane and Blank in their poster at the American Academy of Dermatology meeting in 1946 (Figure 1) remains unchanged, and postulates three pathways for passive diffusion across intact mammalian epidermis: (1) between the cells of the stratum corneum (intercellular pathway), (2) through the cells of the stratum corneum, and (3) through appendages such as hair follicles and sweat glands. Although the relative importance of these routes in various applications remains the subject of discussion, there is consensus that in mammalian interfollicular epidermis, the major part of the permeability pathway is *between* the cells of the stratum corneum, and requires the presence of the component lipids, particularly ceramide (e.g., 19-25).

Stratum corneum is obviously a heterogeneous material. From the perspective of the diffusion of solutes however, the porous medium model would (in the simplest case) divide the SC into two domains, the pore space and the impermeable space, with the solubility of a given solute in the impermeable space being zero. The physical counterparts of these conceptual domains would also be heterogeneous; for example, the possible pore space for water would include fluid regions of intercellular membranes, and associated interlayer spaces in which water can diffuse. Similarly, the impermeable space might include the corneocytes (particularly the cellular envelope), regions of “solid” lipid in intercellular membranes, and any interlayer regions not allowing free diffusion of water (such as interdigitation of apposing hydrogen-bonded headgroups). Thus passive diffusion

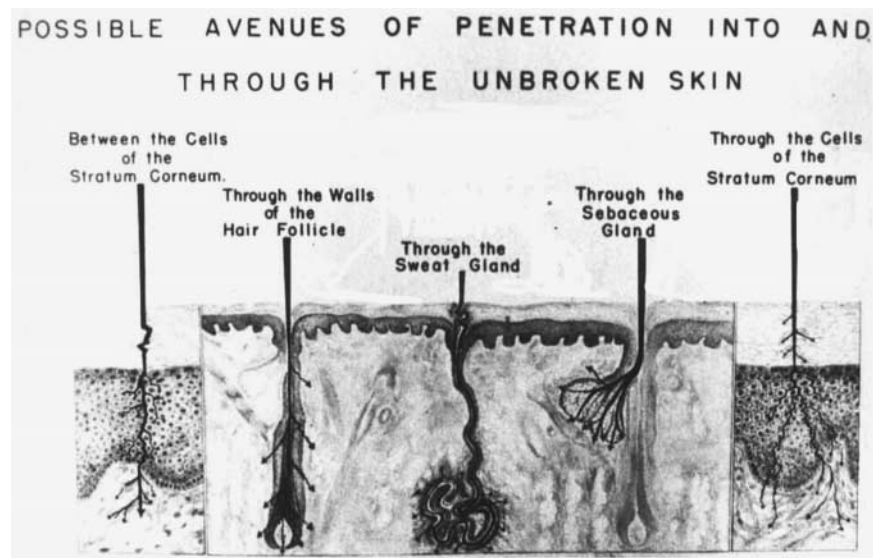


Fig. 1. Reproduction of illustration from Lane and Blank's 1946 poster at the American Academy of Dermatology, "Vehicles, Their Physical and Physicochemical Action On The Skin". Photo kindly supplied by Dr. Irwin Blank.

across and within the SC would depend on the architecture of the percolating "pore space" (which may differ for different solutes; e.g., water vs. hydrocortisone). This is essentially an extension of the "tortuous path" hypothesis of Potts and Francoeur (26), in that the impermeable space is proposed to be heterogeneous both on cellular and subcellular length scales, and to consist of elements additional to impermeable corneocytes (Figure 2).

IMPLICATIONS

In our view, the major gain to be made by considering the SC to be a porous medium is the possibility of linking structure with function. From this perspective, the *physical organization* (particularly porosity) of the SC is the direct determinant of its barrier properties. Thus, relatively small changes in the lipid composition, temperature, water content, pH, ion composition etc. that affect the arrangement of solid and fluid lipid domains, may have disproportionately large effects on permeability. We suggest that in psoriasis for example, the shorter turnover time of epidermis would tend to reduce the proportion of solid (vs. fluid) lipid, and therefore increase permeability. Similarly, the increased proportion of glucosylceramides in the sphingolipid fraction of the SC in the mouse Gaucher model (27) would be expected to increase the proportion of fluid (vs. solid) lipid (28) therefore and also increase permeability. Diverse alterations in formation of the SC might account for variations in permeability among anatomical regions, species, and pathology. An analogy might be the relationship between the composition of feathers (another adaptation of epidermis) and the physics of flight: knowledge of the composition of feathers, or even the gross structure of feathers, cannot be used to explain flight, which is more complicated.

What is needed is a means of examining the "pore space" within the SC (and its various models), and relating this to diffusion. One possible approach is the so-called "pulsed field gradient-spin echo" nuclear magnetic resonance technique which has allowed the determination of pore sizes and connectivities in various porous media. For example, in an aqueous sample of packed monodisperse polystyrene beads of

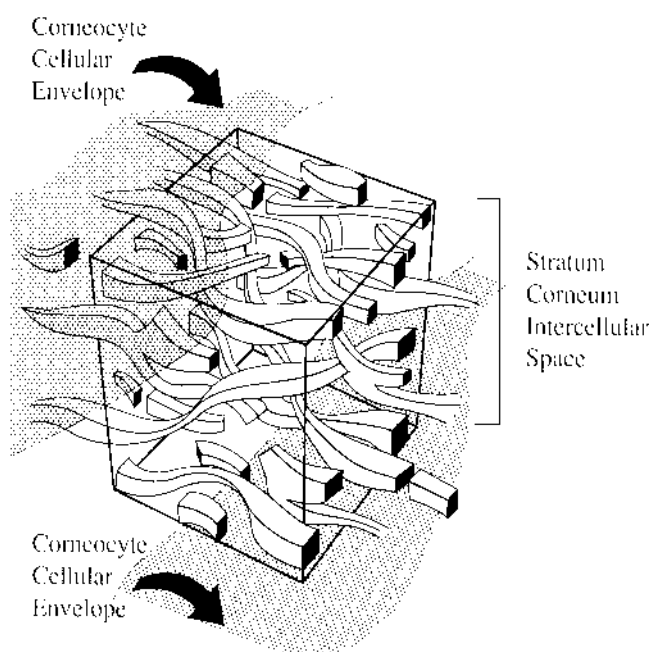


Fig. 2. Cartoon to show the concept of percolating (continuous) and non-percolating (interrupted) pore space through the SC intercellular space. Note that such pore spaces would be composed of more than one biological material. The model assumes completely impermeable corneocytes.

known size, it was possible to measure pore size, bead size (with standard deviation), and the effective diffusion coefficient of water (29). The theoretical basis and technical details of this method are obviously not relevant to our discussion here, but interested readers are referred to the work of Callaghan and colleagues, and references therein (30). However, the validity of the approach has been demonstrated both for water (31) and phospholipids (32) in phospholipid model membranes. Our goal is to apply these methods to sphingolipid dispersions modelled on the SC intercellular membranes, and eventually to the tissue itself. We suspect that not only molecules of interest present in the barrier under

physiological conditions (e.g., fatty acids, water), but also those applied from the external environment, may be susceptible to such analysis.

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