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THE STRUCTURES WHICH LIMIT THE PENETRABILITY OF THE SKIN

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A lecture delivered before a Joint Meeting of the Surface Activity Group, Society of Chemical Industry, and the Society on 20th November 1961.

Evidence is presented that the penetration of substances applied to skin is a physical process, independent of metabolic activity. Penetrants probably pass through the epidermis, not its appendages. The barrier to penetration is the keratinised cell matrix of the stratum corneum, assisted by the surface lipids. Ions probably penetrate around the keratinised cells, not through them.

One of the main functions of skin is to prevent the loss of water from the body and the entrance of extraneous chemicals. In mammals this function is nearly perfect and the rate of movement of substances through the skin surface is very slow. The loss of water through non-sweating skin (insensible water loss) is readily measured by the increase in mass of a water absorber in the air above the skin. It has often been studied in the last twenty years, and the factors affecting it are now reasonably well-known[1-5]. The inward movement of extraneous chemicals is not so easily measured, because the mass of tissue into which the penetrants move is so large that they have to be measured at very low concentrations. Interesting qualitative, or

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semi-quantitative, observations have been made with the techniques of histochemistry[6], autoradiography[7] or auto-assay[8] (i.e. detection of the penetrant by the reaction of the animal) but true quantitative data were lacking until the advent of radio-isotopes, which can be measured at the necessary low concentration.

When a chemical is applied to the skin it penetrates slowly at first ; the penetration rate then rises, approximately exponentially, to reach a steady level (*Fig. 1*). This steady rate is directly proportional to the concentration of the chemical in the solution applied to the surface of the skin[9], so that the

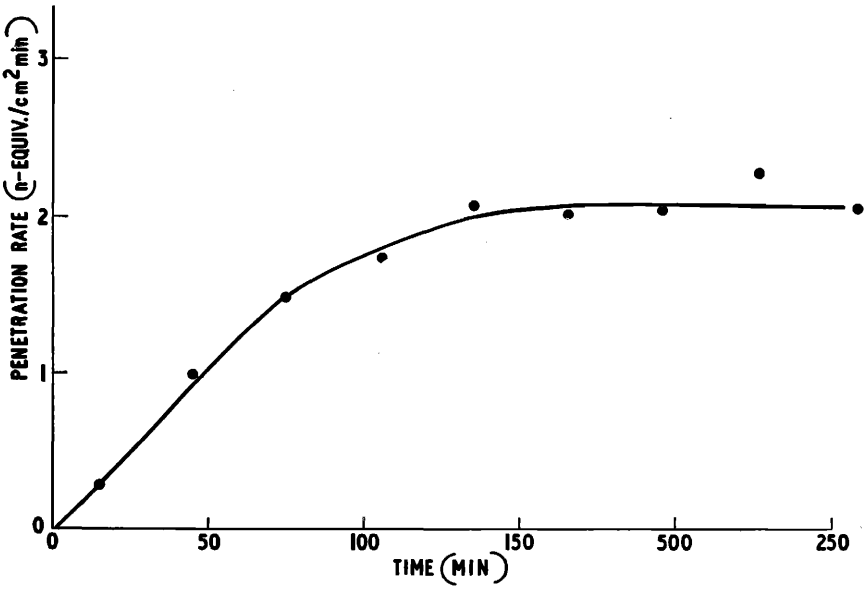

Figure 1. The penetration of sodium ions through excised pig skin at 35°C from an isotonic solution of sodium chloride applied to the epidermal surface.

permeability constant (penetration rate/applied conc.) defines the permeability of skin of a particular species to a particular chemical. The permeability constant of human, pig or rabbit skin to ions is not affected by the presence of the metabolic inhibitors—azide or iodoacetate ion, nor by the storage of excised skin for several days. Similarly, insensible water loss is not critically dependent on the condition of the skin[2,10]. The large resistance to diffusion of skin is therefore apparently a physical property of the tissue, independent of its metabolism.

There are three parallel paths in the skin through which chemicals might diffuse : The epidermis, and the accessory structures that pierce it, the hair follicles, and the sweat glands. The latter are present over the general body

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surface only in man, not in the usual experimental animals (rabbit, rat, pig, guinea-pig). Even in man they are probably unimportant as a route of penetration, for the palm skin is only one-third as permeable to iodide ions as the forearm skin[11], although it contains several times as many sweat glands[12]. In the relatively unhairy species, pig and man, the hair follicles are also probably unimportant, for they cover only a small fraction of the area of the skin, and in the pig are only as permeable to tri*n*butyl phosphate as the epidermis per unit area[13]. In these animals, penetration is probably nearly all through the exposed epidermis. In more hairy mammals so large a fraction of the area is covered by hair follicles that this argument does not apply and much of the penetration may be via them. This does not imply a different mechanism of penetration, for the walls of the hair follicles are composed of an epithelium continuous with the epidermis, and very similar to it in structure.

The stratum corneum consists of several layers of dead cells flattened into plates of denatured protein. Clusters of these cells can be removed by sticking adhesive tape to the skin and pulling it off again. If this process is repeated ten to twenty times, nearly all the dead cells can be removed from the surface of pig or human skin. Skin with the dead cells removed is approximately an order of magnitude more permeable than normal skin[14,15]. The dead cells sometimes come away from human skin on to the tape as an

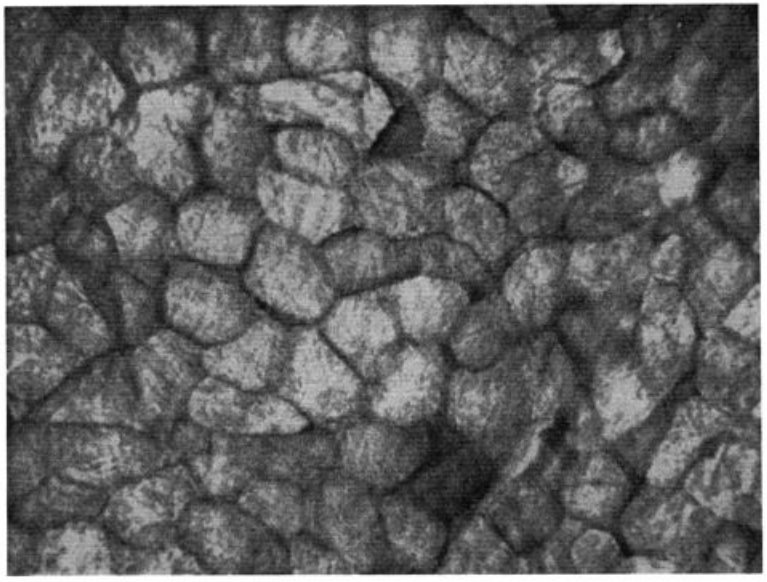

Figure 2. A layer of stratum corneum cells, stained with iron haemotoxylin. This is the innermost layer of a stratum corneum membrane[16] taken from the dorsal surface of the human forearm. Magnification x 500.

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intact membrane, representing a large part of the stratum corneum[16]. The membrane can be removed from the adhesive tape with a latex solvent. It is as impermeable to tri*n*propyl phosphate as whole skin[17]. Both these facts suggest that the diffusional resistance of the epidermis is within the stratum corneum.

The stratum corneum contains ten or more cell layers. The cells of each layer form a crude hexagonal lattice (*Fig. 2*) with the edges of adjacent cells interdigitated[18]. The cells of adjacent layers are connected by small intercellular bodies (desmosomes) of considerable mechanical strength[19]. The cells have an osmiophilic border, suggesting some form of cell membrane (*Fig. 3*). In sections prepared for electron microscopy there is a definite

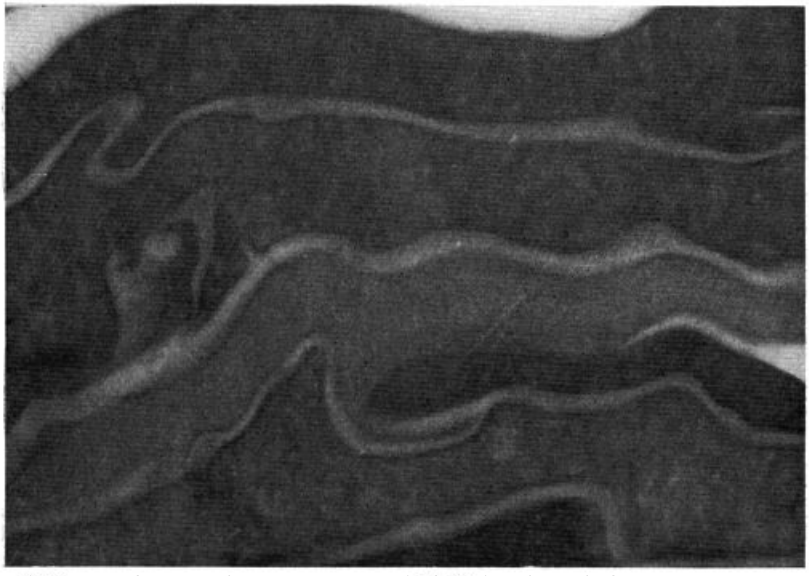

Figure 3. Electron micrograph of a vertical section through the stratum corneum of human thigh skin. Note the osmiophilic borders of the cells, the variable density of the cell contents, the interdigitation of the cell processes, and the intercellular bodies (poorly preserved in this section). Magnification x 20,000.

intercellular space of the order of 100 Å wide. Apart from the denatured protein, which makes up about half the weight of the cells, the stratum corneum contains large amounts of free aminoacids (10-20%) and a variable amount of lipid (2-20%)[20].

The clusters of cells torn off with adhesive tape are only a few cells thick, not the full depth of the stratum corneum[21]. Each stripping with adhesive tape produces an increase in the permeability of the skin to ions[22] (as shown

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by a decrease in the electrical impedance) and to local anaesthetics[15] (as shown by the speed at which they act). It is therefore unlikely that the diffusional resistance is a property of one specialised cell layer. There is, however, a gradation in properties of the cell layers. Those nearest the skin surface do not adhere together as strongly as the lower ones, as shown by their easier removal on adhesive tape[21]. A chemical applied to the skin, either in solution or as an undiluted liquid, is found in disproportionately

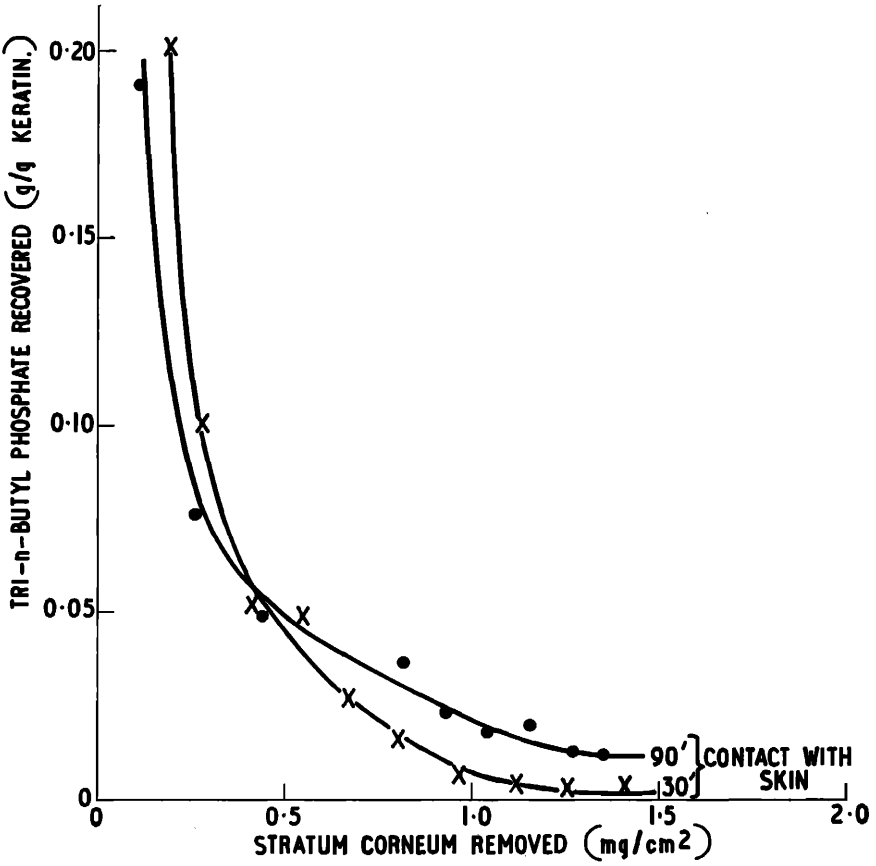

Figure 4. The mass of tri*n*butyl phosphate associated with particles of the stratum corneum which were removed by successive strippings of the skin with adhesive tape after the organic liquid had been in contact with the dorsal surface of the human forearm for 30 (x) or 90 (•) minutes.

large amounts in the upper cell layers (*Fig. 4*)[23], which indicates that the intercellular spaces are wider near the surface.

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Which components of this dead cell layer could produce a large diffusional resistance? The two obvious possibilities are lipid, forcing the penetrant to change phase, and insoluble protein, restricting the area for diffusion within the one phase. The direct relationship between the permeability of rabbit skin to an applied solute and the ether-water partition of that solute[9] indicates that there are lipid membranes through which the penetrant has to pass, and this is supported by the higher permeability of skin to drugs in the free base form rather than the salt form[15,24], and the increase in insensible water loss when the skin is washed with lipid solvents[2]. On the other hand, the low permeability of isolated membranes of stratum corneum even after prolonged immersion in petroleum ether[17] shows that the protein framework alone has a large diffusional resistance. The relatively high permeability of skin to water[1] and ions (*unpublished observations*) is not consistent with the presence of a complete lipid barrier. It is probable that both components, the fat and the protein, combine to produce the observed resistance.

Penetration could be through the cells of the stratum corneum or around them. If entry were through the cells, the amount of an applied chemical recovered from unit mass of the cells (*Fig. 4*) would be a measure of the concentration of the penetrant as it diffused through them. On this hypothesis the results show that much the largest concentration gradient, and hence the largest diffusional resistance, is in the surface layers of the stratum. This is improbable, as the membrane formed from the lower layers is very impermeable and the upper layers are comparatively friable. It follows that entry is between the cells in the upper layers. Ions probably penetrate the entire system in this way, for the electrical impedance of the skin is of the "polarisation impedance" form[25], characteristic of passage by direct current (equivalent to ionic penetration) around obstructions, presumably the cells. Covalent compounds may pass through the cell membranes and hence the cells. This subdivision is, however, highly speculative.

In conclusion, it appears that the route of entry of extraneous chemicals is through the epidermis itself rather than through its accessory structures, and that the resistance to this entry is a physical property of dead cells, uncomplicated by "active processes". The resistance does not seem to be a function of a specialised membrane, nor is it entirely dependent on any one component of the structure. It is a property of the keratinised cell matrix, effective against all molecules so far studied, and not easily destroyed save by actual removal of the cells.

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